

STRUCTURAL GENOMICS OF *Fragaria*—WILD AND CULTIVATED STRAWBERRIES

By

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To:

- my father Vadir Tombolato, who has taught me the importance of moral integrity;
- my mother, Marlene Tombolato, who has, by example, taught me persistence;
- my professor, Kevin Folta, who permitted and encouraged me to exercise those virtues.

‘Yes,’ you will say, ‘but the plank is very long.’ That is true, and so if you do not have a sure foot and a steady eye, and are afraid of stumbling, do not venture down the path.

Jean de Léry, in "History of a Voyage to the Land of Brazil, Otherwise Called America", 1578

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The extensive phenotypic variability and complex genetic makeup of the cultivated strawberry *Fragaria × ananassa* permits advances in plant improvement, a factor breeders have exploited to great benefit. However, the introgression of specific characters is complicated due to the cumbersome genetics and limited knowledge of genome structure and function of genes relevant to traits of interest. The present study represents the first genomics-level insight into strawberry genome structure and explores the hypothesis that a new type of molecular marker, the Gene-Pair Haplotype represents a transferable marker that may hasten linkage mapping in the diploid and octoploid strawberry.

My research presents the findings of four related research activities. First, an efficient and unified method for genomic DNA isolation was derived from over 100 experimental tests and conditions. Next, 1% of the *Fragaria* genome was sequenced and functionally annotated, using a bioinformatics approach and computational tools. Over 120 kb of intergenic regions were sequenced using the Gene-Pair-Haplotype approach, allowing for some initial relationships to be formulated concerning the diploid subgenome contribution to octoploid strawberry. Finally, Gene-Pair Haplotypes were used to add a suite of alleles to the growing *Fragaria* linkage map. These findings provide a starting point for further analyses of the strawberry genome.

CHAPTER 1 STRAWBERRY AND THE GENOMICS ERA

Introduction

The cultivated strawberry, *Fragaria* × *ananassa* Duch, belongs to the family Rosaceae as do the also economically important crops rose, apple, pear, peach, cherry, plum, raspberry, and almond. Linnaeus named the genus *Fragaria* due to its fragrant properties, whereas the odor, taste and berry shape was thought to be similar to pineapple, or “anasas”, in Latin (Darrow, 1966). In 1765, the *F.* × *ananassa* parentage was proposed by Antoine Nicolas Duchesne, whose father worked at the Court of Louis XV (Darrow, 1966). *F.* × *ananassa* was first observed in several countries in Europe since the 1750’s and it originated from a spontaneous hybridization between *F. virginiana* and *F. chiloensis*, both from the American continent. *F. virginiana* is thought to have been imported to Europe by two routes (Wilhelm and Sagen, 1974): to France by the explorer Jacques Cartier during his first expedition to the Quebec Canadian Province in 1534; and to England, by Thomas Hariot, who visited the “New Found Land of Virginia” in 1588. Later, in 1714, *F. chiloensis* was taken to France by the engineer Amédée François Frézier. During his mission to study the defense fortifications of Chile and Peru, Frézier noticed the large-fruited berries at Concepción, Chile, and collected several plants to take back to his country (Darrow, 1966). The result of the accidental cross between the two *Fragaria* species was the basis for the creation of the fruit cultivated and appreciated throughout the world today.

Profitable strawberry production is challenged by several factors: diseases, pests, market competition, and, arguably most importantly, by the phase-out of methyl bromide. This fumigant is considered essential for the production of many crops, including strawberry (Roskopf et al., 2005), but because methyl bromide has great stratospheric ozone depletion ability, the Montreal Protocol mandates that its use be reduced (Anonymous, 1998). Although traditional plant

breeding has been used to remedy several of the above-mentioned challenges, the knowledge of the *Fragaria* genome structure may streamline the variety improvement process, potentially permit discovery of gene function, and ultimately lead to more diverse and hypothesis-based solutions to traditional and contemporary problems not only for the strawberry but also for other Rosaceous crops.

Molecular Markers for Strawberry

The cultivated strawberry has a complex ($2n=8x=56$) (Ichijima, 1926), (Fedorova, 1946) and poorly understood genome. Despite strawberry's commercial value of 1.4 billion dollars as a fruit crop (Folta et al., 2005), substantial knowledge of *Fragaria* structural genomics before this project was virtually nonexistent. Sequence information facilitates the development of molecular markers that can be used for marker-assisted selection (Haymes et al., 1997), (Van de Weg, 1997), (Albani et al., 2004), (Sugimoto et al., 2005), (Haymes et al., 2000), (Lerceteau-Köhler et al., 2002), clone characterization in germplasm banks (Harrison et al., 1997), (James et al., 2003), identification of cultivar proprietary (Arulsekar et al., 1981), (Bringhurst et al., 1981), (Gidoni et al., 1994), (Nehra et al., 1991), (Bell and Simpson, 1994), (Hancock et al., 1994), (Levi et al., 1994), (Parent and Page, 1995), (Landry et al., 1997), (Degani et al., 1998), population genetics studies (Degani et al., 2001), (Harrison et al., 1997), (Graham et al., 1996), (Arnau et al., 2003), (Haddonou et al., 2004), and construction of genetic linkage maps (Williamson et al., 1995), (Yu and Davis, 1995), (Davis and Yu, 1997), (Deng and Davis, 2001), (Lerceteau-Köhler et al., 2003), (Sargent et al., 2003), (Sargent et al., 2004).

Pioneer molecular markers were based on polymorphisms observed on punctual loci or the whole genome: isozymes and intron length polymorphism; Randomly Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), and Amplified Fragment Length Polymorphism (AFLP). More recently, Simple Sequence Repeats (SSRs) have been

employed to address the challenge of marker transferability (Monfort et al., 2005), (Nourse et al., 2002), (Ashley et al., 2003). The present work discusses the creation of a novel marker type that, in addition to responding to the transferability necessity of modern markers, also attaches functional information to markers generated.

The Genomics Era

“Genomics” has been defined as “the study of all nucleotide sequences, including structural genes, regulatory sequences, and noncoding DNA segments, in the chromosomes of an organism.” (The American Heritage®, 2006)

The complexity of plant genomes began to be investigated in the mid- to late-1970’s using quantitative DNA reassociation kinetics (i.e. Cot curves) (Goldberg, 2001). It was determined that plant genomes had families of repetitive sequences and that these repeats varied in copy number and arrangement in the genome (Flavell et al., 1974), (Goldberg, 1978).

By the end of the 1970’s, with the ability to construct cDNA clones, there was the surprising finding that the coding regions of eukaryotic genes were interrupted by introns (Gilbert, 1978), what led to investigation of posttranscriptional splicing mechanisms (Jeffreys and Flavell, 1977).

The first plant gene was cloned in 1979 (Bedbrook et al., 1980), demonstrating that plant DNA was not different from the DNA of other organisms and therefore could be manipulated using the same enzymes, cells, and vector systems. The result was the construction of both plant genomic and cDNA libraries of many plants and organs (Goldberg, 2001).

The demonstration that *Agrobacterium tumefaciens* tumor DNA (T-DNA) integrates into the chromosomes of plant cells (Chilton et al., 1977) created the opportunity to generate transgenic plants, the first one being sunflower cells expressing bean phaseolin seed storage protein gene (Murai and Sutton DW, 1983). In addition to being a vector to foreign genes, T-

DNA began to be used to generate transformed *Arabidopsis* lines with mutant phenotypes to identify and clone important plant genes, such as genes involved in the control of meristem identity and hormone perception (Feldmann, 1991), (Feldmann and Marks, 1987). A second method to clone plant genes was devised upon the isolation of the Ac and Ds transposable elements (Fedoroff et al., 1983).

The beginning of the sequencing era can be attributed to the determination of a bacteriophage RNA gene sequence in 1972 (Min Jou et al., 1972). The first whole-genome sequencing was also from a virus, *Haemophilus influenza*, completed in 1995 (Fleischmann et al., 1995), whereas a draft of the Human Genome was released in 2001 (Venter JC, 2001). The first plant genome sequenced was *Arabidopsis thaliana*, completed in 2000 (The Arabidopsis Genome Initiative, 2000). In 2007, approximately 2300 sequencing projects are being carried out or completed, of which about 130 are plant genomes, according to the Genomes Online database (Liolios et al., 2006). Our collaborators in this *Fragaria* genomics project have successfully completed 1% of the *F. vesca* genome. Recently, *Malus* (apple) was selected for full sequencing by an Italian sequencing effort. Peach also will be sequenced through a US Department of Energy initiative. Although these genomes are much larger than the strawberry genome, their completion will have important ramifications to *Fragaria*, as annotation will provide a list of components that are similar to those in strawberry. The work presented here is a complementary effort to those in other rosaceous crops, providing an initial glimpse into the genome of one of the world's most prized horticultural crops.

CHAPTER 2 DNA EXTRACTION FROM RECALCITRANT SPECIES

Introduction

Strawberry (*Fragaria × ananassa*) is an important crop worldwide, and it supports many regional economies in the United States. However, relatively little is known about the genes that govern agriculturally important traits or their expression. Contemporary genomics tools have the potential to accelerate study of strawberry and bring additional resolution to strawberry gene form and function. Strawberry belongs to the genus *Fragaria*, a genus that includes a number of species of varying ploidy with a small haploid genome size. These facets make strawberry an excellent candidate for genomic studies representing the Rosaceae family. Because it is easily transformable, it is particularly well suited for translational-genomics studies.

Any genomics effort, whether translational, structural or functional, is generally dependent on a reproducible and effective means to isolate quality genetic material. Although protocols have been streamlined over the last several decades, it is challenging to isolate large amounts of quality DNA from strawberry (Manning, 1991; Porebski et al., 1997). A similar problem has been encountered in other species. Plants like cotton (Katterman and Shattuck, 1983; Dabo et al., 1993; Chaudhry et al., 1999; Li et al., 2001), sugarcane (Aljanabi et al., 1999), conifers (Crowley et al., 2003), tomato (Peterson et al., 1997), grape (Collins and Symons, 1992; Lodhi et al., 1994), and the rosaceous chestnut rose (Xu et al., 2004) have been reported to be recalcitrant to DNA extraction. The high content of polysaccharides and polyphenols either limit DNA isolation or inhibit downstream enzymatic reactions.

The DNA Extraction Procedure

A typical DNA extraction is accomplished by three basic steps: lysis of the cell, removal of proteins, and separation of nucleic acids from other cellular compounds. Cell lysis is easily

achieved by removal of membrane lipids with detergents such as sodium dodecyl sulfate (SDS), triisopropyl naphthalenesulfonic acid (TIPS) (Bies and Folta, 2004), and N-laurylsarcosine (sarkosyl) when extracting DNA from bacterial or animal cells; however, because plants have a solid cell wall in addition to the cellular membrane, solvents alone are not enough to expose organelles, and mechanical force must be applied. Samples can be sonicated but generally are either treated with ethyl ether (Watson and Thompson, 1986; Peterson et al., 1997; Folta and Kaufman, 2000; Peterson et al., 2000), lyophilized or frozen in liquid nitrogen to make the material more friable prior to manual grinding. Additional homogenization is performed with a Polytron or comparable tissue disruptor.

Cell lysis is carried out either as a single step, breaking open all cellular compartments simultaneously, or in a stepwise fashion, first rupturing outer membranes to expose the nucleus, then solubilizing the nuclear envelope to free nucleic acids. The first membrane lysis is induced by osmotic pressure generated by 0.35M sorbitol (Fulton et al., 1995; Hanania et al., 2004), 0.35M glucose (Chaudhry et al., 1999) or Triton X-100 (which lyses chloroplasts and mitochondria, but does not solubilize nuclear DNA) (Watson and Thompson, 1986; Peterson et al., 1997), while the second lysis is performed by detergents and ethylenediaminetetraacetate (EDTA). During this perturbation of the cell, DNA-degrading enzymes must be inhibited, which is accomplished by manipulating pH and removing divalent cations. Since DNases act at pH 7.0, Tris is added to raise the pH to between 7.5 and 8.0. The chelation of divalent cations (Ca^{2+} , Mg^{2+}) by EDTA prevents the activity of metal-dependent enzymes.

Cellular and histone proteins can be dissociated by SDS (Kay and Dounce, 1953), proteases, chaotropic agents, chloroform (Sevag et al., 1938), and phenol. Because phenol solubilizes proteins (Cohn and Conant, 1926), it has been used to deproteinize preparations of

carbohydrates (Westphal et al., 1952; Westphal and Jann, 1965) and nucleic acids (Kirby, 1956). Chaotropic agents denature proteins by increasing the solubility of nonpolar substances in water (Voet et al., 1998). Hofmeister (Hofmeister, 1888) defined the series of anions and cations with increasing protein destabilizing properties when he measured the concentration of various salts needed to precipitate proteins from whole egg white (translated by (Kunz et al., 2004)). According to the Hofmeister series, urea, guanidinium, thiocyanate (Sawyer and Puckridge, 1973) and perchlorate (Wilcockson, 1973) are extremely chaotropic agents. Thus, high concentrations of urea (Settles et al., 2004), guanidine hydrochloride (Logemann et al., 1987), and guanidine thiocyanate have been used in isolation of RNA (Cox, 1968; Chomczynski and Sacchi, 1987) and DNA (Chomczynski et al., 1997).

Chemical or physical means such as precipitation by isopropanol, ethanol, butoxyethanol (Manning, 1991), acetone (Vogelstein and Gillespie, 1979), adsorption to silica (Vogelstein and Gillespie, 1979), paramagnetic particles (Anonymous, 1980, 2001; Koller and al., 2001), and ion exchange resin (QIAGEN Anion-Exchange Resin manual) can be utilized to retrieve DNA from solution. The resin is coated with diethylethanolamine (DEAE), and DNA recovery is due to interaction between negatively charged phosphates of the DNA backbone and positively charged DEAE groups. In the case of silica columns, DNA is recovered from solutions because it tends to adsorb to silica in the presence of chaotropic salts, such as sodium iodide (NaI) (Vogelstein and Gillespie, 1979), guanidine thiocyanate, and guanidine hydrochloride. The binding capacity depends on the solution's ionic strength and pH, being higher in concentrated solutions and at $\text{pH} < 7.5$ (GeneClean Manual). Silica columns have been used to eliminate polysaccharide contaminants, and the ratio A_{260}/A_{230} increases as polysaccharides are removed (Abdulova et al., 2002).

DNA Extraction from Plants

Pioneer methods to isolate genetic material of plants used DNA-rich matter such as germ tissue (Lipshitz and Chargaff, 1956; Shapiro and Chargaff, 1960). Early attempts to extract DNA from leaves resulted in degraded product due to the extreme pHs used by the procedure for removal of RNA (Thomas and Sherratt, 1956). The currently most used protocol for plant DNA isolation, developed by Murray and Thompson (Murray and Thompson, 1980), takes advantage of the selective precipitation of DNA by cetyltrimethylammonium bromide (CTAB), a phenomenon observed by Jones during DNA isolation from bacteria (Jones, 1953). CTAB is a cationic detergent that, in high ionic strength solutions (e.g. >0.7M NaCl), complexes with proteins and non-acidic polysaccharides, whereas at low ionic strength it precipitates nucleic acids and acidic polysaccharides, leaving proteins and neutral sugars in solution (Sambrook and Russell, 2001). Multiple variations of Murray and Thompson's protocol have been used by researchers to adapt the original process to different plant species. A protocol designed by Doyle and Doyle (Doyle and Doyle, 1987) is also frequently used for plant DNA extraction and is ultimately a variation of the Murray and Thompson procedure. Doyle and Doyle's protocol uses fresh tissue in place of lyophilized material and a higher concentration of CTAB and salt to compensate for the greater water content of fresh tissue.

Although CTAB is the reagent of choice to purify DNA from organisms that produce many polysaccharides (Sambrook and Russell, 2001), even high quantities of the cationic detergent seem insufficient to free DNA preparations from sugar contamination. In attempt to circumvent this problem, boric acid is added to the extraction buffer. Boric acid forms complexes with polyphenols at pH 7.5 (King, 1971) and with carbohydrates (Gauch and Dugger Jr., 1953), making these complexes more soluble. An additional approach to avoid co-purification of

polysaccharides during DNA isolation is to differentially precipitate the sugars by manipulating the 2-butoxyethanol concentration (Manning, 1991).

Cytoplasmic compounds come into contact with nuclei contents when cells are disrupted and the oxidized polyphenols covalently link to DNA (Loomis, 1974), restraining subsequent DNA manipulation (Katterman and Shattuck, 1983). Reducing agents like β -mercaptoethanol, dithiothreitol, ascorbic acid, sodium bisulfite, and diethylcarbamate can be added to the extraction buffer to inhibit the oxidation process and protect DNA from quinones, disulfates, peroxidases, and polyphenoloxidases. Polyvinylpyrrolidone (PVP) and its insoluble, cross-linked form, PVPP (Gegenheimer, 1990), also protect DNA from phenolics and alkaloids by sequestering them. Additional approaches to avoid problems caused by phenolics like freezing tissue prior to homogenization (Katterman and Shattuck, 1983; Leutwiler et al., 1984), purification by cesium chloride gradient (Travaglini and Meloni, 1962; Williamson, 1969; Murray and Thompson, 1980), and extraction of DNA from isolated nuclei (Hamilton et al., 1972; Katterman and Shattuck, 1983; Watson and Thompson, 1986; Peterson et al., 1997) have been used.

As genomics tools become more common in strawberry research, it is imperative to devise a standard protocol that is effective across cultivars and species of different ploidy levels. Examination of the literature on strawberry (*Fragaria* spp.) indicates that the many published DNA isolation methods are not universally transferable between cultivars or species. An optimal protocol should use readily available plant material (such as mature leaves), be inexpensive, rapid, reproducible, and have high yields of high molecular weight DNA, amenable to downstream manipulation. Of all these traits, quality is most important, yield second in importance, followed by cost and ease of protocol.

Material and Methods

Thirty-three DNA extraction protocols, totaling 103 treatments, were tested using either lyophilized or liquid nitrogen-frozen leaf tissues. A broad range of genotypes were tested, including tissue from *F. nubicola*, *F. vesca* cultivars Yellow Wonder, Alexandria, and Hawaii-4, *F. chiloensis* CA 1367, *F. virginiana* NC 96-35-2, *F. × ananassa* cultivars Sweet Charlie, Tristar, Camarosa, Quinault, Diamante, Strawberry Festival, and the laboratory transformation genotype LF9 (Folta et al., 2006). The detailed protocols can be found in Appendix A, whereas further below is a summary of the approaches adopted. When at least 15µg of DNA were obtained, digestion of 5µg of DNA with at least 2 separate restriction enzymes were carried out. The uncut and enzyme treated samples were loaded on 1% agarose gel for assessment of DNA quality (integrity and amenability to use of restriction enzymes), and correlation to spectrophotometric readings. Phenols are known to absorb at 260nm as does DNA, and high readings may be attributed to the presence of phenols, particularly when the DNA pellet has brown coloration, caused by oxidation of phenolic compounds (phenylpropanoid and flavonoids) to quinones (Loomis, 1974). To further test the quality of the DNA preparations, PCR was carried out using primers for *F. × ananassa* 18S ribosomal DNA. The primers (forward: 5' TAT GGG TGG TGG TGC ATG GC 3'; reverse: 5' TTG TTA CGA CTT CTC CTT CC 3') were designed utilizing as sequence source the accession gi|18448|emb|X15590.1|FA18S. The fragment to be amplified by this primer pair is not large (510bp from cDNA, ~1kb from genomic) and should be easily amplified, since many copies of ribosomal DNA are present in the genome. If a product was observed, a second set of primers (forward: 5' CAC TGC CAA GGA GCG TGG TG 3'; reverse: 5' TCA GTA GGG CAG CTG ATG 3') targeting a single-copy region, the *Leafy* gene, was used to provide a more challenging test. This second primer pair was designed utilizing *F. vesca* 'Pawtuckaway' sequence provided by our collaborator, Dr. Thomas

M. Davis, and encompassed a 770-nucleotide region. Both PCR reactions were carried out for 35 cycles, with 55°C as annealing temperature, and 1min as extension at 72°C.

The original CTAB protocol designed by Murray and Thompson (Murray and Thompson, 1980) is extremely laborious, requiring a long centrifugation period in a cesium chloride (CsCl) gradient. Since the aim of this project was to develop a rapid, practical method to extract DNA, the CsCl step was omitted from all DNA extraction attempts. Further modifications of the protocol were tested systematically to pyramid the beneficial aspects of each preparation into a unified and effective means to generate high-quality DNA for downstream analysis as described below:

- CTAB was tested at 1, 2, 6, and 20%
- Inclusion of one or combinations of the following reagents to prevent DNA oxidation: 0.01% -1% sodium (bi)sulfite, 5mM ascorbic acid, 1-4% PVP
- EDTA concentration from 10mM (as proposed by Murray and Thompson) to 200mM
- Tris concentration ranged from 50mM (as in original protocol) to 200mM. The pH was adjusted to 8.0 by addition of HCl. In cases where boric acid was used to adjust the pH, the Tris-borate solution was brought to pH 7.6 because at that pH, boric acid forms complexes with polyphenols
- The original protocol removes proteins by treating the solution with 24:1 chloroform:octanol. Alternative deproteination methods tested were: 25:24:1 phenol:chloroform:isoamyl alcohol, 1M sodium perchlorate, and 150µg/ml proteinase K
- DNA was recovered by either adsorption to silica, or precipitation by ethanol, isopropanol, 2-butoxyethanol, or 5M potassium acetate. In Murray and Thompson's original protocol, DNA is precipitated by decreasing salt concentration
- Attempts to remove water-soluble contaminants by adsorption to silica column (QIAGEN DNeasy kit) and by dialyses of DNA solution into TE pH 7.0 at 4°C
- Instead of adding buffer subsequent to grinding the plant tissue, an additional tissue/buffer homogenization step was performed. An aliquot of the final buffer was used to either produce a tissue/buffer paste in the mortar and pestle or Polytron homogenizer
- In place of the standard incubation in buffer at 50-60°C for 20-30 minutes, incubation was carried out at 4, 20, 42, and 65°C for 0, 5, 30, and 60 minutes. In order to eliminate

variability that may be induced because of the leaves of various ages, leaves were cut with a hole puncher, mixed, and split into 4 portions, one for each temperature treatment. Enough plant tissue was ground per temperature treatment so that 2 experimental replicates for each time treatment were derived from a single test tube (see figure 2-1).

In addition to variations of the CTAB protocol, other approaches adopted included use of the chaotropes 8M urea, 4M guanidine thiocyanate (alone or in combination with 2% CTAB, simultaneously or sequentially); DNA isolation using kits: QIAGEN DNeasy Plant Mini Kit (charged resin-based), Molecular Research Center DNAzol® Extra Strength (guanidine thiocyanate-based), Epicentre MasterPure™ Plant Leaf DNA Purification, MoBio PowerPlant™ DNA Isolation Kit; 0.5% SDS, Tris-borate extraction buffer; and crude and fine isolations of nuclei prior to DNA extraction. Five DNA extraction procedures, QIAGEN DNeasy kit, 2% CTAB, 2% SDS, 4M guanidine thiocyanate/1% sarkosyl, and 5% SDS/1%TIPS, were tested on Percoll gradient-isolated nuclei. Refer to table 2-1 for all the treatments.

The amount of tissue necessary to obtain the highest DNA extraction efficiency was determined by keeping the volume of buffer constant at 5ml and varying the tissue weights at 50, 200, 500, and 1,000mg. Once the best tissue-to-buffer ratio was determined, an attempt to extract DNA from 10 species within the genus *Fragaria* was made to test the universality of the method. Each treatment had 2 replicates for both experiments. Expanded leaf tissue was ground in liquid nitrogen, added to the buffer, and the mixture was incubated at 4°C for 5 minutes. An equal volume (5ml) of 24:1 chloroform:octanol were added to the tubes after incubation, agitated, and centrifuged at 4,000rpm for 5 minutes. The aqueous phase was transferred to a new tube, and nucleic acids precipitated by 1/10 volume of 5M NaCl and 7/10 volume of isopropanol. After a second centrifugation, the supernatant was decanted, the pellet air-dried, and resuspended in 500µl water. RNase was added to final concentration of 50µg/ml. The solution was transferred to 1.5-ml tubes and DNA was precipitated as described above. The dry DNA pellet was

resuspended in 200µl water and DNA quantities were estimated by a NanoDrop ND-1000 spectrophotometer.

Nucleic acids were extracted from 96 individuals that belong to a diploid *Fragaria* mapping population. Minimal quantities of lyophilized tissue were processed, ranging from 3 to 14mg (average = 6.44mg, standard deviation = 1.98). Because the buffer volume was kept constant, there was an opportunity to further study tissue-to-buffer ratios, under different conditions from those tested above. This time, tissue was macerated in buffer after having been ground in liquid nitrogen and incubated at 65°C for 1 hour. The absorbance values at 230, 260, and 280nm were determined by a NanoDrop to make inferences about nucleic acid purity. Absorbance ratios A260/A230 and A260/A280 are measures of contamination by polyphenols or carbohydrates (Craigie and McLachlan, 1964; Logemann et al., 1987), cited by (Manning, 1991) and protein, respectively. The ultimate usefulness of each sample was determined by PCR with two primer pairs in separate reactions—*leafy* primers amplify a short fragment of 770 nucleotides; 72E18 challenged amplification, for it is a relatively long fragment of 2622 nucleotides. Like primers for *leafy*, primers 72E18 (Fb: GCT AGG GAA AAC AGC TCG TG; Rb: TGG GTT TGG TTT TGG GAT AA) were designed for *F. vesca* cv. ‘Pawtuckaway’ and are transferable to *F. nubicola*.

Results

The majority of the protocols tested either failed to render appreciable amounts of DNA from mature plant leaf tissue, or yielded plenty of material that was not amenable to further manipulations, such as restriction digestion or PCR (data not shown). However, a variable previously considered minor had an unexpectedly great impact in the retrieval of nucleic acids: further maceration of tissue in extraction buffer. Most of these preparations do not separate DNA

from RNA, so quantification is generally a combination of nucleic acids. This is important for two reasons. First, the RNA isolation protocols for strawberry are principally revisions of DNA extraction methods. Those that yield high amounts of RNA also contain proportionate amounts of DNA, and RNA is removed with selective LiCl precipitation. In these preparations RNA and DNA recovery is generally parallel and so quantification of both as “nucleic acids” provides a general measure of DNA recovery. Also, in an attempt to identify an efficacious method, the step of removing RNA, and verifying its removal would limit the number of protocols and experimental conditions that could be tested.

Table 2-1 lists yields from the different DNA isolation protocols described in the Appendix A. Different numbers of treatment replications and amounts of plant tissue were used in the DNA extraction attempts. Therefore, to allow comparison between treatments, values for yield shown in the table are averages of replications, standardized using 1 g of plant tissue as the denominator. Table 2-2 ranks the four methods that had highest nucleic acid returns per g of tissue. Control samples were excluded from the calculation of averages. For example, T85 was a control in protocol 30—tissue was not macerated in buffer. Because the factor in question was the formation of slurry due to maceration, T85 was excluded from the calculation of the average for “slurry” protocols.

Although the “strawberry protocol” permitted extraction of nucleic acids 10 times greater than CTAB-based methods, DNA obtained through the former protocol cannot be digested by restriction enzymes or PCR-amplified by primers for the 18S ribosomal DNA. The DNA remains intractable even after treatment with proteinase K and subsequent dialysis. Similar situations occurred with DNA extracted by CTAB/Tris-borate or guanidine thiocyanate. Only after purifying the guanidine thiocyanate prep utilizing the DNeasy Plant Mini kit, did the DNA

become PCR-amplifiable. It is interesting to note that the difference in spectrophotometer readings before and after the purification was minor (treatments 8 *versus* 10), suggesting that the kit may be a viable alternative to other methods used to purify PCR-recalcitrant DNA.

The 4th highest ranked protocol type in table 2-2 is in fact the only one of the four listed that resulted in tractable DNA. Of the many CTAB protocols that were investigated, the ones that required maceration of plant tissue in buffer cluster together at the top in terms of μg of nucleic acid obtained per gram of tissue (presented later in Figure 2-6).

Components of the “Strawberry Protocol”

Because the strawberry protocol had such high yield relative to the other methods tested, attempts to determine the reason for its superiority were made. The objective was to discover the variable responsible and incorporate it into a protocol that would yield DNA amenable to enzymatic reactions. The factors tested were: i, nucleic acid precipitation by 2-butoxyethanol; ii, boric acid (rather than HCl) used to adjust the pH of Tris for the extraction buffer; iii, second round of extraction from plant tissue after chloroform treatment; iv, dilution of upper phase with Na^+ solution before DNA precipitation.

Treatments T30-T37 (comparing precipitations by isopropanol against 2-butoxyethanol) verified that the latter has a detrimental effect on DNA precipitation. Considering all 4 experimental variables, 65 to 200% more nucleic acids were recovered by isopropanol rather than by 2-butoxyethanol precipitation.

The absolute importance of boric acid to nucleic acid isolation has not been tested, though borate appears to contribute to higher yields when in combination with other factors. In the extractions using guanidine thiocyanate, borate-containing buffer (T36) had on average 10x higher yield than HCl-containing buffer (T8, T9). However, this increase may be attributed to the different tissue-to-buffer ratios among treatments. A second comparison, this time between

CTAB buffers, strengthens the argument for the contribution of borate: T30 (Tris-borate) *versus* T82 (Tris-HCl), where T30 had a tissue-to-buffer ratio = 16mg/ml and T82 had the ratio that was determined to be optimum for DNA extraction (illustrated in figure 2-2). Perhaps borate was at least partially responsible for T30's 25x greater yield than with T82. When used in substitution to Tris, though, boric acid alone was not able to increase the retrieval of nucleic acids. T38 (1M boric acid, no Tris) was a similar treatment to T30, but the yield was 60x lower.

A second round of extraction from plant tissue increased approximately 50% the DNA recovery relative to a single incubation in extraction buffer. T34 and 35 yielded 60 and 45% of single-extraction treatments T32 and T33, respectively. Although this may be a considerable increase, it is not the sole factor responsible for the dramatic advantage of the strawberry protocol (3 times higher yield than the 2nd highest ranked protocol).

The dilution of the aqueous phase also plays an important role in the recovery of nucleic acids. Observing the results for treatments T24, T27: no dilution; T25, T28: dilution by 2.5 volumes of Na⁺ solution (detailed in Appendix A); T26, T29: dilution by 4 volumes, it became apparent that the 2.5 volumes were superior to the other two, in a ratio of 50:125:1 (no dilution : 2.5vol : 4vol).

Optimization of the CTAB Protocol

Protocols containing CTAB in the extraction buffer produced the highest yield of tractable DNA. Therefore, an optimum protocol was devised to further investigate the following factors: leaf tissue state, incubation temperature and duration, tissue-to-buffer ratio, leaf tissue maceration.

Leaf tissue state

DNA was extracted from the same mass of fresh and lyophilized tissues. As expected, yield per gram of sample was generally higher from lyophilized samples. However, this likely is

due to the higher number cells that contained in freeze-dried samples in comparison to the same weight of fresh tissue. While yield from T58 was not different from that of T59, increases of 73 and 50% were observed in T13-T16.

There was concern that the lyophilization process might compromise DNA quality. This was addressed by running uncut genomic DNA on agarose gel, and the integrity of all lyophilized samples (T13, T14, T23, and T57) appeared preserved. Therefore, lyophilization may be a good solution for storing material that does not require immediate DNA extraction, but it is not indispensable.

Incubation temperature and duration

Utilizing fresh ‘Strawberry Festival’ leaf tissue, the effects of temperature and duration of incubation of tissue in extraction buffer were investigated. The treatment that relinquished the most DNA was incubation at 65°C for 1 hour (figure 2-2), which is the treatment specified in most plant DNA extraction protocols. However, the resultant preparation at this temperature is atypically viscous, complicating mechanical and enzymatic downstream manipulations.

Tissue-to-buffer ratio

Tissue-to-buffer ratios were tested for four protocols (2, 5, 14, 23; ratios and yields shown in table 2-1), and yielded inconsistent results. For protocols 2 and 14, the lower the ratio, the higher the yield, whereas for protocols 5 and 23, the opposite was true. Since all of the ratios (10-200 mg/ml) tested did not use the same protocol, a last DNA extraction experiment was conducted using leaf tissue of ‘Strawberry Festival’. Volumes of extraction buffer were kept constant at 5 ml, whereas the treatments were 50, 200, 500, or 1000 mg of fresh tissue. Each treatment included two replicates, and incubation was carried out at 4°C for 5 min. Samples were treated with RNase A, DNA was precipitated by isopropanol and resuspended in deionized

water. Figure 2-3 illustrates the result of the optimization of the tissue-to-buffer ratio, where the optimum observed was at 40 mg of fresh tissue per milliliter of buffer.

Using the optimum tissue-to-buffer ratio determined in the experiment above (40 mg/ml), the procedure of extracting DNA with incubation at 4°C for 5 min was tested on ten strawberry cultivars, 2 replicates each. ‘Strawberry Festival’ was included as a control. DNA recovery was dependent of plant species and cultivars (table 2-3). Plants with rigid leaves, such as *F. chiloensis* and the more *F. chiloensis*-like *F. × ananassa* ‘Diamante’ had negligible yields. Perhaps solely grinding leaves in liquid nitrogen is not sufficient to break down the cells and expose contents to the extraction buffer solvents.

An attempt to determine the optimum tissue-to-buffer ratio for lyophilized tissue was made utilizing material from a *Fragaria* diploid mapping population. Tissue weights varied from 3 to 14 mg, with average of 6.8 mg and standard deviation of 2 mg. Tissue was macerated in liquid nitrogen and, subsequently, in extraction buffer for approximately 30 s. Grinding in buffer was conducted until the material was the consistency of paste. Incubation was performed at 65°C for 1 hour. No correlation between amount of tissue processed and DNA recovered was apparent (figure 2-4).

PCR was performed using 1µl of the extracted DNA at variable nucleic acid concentrations (40ng/µl to 4.5µg/µl) and the primer pairs designed for the *Leafy* gene: FvLFYintron2F (5’ CAC TGC CAA GGA GCG TGG TG 3’) and FvLeafy3’ (5’ TCA GTA GGG CAG CTG ATG 3’). Due to inability to PCR-amplify 50% of the diploid mapping population samples, an effort was made to monitor for correlations between PCR outcomes and i, nucleic acid concentration in the sample (figure 2-4); ii, tissue-to-buffer ratio during DNA extraction (figure 2-4); and iii, A260/A230 ratios (figure 2-5) that could be indicative of carbohydrate contamination. The

absorbance ratios at 260nm and 230nm wavelengths were grouped into seven categories, and the number of samples in each category is indicated in figure 2-5.

No conclusive correlation between success of amplification reaction and any of the three variables cited above could be determined. Although not statistically analyzed, subjective evaluation indicated no need to apply statistical techniques. Surprisingly, there was no pattern suggesting a relationship between template concentration and PCR amplification. This outcome indicates that other factors are contributing to inhibition of the process. In an attempt to dilute a possible polymerase inhibitor, lower tissue-to-buffer ratios were tested. However, no correlation between ratios and PCR outcome was apparent, since all permutations were detected:

amplification was observed for both low and high tissue-to-buffer ratios; lack of amplification was also observed for both low and high ratios. Regarding the A260/230, according to Manning (Manning, 1991), the ratio 1.8 indicates the purest nucleic acid sample. From the samples that were classified in this category (47 samples out of 91), 2/3 of them were amenable to amplification. Amplification was also observed for both extreme A260/230 ratios: 0.6 and 6.2. Therefore, the ratio either is a poor estimator of polysaccharide inhibition, or the polymerase inhibition was caused by polyphenols or other indeterminate factors. These trials indicate that there is no simple measure that serves as an indicator of a sample's potential to be used successfully in downstream applications.

Tissue maceration method

The processes of breaking leaf tissue down solely in liquid nitrogen *versus* preliminary pulverization in liquid nitrogen with subsequent grinding in buffer were compared. Formation of slurry by maceration of tissue in buffer not only increased the yield by many fold (table 2-4 A), but also permitted the extraction of allegedly purer DNA, indicated by the lower absorbance at 230nm (figure 2-6). The most prominent absorbance peak at 260nm was observed for samples

that were processed at 60°C and ground in buffer (figure 2-6). Samples macerated this manner and incubated at 4°C appear to contain many polysaccharide contaminants, as a peak is seen at 230nm. The desired A260:A230 and A260:A280 ratios are equal to 1.80. Samples that were ground in liquid nitrogen only and incubated at 4°C absorbed more at 230nm than 260nm (ratio = 0.61, table 2-4), indicating that they probably had low content of nucleic acids.

Due to the extraordinary increase in DNA content by the maceration procedure, several treatments combining speed (1/2, full) and duration (5, 15, 30, 60, 120 seconds) of homogenization with a Polytron were investigated. Incubations post-homogenization were carried out at 65°C for 1hour. The more aggressive the treatment, the higher the amount of DNA obtained (figure 2-7). None of the samples appeared degraded on 1% agarose gel, DNA was digestible by restriction enzymes and amenable to PCR amplification with *Leafy* primers.

Discussion

The profound effect on nucleic acid yield by the aggressive maceration method suggests that the cell wall plays a major role in preventing DNA isolation. This hypothesis is further substantiated by the lower DNA yields observed for genotypes that contain harder leaves with a glossy, conspicuous cuticle, such as *F. chiloensis* and ‘Diamante’ (table 2-3). However, when the cell wall was removed prior to DNA extraction, DNA extraction from isolated nuclei did not present appreciable yields. It is possible that the isolated nuclei were not pure and therefore the number of nuclei used for DNA extraction was overestimated, explaining the low yield observed.

Guanidine thiocyanate has been used in nucleic acid isolation for a variety of plants. The compound is known to act as protein denaturant by breaking intramolecular hydrogen bonds (Kauzmann, 1954) and, therefore, it causes inhibition of enzyme activity. We hypothesized that the lack of amplification by PCR and digestion by restriction enzymes occurred due to the presence of this chaotropic salt in the DNA preparation. To test this hypothesis, two approaches

were adopted to purify the DNA from the guanidine thiocyanate: DNA adsorption to a silica column and dialysis of the DNA preparation. DNA purified by the first method rendered tractable DNA, whereas DNA remained unsuited for enzymatic reaction after dialysis. When isolated by the “strawberry protocol” proposed by Manning, DNA was also intractable even after treatment with proteinase K and dialysis. Therefore, it is possible that the co-purified guanidine thiocyanate or other inhibitors are retained in the dialysis tube. A modification of DNA during the extraction procedure was considered as a possible explanation to enzyme activity inhibition, but the fact that previously intractable DNA purified by a silica column permits amplification by PCR refutes this idea.

The disappearance of an absorbance peak at 230nm when incubation was carried out at higher temperatures (figure 2-6) may be explained by the solubilization of sugars. At lower temperatures, the sugars are present and are not solubilized by the extraction buffer, therefore are carried throughout the remaining steps of the DNA extraction protocol. Their solubilization in the early phase favors production of a purer product.

When considered together it is clear that many variables have no effect on yield. Whereas many protocols alter CTAB concentration, Na concentration, method of precipitation, additional organic extraction and use of affinity matrices, it is clear that concurrent physical and chemical disruption of cells is the most critical parameter in the generation of pure genomic DNA suitable for downstream manipulations.

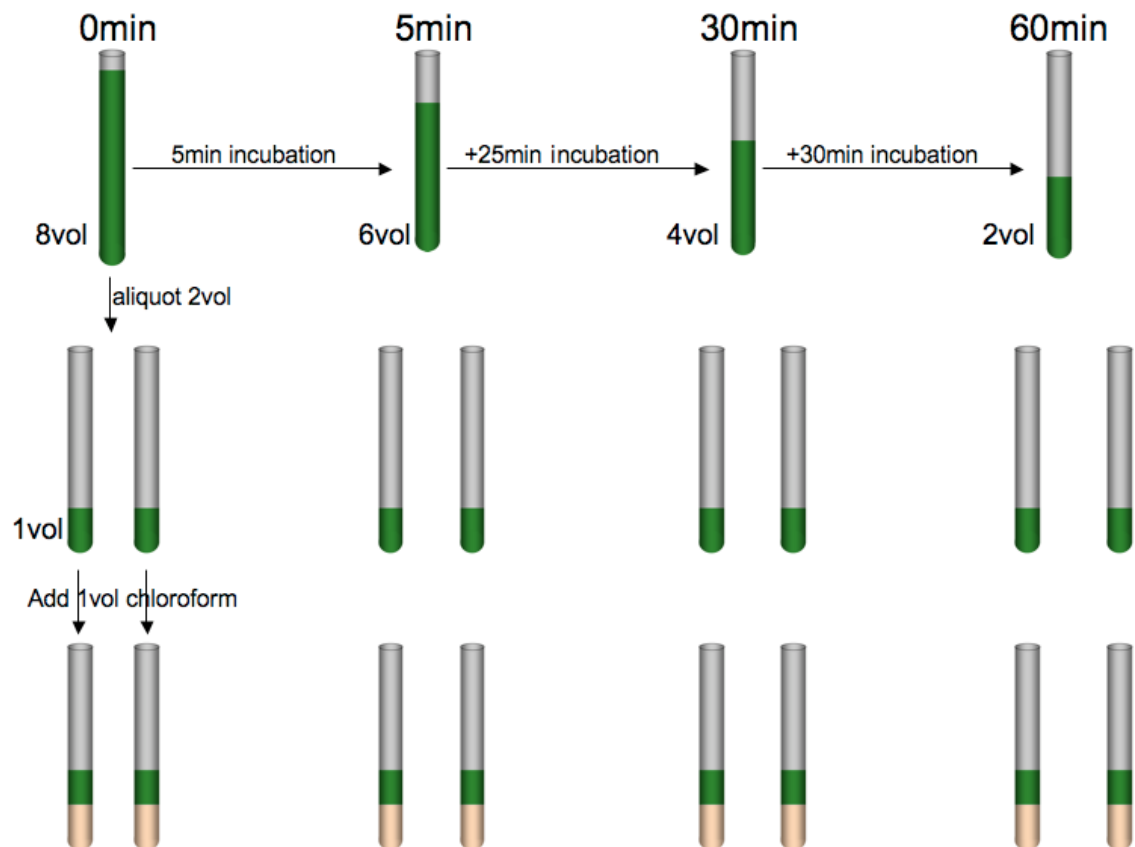


Figure 2-1. Design of incubation temperatures and durations experiment. The scheme illustrated above was followed for each of the incubation temperatures of 4, 20, 42, and 65°C. Samples for a specific temperature were ground and homogenized together to decrease random variation between time points.

Table 2-1. Nucleic acid yields from isolation protocols. P: Protocol number as listed in Appendix A; T: Treatment number; Status: condition of leaves prior to DNA isolation. F: fresh, L: lyophilized; T/B: tissue-to-buffer ratio (mg of tissue per ml of buffer). n/a: not applicable; Yield: μg of nucleic acids obtained if 1 g of tissue had been used for DNA isolation

P	T	Status	T/B	Yield	Brief Description
				$\frac{\mu\text{g}_{\text{nucl ac}}}{\text{g}_{\text{tissue}}}$	
1	1	F	100	0	Nuclei crude isolation
	2	F	200	0	Nuclei crude isolation
	3	F	400	0	Nuclei crude isolation
2	4	F	100	3	PEG
	5	F	100	1	PEG
	6	F	10	235	PEG
	7	F	10	232	PEG
3	8	F	200	112	Guanidine thiocyanate, newly expanded leaf
	9	F	200	774	Guanidine thiocyanate, unexpanded leaf
	10	F	200	96	T8 cleaned by QIAGEN kit
	11	F	200	11	T8 cleaned by dialysis
4	12	F	1000	35	Guanidine thiocyanate, CTAB consecutively
5	13	L	20	450	Guanidine thiocyanate, CTAB simultaneously
	14	L	200	750	Guanidine thiocyanate, CTAB simultaneously
	15	F	20	260	Guanidine thiocyanate, CTAB simultaneously
	16	F	200	500	Guanidine thiocyanate, CTAB simultaneously
6	17	L	66	0	DNAzol® kit by Molecular Research Center, Inc
	18	L	333	0	DNAzol® kit by Molecular Research Center, Inc
	19	F	66	0	DNAzol® kit by Molecular Research Center, Inc
	20	F	333	0	DNAzol® kit by Molecular Research Center, Inc
7	21	F	70	15	Pine tree <i>minus</i> lithium chloride
8	22	F	400	30	Urea
	23	L	50	580	Urea + antioxidants
9	24	F	15	5000	No dilution
	25	F	15	15000	2.5vol dilution
	26	F	15	120	4vol dilution
	27	F	30	8200	No dilution
	28	F	30	18800	2.5vol dilution (not amenable to restriction digestion, even after treatment with proteinase K and dialysis)
10	29	F	30	150	4vol dilution
	30	F	16	5700	Tris-borate, isopropanol
11	31	F	16	3130	Tris-borate, 2-butoxyethanol
	32	F	25	3515	1 st extraction, isopropanol
	33	F	25	1190	1 st extraction, 2-butoxyethanol
	34	F	25	2135	2 nd extraction, isopropanol
	35	F	25	545	2 nd extraction, 2-butoxyethanol
12	36	F	16	4300	Guanidine thiocyanate/Tris-borate, isopropanol
	37	F	16	2600	Guanidine thiocyanate/Tris-borate, 2-butoxyethanol
13	38	F	20	90	1M Boric acid, no Tris
14	39	F	33	50	Epicentre kit

Table 2-1. continued

P	T	Status	Yield		Brief Description
			$\frac{\text{mg}_{\text{tissue}}}{\text{ml}_{\text{buffer}}}$	$\frac{\mu\text{g}_{\text{nucl ac}}}{\text{g}_{\text{tissue}}}$	
	40	F	100	15	Epicentre kit
	41	F	333	5	Epicentre kit
15	42	F	635	0	Mo Bio kit
16	43	F	125	8.5	Qiagen DNeasy kit
17	44	F	2.5	150	Silica
	45	F	25	40	Silica
18	46	F	n/a	18	Isolated nuclei, Qiagen DNeasy kit
	47	F	n/a	5	Isolated nuclei, Qiagen DNeasy kit
19	48	F	n/a	12	Isolated nuclei, CTAB
	49	F	n/a	14	Isolated nuclei, CTAB
20	50	F	n/a	3	Isolated nuclei, SDS
	51	F	n/a	1	Isolated nuclei, SDS
21	52	F	n/a	0	Isolated nuclei, guanidine thiocyanate
22	53	F	n/a	0	Isolated nuclei, SDS, TIPS
23	54	F	14	0	Murray and Thompson + solid CTAB, ppt by low ionic strength
	55	F	70	25	Murray and Thompson + solid CTAB, ppt by low ionic strength
	56	L	14	0	Murray and Thompson + solid CTAB, ppt by low ionic strength
	57	L	70	1300	Murray and Thompson + solid CTAB, ppt by low ionic strength
24	58	F	66	45	Murray and Thompson, 6% CTAB, ppt by low ionic strength
	59	L	66	50	Murray and Thompson, 6% CTAB, ppt by low ionic strength
25	60	L	1.6	1250	Murray and Thompson, precipitation by ethanol
	61	L	8	60	Murray and Thompson, precipitation by ethanol
	62	L	16	100	Murray and Thompson, precipitation by ethanol
26	63	F	250	0	Murray and Thompson, 5% CTAB, ppt by isopropanol
	64	F	250	1	Murray and Thompson, 5% CTAB, ppt by isopropanol
27	65	F	100	0	CTAB + SDS
	66	F	100	0	CTAB + SDS
28	67	F	40	16	4°C, 0min
	68	F	40	80	4°C, 5min
	69	F	40	98	4°C, 30min
	70	F	40	69	4°C, 60min
	71	F	40	34	20°C, 0min
	72	F	40	28	20°C, 5min
	73	F	40	95	20°C, 30min
	74	F	40	142	20°C, 60min
	75	F	40	28	42°C, 0min
	76	F	40	41	42°C, 5min
	77	F	40	34	42°C, 30min
	78	F	40	57	42°C, 60min
	79	F	40	41	65°C, 0min
	80	F	40	25	65°C, 5min
	81	F	40	76	65°C, 30min
	82	F	40	211	65°C, 60min
29	83	F	75	387	Unexpanded leaf
	84	F	75	28	Expanded leaf

Table 2-1. continued

P	T	Status	Yield		Brief Description
			$\frac{\text{mg}_{\text{tissue}}}{\text{ml}_{\text{buffer}}}$	$\frac{\mu\text{g}_{\text{nucl ac}}}{\text{g}_{\text{tissue}}}$	
30	85	F	100	92	Powder
	86	F	100	400	Slurry
31	87	F	50	2476	Slurry, 4°C
	88	F	50	300	Powder, 4°C
	89	F	50	3048	Slurry, 60°C
	90	F	50	700	Powder, 60°C
32	91	F	40	1400	2% CTAB
	92	F	40	1450	6% CTAB
	93	F	40	665	20% CTAB
33	94	F	40	660	No polytron
	95	F	40	1000	½ speed, 5sec
	96	F	40	940	½ speed, 15sec
	97	F	40	1155	½ speed, 30sec
	98	F	40	1605	½ speed, 60sec
	99	F	40	955	Full speed, 5sec
	100	F	40	975	Full speed, 15sec
	101	F	40	1335	Full speed, 30sec
102	F	40	1455	Full speed, 60sec	
103	F	40	2245	Full speed, 120sec	

Table 2-2. Ranking of 4 best nucleic acid extraction protocols

Average μg nucleic acid/g tissue	Treatments included in average calculation	Protocol #	Protocol type
11,750	T24, T25, T27, T28	9	Strawberry
4,415	T30, T31	10	CTAB with tris/borate
3,450	T36, T37	12	Guanidine thiocyanate
1,232	T83, T84, T86, T87, T89, T91-T103	29-33	CTAB with slurry

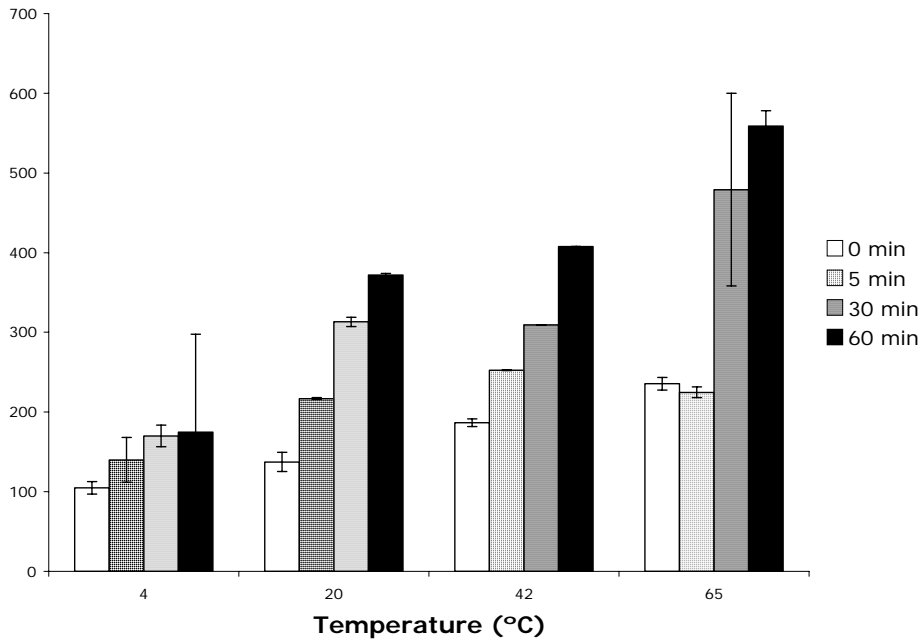


Figure 2-2. Effect of incubation temperature and time on DNA yields. The standard plant DNA extraction procedure of carrying out incubation at 65°C for 1 hour displayed, as expected, superior yields to other incubation time lengths and temperatures.

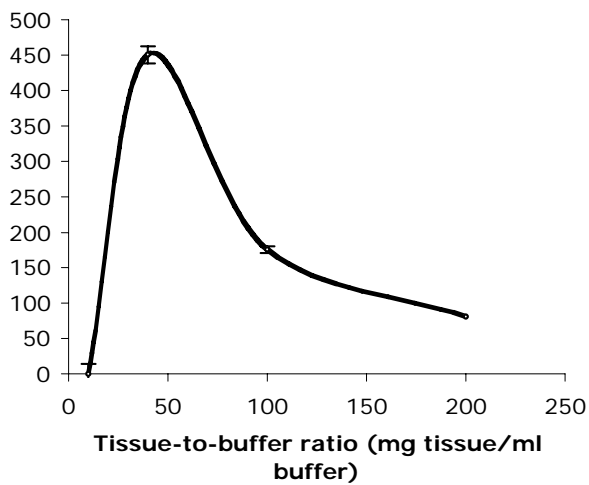


Figure 2-3. Effect of tissue-to-buffer ratios on DNA yields. The optimum ratio for DNA isolation was 40 mg of leaf tissue per ml of extraction buffer. The yield declined rapidly as more tissue was processed by the same volume of buffer.

Table 2-3. DNA yields ($\mu\text{g DNA}$) from ten strawberry genotypes. Plant tissue incubation with the extraction buffer was carried out at 4°C for 5min. Averages of 2 replicates, 200mg tissue each, extracted by 5ml buffer.

Genotype	$\mu\text{g DNA}/200\text{mg tissue}$
<i>F. vesca</i> cv Yellow Wonder	127
<i>F. vesca</i> cv Alexandria	59
<i>F. virginiana</i>	54
<i>F. chiloensis</i>	0.85
<i>F.</i> \times <i>ananassa</i> cv Diamante	0.65
<i>F.</i> \times <i>ananassa</i> cv Strawberry Festival	50
<i>F.</i> \times <i>ananassa</i> Laboratory Festival #9	52
<i>F.</i> \times <i>ananassa</i> cv Camarosa	100
<i>F.</i> \times <i>ananassa</i> cv Sweet Charlie	64
<i>F.</i> \times <i>ananassa</i> cv Quinault	55

Table 2-4. Impact of interactions between maceration methods and incubation temperatures on DNA yield and purity. The ratio between absorbance at 260nm and 230nm ($A_{260/230}$) estimate contamination by polysaccharides, whereas the ratio $A_{260/280}$ estimate contamination by proteins. Pure samples have both ratios equal to 1.80.

	Yield $\mu\text{g DNA}/50\text{mg tissue}$		$A_{260/230}$		$A_{260/280}$	
	4°C	60°C	4°C	60°C	4°C	60°C
slurry	31	38	1.02	1.78	1.71	1.91
no slurry	3.8	8.8	0.61	1.46	1.67	1.95

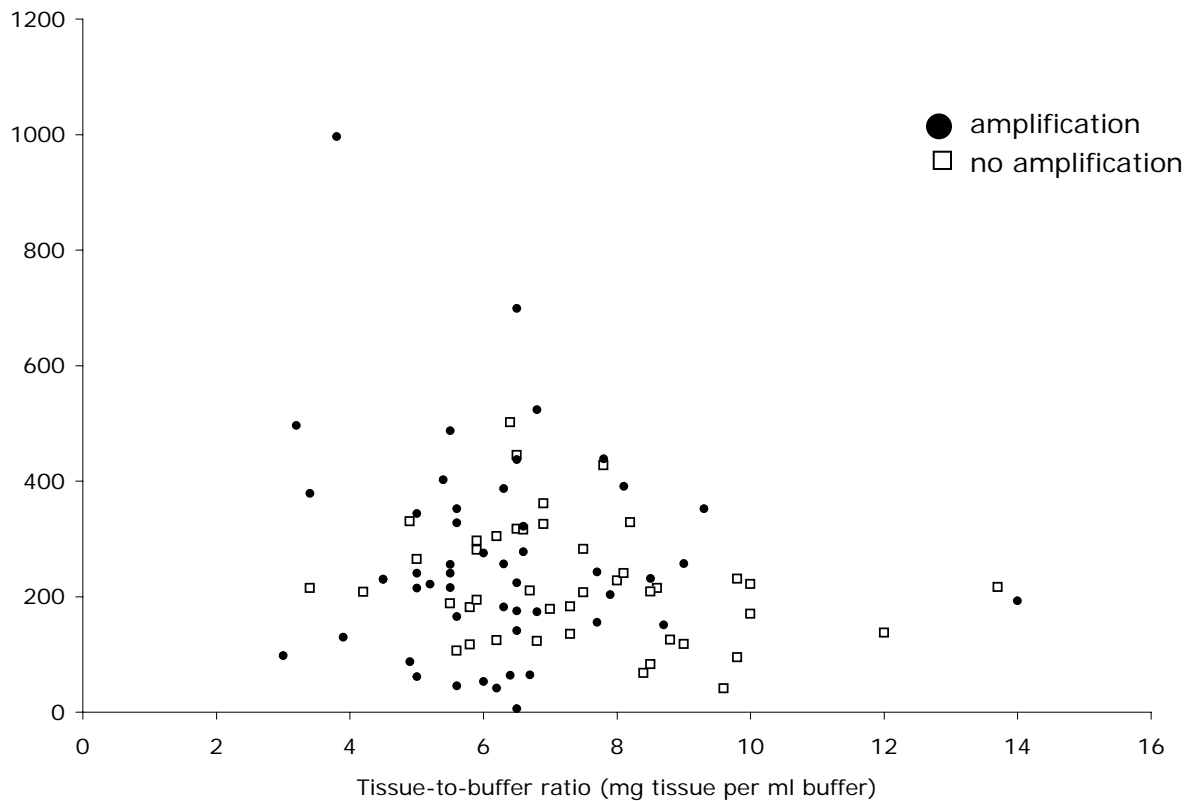


Figure 2-4. Relationships between DNA yield, tissue-to-buffer ratios, and sample amenability to amplification by PCR. DNA was extracted utilizing lyophilized tissue from 94 F2 individuals from a *Fragaria* diploid mapping population. The range of tissue weights was 3-14mg, with average of 6.7mg and standard deviation of 2mg. Because the volume of extraction buffer was kept constant at 1ml, the tissue-to-buffer ratios also represent the amount of tissue (in mg) processed per sample. Correlations between amount of tissue processed, tissue-to-buffer ratio, DNA yield, and PCR outcomes were not apparent.

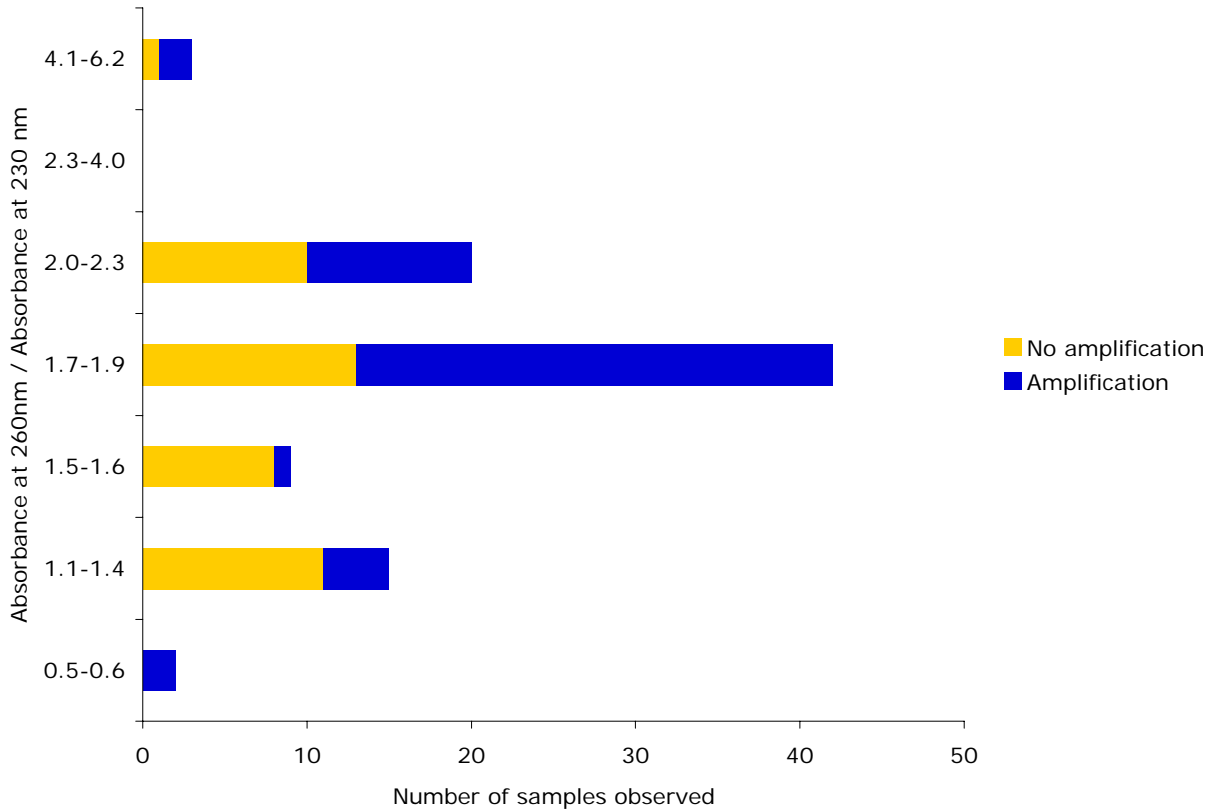


Figure 2-5. DNA contamination by carbohydrate (estimated by the ratio between absorbance at 260nm and 230nm) and its influence on PCR outcome. Absorbance at 230nm and 260nm wavelengths were observed for 94 samples from a genetic linkage mapping population. The A260/230 ratio was calculated for each sample and the ratio data were grouped into 7 categories, varying from 0.5 to 6.2. Most samples presented ratio in the 1.7-1.9 range (1.8 is the optimum for DNA purity from carbohydrates). However, even within the purest DNA category, amplification by PCR was not observed for 1/3 of the samples. Therefore, contamination by carbohydrates may not be considered the sole responsible for the polymerase inhibition.

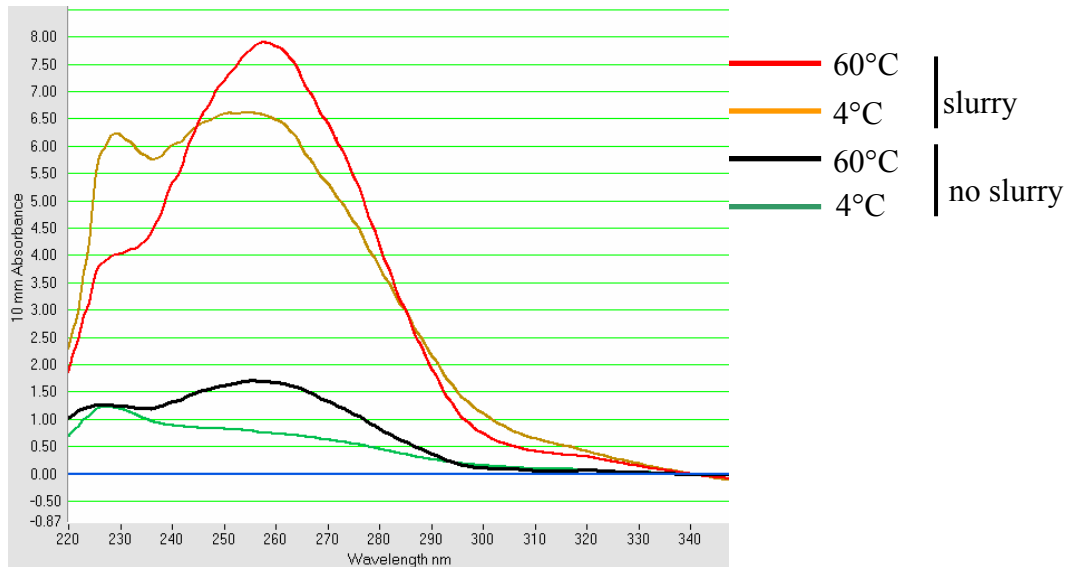


Figure 2-6. Effect of interactions between maceration method and incubation temperature in the absorbance at 220-340nm. The most desirable product from a DNA isolation procedure has a peak at 260nm. A peak at 230nm indicates contamination by polysaccharides. The more aggressive maceration method, combined with higher temperatures, appears to be the best combination of treatments.

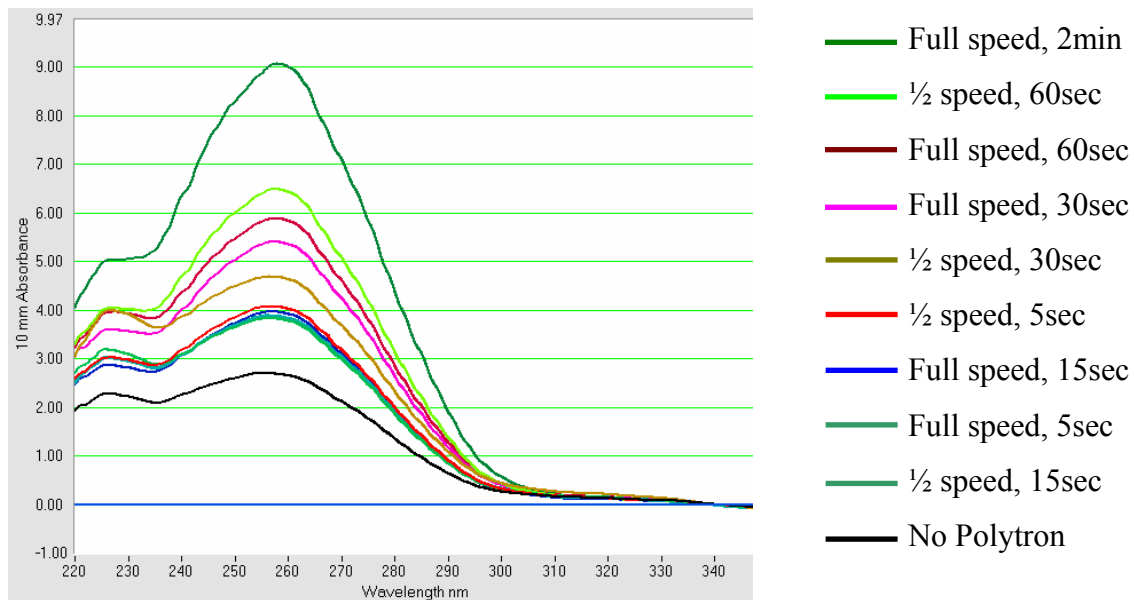


Figure 2-7. The effect of Polytron homogenization on nucleic acid recovery. Leaf tissue was ground in liquid nitrogen and further blended with buffer by utilization of a Polytron. The full uniformization promoted by higher speeds and prolonged durations yielded the best results on DNA isolation.

CHAPTER 3
PRIMARY ANALYSES OF *Fragaria* GENE DISTRIBUTION

Introduction

Although the cultivated strawberry genome is complex and polyploid, its monoploid genome is particularly small and tractable (approximately 200 Mb (Folta and Davis, 2006)). When compared to other rosaceous species, the strawberry genome is exceptionally well suited for rapid elucidation of its sequence, leading to meaningful descriptions of gene distribution and content. Here, small portions of the genome may be sampled and annotated to describe the basis of the *Fragaria* genome. These studies may then be extended to other rosaceous species or utilized in comparative genomics efforts. The goal of the research described in this chapter is to provide a basic description of the first expanses of the *Fragaria* genome. The sequences obtained originate from a fosmid library constructed by Dr. Thomas M. Davis. Individual fosmids were selected by hybridization to genes of interest, and some were randomly selected. These studies provide a primary characterization of the *Fragaria* genome, revealing an understanding of gene content and placement as well as other features of the genome of strawberry.

Genome annotation has been defined as “the process of taking the raw DNA sequence produced by the genome-sequencing projects and adding the layers of analysis and interpretation necessary to extract its biological significance and place it into the context of our understanding of biological processes.” (Stein, 2001) The first challenge to annotate any genomic sequence information is to discriminate between two types of sequences: coding (DNA sequences encoding a protein) and non-coding (DNA is not transcribed into RNA or it is transcribed but not translated into a protein). Regulatory sequences such as promoters and enhancers are examples of non-coding DNA sequences. Other non-coding DNAs are transfer RNA, ribosomal RNA,

small RNAs (snoRNAs, microRNAs, siRNAs, piRNAs), and long RNAs (Xist, Evf, Air, CTN, PINK).

The second challenge for annotation is to ascertain or predict gene function, how gene products might interact, and how they are regulated (Salamov and Solovyev, 2000). Gene finding can be accomplished by similarity-based or *ab initio* gene prediction software. Similarity is defined by the NCBI glossary as “the extent to which nucleotide or protein sequences are related.”

Similarity-based algorithms provide information on alternative transcription (Li et al., 2006), translation start sites, and slicing and are more specific than *ab initio*. However, the latter is more sensitive than the former because it does not bias findings based on prior descriptions (Birney et al., 2004).

Similarity-based algorithms like GeneWise (Birney et al., 2004) predict genes by testing putative translation products for similarity to known proteins. A nucleotide comparison against cDNA, to an expressed sequence tag (EST), or a protein database using the Basic Local Alignment Search Tool (BLAST) are also similarity-based gene predictions. Non-coding rRNAs are also identified using this approach (Stein, 2001). In contrast, the *ab initio* approach attempts to predict genes from sequence data without prior information on gene characterization. Most gene predictors attempt to define a gene using neural networks (modeled according to the learning process in cognitive systems), rule-based systems (algorithms that use an explicit set of rules to make decisions), or hidden Markov models (HMMs). HMMs are statistical algorithms typically utilized in natural language processing. In gene prediction, they are trained with known gene structures (Stein, 2001; Yandell and Majoros, 2002). A Markov model is a statistical model in which the system being modeled is assumed to be a Markov process, i.e., a stochastic

(random) process in which the conditional probability distribution of future states of the process depends on previous states. While in the Markov model one or more states can be directly observed, in the *hidden* Markov model, they cannot. HMMs are popular because they are relatively simple, and efficient methods that exist for training and testing HMMs, these being the Baum-Welch and the Viterbi algorithms, respectively (Mark D. Skowronski, *personal communication*). For a review on HMMs, refer to Rabiner, 1989 (Rabiner, 1989). Examples of *ab initio* HMM gene prediction software are GenScan (Burge and Karlin, 1997), GeneMark (Besemer and Borodovsky, 1999), and FGENESH (Salamov and Solovyev, 2000). When used to annotate the rice genome, FGENESH was more sensitive and more specific than GeneMark and GenScan (Yu et al., 2002).

Plant genomic annotation mechanisms gained favor in the year 2000, shortly after the completion of sequencing of *Arabidopsis thaliana*, a widely used genetic, developmental and physiological model for plants (The Arabidopsis Genome Initiative, 2000), followed by rice in 2002 (Yu et al., 2002). The initial annotation of the *Arabidopsis* genome was submitted by numerous centers, each of them utilizing their own annotation method and terminology. The genome has been re-annotated and classified using Gene Ontology terms as a solution to the cumbersome handling of the information that had resulted from non-centralized annotation (Haas et al., 2005).

Since the completion of the first draft of the rice genome, sequencing of many plants has progressed: high-quality finishing of rice and deep draft coverage of maize, alfalfa (*Medicago truncatula*, the model legume), tomato (*Lycopersicon esculentum*) (National Plant Genomics Initiative, 2002), and black cottonwood (*Populus trichocarpa*) (Tuskan and Difazio S, 2006). Despite the high commercial value of strawberries, there is extensive more nucleotide sequence

information for the above-mentioned species than for *Fragaria*. The availability of strawberry nucleotide sequences was so scarce in 2004 that, if one searched for “*Fragaria*” in public databases, only 58 gene sequences were retrieved (Folta and Davis, 2006). In 2007, this number jumped to over 20,000 sequences, of which 50% are Expressed Sequence Tag (EST) sequences. Collaborative work between the laboratories of Drs. Thomas M. Davis (University of New Hampshire), Kevin M. Folta (University of Florida), Jeffrey L. Bennetzen (University of Georgia), and Phillip SanMiguel (Purdue University) have added an additional 50 genomic DNA sequences, constituting slightly less than 2 megabases of genomic information. The sequences are derived from a *Fragaria vesca* ‘Pawtuckaway’ genomic library and represent 1% of *F. vesca*’s 200Mbp haploid genome (Folta and Davis, 2006). Due to its minute genome size and to the facts that *F. vesca* is the most geographically predominant diploid *Fragaria* species (Folta and Davis, 2006) and it is a plausible ancestor of the cultivated, octoploid strawberry (Ichijima, 1926; Davis and DiMeglio, 2004), this diploid serves as a valuable model for development of molecular markers and comparisons amongst several *Fragaria* species, as well as other genera of the Rosaceae family.

This study aimed to annotate the newly sequenced parcels of the *F. vesca* genome. This represents the first opportunity to explore the gene distribution and the composition of the *Fragaria* genome, which, at 200 Mbp, is comparable to the 157 Mbp (Bennett et al., 2003) genome size of the model plant *A. thaliana*.

Materials and Methods

Dr. Thomas M. Davis at the University of New Hampshire used fosmids (CopyControlTM pCC1FOSTM from Epicentre) as vectors to produce a *F. vesca* genomic library with 8x coverage. The theory is that if the genome was digested into 35kb fragments, approximately 45,000 colonies would be necessary to represent the 200Mbp haploid genome 8 times. Fosmid vectors

were developed by Kim *et al.* (Kim et al., 1992) to address undesirable recombination during cloning in multicopy cosmid vectors. Due to the single-copy F-factor replicon, DNA inserted into fosmid vectors underwent a lower rate of rearrangements and deletions than did fragments inserted into cosmids.

In order to annotate the newly available *F. vesca* sequence, a complement of *ab initio* and similarity-based approaches was utilized. Preliminary screening for putative genes was executed by using the gene prediction software FGENESH (accessible at <http://www.softberry.com>) for each of 26 fosmid insert sequences, using *Medicago* as the gene model. Subsequently, a series of different types of sequence similarity searches were performed using BLAST algorithms (<http://www.ncbi.nlm.nih.gov/BLAST/>), as illustrated in figure 3-1.

The amino acid sequences from each gene predicted by FGENESH were used as query sequences against the non-redundant protein sequences database for “all organisms” using the BLASTP algorithm. Significant similarities between a query sequence and a sequence in the database, termed “hits”, were indicated by an expectation value (E value) lower than 10^{-15} . (The lower the E value, the more significant is the score because the E value ultimately represents how likely two sequences are of being similar by chance alone.) The threshold of 10^{-15} was defined based on thresholds used in the *Arabidopsis* genome annotation (The Arabidopsis Genome Initiative, 2000), where BLASTP E values $< 10^{-20}$ and 10^{-10} were adopted to identify protein families and functional roles between different organisms, respectively.

The BLASTP results that produced significant hits were used to guide the subsequent BLAST interrogations because they determined which nucleotide fragments should be further analyzed. Though the entire 30-45kb sequence could conceivably be analyzed at once, it is more convenient to do the analysis in sequence parcels. The response to a BLAST submission of

sequences larger than 12kb may require protracted time frames and the process may get aborted before the result is retrieved (T. M. Davis, *personal communication*). A second reason to perform searches in parcels is that if two genes are contained in the large query nucleotide sequence and one of them has very high similarity to more than 100 hits, this condition may mask the similarity results to the second gene, appearing as if the second gene was non-coding sequence.

Similarity searches with BLASTX were performed using sequence segments for which BLASTP detected amino acid matches. The translated nucleotide query was delimited to sequence fragments of 8kb whereas the non-redundant (nr) amino acid database against which the *F. vesca* sequences were compared was confined to green plants (green algae and embryophytes) “Viridiplantae”. BLASTX was carried out to determine coding sequence orientation, to assign tentative gene identification and function to the query sequence, and to note the accession and locus tag numbers for the best *Arabidopsis thaliana* orthologs. The *Arabidopsis* loci are sequentially tagged according to their physical position in the genome. Therefore, the tag numbers could be used to assess microcolinearity between *Arabidopsis* and *F. vesca*.

The BLASTN algorithm was utilized in separate searches against the EST and the “nr” nucleotide collection databases. The query sequences originated from fragments for which a gene had been predicted by FGENESH. EST databases searched were delimited to the Rosaceae, in an attempt to detect homologs (sequences that display similarity due to their shared ancestry) and the best *Fragaria*, *Malus*, *Prunus*, *Rubus*, and *Rosa* hits were noted. When no identities were detected within this botanical family, the search was expanded to the Viridiplantae database to detect ESTs that would facilitate detection of genes in the genomic sequence. BLASTN against the “nr” database was executed to detect repetitive elements and non-translated sequences

features such as rRNA, tRNA, and was also useful to detect duplications within the query sequence. If two different regions from a single query were similar to single subject from the database, that indicated a duplication in the fosmid insert sequence under investigation.

To address the sensitivity aspect of the FGENESH gene predictor software, a second search utilizing the BLASTN algorithm was carried out. This time, the query sequences were 8-12kb fragments of genomic sequence (regardless of whether or not genes were predicted in that segment), compared against Rosaceae ESTs. The objective was to determine if *Medicago* suffices as a gene model for gene prediction in *Fragaria*.

A survey of the simple sequence repeats (SSRs) present in the newly accessible *F. vesca* sequences was carried out and their location, composition and predominance were noted. The tool used, SSRIT (Temnykh et al., 2001), is available online at the Gramene website: <http://www.gramene.org/db/searches/ssrtool>.

Results

The average fosmid insert fragment size was 35kb and FGENESH predicted 235 genes from the 26 fosmid insert sequences. Of the total number of nucleotides, 42% were predicted to belong to genic sequences. A list of the numbered predicted genes and their corresponding BLASTX results is available in Appendix B. The software specificity was 55%, since out of the 235 genes predicted, 129 had hits in the amino acid database having as threshold $E < 10^{-15}$.

Enzymes related to mobile elements like transposase, integrase, polyprotein, retrotransposon polyprotein, transcriptase, and reverse transcriptase were putatively present ubiquitously: 14 out of 26 fosmids contained at least one of those types of enzymes. In some cases, several of these enzymes were present in tandem, as depicted for fosmid 18A19 in figure

3-2. The second fosmid diagrammed in figure 3-2, fosmid 34D20, contained putative protein-encoding sequences, including inverted repeats of a gene next to a transposase.

Expressed Sequence Tags (ESTs)

ESTs facilitate genome annotation (The Arabidopsis Genome Initiative, 2000) because they are strong evidence that a sequence is transcribed. In the case of *Fragaria*, only a small percentage of protein hits was supported by EST hits (32 of 129), exacerbating the need for more rosaceous ESTs. Three classes of ESTs were identified (figure 3-3): i, those that displayed identity to predicted, putative protein-encoding genomic sequence; ii, those that were FGENESH-predicted genes, but for which there was no protein hit; and, more interestingly, iii, those that were identified spanning DNA sequences for which no ORF was predicted.

Simple Sequence Repeats (SSRs)

Due to their widespread presence, SSRs have been used to construct a linkage map in diploid strawberry (Sargent et al., 2004). Here, SSRs were identified in all fosmid insert sequences, except three: 11D02, 15B13, and 32L07. It is interesting to note that these fosmids putatively contain plastid and RNA genes and belong to the 50% class that did not contain any putative transposon-related enzymes.

A total of 195 SSRs containing at least 5 motif repetitions were identified. Of the nearly 4,000 nucleotides contained in the SSRs, 71% occurred in regions that were predicted to be intergenic. The great majority (92%) of the repeats were dinucleotides (table 3-1). The numbers of times a specific motif was observed are listed in table 3-2.

Discussion

Amplification of repetitive elements, together with polyploidy, are the mechanisms responsible for genome expansion (Bennetzen and Kellogg, 1997). Evolutionary mechanisms for genome downsizing also exist, though they are less well characterized. Bennetzen *et al.*

(Bennetzen et al., 2005) proposed that retrotransposon removal as well as small deletions caused by unequal homologous recombination and illegitimate recombination, lead to genome shrinkage. Grasses like rice, maize, sorghum (Bennetzen et al., 1998), and wheat (Li et al., 2004) are known to have large gene-empty regions and abundant transposons in the intergenic sequences of gene clusters (Barakat et al., 1998). Fosmid insert 38H05 appeared to be one such gene-empty space, since the only similarity detected between its 32kb sequence and the protein database was to polyprotein, which comprised only a small portion of the fosmid sequence.

The pattern of gene distribution was more similar to *Arabidopsis* than to grasses. *Arabidopsis* has been determined to have 15 to 32 Open Reading Frames (ORFs) per 100 kb (Barakat et al., 1998), or 1 gene per 3-6.6kb, whereas rice has one gene per 6.46 kb (Yu et al., 2002) and barley has one gene per 15-20 kb (Keller and Feuillet, 2000). The *Fragaria* average gene distribution was calculated as 1 gene/4kb or 1 gene/9kb, depending on the prediction method used: *ab initio* gene prediction software FGENESH or BLASTX similarity-based approach at $E < 10^{-15}$, respectively. In either case, strawberry ranks among the more gene-dense species. Since a portion of the fosmid sequences analyzed arose from non-random, “gene of interest” selections, it is possible that the sample was biased toward genic regions, and that the number of kb containing one gene will increase as more random expanses of the genome are sequenced.

The number of putative genes per fosmid ranged from 6 to 15 (identified by *ab initio*) or from 1 to 11 (according to homology to protein database). The discrepancy between the numbers from the two methods may be attributed to the following possibilities: i, the gene structure used for prediction was from *Medicago*, not *Fragaria*. There is a possibility that the gene structures between these two organisms are distinct enough that a sequence encoding a protein in *Medicago*

is not coding in *Fragaria*; ii, the gene prediction is correct, but the putative amino acid sequence is not represented in the protein database because the transcript is not translated (RNA genes in fosmid 15B13, for example), or because the protein has not yet been described; iii, the amino acid sequence is indeed represented in the database, but it is not conserved with *Fragaria*, so the E value threshold chosen as a threshold is too stringent. If a less stringent threshold is used (E value 10^{-10} , rather than 10^{-15}), the number of BLASTX hits increases from 129 to 166 and, therefore, software specificity rises from 55 to 70%.

Half of the ESTs that were identified in genomic regions for which no gene was predicted (figure 3-3) were detected in fosmids that either contained sequence similar to chloroplast DNA (11D02 and 32L07) or to ribosomal RNA (26S in fosmid 15B13). One of the ESTs displayed identity starting in the first nucleotide of the fosmid insert. Perhaps the gene predictor failed to perceive this ORF because the query sequence did not contain transcription initiation signals. The other half of the ESTs that were identified but not predicted was similar to genomic sequences from other species, and the reason why the gene prediction software failed to predict them is not clear. This may suggest some facet of *Fragaria* gene structure that is not recognized by other conditioned algorithms. The detection of putative genes through homology-based similarity search reveals the need to utilize various homology search methods in combination to *ab initio* gene prediction for the optimum genome annotation. This finding is exceedingly important as the genomes of peach and apple will soon be sequenced. Accurate genome annotation will depend on the capacity to adapt current gene prediction methods to these genomes.

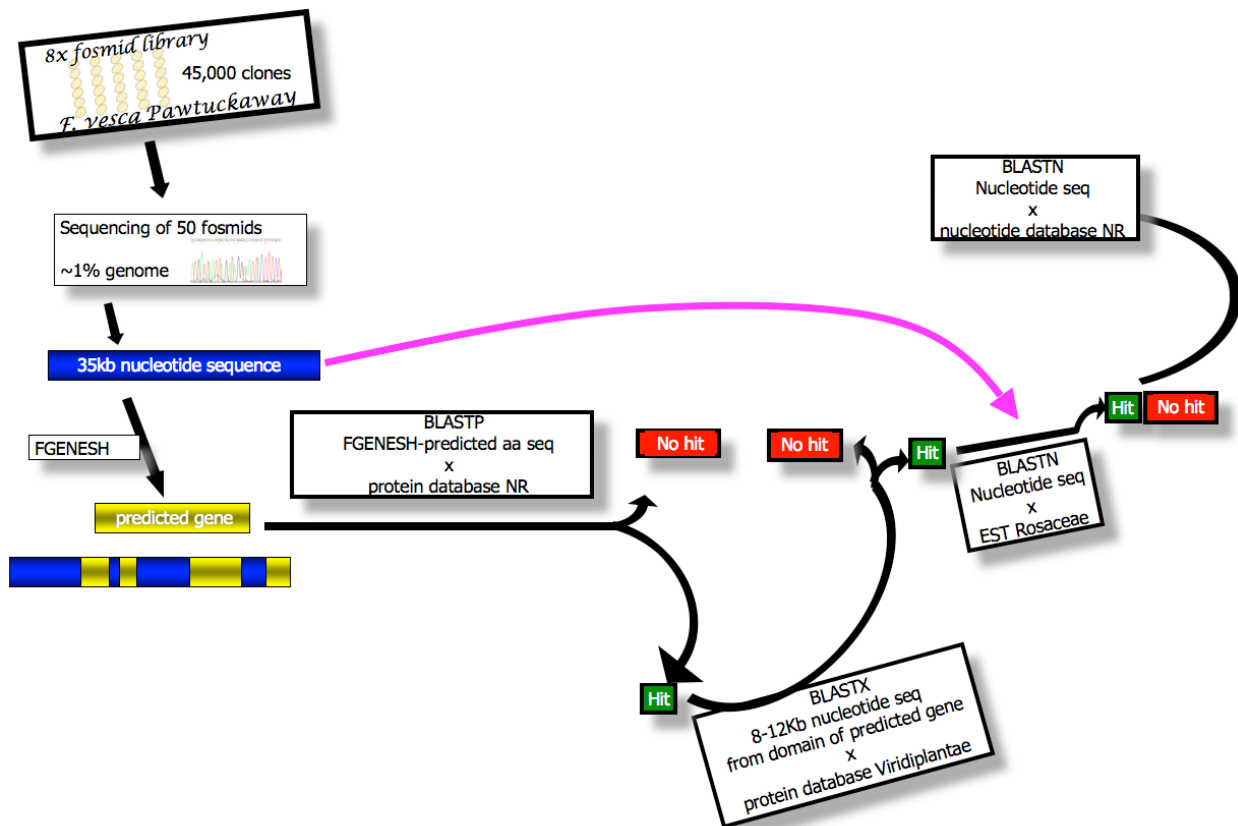


Figure 3-1. Flowchart of genomic DNA sequence annotation scheme. The software FGENESH was used with *Medicago* as the gene model to predict possible gene positions in the genomic sequence. BLASTP algorithm was utilized as preliminary validation FGENESH prediction, whereas BLASTX was used to determine coding sequence orientation and assign tentative gene function. Putative homologs within Rosaceae, conservation amongst various taxonomical families, as well as sequence repeats and duplications were detected by different homology searches utilizing BLASTN. Finally, putative genes that had not been predicted by FGENESH were identified by searching similarities between large fragments of genomic sequence (containing or not FGENESH-predicted genes) and Rosaceae EST.

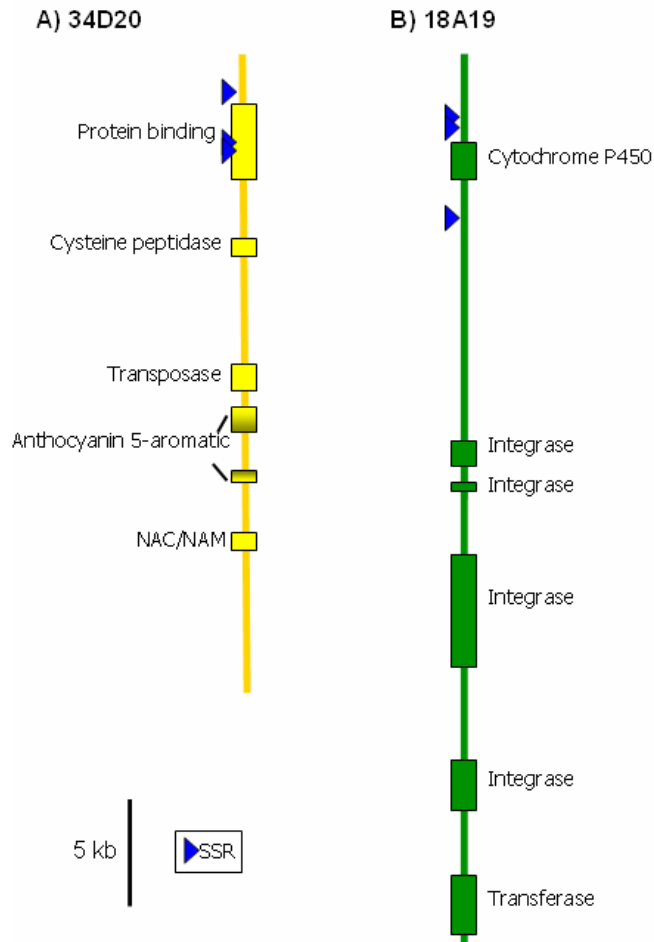


Figure 3-2. Diagram of two fosmid inserts of variable length, with their putative proteins and Simple Sequence Repeats (SSRs). Fosmid 34D20 contained an inverted repeat of an anthocyanin gene next to a transposase, in addition to other protein-encoding genes. Fosmid 18A19 contained mostly transposon-related enzymes, integrase and transferase. SSRs were identified both in genic and intergenic spaces.

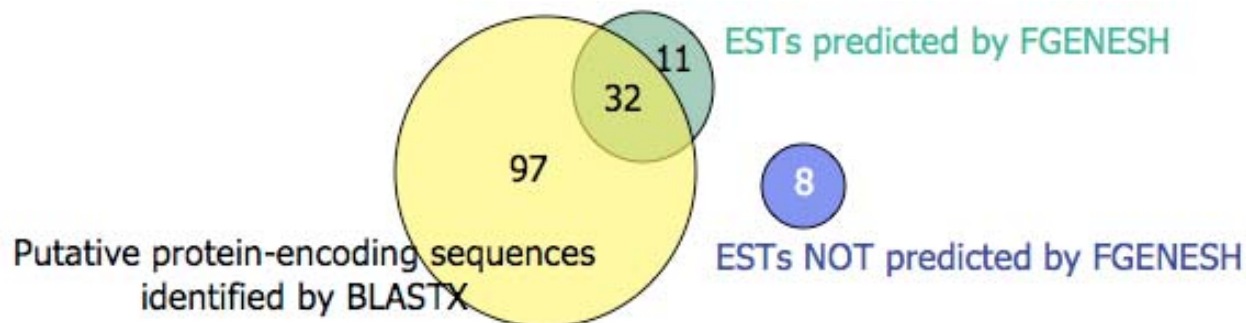


Figure 3-3. EST classes identified by homology searches between large genomic *F. vesca* sequence and Rosaceae ESTs. BLASTX similarity searches were carried out between genomic sequence and the Viridiplantae protein database. A fraction (25%) of the protein matches was validated by BLASTN-detected similarities to the Rosaceae EST database. Other 19 ESTs present no functional information, since no similar amino acid was identified. Of these, a set of 8 ESTs belong to genomic sequence for which there were no genes predicted by FGENESH utilizing *Medicago* as the gene model.

Table 3-1. Number of simple sequence repeats (with a minimum of 5 repeats) observed in *Fragaria vesca* genomic sequence

Motif length	Number of Repeats	Frequency
2 bp	1864	92.1%
3 bp	149	7.4%
4 bp	10	0.5%

Table 3-2. Different types of dinucleotide and trinucleotide repeats observed in *Fragaria vesca* genomic sequence

Motif	Number of Repeats	Frequency
AG/GA/CT/TC	1105	54.9
AT/TA	658	32.7
AC/CA/TG/GT	91	4.5
AGA/GAA/CTT/TCT	79	3.9
CAC/GGT/GTG/TGG	21	1.0
AAC/ACA/GTT/TGT	19	0.9
AAT/TTA	15	0.7
GC/CG	10	0.5
ATG/CAT/TGA//TCA	10	0.5
AGG/CCT	5	0.2

CHAPTER 4
GENE-PAIR HAPLOTYPES: NOVEL MOLECULAR MARKERS FOR INVESTIGATION
OF THE *Fragaria* × *ananassa* OCTOPLOID GENOME

Introduction

The cultivated strawberry, *Fragaria* × *ananassa*, contains 8 copies of a set of 7 chromosomes ($2n=8x=56$). The amount of DNA contained in a single complete strawberry chromosome set is approximately 200 million bases (Nehra et al., 1991; Akiyama et al., 2001; Folta and Davis, 2006), a very small genome size relatively to other angiosperms. There is some controversy as to which angiosperm contains the lowest C-value (or “Cx-value”, terminology proposed by Greilhuber (Greilhuber et al., 2005) to specify the monoploid genome of polyploids), due to different size standards used among various flow cytometry studies. However, likely candidates to the smallest genome are *Arabidopsis thaliana* with 157Mb (Bennett et al., 2003), and perhaps the green strawberry *Fragaria viridis* with 0.108pg (Antonius and Ahokas, 1996). If the formula proposed by Doležel (Doležel et al., 2003) (where 1pg = 978 Mb) is applied, the estimate for *F. viridis* genome size is 105Mb. However, according to the correction proposed by Bennett (Bennett et al., 2003), *F. viridis* current C-value estimate is 206 Mb (Folta and Davis, 2006). Considering that angiosperm C-value varies approximately 1000-fold between species (Bennett and Leitch, 2005), the difference in monoploid genome sizes between *F. × ananassa* and *A. thaliana* is negligible.

Although strawberry’s small basic genome size makes *Fragaria* species attractive organisms for genomic studies, the process of sorting out segregation in an octoploid background is an extremely complex task, posing a formidable barrier to development of molecular markers and genetic linkage mapping. In a polyploid where reassortment amongst all homologous

chromosomes occurs, the number of possible genotypes for a single locus would be $1 + \frac{1}{2} \left(C_{\frac{a}{2}}^a \right)^2$,

where a = number of distinct alleles. For an octoploid containing 8 different alleles for a single locus, the number of different combinations would be 2,451. However, this estimate is artificial, since most polyploid plants are considered to be allopolyploids, and therefore display a degree of fixed, non-segregating heterozygosity (Soltis and Soltis, 2000).

The *F. × ananassa* genome structure is not well understood. The first proposed genome structures were derived from cytological analyses of meiotic pairing chromosomes. The genome composition was first described as AABB³BCC (Fedorova, 1946), whilst the model AAA'A'BBB'B' (Bringhurst, 1990) is currently the accepted one. More evidence gathered through the use of molecular markers (Arulsekhar et al., 1981; Haymes et al., 1997; Viruel et al., 2002; Ashley et al., 2003) supports the fully diploidized model. In a single study using molecular markers (Lerceteau-Köhler et al., 2003), the authors have observed some polysomic inheritance in a F1 octoploid population. However, the deviations from disomic ratios observed may not be due to polysomic inheritance, as segregation distortions have also been observed in diploid segregant populations (Davis and Yu, 1997; Sargent et al., 2004; Sargent et al., 2006).

The identification of genome-specific polymorphisms may permit the monitoring of segregation of each genome in the complex polyploid background. The “Gene-Pair Haplotype” (GPH) is a tool developed to fingerprint the alleles present in the contributing genomes in the octoploid strawberry. It is defined as a suite of intergenic polymorphisms—Simple Sequence Repeats (SSRs), Single Nucleotide Polymorphisms (SNPs), insertion or deletions (InDels), and changes in restriction sites (RFLP) that present a complex genetic marker for a given locus within the diploid genomes. The types of polymorphisms likely to be detected in a GPH locus and their respective expected location in the genome (within versus between genomes) are summarized in figure 4-1.

GPH markers were also used to investigate polymorphisms in diploid *Fragaria* species, in an attempt to identify genome contributors and trace the diploid ancestors. The genus *Fragaria* contains 23 species of different ploidy numbers. Most of the *Fragaria* species are represented in figure 4-2, with locations based on maps and descriptions published elsewhere (Hancock et al., 2004) (Darrow, 1966) (Staudt, 1973) (Hummer et al., 2005) (Staudt, 2003) (Staudt, 2005). *F. × ananassa* is not included in the figure, as cultivated strawberry is ubiquitous.

According to T. M. Davis, “Lake Baikal marks a major geographical boundary for strawberry distribution. *F. vesca* and *F. viridis* are not found in India, Tibet, China, Japan, or southeast Asia. Likewise, no Asian species grow to the west of Lake Baikal.” (<http://www.strawberrygenes.com/map.html>)

Fragaria species have been cultivated for a long time. The French started transplanting fraise des bois, or the wood strawberry *F. vesca* (“vesca” means “little”, in Latin (Fay, 1903)) from the wilderness to gardens in the 1300’s, whereas the hexaploid *F. moschata*, the “musky” strawberry, was common in gardens in the 1700’s (Darrow, 1966). The modern cultivated strawberry has a very well documented history. It was first cited by Philip Miller in the 1759 edition of the *Gardener’s Dictionary* (Darrow, 1966) and it received the name of *F. × ananassa* due to its resemblance to pineapple in odor, taste, and berry shape. In 1765, Duchesne correctly proposed that the new species’ parents were *F. virginiana* and *F. chiloensis*. Although both parents are native to America, the spontaneous hybridization occurred in Europe. *F. virginiana* with its rather small fruits was transported overseas in the 1600’s. Because of its relatively large fruits, *F. chiloensis* was collected by the Frenchman Amédée François Frézier, during a reconnaissance mission to the Spanish West Indies ordered in 1714 by the king Louis XIV. Disappointingly, no fruits were observed during the first years, probably because, in an attempt

to collect only the largest-fruited plants, Frézier imported only female plants. About 50 years later, the product of the pollination of *F. chiloensis* by *F. virginiana* was observed in Germany, Switzerland, Holland, and the Trianon gardens in France (Darrow, 1966).

F. × ananassa's nuclear genomic content can be traced to fifty-three founding clones (Sjulin and Dale, 1987), whereas as few as seventeen cytoplasm donors are represented in the cultivated strawberry (Dale and Sjulin, 1990). Wild accessions from the octoploid parents have been used relatively recently in strawberry breeding programs for introgression of various characteristics (Hancock, 1999), including day neutrality into California cultivars (Ahmadi et al., 1990).

Although the identities of the direct ancestor of *F. × ananassa* are known, their genome constitutions and evolution are not. The present research investigated polymorphisms in the intergenic regions of diploid species, as well as the cultivated octoploid to attempt to trace ancestry and make inferences about the octoploid genome mode of inheritance.

Materials and Methods

Before the commencement of this study in the year 2004, virtually no *Fragaria* genomic sequence was available. Therefore, it was necessary to develop a means to capture useful sequences for analysis. Two different approaches were adopted: i, inference of gene adjacency by putative micro-colinearity between *F. × ananassa* and *Arabidopsis thaliana*; ii, construction and annotation of a *F. vesca* genomic library (discussed in Chapter 3).

Potential micro-colinearity was detected using the approach described in figure 4-3. This approach was possible because the genome of *Arabidopsis* has been completely sequenced and the genes were numbered in such fashion that their locus tags indicate their position on the chromosomes. The hypothesis was that if two genes were adjacent in *Arabidopsis*, they would also be adjacent in *Fragaria*. Similarity between *F. × ananassa* ESTs and *A. thaliana* transcripts

was tested using the FASTA software available at The *Arabidopsis* Information Resource (TAIR)'s website (<http://arabidopsis.org/cgi-bin/fasta/nph-TAIRfasta.pl>) and the best match for *Arabidopsis* was recorded. The sequences of each of the *Arabidopsis* genes adjacent to the *Arabidopsis* matches were retrieved from the Salk Institute Genomic Analysis Laboratory (SIGnaAL) "T-DNA Express" *Arabidopsis* Gene Mapping Tool website (<http://signal.salk.edu/cgi-bin/tdnaexpress>). The next step was to detect *Fragaria* sequences that were similar to each of the *Arabidopsis* gene sequences retrieved. The Basic Local Alignment Search Tool (BLAST) was used to search the *Fragaria* translated nucleotide database using the *Arabidopsis* translated nucleotide query, since TBLASTX is the most sensitive algorithm to detect sequence similarities. Results with an E-value $< 10^{-4}$ were considered positive hits and primers were designed for the putative gene pair to amplify the presumably intergenic sequence flanked by the conserved *Fragaria* and *Arabidopsis* primers. In addition, forward and reverse primers (table 4-1) were designed to amplify at least 100 bp of the EST. This allowed validation that the amplification sequenced was specific to the target regions.

A second approach was adopted to increase the micro-colinearity detection level. Two-hundred and fifty *F. × ananassa* EST sequences were randomly selected for similarity searches against *Arabidopsis* utilizing FASTA and all (rather than only the best match) of the *Arabidopsis* sequences that had a similarity E-value $< 10^{-4}$ were considered for further analysis. The loci tags were recorded on two separate tables, one table keeping the correspondence between *F. × ananassa* and *Arabidopsis* similar sequences, and the other table had the *Arabidopsis* loci tag sorted in crescent order. When the difference between two consecutive *Arabidopsis* loci tag numbers was equal to or lower than 10, a putative gene pair was detected and the *F. × ananassa* EST sequences were retrieved from the non-sorted table.

In addition to the amplification of unknown regions, sequences were gathered by sample sequencing genomic DNA. Both random and targeted sequences were studied in a *F. vesca* fosmid library. The annotation scheme is described in Chapter 3 of this dissertation. Forty combinations of PCR primer pairs were tested to amplify 18 loci, since different primer combinations were required to amplify some of the loci. The primer pairs generated for the putative intergenic regions are listed in table 5-1 of Chapter 5.

Following determination of location and design of PCR primer pairs, PCR was carried out to amplify 28 loci, of which 10 gene pairs (listed in table 4-1) were inferred by the *F. × ananassa/Arabidopsis* micro-colinearity approach and 18 gene pairs (listed in table 5-1) were inferred from gene prediction from *F. vesca* ‘Pawtuckaway’ genomic sequence. The optimizations of PCR conditions were carried out utilizing as template DNA from the species for which primers had been designed: *F. × ananassa* and *F. vesca* ‘Pawtuckaway’ for micro-colinearity- and genomic-DNA-based approaches, respectively. Once optimum conditions were determined, the reaction was carried out for seven *Fragaria* species, which included the respective control species: *F. × ananassa* ‘Strawberry Festival’, *F. vesca* ‘Pawtuckaway’, FRA341 *F. viridis*, FRA377 *F. iinumae*, FRA520 *F. nubicola*, FRA1318 *F. nilgerrensis*, and FME *F. mandshurica*.

The PCR products were cloned using the plasmid cloning vectors pJET1 (GeneJet™ PCR cloning kit by Fermentas Life Sciences) or pCR®2.1-TOPO® (Invitrogen Life Technologies™). The ligation reaction was carried out according to manufacturer’s directions and 1µl of the ligation reaction was used to transform 50µl of competent cells. The chemically competent *Escherichia coli* bacterial cells (Invitrogen One Shot® TOP10) were purchased with the TOPO cloning kit whereas XL1-Blue competent cells (Bullock et al., 1987) were prepared in the

laboratory using and the rubidium chloride method (Hanahan, 1985). The putative recombinant plasmids and competent cells were gently mixed, iced for 30min, heat-shocked at 42°C for 2min, and immediately iced again for at least 5min. XL1-Blue cells with no vector were included in each transformation round as a negative control. When large fragments were cloned, a separate treatment with a smaller fragment for which transformation had been successful before was included as a positive control. Two-hundred μ l of Luria-Bertani (LB) broth (10g tryptone, 5g yeast extract, 10g NaCl, per liter of deionized water) were added to the transformed cell and were incubated in a shaker for 1 hour at 37°C, with agitation of 220rpm, after which 100 μ l of cells were spread onto LB-agar plates containing 50 μ g ampicillin/ml medium. The TOPO vector has the β -galactosidase reporter gene. Therefore, when this vector was used, an overlay of 50 μ l of the chromogenic substrate 5-bromo-4-chloro-3-indolyl- β -D-galactoside (X-gal) at 20mg/ml dissolved in N-N'-dimethyl-formamide and 10 μ l of the filter-sterilized inducer isopropylthiogalactoside (IPTG) at 1M were added to the LB-agar plates before the transformed cells were plated. IPTG and X-Gal were not added to the LB plates when the pJET1 vector was used. This vector contains a gene for a restriction endonuclease in the cloning site. If disrupted by an insert, the lethal endonuclease is not expressed and the transformants are able to propagate. After the cells were plated, they were incubated at 37°C overnight and single colonies were selected for screening for transformants—white colonies for TOPO and, supposedly, any colony for pJET1. The screening procedure was carried out by setting up individual PCR reactions for each colony using annealing temperature of 55°C and primers specific for the vector (pJET1F: 5' GCC TGA ACA CCA TAT CCA TCC 3', pJET1R: 5' GCA GCT GAG AAT ATT GTA GGA GAT C 3'; TOPO, M13F: 5' GTA AAA CGA CGG CCA GTG AAT TGT A 3'; M13R: 5' CAG GAA ACA GCT ATG ACC ATG ATT AC 3'). Approximately 10 colonies were initially

selected from each plate with transformants containing PCR products from diploids. Because several different alleles were sought for the octoploid, 30 colonies were selected from the plates that had transformants with inserts amplified from 'Strawberry Festival'. The tested colonies were streaked on a separate LB/ampicillin plate during the set up of the colony PCR reactions. The PCR products were resolved in 0.8% agarose gel with 1x TAE buffer. PCR-confirmed transformants were grown in 3ml LB broth containing 50µg ampicillin/ml for approximately 4 hours at 37°C, with agitation at 220 rpm. Plasmids were extracted from 1.5ml culture by the alkaline lysis method, followed by 24:1 chloroform extraction. Isolated plasmids were resuspended in 50µl of deionized water and 5 µl were digested with 1 unit of restriction enzymes: EcoRI or XbaI/XhoI for amplicons ligated to TOPO or pJET1, respectively. Transformants carrying distinct alleles were detected by digestion with EcoRI. The digested bands were resolved in 2% Metaphor/1xTBE or 2% agarose/1xTAE. Clones with similar restriction patterns were grouped into different classes and a representative clone of each class was sent to DNA sequencing facilities. A list of primers generated for sequencing reactions can be found in Appendix C, whereas the sequences generated are included in Appendix D.

Sequences obtained were analyzed for conservation between diploid and octoploid alleles. Alignments were performed using the global alignment tool ClustalW available at the European Bioinformatics Institute's website at <http://www.ebi.ac.uk/clustalw/>. Except for the penalty for gap extension, which was set at 0.05 instead of the default 6.66, all other penalty settings were the default ones: gap open: 15; end gap: -1; gap distance: 4.

Results

Considering the number of *Fragaria* ESTs available at the time this study was initiated (approximately 1,500 ESTs), and the estimated 26,000 genes in the *Arabidopsis* genome (Sterck et al., 2007), if micro-colinearity indeed existed, the chance that two adjacent genes would be

detected in the pool of 1,500 was approximately 10% as calculated by

$1,500 \times \left(\frac{1}{25,999} + \frac{1}{25,998} \right)$. Therefore, the amplification of 2 loci out of the 10 investigated (table

4-2) may be regarded as fairly successful. A third set of primers (GPH4) permitted amplification, though after fragment cloning and sequencing, amplification was determined to be unspecific.

There was no similarity between sequences obtained and the 770bp from the template sequences for primer design.

The gene prediction from *F. vesca* genomic sequence enabled detection of 18 potential gene pairs. Of those, primers designed for 11 loci rendered amplification of at least the positive control DNA template of *F. vesca*.

Table 4-2 summarizes the results of PCR amplification using primers designed through both gene-pair detection approaches, as well as results on cloning amplicons and sequencing inserts. The “clone #” in the table is in most cases the PCR reaction number, followed by the colony that was determined to be a transformant by PCR and/or restriction enzyme digestion.

The hypothesis that a fingerprint for each allele belonging to the octoploid ‘Strawberry Festival’ would correspond to alleles from different diploids could be tested by GPHs 5, 23, 10, 27F10, 34D20, and 72E18. The full alignments for every GPH characterized in this dissertation can be found in appendix E.

Data of Individual Loci

GPH5

GPH5 was detected by the micro-colinearity search approach. The adjacent genes in *Arabidopsis* were At3g07320 (E value of 9×10^{-21} , encoding a glycosyl hydrolase family 17 protein) and At3g07330 (E value of 2×10^{-60} , encoding a glycosyl transferase family 2 protein).

GPH5 is a particularly interesting locus, since amplification was observed for all diploids and 2

alleles of the octoploid were detected by *EcoRI* digestion. The following polymorphisms were identified in the 2.8kb analyzed: short indels of 4-12bp (9 bp insertion in *F. vesca*; 12 bp deletion in *F. iinumae*, shown in figure 4-4), 180 SNPs, of which 125 are ambiguous (may be sequencing or polymerase errors) and 55 likely true SNPs, because the base change occurs in more than a single clone. Most of the likely SNPs delineate the octoploid clones from the diploid ones. It is interesting to note that the octoploid alleles are grouped separately from diploid alleles not only for their SNPs, but also for small indels. Two SSR motifs were identified (AAG and AT), with 4 repeats each, for every clone. Therefore no polymorphism in the number of repeats was detected.

GPH23

At1g23740 (oxidoreductase, zinc-binding dehydrogenase family protein) and At1g23750 (DNA-binding protein) were similar to *F. × ananassa* with E values of 3×10^{-64} and 2×10^{-57} , respectively.

Only *F. mandshurica*, *F. iinumae*, and *F. × ananassa* were amplified by the primers designed for this region. Larger deletions than those observed for other loci investigated, and different alleles from the diploids were observed for GPH23. Figure 4-5 illustrates the polymorphisms detected. After preliminary sequence alignment, the putative SNPs were verified by observation of unambiguous peaks in the chromatograms. Therefore, for this locus, a SNP is only an artifact if it was introduced during amplification by the polymerase. (CTC)₄ SSRs were detected and occurred in equal number of repeats for every clone, in the same position when aligned. The implications of the polymorphisms are discussed below.

GPH10

Primers GPH10A and GPH10C were utilized to amplify a 4.4kb fragment from the octoploid 'Strawberry Festival'. Four categories of polymorphic clones were detected by *EcoRI*

restriction digestion (figure 4-6) and sequence was obtained for the full clones (figure 4-7). The primers flanking the most polymorphic region (10PPR1 and 10AB#22) were utilized to amplify that region from all six diploids included in this study. A cladogram based on this polymorphic region is shown in figure 4-9.

72E18

72E18 was the only GPH sequenced that presented polymorphism in the number of repeats in SSRs.

Estimations of Relatedness from Sequence Variation

Cladograms are branching diagrams assumed to be an estimate of a phylogeny where the branches are of equal length. Therefore, cladograms show common ancestry, but do not indicate the amount of evolutionary "time" separating taxa (information from the <http://www.ebi.ac.uk/> website). In this study the use of cladograms generated from multiple sequence alignments provide an outstanding means to gauge the relatedness between strawberry genomes. When compared against each other, the use of cladograms depicts the relative divergence between similar sequences, and thus is a useful estimate of SNP frequency between the alleles in *F. × ananassa* and the putative diploid subgenome donors. The following cladograms derive from all GPH that contained at least one allele representing the octoploid strawberry compared to all cases where products could be amplified from diploids. The results indicate that octoploid alleles cluster together, as do diploid alleles. The most related diploid to octoploid alleles is consistently *F. iinumae*, and surprisingly, alleles closely matching *F. vesca* were not detected for any of these GPH loci.

Relatedness may also be assessed by studying the order of insertion-deletions and SSRs. Presumably, a set of similar indels or SSRs may be conserved between the diploid subgenome donors and the modern cultivated octoploid. The presence and order of these features provides

evidence of relatedness. Table 4-3 represents the length and position of indels and SSRs identified in the sequenced clones. In this table, indels and SSRs are presented as variable “features” in genomic sequence as it is parsed from 5’ to 3’. With this method the size and position can be best described, presenting evidence of relatedness. In this table the variable features present in all genomes are revealed. When two or more values in the same column are shown, this represents indels present in the same region of a given locus, as the corresponding genotype deviates from a consensus sequence compiled from multiple sequence alignment of all sequences. A blank box indicates agreement with consensus in a given region. The corresponding genotype does not deviate from consensus. The sequence of the clones listed in the table is conserved with the sequence as they appear in the cladograms of figure 4-9 to facilitate the perception of relatedness.

Discussion

Synapsis between *F. vesca* and *F. virginiana* chromosomes has been shown to occur (Ichijima, 1926). This is regarded as the first evidence that *F. vesca* is a likely genome donor to *F. × ananassa*, since *F. virginiana* is the pollinating parent to *F. × ananassa*. Another study published a year later showed that the crosses between *F. vesca* × *F. chiloensis* and *F. vesca* × *F. virginiana* produced sterile hybrids (Mangelsdorf and East, 1927). The occurrence of natural hybrids between *F. chiloensis* and *F. vesca* (Bringhurst and Gill, 1970), the geographical predominance of *F. vesca*, and a recent study on chloroplast DNA showing that the *F. vesca* is closely related to *F. × ananassa* (Potter et al., 2000), support the hypothesis that *F. vesca* is a contributor to the genome of octoploid strawberries. In this study large intergenic regions were sequenced from a series of octoploid and diploid alleles to assess the relatedness between the cultivated strawberry and potential subgenome donors. Two central methods were used to detect

relatedness, both based on multiple sequence alignments. The first used cladograms to display consolidation of single nucleotide polymorphisms (Table 4-2). The second method was as assessment of multinucleotide polymorphisms, detected as indels or SSRs that varied between accessions and a consensus sequence. The use of these complementary methods provides two levels of resolution that describe relatedness between alleles.

Contrary to the expected, however, data from five characterized loci and the inability to PCR-amplify *F. vesca* using primers designed for *F. × ananassa* (GPH23), display *F. vesca* ‘Pawtuckaway’ as the most unrelated sequence to any of the sequenced octoploid alleles. From the few loci studied, it does not appear that *F. vesca* is a more likely A-genome donor than any of the other diploids studied. This surprising finding contrasts directly with cytological evidence and suggests that *F. vesca* may not be a contributor to at least the ‘Strawberry Festival’ cultivar.

F. iinumae, on the other hand, was confirmed as one of the most distinct diploid. Table 4-3 shows that *F. viridis* and *F. iinumae* had the most dramatic changes in relationship to the other four diploids concerning size of their indels. *F. viridis* displays large indels: 44, 500, and 800bp in loci 11D02, 27F10, and 32L07, respectively. None of the deletions, however, corresponded to any of the *F. × ananassa* alleles sequenced. In the case of 32L07, no octoploid allele was sequenced because PCR amplification could not be detected for any of the following octoploids: ‘Strawberry Festival’, ‘Carmine’, ‘Diamante’, ‘Rosa Linda’, and ‘Sweet Charlie’.

F. iinumae has a deletion greater than 500bp in the fragment 10PPR1AB22 of GPH10, five indels of approximately 30 bp (three in 11D02, and two in 34D20), and one of approximately 50 bp in 72E18. The indels in 34D20 and 72E18 from *F. iinumae* coincided with *F. × ananassa*, suggesting that *F. iinumae* is indeed a genome donor to the diploid. The cladograms from figure 4-9 suggest that in every locus studied, *F. iinumae* was the most similar diploid haplotype to the

octoploid alleles. Phylogenetic analysis of the intron-containing region of the Adh gene of 20 *Fragaria* species identified two major clades, and pointed to *F. iinumae* as the best B clade candidate for Adh allele donor to octoploids (Davis and DiMeglio, 2004).

The data identified here provide further evidence to support the hypothesis that *F. iinumae* is a subgenome donor to the modern octoploid. In all comparisons herein where octoploid sequence was obtained, the octoploid related more closely to the *F. iinumae* haplotype. Thus, one conclusion that can be made is that *F. × ananassa* cv. Strawberry Festival contains clear evidence of the *F. iinumae* characters within its subgenome composition. But what about the A genome? The B genome donor has been disputed, but almost 100 years of evidence implicates *F. vesca* as an A-genome donor. In this data set, little evidence of the A-genome exists. There are several ways to reconcile this discrepancy, although all of them are speculative. The one important caveat is that ‘Strawberry Festival’ is only one octoploid accession and was used almost exclusively as the octoploid representative. ‘Strawberry Festival’ has a broad east-coast, west-coast lineage, so in many ways it is an excellent representative for this study. It is possible, albeit unlikely, that the allelic content of ‘Strawberry Festival’ is skewed to the B-genome *F. iinumae* components and somehow the A-genome is not being detected. This is surprising because the primers that detect the B genome variants were derived from the A genome donor. One alternative explanation is that perhaps the A genome underwent extensive modification, such as expansion, therefore preventing amplification of octoploid sequences by PCR. Alternatively, these regions could have been deleted from the octoploid genome, as the octoploid genome is smaller than four diploid genomes, indicating a loss of genetic material (Folta and Davis, 2006). A final explanation is that not all diploid species, including many *F. vesca* accessions, were tested, so the A genome may be represented by a genotype not tested in this

study. There is no simple answer, and this finding may indicate that some higher-order mechanism is at work to limit the presence of subgenome sequence in the polyploid. Polysomic inheritance has been documented (Lerceteau-Köhler et al., 2003). If polysomic inheritance led to a trait of interest early on, it may have been selected as beneficial in breeding populations and fixed in subsequent lines.

Another unlikely explanation is that changes in *F. iinumae* paralleled those in *F. × ananassa* in two separate and unrelated instances. Probability suggests that this cannot be the case, yet it remains a formal possibility, especially if the changes induced result in regulatory alterations that affect gene expression, biological function and possibly selection. It is also possible that cultivation and selection have important consequences in skewing subgenome representation. It has been demonstrated that *F. iinumae* is a robust plant, with more vigorous growth than *F. vesca* (Sargent et al., 2004). These characters may have lent themselves to the wild octoploids and were attractive to potential early breeders. These alleles may dominate certain selections, like ‘Strawberry Festival’. Other cultivars need to be tested to assess allelic composition to further query this hypothesis.

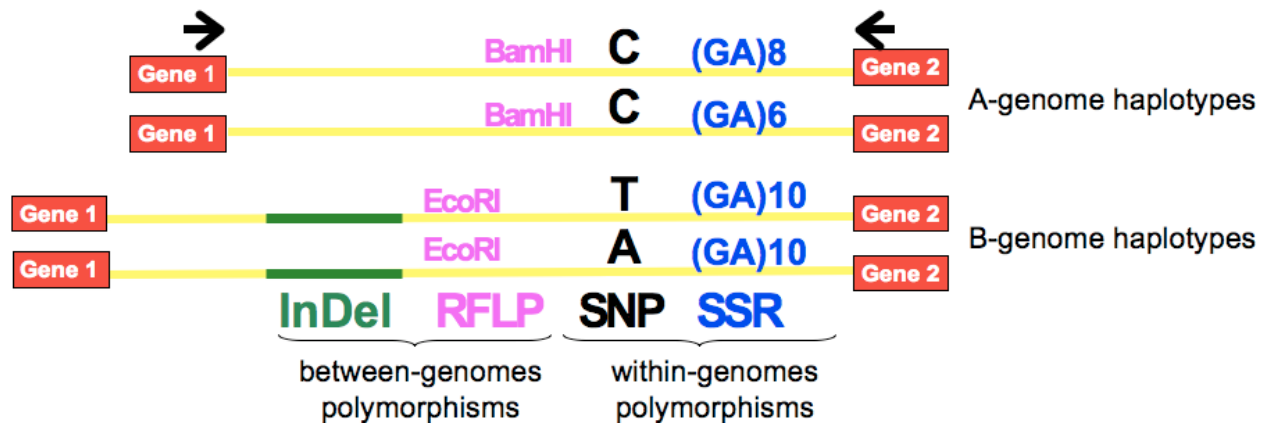


Figure 4-1. An idealized GPH locus. Arrows represent primers designed to amplify the intergenic spaces of a GPH. The combination of polymorphisms within (SSR, SNP) and between subgenomes (InDels, change in restriction sites) define each haplotype.

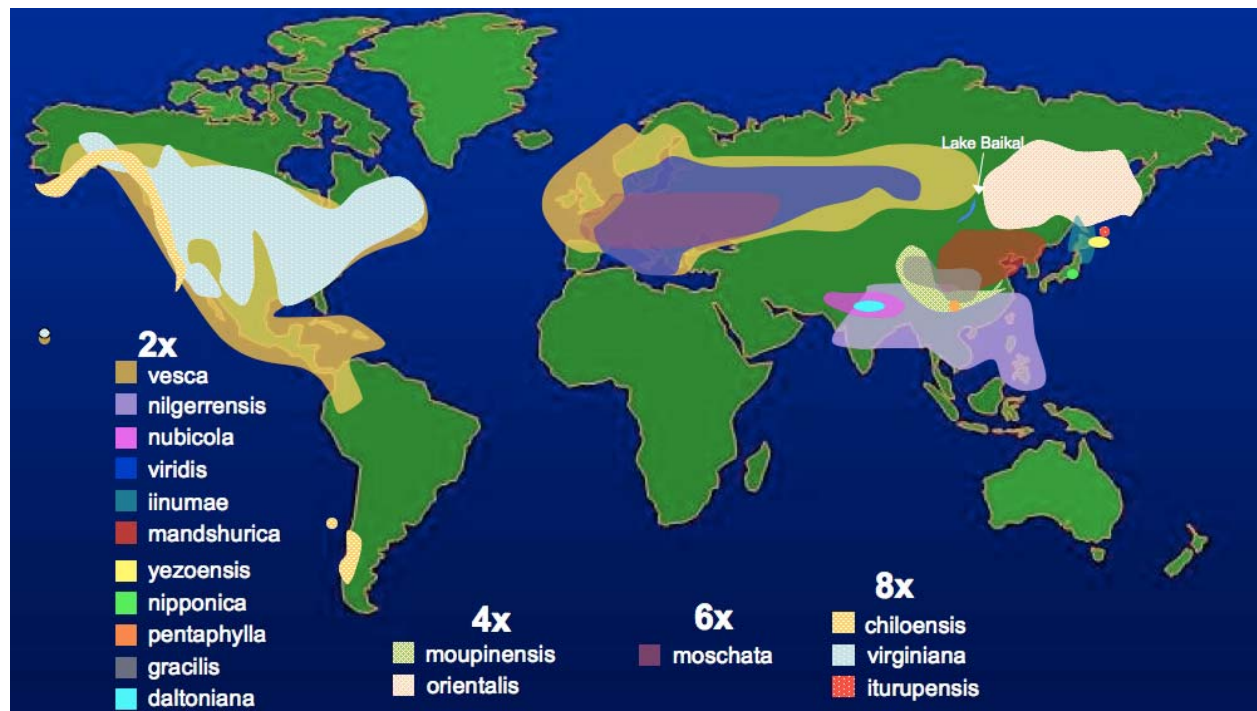


Figure 4-2. *Fragaria* species and their geographical locations

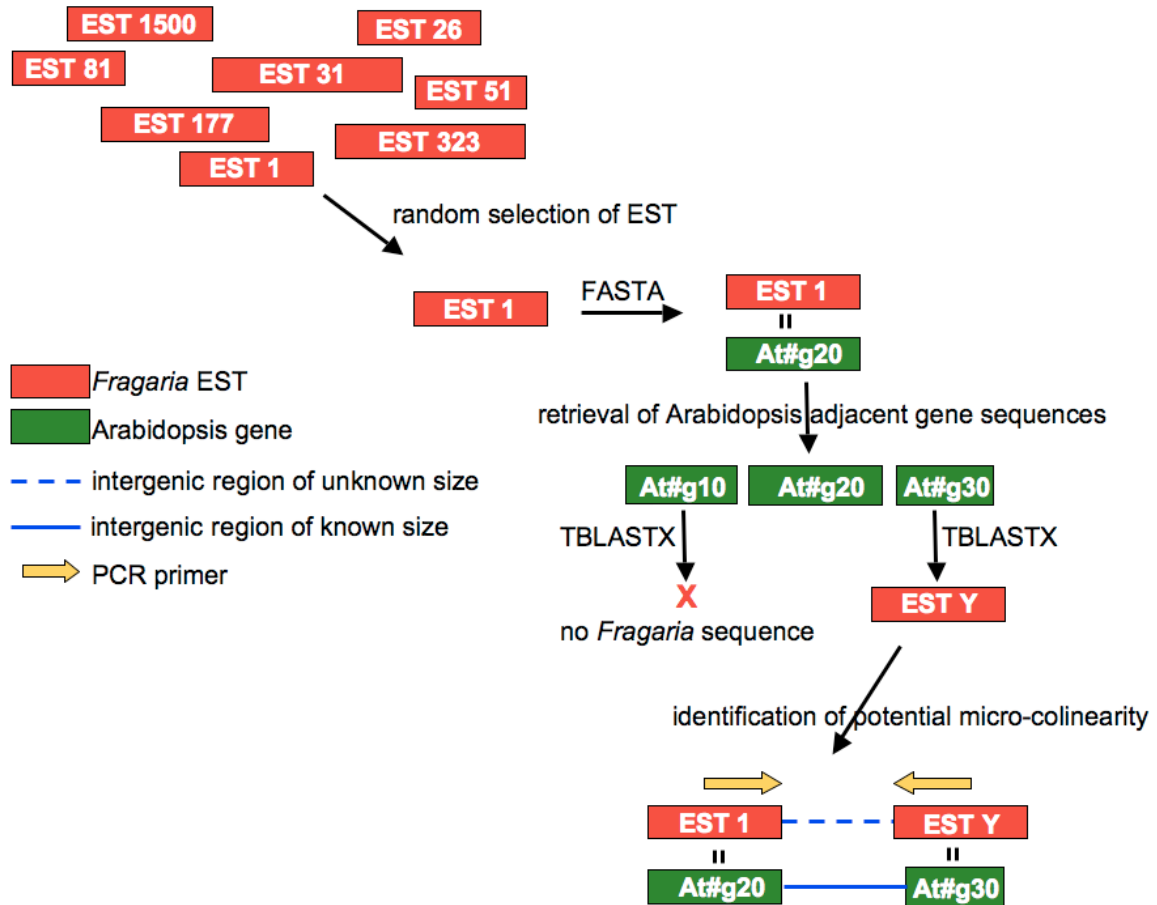


Figure 4-3. GPH design upon comparison between strawberry ESTs and *Arabidopsis* database. When the quest for homologies culminates in the detection of potentially neighboring genes, primers are designed in the strawberry EST and the intergenic region is amplified if the adjacency is true, the gene space is smaller than 4kb, and the gene orientations are conserved.

Table 4-1. PCR primers designed for amplification of micro-colinearity-inferred putative intergenic fragments

Primer name	<i>Fragaria</i> EST	<i>Arabidopsis</i> gene	Intergenic fragment size in <i>Arabidopsis</i> (bp)	Primer sequence
GPH4a	FA_SEa0007-G07	At2g20120	3,415	acgagggccttggaaagaaagg
GPH4b	FA_SEa0015-B08	At2g20140		gccaacaacagaaagacc
GPH5a#2	FA_SEa0002C03r	At3g07320	2,196	caatgccatgggtctccggtc
GPH5b#2	FA_SEa0018E10r	At3g07330		tgccgttgccacacaccttc
GPH20	FA_SEa0012D10r	At5g13440	1,043	gagggtaacgctcatgggt
GPH20	FA_SEa0012E08r	At5g13450		gtctccttcaattctttctcctc
GPH21a	AY679587	At5g06750	1,509	tgacatcccataagccatca
GPH21b	DQ011163	At5g06760		gggaggactacggcacataac
GPH21c	DQ011163	At5g06760		atcagatgtcggcactgc
GPH22	gi 48249442	At5g11250		tttcagctcagcaagcaagg
FaSCH6rgene GPH22 FaHy5	FA_SEa0004E09r	At5g11260	1,632	gctcccaggaccaaacca
GPH23F	FA_SEa0013H07r	At1g23750	982	cttgagggccatcagcac
GPH23R	V01014C10_558132	At1g23740		tacaccacgccttcatctc
GPH27F	FA_SEa0014E11r	At1g74260	2,300	tgccgctgccatcttctct
GPH27R	FA_SEa0011H07	At1g74280		ccatgctcttgataggccaaat
GPH31F	FA_SEa0014B05r	At2g30100	724	aatggagctgatgggttctgat
GPH31R	FA_SEa0016G12r	At2g30110		aaggatgatgacacgaactatca
GPH51F	CX662192	At3g176600	1,867	ggacacatggctcccaga
GPH51R	AY961594	At3g17670		caagacagcgggagcagt
GPH56F	AB211167	At4g38970	3,017	ccagggacgatgttttgctc
GPH56R	AJ414709	At4g38990		ggtggattacatcttgggtgaca
GPH56R2	AJ414709	At4g38990		ttcaagctttggacaactaacg

Table 4-2. PCR primers that allowed amplicon generation. A “minus” sign in the “amplification” and “clone #” columns signify, respectively, no amplification and no transformants were observed.

Primer name	Template	PCR product size (kb)	Clone #	Vector	<i>E. coli</i> strain	Sequence obtained from forward end (bp)	Sequence obtained from reverse end (bp)	
GPH4	‘Strawberry Festival’	2.0, 1.0,	13	TOPO	TOP10	1,109	-	
		0.5	15	TOPO	TOP10	638	-	
GPH5	<i>F. vesca</i>	2.8	21	TOPO	TOP10	1,268	1,299	
		2.8	5	TOPO	TOP10	1,249	1,287	
		2.8	5	TOPO	TOP10	1,256	1,298	
		2.8	7	TOPO	TOP10	1,261	1,263	
		2.8	19	TOPO	TOP10	1,262	1,295	
		2.8	1	TOPO	TOP10		Full clone	
		2.8	2	TOPO	TOP10	1,257	1,306	
‘Strawberry Festival’	2.8	6	TOPO	TOP10	749	755		
	2.8	7	TOPO	TOP10		Full clone		
GPH23	<i>F. vesca</i>	-	-	-	-	-	-	
		-	-	-	-	-	-	
		2.0	2	TOPO	TOP10		Full clone	
		2.0	5	TOPO	TOP10		Full clone	
		-	-	-	-	-	-	-
		-	-	-	-	-	-	-
		2.0	3	TOPO	TOP10		Full clone (2,024)	
‘Strawberry Festival’	2.0	3	TOPO	TOP10		Full clone (2,081)		
	2.0	4	TOPO	TOP10		Full clone (2,111)		
	4.4	2	TOPO	TOP10		Full clone		
	4.4	7	TOPO	TOP10		Full clone		
GPH10	‘Strawberry Festival’	4.4	18	TOPO	TOP10		Full clone	
		4.4	19	TOPO	TOP10		Full clone	
		4.4	20	TOPO	TOP10		Full clone	
		0.728	2	TOPO	TOP10		Full clone	
		0.726	7	TOPO	TOP10		Full clone	
10PPR 1/10A B22*	<i>F. vesca</i>	0.266	18	TOPO	TOP10		Full clone	
		0.722	19	TOPO	TOP10		Full clone	
		0.652	19	TOPO	TOP10		Full clone	
		0.724	20	TOPO	TOP10		Full clone	
		528	2				Subset of GPH10 sequence	
		644	7				Subset of GPH10 sequence	
		584	18				Subset of GPH10 sequence	
		584	19				Subset of GPH10 sequence	
		643	20				Subset of GPH10 sequence	
		11D02	<i>F. vesca</i>	1.6	library			
1.6	2031-1			pJET1	XL1-Blue	1,403		
1.6	2032-1			pJET1	XL1-Blue		Full clone	
1.6	2033-1			pJET1	XL1-Blue	1,299		
1.6	-							
1.6	-							
1.6	-							

* 10PPR1/10AB22 is a locus within GPH10

Table 4-2. continued

Primer name	Template	PCR product size (kb)	Clone #	Vector	<i>E. coli</i> strain	Sequence obtained from forward end (bp)	Sequence obtained from reverse end (bp)
17O22	<i>F. vesca</i>	1.4	library				
	<i>F. viridis</i>	1.4	678	pJET1	XL1-Blue		Full clone
	<i>F. iinumae</i>	1.4 & 1.0	668	pJET1	XL1-Blue		Full clone
	<i>F. nubicola</i>	1.4	653	pJET1	XL1-Blue		Full clone
	<i>F. nilgerrensis</i>	-					
	<i>F. mandshurica</i>	1.4	655	pJET1	XL1-Blue		Full clone
27F10	'Strawberry Festival'	1.5 & 1.4	-				
	<i>F. vesca</i>	1.0	library				
	<i>F. viridis</i>	1.5	2039-1	pJET1	XL1-Blue	537	582
	<i>F. iinumae</i>	1.0	2040-1	pJET1	XL1-Blue		Full clone
	<i>F. nubicola</i>	1.0	2041-1	pJET1	XL1-Blue		Full clone
	<i>F. nilgerrensis</i>	1.8	-				
29G10	<i>F. mandshurica</i>	1.0	2043-1	pJET1	XL1-Blue		Full clone
	'Strawberry Festival'	1.0	2046-1	pJET1	XL1-Blue		-
			2046-2	pJET1	XL1-Blue		Full clone
	<i>F. vesca</i>		library				
	<i>F. viridis</i>	-					
	<i>F. iinumae</i>	-					
32L07	<i>F. nubicola</i>	0.7	2049-1	pJET1	XL1-Blue		Full clone
	<i>F. nilgerrensis</i>	0.7	2050-1	pJET1	XL1-Blue		Full clone
	<i>F. mandshurica</i>	0.7	2051-1	pJET1	XL1-Blue		Full clone
	'Strawberry Festival'	-					
	<i>F. vesca</i>	2.7	library				
	<i>F. viridis</i>	1.9	640 1090-11	pJET1 TOPO	XL1-Blue TOP10		Full clone
34D20	<i>F. iinumae</i>	-					
	<i>F. nubicola</i>	2.7	647	pJET1	XL1-Blue	No seq	
	<i>F. nilgerrensis</i>	2.7	993	TOPO	TOP10	No seq	
	<i>F. mandshurica</i>	2.7	1000	TOPO	TOP10	No seq	
	'Strawberry Festival'	I attempted to amplify fragment from the octoploids 'Carmine', 'Diamante', 'Rosa Linda', and 'Sweet Charlie', but amplification was not observed for any of them					
	<i>F. vesca</i>	2.0	1826-3	pJET1	XL1-Blue		library
40M11	<i>F. viridis</i>	2.0	1827-3	pJET1	XL1-Blue		Full clone
	<i>F. iinumae</i>	2.0	1828-4	pJET1	XL1-Blue		Full clone
	<i>F. nubicola</i>	2.0	1829-1	pJET1	XL1-Blue		Full clone
	<i>F. nilgerrensis</i>	2.0	1830-3	pJET1	XL1-Blue		Full clone
	<i>F. mandshurica</i>	2.0	1831-5	pJET1	XL1-Blue		Full clone
	'Strawberry Festival'	2.0	1832-2	pJET1	XL1-Blue		Full clone
40M11	<i>F. vesca</i>	3.1	library				
	<i>F. viridis</i>	3.1	-				
	<i>F. iinumae</i>	2.9	-				
	<i>F. nubicola</i>	3.1	-				
	<i>F. nilgerrensis</i>	3.1	-				
	<i>F. mandshurica</i>	3.1	1088-1	TOPO	TOP10	884	
'Strawberry Festival'	2.9	-					

Table 4-2. continued

Primer name	Template	PCR product size (kb)	Clone #	Vector	<i>E. coli</i> strain	Sequence obtained from forward end (bp)	Sequence obtained from reverse end (bp)
63F17	<i>F. vesca</i>	1.2	library				
	<i>F. viridis</i>	1.2	2	pJET1	XL1-Blue	Full clone	
	<i>F. iinumae</i>	1.2?	3	pJET1	XL1-Blue		
	<i>F. nubicola</i>		-				
	<i>F. nilgerrensis</i>		-				
	<i>F. mandshurica</i>	1.2	3	pJET1	XL1-Blue	Full clone	
	'Strawberry Festival'	1.2	1	pJET1	XL1-Blue		570
72E18	<i>F. vesca</i>	2.6	library	TOPO	TOP10		
	<i>F. viridis</i>	2.6	1096-4	TOPO	TOP10	1,052	1,515
	<i>F. iinumae</i>	2.6	1097-1	TOPO	TOP10	1,000	973
	<i>F. nubicola</i>	?	-				
	<i>F. nilgerrensis</i>	2.5	1099-1	TOPO	TOP10		1,958
	<i>F. mandshurica</i>	2.6	1100-6	TOPO	TOP10	Full clone	
	'Strawberry Festival'	2.5	1101-10	TOPO	TOP10		1,170
73I22	<i>F. vesca</i>	-					
	<i>F. viridis</i>	3.0	1834-16	pJET1	XL1-Blue	No seq	
	<i>F. iinumae</i>	-					
	<i>F. nubicola</i>	3.0	1836-1	pJET1	XL1-Blue	No seq	
	<i>F. nilgerrensis</i>	-					
	<i>F. mandshurica</i>	3.0	1838-5	pJET1	XL1-Blue	No seq	
	'Strawberry Festival'	-					

```

GPH5_ananassa_clone2      CAGAAGGTAATATGCATGATATAAATATCAAGTTAATTGTACAATGATATTATTTGTAATA 582
GPH5_ananassa_clone7      CAGAAGGTAATATGCATGATATAAATATCAAGTTAATTGTACAATGATATTATTTGTAATA 582
GPH5_viridis              TAGAAGGTAATATGCATGATATAAATATCAAGTTAATTGTACAAGTGTATAT---TTGTAACC 576
GPH5_iinumae              TAGAAGGTAATAT-----ATCAAGTTAATTGTACAATAATAT---TTGTAATC 566
GPH5_nilgerrensis         CAGAAGGTAATATGCATGATATAAATACCAAGTTAATTGTACAATGATAT---TTGTAATC 579
GPH5_mandshurica         TAGAAGGTAATACGCATGATATAAATATCAAGTTAATTGTACAATGATAT---TTGTAATC 578
GPH5_nubicola            TAGAAGGTAATACGCATGATATAAATATCAAGTTAATTGTACAATGATAT---TTATAATC 583
GPH5-vesca                TAGAAGGTAATATGCATGATATAAATATCTAGTTAATTGTACAATGATAT---TTGTAACC 579
                        ***** * * ***** * **** ** **

GPH5_ananassa_clone2      GGAACAAGAAGTAGCACCTCCCAAGAAGAAGAAGAAAAATGGGATCTACAGAAAAGAGCT 240
GPH5_ananassa_clone7      GGAACAAGAAGTAGCACCTCCCAAGAAGAAGAAGAAAAATGGGATCTACAGAAAAGAGCT 240
GPH5_viridis              GGAACAAGAAGTAGCACCTCCCAAGAAGAAGAAGAAAAATGGGATCTACAGAAAAGAGCT 240
GPH5_iinumae              GGAACAAGAAGTAGCACCTCCCAAGAAGAAGAAGAAAAATGGGATCTACAGAAAAGAGCT 240
GPH5_nilgerrensis         GGAACAAGAAGTAGCACCTCCCAAGAAGAAGAAGAAAAATGGGATCTACAGAAAAGACT 240
GPH5_mandshurica         GGAACAAGAAGTAGCACCTCCCAAGAAGAAGAAGAAAAATGGGATCTACAGAAAAGAGCT 240
GPH5_nubicola            GGAACAAGAAGTAGCACCTCCCAAGAAGAAGAAGAAAAATGGGATCTACAGAAAAGACT 240
GPH5-vesca                GGAACAAGAAGTAGCACCTCCCAAGAAGAAGAAGAAAAATGGGATCTACAGAAAAGAGCT 240
                        ***** **

```

Figure 4-4. Subset of the alignment of GPH5 octoploid and diploid clones. Single Nucleotide Polymorphisms (SNPs) are in bold font. Same base changes that appear in a determinate position for more than one clone are likely to reflect real differences and are colored red. Hyphens signify indels whereas SSRs are magenta-colored.

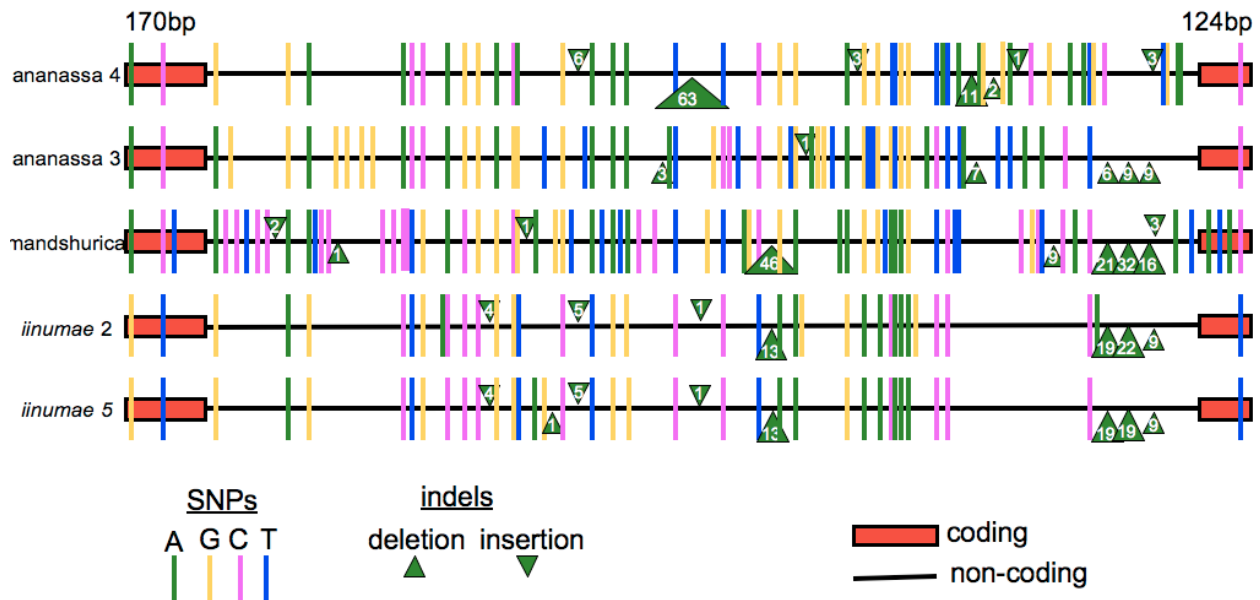


Figure 4-5. Diagrammatic representation of alignment of full GPH23 clones, depicting all polymorphisms identified, such as Single Nucleotide Polymorphisms, insertions, and deletions. Numbers in triangles indicate the length of indels.

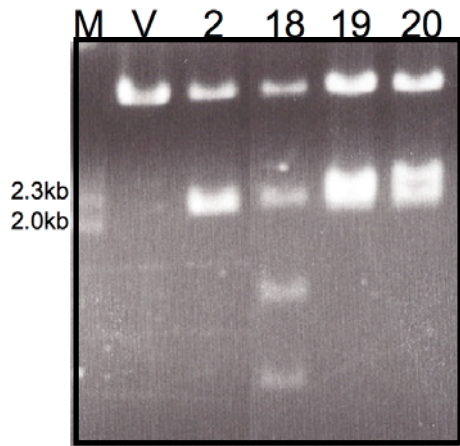


Figure 4-6. *EcoRI* Restriction patterns observed for GPH10 clones from the octoploid ‘Strawberry Festival’, indicating four different allele classes. M: molecular weight marker; V: empty vector; 2-20: polymorphic clones

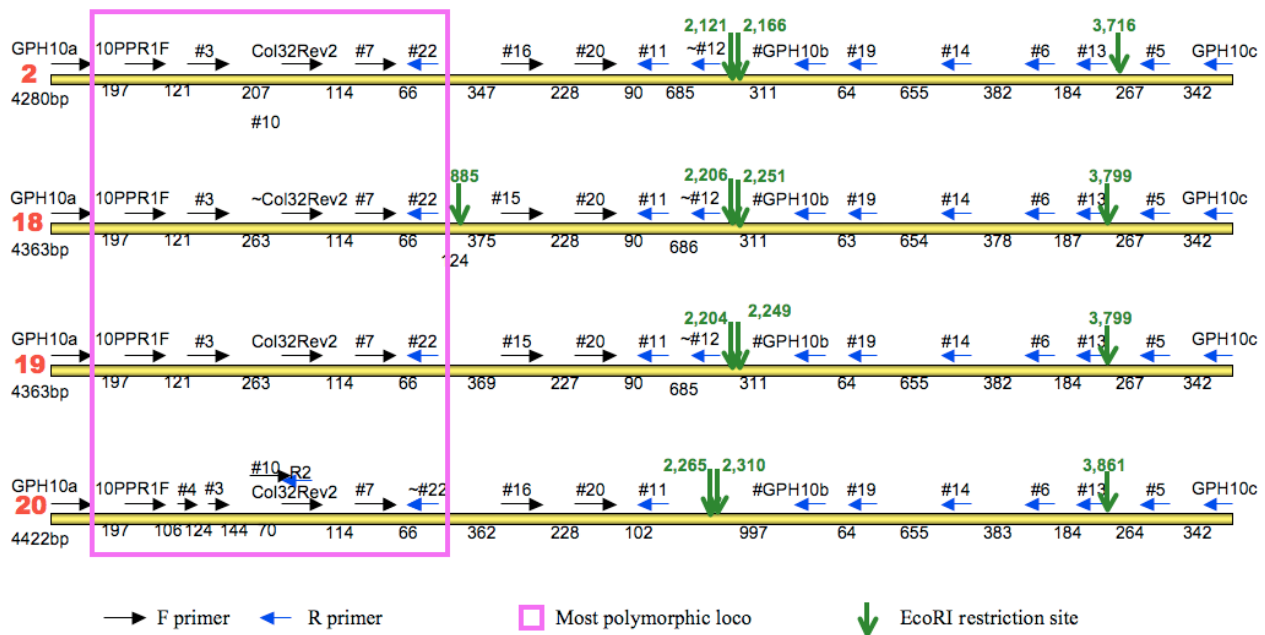


Figure 4-7. GPH10 clones, 4 alleles from the octoploid *Fragaria* × *ananassa*, detected by distinct *EcoRI* (green, vertical arrows) restriction patterns. The primers designed to amplify and sequence all 4.4kb clones are represented by black and blue arrows. The numbers between primers are the distances (in bp) between primers. The boxed region contained most of the polymorphism observed for GPH10, and it is comprehended between primers 10PPR1 and 10AB#22.

```

72E18_vesca          GAAAA-AAAGAGAGAGA--AAATTACAGATTTAAAGCGACGAACAA-TGAAAAGGAATGA 601
72E18_mandshurica   GAAAA-AAAGAGAGAGA--AAATTACAGATCTAAAGCGACGAACAG-TGAGAAGGAATGA 594
72E18_nilgerrensis  NNAAA-AAAGAGAGAGAGA---TTACAGATCTAN-GCGACGAACAA-TGAGAAGGAATGA 593
72E18_viridis       AGAAATAAAGAGAGAGA--AAATTACAGATCTAAAGTGACGAACAA-TGAGAAGGAATGA 604
72E18_iinumae       GAAAAAAGAAGAGAGA--AAATTACAGATCTAAAGCGACGAACAAATGAGAAGGAATGA 658
72E18_ananassa      GAAAAAAGAAGAGAGAGAAAATTACAGATCTAAAGCGACGAACAA-TGAGAAGGAATGA 638
                    *** **  ** ***** * ***** * * ***** *** *****

72E18_vesca          GAGGCAAAGAGAAGAGATGAGGAAGTTGACCTTTGTGAATGAGAGTGAGTGAGGGAGAGA 661
72E18_mandshurica   GAGGCAGAGAGAAGAGATGAGGAAGTTGACCTTTGTGAATGAGAGTGAGTGAGGGAGAGA 654
72E18_nilgerrensis  GAGGCAGAGAGAAGAGATGAGGAAGTTGACCTTTGTGAATGAGAGTGAGT-----GA 645
72E18_viridis       GAGGCAGAGAGAAGAGATGAGGAAGTTGACCTTTGTGAATGAGAGTGAGTGAGG--GAGA 662
72E18_iinumae       GAGACAGAGAGAAGAGATGAGGAAGTTGACCTTTGTGAATGAGAGT-----GAGAGA 710
72E18_ananassa      GAGGCAGAGAGAAGAGATGAGGAAGTTGACCTTTGTGA-----GTGAGGGAGAGA 688
                    *** *  ***** ***** ***** *** **

72E18_vesca          GAGAGAGAGATCGACGACGAAGCAGAGCGAAAGAGACGAGTGTGGTGTGTTGTGAGTTGAG 721
72E18_mandshurica   GAGAGAGAGATCGACGACGAAGCAGAGCGAAAGAGACGAGTGTGGTGTGTTGTGAGTTGAG 714
72E18_nilgerrensis  GAGAGAGAGATCGAAGACGAAGCAGAGCGAAAGAGACGAGTGTGGTGTGTTGTGAGTTGAG 706
72E18_viridis       GAGAGAGAGATCGAAGACGAAGCAGAGCGAAAGAGACGAGTGTGGTGTGTTGTGAGTTGAG 722
72E18_iinumae       GAGAGAGAGATCGAAGACGAGGCAGAGCGAAAGAGACGAGTGTGGTGTGTTGTGAGTTGAG 771
72E18_ananassa      GAGAGAGAGATCGAAGACGAAGCTGAGCGAAAGAGACGAGTGTGGTGTGTTGTGAGTTGAG 749
                    ***** ***** ** *****

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Figure 4-8. Subset of GPH72E18 alignment displaying SSR polymorphisms.

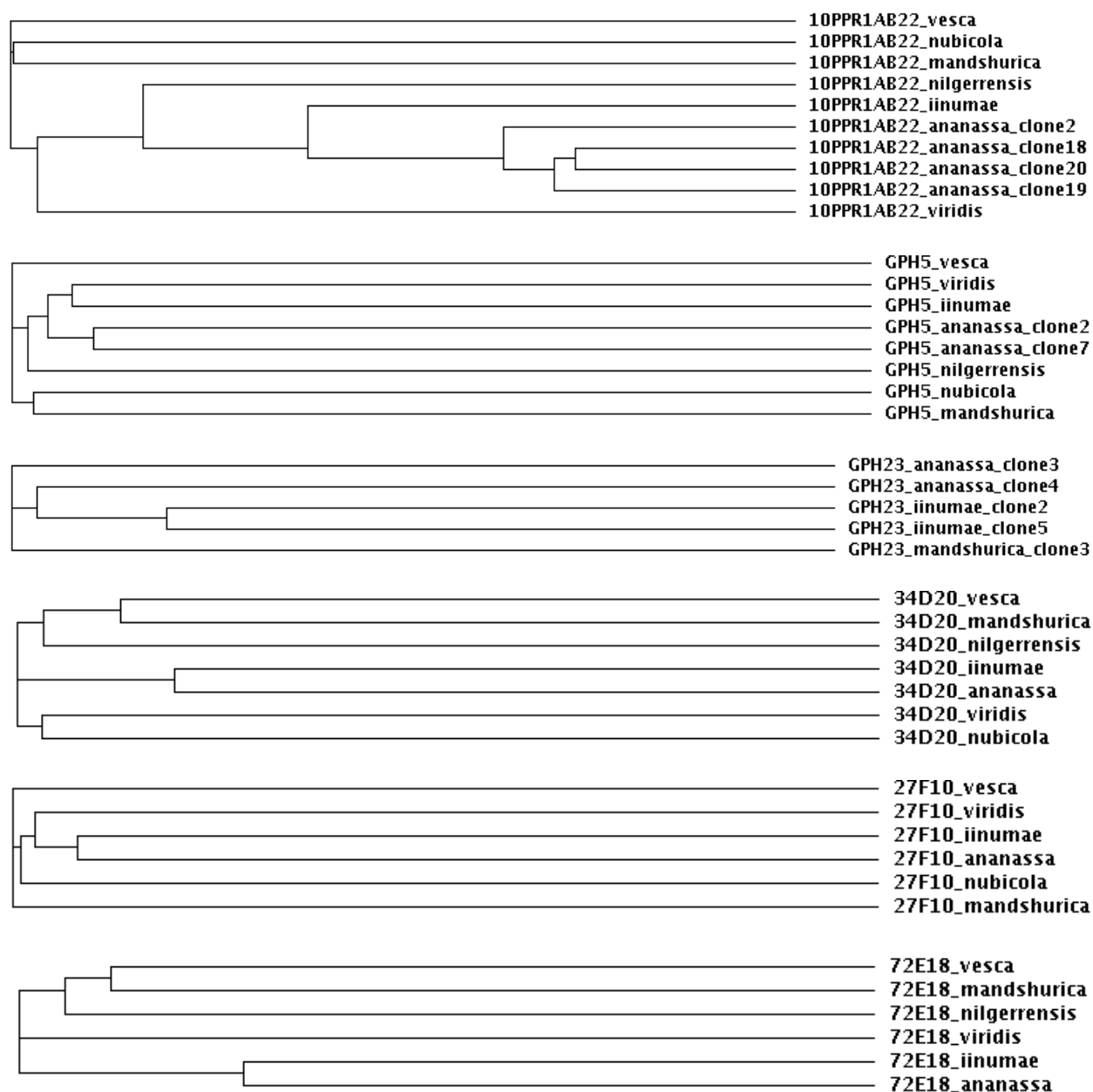


Figure 4-9. Cladograms of *F. × ananassa* and diploid alleles for six independent GPH loci. Amplified loci were sequenced, aligned with ClustalW, and their relatedness represented through cladograms. The *F. iinumae* clones were the most related diploid to *F. × ananassa* clones in every locus analyzed. *F. vesca* clones, on the other hand, were the furthest from the octoploid, contrary to prediction based on data of other author's previous studies.

Table 4-3. Overview of insertions and deletions detected through alignment of all sequenced clones. Each column represents an aligned region within haplotypes of a specific locus. The aligned regions where an indel or SSR were identified were named with Roman numbers. No relationship between clones of different loci is implied by the utilization of the same Roman number, as each locus was analyzed independently from the others. The Arabic numbers signify the number of bases in the deletions or insertions (*minus* or *plus* signs, respectively) in relationship to the consensus observed. White boxes represent accordance to the consensus sequence for the region in focus.

Clone	Indels and SSRs in Polymorphic Loci													
10PPR1AB22	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
<i>nubicola</i>		-5						6 TA						
<i>mandshurica</i>		-5						5 TA						
<i>vesca</i>		-5						8 TA						
<i>viridis</i>		-5						7 TA						
<i>nilgerrensis</i>			-4						+6			+4	-44	
<i>ananassa_18</i>			-4				+36				-15		-176	
<i>ananassa_20</i>			-4	+19	+3	+7	+71		+6	-12	-15		-181	
<i>ananassa_19</i>			-4				+36				-15		-181	
<i>ananassa_2</i>			-4						+6	-20	-15		-181	
<i>iinumae</i>	+5		-4							-566				-8

11D02	I	II	III	IV	V	VI
<i>viridis</i>	-4		+8	+44		
<i>nubicola</i>					+5	
<i>vesca</i>					+5	
<i>iinumae</i>		-28		+32		+27

17O22	I	II	III	IV
<i>vesca</i>			-36	
<i>mandshurica</i>				
<i>viridis</i>		+5		-5
<i>nubicola</i>	-5		-16	
<i>iinumae</i>				

27F10	I	II	III	IV	V	VI	VII	VIII	IX	X
<i>vesca</i>	+2							-2		
<i>mandshurica</i>	+2				-2			-2		
<i>nubicola</i>					-9			-2		-3
<i>iinumae</i>		-7	+6	+12	-5					-3
<i>ananassa</i>		-14	+6	+12	-26	+8			+3	-3
<i>viridis</i>							+505			

29G10	I	II	III	IV	V
<i>vesca</i>					
<i>mandshurica</i>					
<i>nubicola</i>	-1	+2	-13	+7	
<i>nilgerrensis</i>					-1

Table 4-3. continued

Clone	Indels and SSRs in Polymorphic Loci											
32L07	I	II	III	IV	V	VI						
<i>vesca</i>				-9								
<i>viridis</i>	-792		-4		-9	-5						
34D20	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	
<i>vesca</i>		+4					+38					
<i>mandshurica</i>		+4					+38					
<i>nilgerrensis</i>			-7		-13			-11	-3	+3	+15	
<i>iinumae</i>	-28		-2	-30		-3						
<i>ananassa</i>			-2	-30		-3			-3		+15	
<i>viridis</i>			-2									
<i>nubicola</i>			-2		-13							
63F17	I	II	III	IV	V	VI						
<i>vesca</i>						-2						
<i>mandshurica</i>				-4	-6							
<i>viridis</i>	-6	+2	-4		-12							
<i>ananassa</i>						-2						
72E18	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	
<i>vesca</i>				4 GA	8 GA							
<i>mandshurica</i>	-8			4 GA	8 GA							
<i>nilgerrensis</i>				5 GA	6 GA	-11	-13	-18	-10	-44		
<i>viridis</i>				4 GA	7 GA						+3	
<i>iinumae</i>			+53	3 GA	8 GA							
<i>ananassa</i>		-11	+45	4 GA	8 GA	-96						

CHAPTER 5
GENE-PAIR HAPLOTYPES: FUNCTIONAL AND TRANSFERABLE MARKERS AS
NOVEL ADDITIONS TO THE DIPLOID *Fragaria* GENETIC LINKAGE REFERENCE MAP

Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) is an economically valuable fruit crop, with average consumption of over 7.3 pounds *per capita* in 2005 in the United States (FAO STAT). The demand tends to increase due to public awareness of the potential health benefits of strawberry: small fruits have been shown to have high content of antioxidants (Wang, 2006), polyphenols and micronutrients that may play a role in human health.

Despite of the great importance of strawberry, knowledge of its genetic composition is very modest. The cultivated strawberry is octoploid, complicating development of molecular markers and construction of genetic linkage maps. Researchers have resorted to utilizing wild diploid strawberries to generate the first linkage relationships, in the hope of extending the findings to octoploid genomes. The first genetic linkages identified showed relationships between fruit color (Williamson et al., 1995) and runnering (Yu and Davis, 1995) to the shikimate dehydrogenase and phosphoglucoisomerase loci, respectively. These associations were shown in *Fragaria vesca*, a diploid that has been proposed to be a possible “A type” genome donor to the cultivated strawberry (Potter et al., 2000). The first indirect evidence of *F. vesca* as a genome contributor to the cultivated octoploid comes from cytological studies by Ichijima in 1926, where he showed the formation of 21 bivalents and 7 univalents during the pairing between *F. vesca* (then called *F. bracteata*) and *F. virginiana*, the pistillate parent to *F.* × *ananassa*.

The first genetic linkage map developed for strawberry was constructed using Randomly Amplified Polymorphic DNA (RAPD) markers developed for an F2 population derived from a cross between two subspecies of the diploid *F. vesca*: ssp. *vesca* ‘Baron Solemacher’ (red-

fruited, runnerless) and ssp. *americana* wild accession WC6 (Davis and Yu, 1997). The map was populated with 3 isozymes and 75 RAPD markers, of which 11 were codominant. This was possible due to a novel approach to Polymerase Chain Reactions (PCR), using mixed DNA templates for formation of heteroduplex bands (Davis et al., 1995). The locations of six genes involved in the anthocyanin pathway were assigned into this map later (Deng and Davis, 2001).

The second strawberry linkage map was developed for *F. × ananassa*, to increase the knowledge of the octoploid genome and to address questions on inheritance patterns in strawberry (if disomic or polysomic) (Lerceteau-Köhler et al., 2003). Amplified Fragment Length Polymorphism (AFLP) markers were used to generate separate maps for the male and the female parents, with 235 markers in 30 linkage groups, and 280 markers in 28 linkage groups, respectively. Though the study generated very detailed maps, with a total of 789 markers, AFLP markers are not easily transferable between species or even populations. The density of markers did add evidence of polysomic inheritance, since genes apparently crossed between subgenomes with some frequency.

A third map was constructed for strawberry (Sargent et al., 2004) addressing RAPD and AFLP transferability issues through the use of microsatellite markers or polymorphic Simple Sequence Repeats (SSRs). The map was based on a polymorphic F2 population generated from a wide inter-specific cross between the diploids *F. vesca* ssp. *vesca* f. *semperflorens* FDP815 (pistillate parent) and *F. nubicola* FDP601 (pollinating parent). These diploids have been shown to be the most closely related diploid relatives to the cultivated octoploid species (Potter et al., 2000). The creation of a reference map using a diploid relative is an approach commonly used to map genetically complex polyploids. Examples of polyploids for which reference maps have been constructed are wheat (Kam-Morgan et al., 1989), alfalfa (Diwan et al., 2000), and potato

(Milbourne et al., 1998). The map published in 2004 had 78 markers and new microsatellite loci were added later, totaling 182 markers (Sargent et al., 2006).

Strawberry belongs to the Rosaceae family, to which the horticulturally important peach, cherry, apple, raspberry, and rose also belong. Although SSRs are markers transferable between mapping progenies within and between species (Dirlewanger et al., 2002) (Hadonou et al., 2004), they are generally not transferable between genera. The challenge in developing transferable markers resides in the fact that markers are, by definition, placed on polymorphic regions of the DNA and, to be transferable, such markers are must be located on conserved regions. A recent study (Sargent et al., 2007) explored intron length polymorphisms, having PCR primers anchored in flanking exons that were conserved across *Prunus* and *Malus*, and thus generated highly transferable markers. In addition, because these markers were gene-linked, they also provided functional information.

A new approach to development of transferable and functional markers was explored by this research. The innovative mapping tool, named “Gene-Pair Haplotype” (GPH) consists of a stretch of intergenic space and takes advantage of its rich polymorphism for the development of markers. GPHs are PCR-amplifiable, with PCR primers anchored to exons of adjacent genes, making these makers transferable between species where microcolinearity is maintained. A significant degree of conservation between *Fragaria*, *Medicago* and *Arabidopsis* has been demonstrated (T. M. Davis, *personal communication*) suggesting that these same intervals might be easily transferable between rosaceous crops.

This investigation aimed to introduce the gene-pair haplotype concept as an innovative mapping tool, thereby increasing the number of transferable and functional markers genetically linked to the existing *F. vesca* x *F. nubicola* diploid reference map.

Materials and Methods

The diploid mapping population generated by Sargent *et al.* (Sargent et al., 2004) (a cross between *F. vesca* ssp. *vesca* f. *semperflorens* FDP815 and *F. nubicola* FDP601) was used in this study. Lyophilized tissue was received from the rosaceous genomics research group in East Malling Research station, in Kent, England. DNA was extracted as described in protocol #29, appendix A. Approximately 7 mg of lyophilized tissue was frozen in liquid nitrogen and ground in a mortar to a fine powder. After addition of 1ml of extraction buffer (2% CTAB, 1.4M NaCl, 100mM Tris-HCl pH 8.0, 20mM EDTA pH 8.0, 1% 2-mercaptoethanol), the tissue was further macerated until no defined leaf particles were observed. The volume was split into two 1.5- μ l tubes, samples incubated at 65°C for 1h, and 1 vol of 24:1 chloroform:octanol was added to each tube. After mixing the organic solvents with the extraction buffer and plant tissue, the samples were centrifuged at 13,000 rpm for 5 min. The upper phases were transferred to new tubes, and the nucleic acids precipitated by equal volume of isopropanol, centrifuged, the supernatant discarded, the pellet air-dried, and resuspended in 50 μ l TE pH 8.0. The DNA concentration varied from 40ng/ μ l to 4,543ng/ μ l.

Target regions for marker development were derived from the *F. vesca* ‘Pawtuckaway’ sequence annotation described in Chapter 3. Similarities between the *F. vesca* genomic sequence and either proteins or ESTs were sought for each of the 26 fosmid insert sequences. Within each fosmid clone, the most suitable pair of genes for PCR amplification was determined according to the following criteria:

- i, The putative intergenic space should be large enough to permit detection of polymorphisms, but not larger than 3.5 kb due to technical limitations of amplification by PCR. Putative genes in fosmids 15B13 and 22L05 were separated by > 4kb, therefore these clones were excluded from the potential GPH pool.

ii, Tandem and non-tandem duplications were avoided as potential targets for PCR primer design. Target sequences should be unique to yield locus-specific amplification, since the assessment of more than one locus at a time would complicate data scoring. Tandem duplications were detected when adjacent *F. vesca* query sequences that had the same BLASTX hit, which appeared to indicate gene family clusters (e.g.: putative genes in fosmids 05N03, 13I03, and 18A19). An exception was made for the chalcone synthase (CHS) gene, which was included in the study although tandemly duplicated. The intergenic region is ~2,300 bp in *F. vesca* 'Pawtuckaway', but varies from 2 kb to over 8 kb in different rosaceous species tested, making this marker transferable across genera (T. M. Davis, *personal communication*).

Non-tandem duplications required indirect evidence, since only 1% of the *F. vesca* genome's sequence was available for analyses. If nucleotide identity was detected through BLASTN between a *F. vesca* sequence and more than one locus belonging to a single organism, that was regarded as evidence of potential duplication in *F. vesca*.

iii, Some fosmid clones did not appear to contain gene pairs when similarity to database-deposited protein sequence was the criterion adopted to classify a sequence as a putative gene. In those cases, a potential gene pair was inferred by two sequences displaying similarities, one to proteins and the other to ESTs.

Once apparent single copy, PCR-amplifiable putative gene pairs were identified, the software Primer3 (Rozen and Skaletsky, 2000) was used to facilitate design of PCR primers. For each primer pair, the forward primer was designed on the 3' end of a putative exon sequence of a gene, whereas the reverse primer was designed in the 5' end of the putative exon sequence of the downstream gene. In some cases, more than one primer pair was designed to generate a single band product, polymorphic between the parents—before or after restriction digest. In those

cases, a primer with the fosmid clone name and orientation (F or R) had a suffix added to indicate another set. Figure 5-1 illustrates the case of primers designed for fosmid 40M11.

The PCR amplification 50 μ l-reaction components and conditions for the parental DNA and for the 94 F2 samples were: 1x buffer (at 10x concentration, composition was 35mM MgCl₂, 37.5 μ g/ml BSA, 160mM KCl, 400mM Tricine-KOH pH 8.0), 0.2mM each dNTP, 0.2 μ M each primer, 0.05unit Taq polymerase, 1 μ l DNA template, at variable concentrations (40ng/ μ l to 4.5 μ g/ μ l). Initial denaturation: 94°C, 2 min, followed by 35 cycles of: denaturation at 94°C for 15 sec, annealing for 45 sec, and extension at 72°C. A last extension of 5min at 72°C after the 35 cycles was executed. Table 5-1 contains functional information of the gene pair amplified, as well as annealing temperatures and the extension times dependent on the primer pair used. In general, extension was carried out for 1 minute per kb amplified, and the annealing temperature was primarily based on the primer melting temperature (T_m) calculated by Primer3 (Rozen and Skaletsky, 2000) using the formula described in (Rychlik et al., 1990) (though in many cases a range of temperatures had to be tested). Positive control primers FvLFYintron2F/ FvLeafy3' are anchored to the exons that flank Leafy gene's second intron (P. J. Stewart, *personal communication*). This intron size is variable among diploid species, being 770bp-long in *F. vesca* 'Pawtuckaway'. The annealing temperature and extension time were variable, since the primer pair under investigation was their determinant.

Following successful PCR amplification, 10 μ l of each single-band amplicons were digested with 1unit of different restriction enzymes (table 5-2) in a total volume of 20 μ l. Amplicon polymorphisms were resolved in 2% agarose gel, 1x TAE buffer, 0.5 μ g/ml ethidium bromide, at 80V, during variable times that were a function of the size of the digested fragments. The gel was exposed to 300-nm UV light for visualization of DNA fragments.

In order to obtain the most precise linkage, analyses were performed against the data set presented in Sargent *et al.* (Sargent et al., 2006), including new information available since the last publication. The novel GPH markers were assigned into linkage groups utilizing the software JoinMap 3.0 (Van Ooijen and Voorrips, 2001) with the application of the Kosambi mapping function and a minimum LOD score threshold of 3.0. The maps presented were constructed using MapChart software (Voorrips, 2002).

Results

Amplification was observed for all primer pairs, though not all were suitable for mapping purposes. Eight GPH primer pairs produced single-band amplicons that were scorable after restriction digest. The remaining primer pairs were not scored for the population for a variety of reasons. Primer pairs 01L02Fb/Rb, 01L02Fb/Rc, 22H18F/R, 22H18F/Rb, 30I24F/R, 32A10F/R, 32A10Fb/R, and 38H02F/R amplified multiple bands even at stringent annealing temperatures and restrictive extension times. The banding pattern for 01L02Fb/Rb appears to be due to a duplication, since two major bands are detected, one of the expected size, the other with higher molecular weight. Amplification by the other primer pairs displayed multiple bands, similar to non-specific amplification. There were primer pairs for which amplifications were observed, but they were not polymorphic (e.g. 10B08Fb/Rb). For others, amplicons were polymorphic, but only a few members of the F2 population were amplifiable. This was the case of both 10B08 (GPHleafy/GPHacs, which amplified a 3.8 kb region that was polymorphic when digested with *EcoRI*) and 32L07F/Rb, polymorphic after treatment with *HaeIII*.

Both parents, when amplified by primers for 34D20, produced amplicons that were the same size. Restriction digestion revealed a rather complicated banding pattern. All of the digested amplicon fragment sizes < 700bp observed for 34D20 were expected, according to the

predicted restriction pattern for *F. vesca* ‘Pawtuckaway’. An unexpected fragment of 1249 bp was observed for *F. vesca*, raising a concern that the putative single locus was in fact two loci. The other possibility was that the higher molecular weight band was a different *F. vesca* allele from the same locus. Had that been the case, a heterozygote should have been observed containing the female allele (1249, 300, 251bp) and the male allele (702, 335, 308, 251 bp). Such an individual was not observed, as 1249bp band cosegregated with the 758 and 429 bp bands. The presence of the 1.25 kb band was attributed to partial restriction digestion and the scoring was therefore carried out based solely on the expected 758 and 429bp bands *versus* the 702 and 335bp bands. Figure 5-2 shows banding pattern for digested amplicons of 34D20 and 72E18.

GPH40M11 is a dominant marker and amplifies a band only for the pistillate parent, *F. vesca*. Since the PCR amplification was precluded for half of the F2 population for some reason, this raised a concern about wrongly scoring individuals as homozygous *F. nubicola*. Thus, amplification patterns for all other 7 loci were compared, using a primer pair as positive control. Individuals for which amplification was observed in all those primer pairs but not observed for 40M11 were scored as homozygous for the *F. nubicola* allele.

The majority of the GPHs investigated were assigned to linkage group VII, as shown in figure 5-3.

Discussion

Gene pair haplotypes are intergenic, multiple character signatures that define suites of variability between two genomes. The purpose for these markers is to provide a complex field of discrete variation that can be related to a specific subgenome donor with the goal of eventually mapping genes to specific subgenomes of the octoploid strawberry. This chapter outlines the

first step in this process, that is, to test if intergenic variability could be used to assign GPH loci to the diploid linkage map.

In all cases the GPH loci were assigned to the linkage map using a CAPS marker approach. Here amplicons were digested with a restriction enzyme that corresponded to sequence variation in the parental lines. A mapping population was treated with identical conditions to reveal the genotype of the specific F2 plant. Analysis of segregation with isozyme, morphological and molecular markers allowed assignment of these GPH loci to the diploid linkage map.

The assignment of these loci to the current map is important for two reasons. First, it demonstrates that the GPH is a viable marker- in this case based on a single restriction site. Other variable characters certainly exist in these regions that will complement the detection noted by this restriction site. In the future, these GPH loci will likely serve as anchors for the octoploid linkage map, because their likely variability supercedes that which is possible from a simple SSR or other marker used for diploid mapping.

This study places markers on linkage groups I, VI, and VII, with several independent markers in the latter. The next step is to translate these markers to an octoploid mapping population. This will immediately bring relevance to the endeavor because GPH loci stem from or are located near genes of known function. In this study GPH 17O22 is localized near F3H whereas 73I22 associates with chalcone synthase, two genes necessary for fruit color production and protective leaf pigments. A breeder with an interest in improving fruit color or possibly increasing plant survival in high light environments may find such loci useful in breeding selections.

The localization of the CHS gene determined by the GPH approach was different from the linkage group to which the gene was assigned when intron length was used to map it in a *F*.

vesca ssp. *bracteata* DN1C x *F. vesca* ssp. *vesca* ‘Yellow Wonder’ F2 population (Deng and Davis, 2001). This may be evidence of multiple copies of the CHS gene in the *Fragaria* genome. While described as a single-copy gene in Brassicaceae (Koch et al., 2000), CHS is a multigene family in many plant species (Jin-Xia et al., 2004). The CHS gene family is comprised of at least seven members, which, at least in petunia and poplar, are mapped to different linkage groups: II and V (Koes et al., 1987), and I and III (Tsai et al., 2006), respectively. It is possible that the different localizations in the genome correlate with different gene functions. In common morning glory (*Ipomoea purpurea*, Convolvulaceae) (Durbin et al., 1995) as well as in *Gerbera hybrida* (Asteraceae) (Helariutta et al., 1996), different family members have shown to have functional divergence.

The experimental outcomes of this chapter validate the use of GPH loci for mapping in the diploid strawberry and suggest great utility in application to octoploid mapping and breeding populations. Their complex characters, ease of detection, coupled to apparent disomic inheritance within octoploid subgenomes, indicate that these may be implemented in practical breeding scenarios.

Conclusions

The experimental trials outlined in this work test various aspects of strawberry structural genomics. From difficult honing of protocols to hasten DNA preparation from recalcitrant tissue, to computational analyses, development and proof-of-concept assessment of a novel molecular marker, these trials present new facets of understanding the complicated genome of the cultivated strawberry.

Recalcitrance to DNA extraction from plants is commonly attributed to their polyphenol and carbohydrate contents. Strawberry appears to be recalcitrant not only due to high sugars and phenols, but also because of strong physical barriers that guard the DNA. The results of over 103

systematic tests of various experimental conditions indicate that the most important consideration is complete disruption of the tissue via maceration, and that this process may be greatly enhanced by co-application of chemical lysis to disrupt tissue. My study provides a comprehensive evaluation of all published techniques and provides a unified protocol that works to some degree in all strawberry cultivars and species tested.

The importance of sequence information as a foundation for functional genomics studies in strawberry has been revealed by the discovery of enzymes associated with flavor (Wein et al., 2002) and fruit firmness (Llop-Tous et al., 1999) (Benitez-Burraco et al., 2003). This project represents the first efforts to examine the genome structure of *F. vesca*. The data indicate that the small genome of *F. vesca* maintains a character and composition similar to other model plant species, suggesting that this species will have utility in answering questions within the Rosaceae family.

Annotation of fosmid inserts leads to the understanding of gene content and distribution, and permits marker generation for linkage mapping. More importantly, this initial survey of the strawberry genome is the first opportunity to compare strawberry to sequences to those of other organisms. Here relationships between the general properties of the genome have been deciphered. Strawberry is a gene-dense organism that maintains a significant content of mobile elements, and microcolinearity with other known genomes (T. M. Davis, *personal communication*).

Detection of gene pairs by searching for micro-colinearity between *F. × ananassa* and *Arabidopsis* is a clever approach, but it needs to be automated to increase the chances of finding adjacent genes. This approach has the advantage that it is not based on *F. vesca* sequence. Therefore, amplification of haplotypes is not biased towards *F. vesca*-like alleles. In addition,

because sequences utilized for similarity search were from *F. × ananassa*, this method is better than the annotation of *F. vesca* genome method to address questions of diploid subgenome contributions to the octoploid. Primer pairs designed for gene pairs detected through this method amplified the octoploid, whereas most (8 out of 11) of the primer pairs generated through *F. vesca* genomic sequence did not amplify alleles from the cultivated strawberry. This study further supports the likelihood of *F. iinumae* as the B genome donor to the octoploid.

The approach based on gene prediction to identify gene pairs, had a higher amplification success rate and it is useful to characterize intergenic regions, serving as a tool to detect polymorphisms between diploids. Chapter 5 showed how this approach was successfully employed to create molecular markers in the *Fragaria* diploid reference map.

We have described the development and mapping of 8 markers, linked to at least one gene of known function. Therefore, this investigation proved the concept that putative intergenic regions may be used as functional markers. In addition, because the markers are designed for conserved sequences across different taxa in Viridiplantae, there is great potential for transferability and use on comparative mapping to appreciate Rosaceae structural genomics.

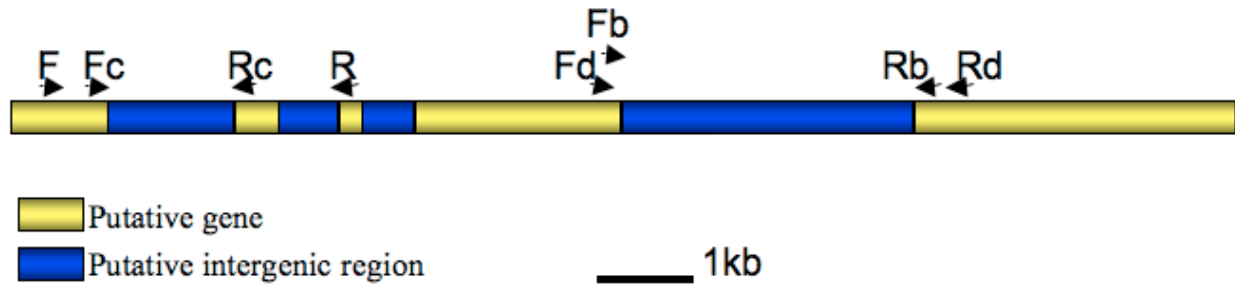


Figure 5-1. Fosmid 40M11 with primers designed on exons of FGENESH-predicted genic regions.

Table 5-1. PCR primer pairs and amplification conditions used in this study

Primer	Putative Gene Function or EST gb number	Sequence 5' to 3'	T _{annealing} (°C)	Extension Time
Control F FvLFYintron2F	Leafy	CACTGCCAAGGAGCGTGGTG	variable	variable
Control R FvLeafy3'	Leafy	TCAGTAGGGCAGCTGATG		
01L02Fb	EST AY573376	GAACCGTTC AAGTTCATAATTGG	54-65	1'30'' - 2'30''
01L02Rb	unknown protein	AAGGGAGGACGTTCAATGTG		
01L02Rc	unknown protein	ACGGAGATCGGGGACTTGT	54-58	2'30''
10B08F	Leafy protein	GGGCCAACTACATCAACAAGC	58-63	3' - 4'
10B08R	ACC synthase	TGTTCTGTTGGGTGGACATGA		
10B08Fb	ACC synthase	TGCCATCGTTTCCATCAGTA	52	1'
10B08Rb	ribosomal protein	CGCGAAGATCATGAAGAACA		
11D02F	EST BQ105541	GAGCTGCTGTGTGAACCAA	56-60	2'30''
11D02R	heat shock binding protein	GTTCAACTCCAGATGAAGTGAGG		
17O22F	Oligopeptidase	AAAATGGGTTGCACGAGTTC	60	2'
17O22Rb	Putative protein	GGGTTTCCTCACAACTTCG		
17O22Fb	Oligopeptidase	GGTACCTCCAATGCAAGGAA	53-60	1'30'' - 2''
17O22R	Putative protein	TTCATCAGAGAAGGCGGACT		
22H18F	EST DY646954	ACCAATGCTTGGACACACAC	52-65	2'30''
22H18R	unknown protein	GATGAAATTCATGCTTGTGAC		
22H18Rb	unknown protein	GGACTCCATGTAACACGGCTA	56-65	2'30''
27F10F	kinase	CCTGCAGGGTTTTTCATCAT	59	1'
27F10R	hypothetical protein	TGGAATGTATTCTGGTTCTCC		
29G10F	phenylacetaldehyde synthase	TGGCCTTGTTTCCTAAACTCTT	59	1'
29G10R	unknown protein	AGAAGAAGGCAGCACCCAAT		
30I24F	transferase	TTGAGAGAGGTCTCCAAGCTC	54, 59	4'
30I24R	chromating remodeling factor	CGGAAGATGGCAAGCTATTG		
32A10F	isomerase (E > 10 ⁻¹²) pathogenesis-related protein	CGGAGAGAACGATGGAGTTG	52-62	1
32A10Fb		CCAAATGAATCAAGCTCAAGTG		
32A10R		ATTGTGACAGTGCAGCAA		
32L02F	SMC2 (Structural maintenance of chromosomes)	GAGTTGAAAAACGGGTCGAA	53-61	2'
32L03Fb		CCTTCCAAGGTCACCTCCTT		
32L02Fc		TTAGCCCGGTTATGGAGTTG		
32L02R		GAAGGTTCAAGGAGCATGGA		
32L02Rb	Exostosin	AGGAAAATGCGGGAGAAAGT		
32L02Rc		GAACGATTTCCGAGGTGTGT		

Table 5-1. continued

Primer	Putative Gene Function or EST gb number	Sequence 5' to 3'	T _{annealing} (°C)	Extension Time
34D20Fb 34D20Rc	RNA recognition motif cysteine-type peptidase	GCAGAAAGAAACTGATGTGCTT CGCAGTCGTAAAAATTTCGTCT	60	3'30''
38H02F 38H02R	serine/threonine kinase exportin	CCAGGCCTAAGCTTGTTCATC AAGGCATTGAAATCATTCTACCA	53, 54, 60	4'
40M11F 40M11Fc 40M11R 40M11Rc 40M11Fb 40M11Fd 40M11Rd 40M11Rb	F-box protein transposase (E > 10 ⁻¹³) expressed protein (E > 10 ⁻⁹) secretory protein SEC14 ATPase	ACACAGGTCATTGGGTCCAT TTGACCCGGATAACATGGAT GTGTTGCACAAGTCCATTTCG CTGACAGCGAATCAATCTGC GGCCTTCTTGACATTCCAGT CAACATTTTGGTGGCCTTCT CGGCCTATGAAACCACAGTT TGGGGTTGTTGGAAGAGAG	60	4'
63F17F 63F17R	phospholipase D unknown protein	CGCTCTATGGAAGGGACAAG TTAAGGGGTCTGTTGATGTGC	59	1'
72E18Fb 72E18Rb	actin elongase	GCTAGGGAAAACAGCTCGTG TGGGTTTGGTTTTGGGATAA	60	2'30''
73I22F 73I22R	chalcone synthase A chalcone synthase B	CAAGCCTGAGAAGTTAGAAGC GAAAGTAGTAGTCGGGGTATGT	62	5'
GPH10a GPH10b GPH10c	unknown protein unknown protein unknown protein	GGCTTCTTCTTGTCCGGCAGC GAACTCCAGGTCAGATCTTCG CTCGCTGCAAATCAGCTACC		2'30'' 4'

Table 5-2. Fragment sizes of parental amplicons digested with restriction enzymes

Locus	Restriction Enzyme	Amplicon estimate fragment sizes (bp)		
		Non-digested	Digested	
			<i>F. vesca</i>	<i>F. nubicola</i>
17O22FRb	<i>RsaI</i>	1,374	486, 413, 292, 83, 67, 28, 5	511, 414, 293, 83, 67, 28, 5
34D20FbRc	<i>AluI</i>	2,050	1249, 758, 429, 300, 251, 107, 69, 48, 26	702, 335, 308, 251, 107, 69, 48, 45, 41, 26
40M11FdRd	Dominant marker	3,100	present	absent
63F17	<i>HaeIII</i>	1,266	992, 234, 40	840, 234, 40
72E18FbRb	<i>HhaI</i>	2,620	1,400, 800, 300	2,300, 300
73I22	<i>PvuII</i>	3,000	2,200, 1,000, 600	2,200, 1,500

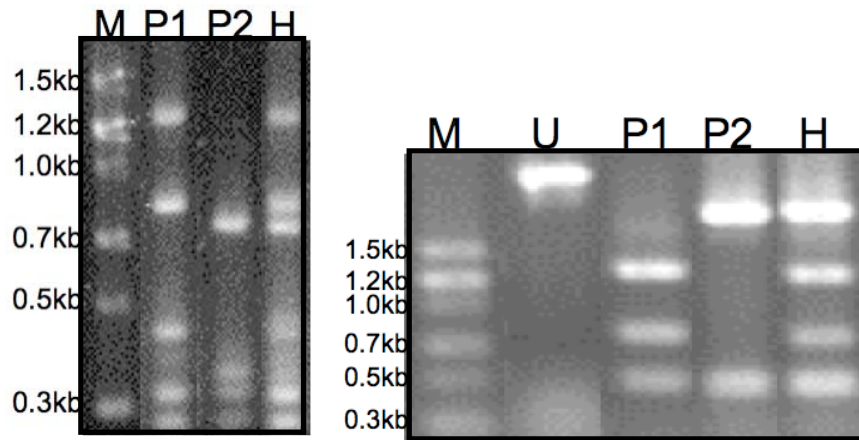


Figure 5-2. Amplicon restriction patterns for GPHs 34D20 and 72E18. M: molecular weight marker; U: uncut amplicon; P1: female parent, *F. vesca*, P2: male parent, *F. nubicola*; H: heterozygote.

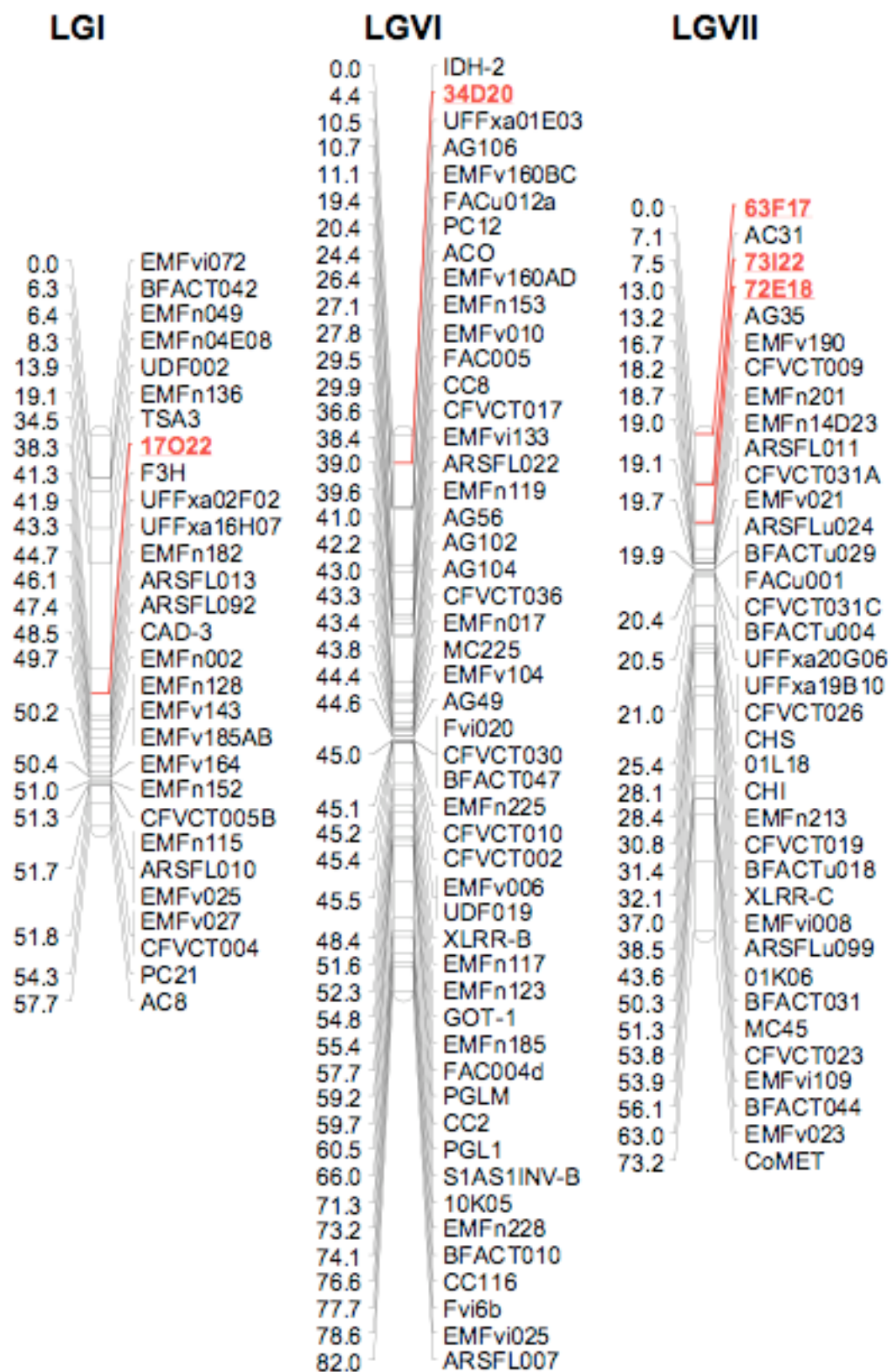


Figure 5-3. Gene-Pair Haplotypes assigned to linkage groups of the reference *Fragaria* map.

APPENDIX A DNA EXTRACTION PROTOCOLS

The numbered items below represent different protocols, whereas numbers preceded by a “T” signify treatment number and correlate with the treatment numbers used in Table 2-1.

In all protocols that used either 2-mercaptoethanol, sodium bisulfite or sulfite, these reducing agents were added just prior to use of buffers. Most procedures included at least one 25:24:1 phenol:chloroform:isoamyl alcohol deproteinization step followed by one 24:1 chloroform:octanol extraction. When RNase-treated, the enzymatic reaction was carried out at 50µg/ml. Precipitation of DNA was executed by adding 0.7 to 1 volume of isopropanol or by sodium acetate to reach final concentration of 0.3M plus two volumes of absolute ethanol, then washed with 70% ethanol, dried, and resuspended in sterile, deionized water. Except for buffers that involved guanidine thiocyanate, which were kept at room temperature, plant material in buffer was incubated 30-60 minutes at 65°C, unless otherwise stated. When product was obtained, 5-10 µg of DNA were digested with 2-4 restriction enzymes. Below is a brief description of each protocol.

DNA Extraction from Leaves

1. Tomato [Fulton, 1995]: utilizes a combination of a DNA extraction buffer (0.35M sorbitol, 0.1M Tris-base, 5mM ethylenediaminetetraacetic acid, EDTA, pH 7.5) and a nuclei lysis buffer (0.2M Tris, 0.05M EDTA, 2M NaCl, 2% CTAB) to make the micro prep buffer (42% extraction buffer, 42% nuclei lysis buffer, 16% sarkosyl 5%, and 0.02% sodium bisulfite). Used 0.5g (T1), 1g (T2), and 2g (T3) of ‘Strawberry Festival’ fresh mature leaf tissue, extracted by 5ml buffer.

2. Woody plants [Kobayashi, 1998], modified by A. M. Hadonou. Two extraction buffers are consecutively used, buffer 1 being used twice and the buffer 2 only once. Following centrifugation with buffer 1 (50mM Tris-HCl pH 8.0, 5mM EDTA, 0.35M sorbitol, 0.3% 2-mercaptoethanol, 10% polyethelene glycol, PEG), the supernatant is discarded before adding buffer 2 (50mM Tris-HCl pH 8.0, 5mM EDTA, 0.35M sorbitol, 0.3% 2-mercaptoethanol, 1% sarkosyl, 0.7M NaCl, 0.1% CTAB). Used 0.1g of two cultivars of *F. vesca* ssp. *vesca* f. *semperflorens*: Yellow Wonder (T4, T6) and Alexandria (T5, T7); fresh expanded leaf tissue, extracted by 1ml (T4, T5) or 10ml (T6, T7) of buffer.

3. Guanidine thiocyanate [Chomczynski, 1987]: The incubation was carried out for 5-15 minutes only and at room temperature instead of 65°C. Buffer composition: 4M guanidine thiocyanate, 100mM Tris-HCl, 10mM EDTA, 0.5M NaCl, 1% sarkosyl, 1% sodium sulfite. Newly expanded (T8) and unexpanded (T9) leaves of ‘Sweet Charlie’ were used for extraction from 100mg tissue in 100µl buffer. Further treatments to aliquots of the product of this prep were performed, aiming removal of contaminants: adsorption to a column from the DNeasy Plant Mini kit (T10) or dialyses into TE pH 7.0 at 4°C (T11). Dialyses was performed overnight, TE buffer replaced by fresh buffer, and dialyzed again for another day. Sample was 50µg/ml RNase- and 150µg/ml proteinase K-treated. DNA isolation was continued with phenol extraction and standard downstream steps.

4. Guanidine thiocyanate and CTAB utilized consecutively (T12): DNA isolation according to Chomczynski [Chomczynski, 1987], and resuspension of the ethanol-precipitated DNA in CTAB buffer described in Chang [Chang, 1993] for re-extraction, an attempt to rid

DNA prep of polysaccharides. The incubations were carried out at room temperature and 65°C with guanidine thiocyanate and CTAB, respectively.

5. Guanidine thiocyanate and CTAB used simultaneously: extraction buffer kept at room temperature, 15 minutes: 4M guanidine thiocyanate, 100mM Tris-HCl, 10mM EDTA pH 8.0, 0.5M NaCl, 1% sodium sulfite, 1% sarkosyl, 2% CTAB, 1% PVP, 2% 2-mercaptoethanol. Treatments included extraction from 10mg (T13, T15) and 100mg (T14, T16) of lyophilized (T13, T14) or fresh (T15, T16) tissues.

6. DNAzol® Extra Strength kit [Chomczynski, 1997]: incubation at room temperature, as suggested by manufacturer. Exact composition of buffers is cryptic, though it is known to contain a guanidine detergent. Tested extraction from 100mg (T17, T19) and 500mg (T18, T20) of lyophilized (T17, T18) or fresh (T19, T20) tissues.

7. Pine tree [Chang, 1993]: this protocol was originally designed for RNA extraction and was adapted here to DNA extraction by omitting the lithium chloride step. Buffer: 2% CTAB, 2% polyvinyl pyrrolidone (PVP), 100mM Tris-HCl, 25mM EDTA, 2M NaCl, 0.5g/L spermidine, 2% 2-mercaptoethanol. After the addition of equal volume of chloroform, samples were homogenized using a Polytron for 1 minute, at 9/10 of maximum speed. Tissue: 0.5g in 7ml buffer (T21).

8. Urea [Settles, 2004]: Phenol deproteination step was done together with incubation with extraction buffer, at room temperature for 20 minutes in 8M urea, 0.4M NaCl, 60mM Tris-HCl pH 8.0, 25mM EDTA pH 8.0, 1.5% sarkosine (T22). A variant of the buffer was also experimented, which consisted of supplementation with 1% sodium sulfite and 1% PVP to prevent oxidation of phenols (T23).

9. Strawberry (Manning, 1991): Buffer: 0.2M Tris, pH adjusted to 7.6 using boric acid (which forms complexes with polyphenols at pH 7.5 (King, 1971) and with carbohydrates (Gauch and Dugger Jr., 1953)), 10mM Na₂EDTA, 0.5% SDS, 2% 2-mercaptoethanol. After a 10-minute incubation at room temperature, equal volume of 25:24:1 of phenol:chloroform:isoamyl alcohol was added, mixed, and centrifuged for 10 min at 3,500rpm. Upper phase was transferred to a new tube (called "Tube A" here). "Tube B" contained inter- and lower phases from this first round of chloroform extraction. A second volume of extraction buffer was added to Tube B and a second round of chloroform extraction took place. The new upper phase from Tube B was combined with Tube A and split into 6 aliquots. Two aliquots (T24, T27) had polysaccharides precipitated by addition of 0.4 volume of 2-butoxyethanol, iced for 30 minutes, and centrifuged at 3,500rpm for 10 minutes. The other four aliquots were diluted by 2.5 (T25, T28) and 4 volumes (T26, T29) of a combination of 1M Na acetate buffer (pH adjusted to 4.5 by acetic acid) and water. The relative volumes of water and 1M Na acetate/acetic acid buffer were calculated to raise the Na concentration to 80mM. Considering that at this point each treatment had a volume of 3.3ml, the dilution by 2.5 volumes brought the volume to 8.3ml. Therefore, 664µl of the 1M Na acetate/acetic acid buffer and 4.3ml of water were required to reach the desired concentration of 80mM Na⁺. In the case of the dilution by 4 volumes, and still considering initial volume as 3.3ml, the final volume was 13.2ml. The sample received 10ml of (water+ sodium buffer), of which 9.2ml were water and 800µl were the 1M Na acetate/acetic acid buffer. After dilutions were made, T25, T26, T28, and T29 were precipitated as before: 2-butoxyethanol, were iced, and centrifuged. The goal of the centrifugation here is to precipitate polysaccharides, not nucleic acids. The six supernatants were transferred to new tubes and equal volumes of 2-butoxyethanol were added to precipitate nucleic acids. After icing for 30 minutes, the tubes were centrifuged for 10 min at 3,500rpm, the supernatant discarded, and the pellet

washed with a 1:1 solution of 0.2M boric acid/Tris, 10mM Na₂EDTA (pH 7.6) : 2-butoxyethanol. Pellets were washed with 70% ethanol, 0.1M acetate/acetic acid (pH 6.0), then with absolute ethanol. After dry, pellets were resuspended in 1ml water, and 10µg DNA digested with restriction enzymes. An aliquot of one of the treatments (T28) was EcoRI-digested before and after treatment with 150µg/ml Proteinase K and with phenol:chloroform. A second attempt to isolate digestible DNA using the strawberry protocol was made, adding antioxidants 4% PVP and 5mM ascorbic acid to the extraction buffer (T32-T35).

10. Several attempts were made to determine which isolated variable in the strawberry protocol plays the major role in DNA yield. The possibilities raised were: i, the SDS, rather than CTAB, nature of the protocol. Treatment numbers T16, T18, and T24 used SDS, therefore testing this variable; ii, the boric acid, instead of HCl, used to adjust the pH of Tris; iii, the re-extraction of interphase formed after chloroform treatment; iv, the dilution that raised Na concentration to 80mM prior to DNA precipitation; v, precipitation by 2-butoxyethanol in place of isopropanol or ethanol. The isolated roles of boric acid and 2-butoxyethanol in DNA isolation were addressed by using a buffer similar to the one proposed by Murray and Thompson, but adjusting the pH of Tris to 7.6 with boric acid, rather than HCl (buffer: 200mM Tris/borate, 200mM EDTA, 2.2M NaCl, 2% CTAB, 2% 2-mercaptoethanol, 2% PVP), and precipitating one treatment with isopropanol (T30) and the other with 2-butoxyethanol (T31).

11. The strawberry protocol suggests two different dilutions (2.5 volumes or 4 volumes) to elevate the Na⁺ concentration to 80mM. The chosen dilution here was the 2.5vol. An experiment was set up to test the merits of the combinations of two factors: i, re-extraction of the interphase by extraction buffer and chloroform; and ii, DNA precipitation by 2-butoxyethanol. The former factor was tested by keeping each, the first and the second extraction rounds, as separate treatments, therefore determining the gain in DNA yield given by the second extraction. The latter factor contrasted the use of isopropanol *versus* 2-butoxyethanol, where T32=first extraction round/isopropanol; T33=first extraction round/2-butoxyethanol; T34=second extraction round/isopropanol; T35=second extraction round/2-butoxyethanol.

12. Finally, 2-butoxyethanol was used in the guanidine thiocyanate protocol (number 3). The treatments were essentially the same as described for T8 in protocol number 3, except that 2% 2-mercaptoethanol was added to the extraction buffer and the Tris was adjusted by boric acid, not HCl. 100mg of tissue processed by 6ml buffer. Nucleic acids precipitations were done by isopropanol (control, T36), and 2-butoxyethanol (T37).

13. According to an article that proposes a method to isolate DNA from cashew (Rout et al., 2002), boric acid can be used in replacement of Tris, instead of assuming the role of simply adjusting the pH of a Tris solution. The buffer composition used in treatment T38 was 1M boric acid pH 8.0, 2mM EDTA, 1.4M NaCl, 4% CTAB, 0.2% 2-mercaptoethanol.

14. Epicentre kit. Used 10mg (T39), 30mg (T40), 100mg (T41) of 'Strawberry Festival' leaf tissue with 300µl buffer.

15. PowerPlant DNA Isolation kit from MO BIO (T42). A leaflet (350mg) of fresh FRA520 (*F. nubicola*) was ground with liquid nitrogen in microfuge tube. The remaining steps were carried out according to manufacturer's directions.

16. Qiagen DNeasy Plant Mini kit (T43). Followed company's directions for fresh tissue.

17. Silica-based DNA extraction. Nucleic acids tend to adsorb to silica in the presence of chaotropic salts, such as sodium iodide (NaI) (Vogelstein and Gillespie, 1979), guanidine thiocyanate, and guanidine hydrochloride. The binding capacity depends on the solution's ionic strength and pH, being higher at concentrated solutions and pH<7.5 (GeneClean Manual). Silica

columns have been used elsewhere to eliminate polysaccharide contaminants, which is verified by increase of the ratio A260/230 (Abdulova et al., 2002). The protocol used here was based on Rogstad's article (Rogstad, 2003), which uses a CTAB extraction buffer and describes the preparation of the silica binder. CTAB extraction buffer: 2% CTAB, 1.4M NaCl, 100mM Tris-HCl pH 8.0, 20mM EDTA pH 8.0, 1% 2-mercaptoethanol. 'Strawberry Festival' leaves were ground (10mg—T44 and 100mg—T45) and 5 ml of extraction buffer were added. Incubation was carried out at room temperature for 30 minutes. Equal volume of chloroform:octanol was added, samples were centrifuged, the upper phase was transferred to a new tube, and 2.5ml of silica binder were added. The mixture was agitated thoroughly for 5 min, then centrifuged. The supernatant was discarded, and 4ml of silica wash (25% isopropanol, 25% ethanol, 100mM NaCl, 10mM Tris-HCl pH 7.4, 2mM EDTA pH 8.0) were added, vortexed to resuspend the silica. Samples were centrifuged, supernatant discarded, and a second wash took place. The silica pellet was dried for 2 hours at 37°C, and the DNA was eluted by 1ml of ultra pure water, vortexed, and incubated at 65°C for 5 min. After centrifugation, the upper phase was transferred to a new tube, RNase-treated, then DNA was precipitated by isopropanol.

The following protocols (18-22) attempted to extract DNA from nuclei isolated from leaf tissue. Protocols 23-33 consist of variations of the protocol by Murray and Thompson and utilized leaves (rather than isolated nuclei) for DNA extraction.

DNA Extraction from Isolated Nuclei

Nuclei were purified according to the procedure described by Folta and Kaufman [Folta, 2000] and nuclei were recovered from the 35/80 interphase of percoll gradients. Nuclei were incubated with each extraction buffer at 65°C for at least 10 minutes. The following buffers were mixed to 50-150µl of purified nuclei in storage buffer as an attempt to extract DNA:

18. Qiagen DNeasy Plant Mini kit. Different volumes (50µl—T46 and 150µl—T47) of isolated nuclei were processed according to manufacturer's directions.

19. Fulton's nuclei lysis buffer [Fulton, 1995], supplemented with 0.5% sodium bisulfite: 200mM Tris pH 7.5, 50mM EDTA pH 8.0, 2M NaCl, 2% CTAB. Two tubes, one 50µl nuclei (T48) and the other containing 75µl nuclei (T49), were incubated with 200 and 75µl of nuclei lysis buffer at 65°C for 45min. Phenol:chloroform followed by chloroform extractions took place, the upper phase transferred to a new tube, and DNA precipitated by isopropanol.

20. Peterson's procedure [Peterson, 1997]: 20% SDS was added to a final concentration of 2% and mixed with 50µl nuclei (T50) or 150µl (T51) by gentle inversion to lyse the nuclei. The mixture was incubated in water bath at 65°C for 10 minutes, cooled to room temperature, then 5M sodium perchlorate was added to reach final concentration of 1M. Sodium perchlorate is used to dissociate nucleic acid-protein complexes [Wilcockson, 1973]. Following centrifugation, the upper phase was transferred to a new tube using a large-bore tip. After a phenol deproteinization step, the aqueous phase was dialyzed twice, the first overnight and the second for an entire day, both into TE pH 7.0 at 4°C. Samples were consecutively treated with 50µg/ml RNase for 1 hour and with 150µg/ml proteinase K. After extractions with phenol:chloroform/isoamyl alcohol and chloroform/isoamyl alcohol, DNA was precipitated and resuspended.

21. Guanidine thiocyanate buffer (4M guanidine thiocyanate, 100mM Tris-HCl, 10mM EDTA, 0.5M NaCl, 1% sarkosyl, 1% sodium bisulfite) was used (750µl) to extract DNA from 50µl nuclei (T52). The buffer/nuclei were incubated at room temperature for 10min and were

followed by phenol:chloroform and chloroform extractions. DNA was precipitated by isopropanol.

22. Use of triisopropyl naphthalenesulfonic acid (TIPS) as a hydrotrope in a surfactant system (Bies and Folta, 2004). Hydrotropes stabilize surfactants (e.g. SDS) to allow them to remain soluble. Nuclei (150 μ l) were incubated with 1200 μ l of extraction buffer 1 (10mM EDTA, 10mM Tris, 1%SDS) at 65°C for 20min (T53). The sample was treated with Proteinase K for 1h at 37°C. After a phenol:chloroform extraction and centrifugation, the interphase was re-extracted with 5 volumes of extraction buffer 2 (50mM Tris-HCl pH 8.0, 5% SDS, 1%TIPS, 2% 2-mercaptoethanol, 4% PAS—p-aminosalicylic acid). The supernatants of both extractions were combined and nucleic acids precipitated by isopropanol.

Modifications of Murray and Thompson DNA Isolation Protocol

A series of modifications of the protocol proposed by Murray and Thompson were tested. Though the original protocol included cesium chloride gradient, this step was suppressed for all variations tested.

23. Extraction buffer: 200mM Tris, 2M NaCl, 50mM EDTA, 2% CTAB, 2% PVP, 2% 2-mercaptoethanol. After initial 45min incubation at 65°C, solid CTAB was added to extraction buffer, raising CTAB concentration to 6%. Further incubation was necessary dissolve the CTAB. Both fresh (T54, T55) and lyophilized (T56, T57) were used, in 100mg (T54, T56) and 500mg (T55, T57) amounts. A chloroform:octanol deproteination step takes place, then the upper phase receives 0.1 volume of 10% CTAB. After a second chloroform:octanol extraction and transfer of the upper phase to a new tube, 3 volumes of 50mM Tris-HCl pH 8.0, 10mM EDTA, 1% CTAB were added to the aqueous phase. The concentration of CTAB here is maintained, but, since not salt was added, the ionic strength of the solution decreases from 2M NaCl to 0.5M. In low ionic strength, CTAB precipitates nucleic acids during a 30-minute incubation. The pellet formed after the incubation and successive centrifugation, the supernatant is discarded and the pellet dissolved in 0.5 volume of 1M NaCl. Prep was treated with RNase and downstream stages of DNA precipitation by alcohol followed as the standard procedure cited above.

24. Increase in CTAB concentration to 6% as above, with the difference that here CTAB was not added as powder, instead as equal volume of 10% CTAB, 2 M NaCl. For DNA precipitation, 3 volumes of 6% CTAB, 100mM Tris-HCl, 25mM EDTA were used, decreasing concentration of NaCl to 0.5M. Both fresh (T58) and lyophilized (T59) leaves were used.

25. Pea: extraction buffer: 0.7M NaCl, 1% CTAB, 50mM Tris-HCl pH 8.0, 10mM EDTA pH 8.0, 1% 2-mercaptoethanol, 0.01% sodium bisulfite. Departs from Murray and Thompson protocol in that DNA precipitation is achieved only by addition of ethanol, and not by decreasing salt concentration. All protocols below counted with precipitation methods that differ from the first proposed by Murray and Thompson. Different tissue-to-buffer ratios were tested by extracting DNA from 10mg (T60), 50mg (T61), and 100mg (T62) of tissue, keeping the extraction buffer volume constant at 7ml.

26. Sugarcane [Aljanabi, 1999]: 200mM Tris-HCl, 50mM EDTA, 2.2M NaCl, 2% CTAB, 0.06% sodium sulfite, pH 8.0; after homogenization of the tissue and buffer, 0.5 volume of each 5% sarkosyl, 10% PVP, and 20% CTAB were added, elevating the CTAB concentration from 2% to 5% and decreasing NaCl concentration to 0.8M. Plant tissue: 'Strawberry Festival', fresh, mature, leaves of 'Strawberry Festival' (T63) or 'Sweet Charlie' (T64), 3.5g, 4ml buffer/g tissue.

27. Cacti (de la Cruz et al., 1997). Combination of CTAB and SDS extraction buffers. CTAB buffer: 100mM Tris-HCl pH 8.0, 20mM EDTA pH 8.0, 4% CTAB, 1.7M NaCl, 4% PVP,

5mM ascorbic acid, 10mM 2-mercaptoethanol. STE buffer: 100mM Tris-HCl pH 8.0, 50mM EDTA pH 8.0, 100mM NaCl, 10mM 2-mercaptoethanol. Fresh 100mg of ‘Strawberry Festival’ leaf tissue were ground in liquid nitrogen and subsequently incubated for 10 min at 65°C with 1ml CTAB buffer. STE buffer (4ml) and SDS (to final concentration of 2%) were added and shaken vigorously for 7 minutes. A second 10-min incubation at 65°C was carried out; 1.25ml of cold 5M KOAc was added and incubated in ice for 40 min, centrifuged at 3,500rpm for 10 min (T65). The upper phase was transferred to a new tube and the nucleic acids precipitated by isopropanol. An alternative method (T66) substituted the addition of KOAc and ice incubation by addition of equal volume of 24:1 chloroform:octanol, keeping steps after centrifugation the same.

28. Extraction buffer/plant material Incubation temperatures and durations were tested: 4°C (T67-T70), 20°C (T71-T74), 42°C (T75-T78), 65°C (T79-T82), and 0min (T67, T71, T795 T79), 5min (T68, T72, T76, T80), 30min (T69, T73, T77, T81), 60min (T70, T74, T78, T82). CTAB buffer (2% CTAB, 1.4M NaCl, 100mM Tris-HCl pH 8.0, 20mM EDTA pH 8.0, 1% 2-mercaptoethanol) was incubated in water baths with the various temperature treatments. When the buffer reached temperature equilibrium with the water baths, each tube received 1.6g of liquid nitrogen-ground strawberry leaves and the mixture was incubated at the various duration treatments. When incubation duration was reached, an aliquot of 10ml of the temperature treatment was mixed with chloroform. For incubation time “zero”, an aliquot was taken right after buffer and ground tissue were mixed and chloroform was added. Samples were centrifuged at 4,000rpm for 10min. The upper phase was transferred to a new tube and nucleic acids were precipitated by isopropanol. After centrifugation and discard of the supernatant, the dry pellet was resuspended in water and treated with RNase. The precipitation steps were repeated to obtain virtually RNA-free DNA. DNA was quantified with aid of a NanoDrop ND-1000 spectrophotometer. This experiment was repeated 3 times.

29. Tissue was ground in liquid nitrogen, and an aliquot of the extraction buffer (2% CTAB, 1.4M NaCl, 100mM Tris-HCl pH 8.0, 20mM EDTA pH 8.0, 1% 2-mercaptoethanol) was combined to the ground tissue to undergo further grinding and formation of slurry. The tissues tested were unexpanded (T83) and expanded (T84) leaves from the *F. nubicola* FRA520. Following formation of slurry, equal volume of 24:1 chloroform:octanol was added, vortexed, samples were centrifuged, and upper phase transferred to a new tube. Nucleic acids were precipitated by addition of 70% isopropanol, the alcohol was decanted, and the dry pellet resuspended in water.

30. An experiment was designed to contrast the traditional method of grinding tissue in liquid nitrogen, then adding the powder to buffer (T85) *versus* grinding tissue in liquid nitrogen, then adding the buffer (described immediately above) to the tissue and further grind until slurry is formed (T86). The 100mg per treatment of FRA520 plant material was mixed before nucleic acid isolation to eliminate the leaf age factor. A NanoDrop was used to quantify the nucleic acid content.

31. A factorial experiment tested interactions between formation (T87, T89) or not (T88, T90) of slurry and incubation temperatures of 4°C (T87, T88) and 60°C (T89, T90). After grinding the tissue (50mg per grinding method) in one of the two fashions tested, the material was split to be incubated for 1 hour in the two different temperatures. The downstream steps were followed as described above, including quantification of nucleic acids and absorbance at 230nm and 280nm by a NanoDrop ND-1000 spectrophotometer.

32. CTAB buffer concentrations of 2% (T91), 6% (T92), and 20% (T93) were tested. The slurry was formed by breaking down 400mg of tissue in liquid nitrogen first, then adding 2 ml of buffer for further grinding. Once homogenization was achieved, another 8ml of buffer were added and the mixture was incubated at 65°C for 30min. The 10ml of buffer were split into 2 tubes (treatment replications) and 5 ml of chloroform:octanol were added to each tube. Nucleic acids from centrifugation upper phase were precipitated by isopropanol and the dry pellet resuspended in 1ml TE pH 8.0. Samples were quantified by NanoDrop.

33. Because homogenizing tissue in buffer seemed to have a positive effect on DNA recovery, an experiment was set up to test Polytron homogenizer speeds (half maximum speed—T95-T98; full speed—T99-T103) and duration of homogenizing treatment (no polytron—T94; 5 seconds—T95, T99; 15 seconds—T96, T100; 30 seconds—T97, T101; 60 seconds—T98, T102; 120 seconds—T103). Enough Laboratory Festival #9 tissue for all treatments (2g) was ground in liquid nitrogen and, by adding an aliquot of the buffer, ground to a paste consistency. The paste was divided into 10 tubes and enough buffer to reach 5ml was added to each tube just prior to treatment with Polytron. Samples were incubated at 65°C for 30min. Downstream steps from incubation were as described above.

The final strawberry DNA extraction protocol is listed below.

Strawberry DNA Extraction Protocol

CTAB extraction buffer – 100ml

2% CTAB	2g
1.4M NaCl	28ml of 5M NaCl
100mM Tris-HCl, pH 8	10ml of 1M Tris
20mM EDTA pH8	4ml of 0.5M EDTA
1% BME	1ml
diWater	to 100ml

Tissue-to-buffer ratio = 40 mg/ml. For 12-ml tubes, maximum tissue processed is 200 mg.

- Grind 200 mg of liquid-nitrogen frozen leaves (young or unexpanded) in mortar-and-pestle
- Add 2 ml extraction buffer to ground sample, macerate in mortar until consistency of paste is achieved. Transfer the paste to a 12-ml tube, and add 3 ml buffer
- Homogenize utilizing a Polytron at full speed for 2 min
- Incubate for 1h at 65°C, with intermittent agitation
- Add equal volume (5ml) of 24:1 chloroform:octanol
- Mix by shaking vigorously
- Centrifuge at 4,000 rpm, 5 min
- Transfer the upper, aqueous phase to a new 12-ml tube
- Precipitate DNA with equal volume of 70% isopropanol
- Mix by inverting the tube several times
- Centrifuge at 4,000 rpm, 5 min
- Discard the supernatant
- Air-dry nucleic acids pellet

- Resuspend pellet in 500ul to 1 ml (depending on the amount required to dissolve the pellet) of deionized water or TE pH 8.0.

APPENDIX B

In silico ANNOTATION AND DISTRIBUTION OF *Fragaria vesca* GENES

Under each fosmid name is a list of numbered potential genes predicted by FGENESH. The nucleotide intervals that had protein hits by BLASTP were used for a similarity search against the non-redundant Viridiplantae, protein database using BLASTX. The best matches identified by the algorithm are listed under “Protein Hit”. Threshold value was 10^{-15} . Letter “X” under “Protein Hit” denotes no similarity was detected in the protein database. Under “Orientation”, “+” signs signify that the query sequence is translated in the same direction it was input, where negative orientation signifies that the complement strand is translated. “EST Hits” are sequences of DNA for which an EST was detected within Rosaceae, with a minimum length of 100 nucleotides and 95% identity.

Gene distributions were calculated by dividing each fosmid insert size by the number of genes either predicted by FGENESH or identified by similarity to the non-redundant Viridiplantae protein database.













Simple Sequence Repeats (SSRs) with at least 5 repeats of a motif are represented by color-coded triangles:

△ in FGENESH-defined genic sequence; ▲ in FGENESH-defined intergenic sequence

Fosmid	Predicted Number of Genes		Protein Hit	Orientation	EST Hits (gb no.)	Fosmid Insert Size (bp)	Putative Gene Distr (kb between genes)	
	<i>ab initio</i>	Similarity					<i>ab initio</i>	Similarity-based
01L02	13	7				40,302	3.1	5.8
1			unknown	-				
2	▲		X					
3	△		pectin lyase	+				
4			unknown	-				
5			beta-glucan binding	-				
6			enolase	-				
7	▲		X					
8	▲▲▲▲		X					
9	△△		unknown	+	DV440 436.1			
10	▲		X					
11	▲		X					
12	▲		X					
13			unknown	-	DY670 952.1			
05N03	8	5				34,611	4.3	6.9
1	▲		ATP binding/adenylate cyclase	+				
2			X					
3	▲		Senescence-associated	+	CX6614 21.1			
4			hypothetical	-	DW248 990.1			

Fosmid	Predicted Number of Genes		Protein Hit	Orientation	EST Hits (gb no.)	Fosmid Insert Size (bp)	Putative Gene Distr (kb between genes)	
	<i>ab initio</i>	Similarity					<i>ab initio</i>	Similarity-based
5			X		CX6616 57.1			
6			X		CO8169 31.1			
7			peroxidase	-				
8			unknown	-				
11D02	9	3				37,961	4.2	12.7
1			ATP synthase, mitochondrial	-	DY668 653.1			
2			X					
Not predicted			X		DW342 667.1			
3			X		DW344 738.1			
4			Release Factor 2, chloroplast	+	DW346 600.1			
5			X					
6			X					
7			X					
8			X		BQ1055 41.1			
9			heat shock binding	-				
13I03	8	8				37,707	4.7	4.7
1			hydrolase	+				
2			leucyl-tRNA synthetase	-				
3			leucyl-tRNA synthetase	-				
4			leucyl-tRNA synthetase	-				
5			leucyl-tRNA synthetase	-				
6			zinc finger family	+				
7			2OG-Fe(II) oxygenase	-	DY670 360.1			
8			integrase	+	DY671 649.1			
15B13	7	2				23,212	3.3	11.6
1			senescence-associated	-	CX6613 47.1			
Not predicted			26S ribosomal RNA (not a protein)		CA8540 88.1			
2			X					
3			senescence-associated	-	CX6613 47.1			
4			X					
5			X		CX6614 21.1			

Fosmid	Predicted Number of Genes		Protein Hit	Orientation	EST Hits (gb no.)	Fosmid Insert Size (bp)	Putative Gene Distr (kb between genes)	
	<i>ab initio</i>	Similarity					<i>ab initio</i>	Similarity-based
6			X					
7			X		CX6616 57.1			
17O22	9	6				34,090	3.8	5.7
1			homeodomain	+				
2	△△		X					
3	△		X					
4			oligopeptidase	+	BQ1046 55.1			
5			hypothetical	-	DY675 330.1			
6			X					
7	△		unknown	-				
8	△		lectin protein kinase	+				
9	△		hypothetical	-				
18A19	7	6				40,908	5.8	6.8
1			cytochrome P450	-				
2			X					
3			integrase	-				
4			integrase	-				
5			integrase	+				
6			integrase	-				
7			transferase	+				
22H18	8	4				37,851	4.7	9.5
1			X					
2			X					
3			polyprotein	-				
4	△ △		X					
5			hypothetical	+				
6			X					
7	△		unknown	+				
8	△		pre-mRNA processing factor 38	+				
22L05	8	3				35,112	4.4	11.7
1			X					
2			X					
Not predicted			X		DY674519.1 EST starts upstream of predicted gene 3, and spans oxidoreductase			
3			oxidoreductase	+	DY671 565.1			
4	△		oxidoreductase	+				
5			sulfate transporter	-				
6			X		CO3800 67.1			
7			X					





Fosmid	Predicted Number of Genes		Protein Hit	Orientation	EST Hits (gb no.)	Fosmid Insert Size (bp)	Putative Gene Distr (kb between genes)	
	<i>ab initio</i>	Similarity					<i>ab initio</i>	Similarity-based
8			X					
27F10	11	8				37,110	3.4	4.6
1			kinase	-	DY675 883.1			
2			hypothetical	-	CX6613 86.1			
3			unknown	-	DV438 706.1			
4			integrase	-				
5			integrase	-				
6			integrase	-				
7			unknown	+				
8			X					
Not predicted			X		CO3787 00.1			
9			unknown	-				
10			X					
11			X					
29G10	10	4				31,681	3.2	7.9
1			transposase	-				
2			X					
3			flavin-binding monooxygenase-like	+	DY673 408.1			
4			X					
5			X					
6			X					
7			X					
8			phenylacetaldehyde synthase	-				
9			unknown	+				
10			X					
30I24	7	5				37,599	5.4	7.5
1			X					
2			wall-associated kinase	+				
3			X (E value=1e-10) arabidopsis response regulator 12					
4			chitinase	+	CX6615 29.1			
5			arabidopsis response regulator 12	+	DY671 913.1			
6			transferase	-				
7			PICKLE chromating remodeling factor	-				

Fosmid	Predicted Number of Genes		Protein Hit	Orientation	EST Hits (gb no.)	Fosmid Insert Size (bp)	Putative Gene Distr (kb between genes)	
	<i>ab initio</i>	Similarity					<i>ab initio</i>	Similarity-based
32A10	15	4				33,577	2.2	8.4
1			catalytic/ hydrolase	-	DY667 800.1			
2	△△		X					
3			X					
4			X					
5			X					
6			X					
7			copper ion binding	+				
8	△△		X					
9			MADS-box	-				
10			X					
11			X					
12			X					
13			pathogenesis-related	-				
14			X					
15			X					
32L07	6	4				32,951	5.5	8.2
Not predicted			X		DY668 002.1			
1			hypothetical	-				
2			SMC2	-				
3			disease resistance	-	DY666 677.1			
4			X					
5			exostosin-like	-	CX6620 49.1			
6			X					
34D20	8	6				30,034	3.8	5.0
1	△△		RNA recognition motif	+				
2			cysteine-type peptidase	+				
3			X					
4			transposase	+				
5			anthocyanin 5-aromatic	-				
6			X (E value = 8e-14) anthocyanin malonyltransferase <i>FGENESH missed EST</i>	+				
7			NAC domain NAM	-				
Not predicted			X		DV438 498.1			
8			X					

Fosmid	Predicted Number of Genes		Protein Hit	Orientation	EST Hits (gb no.)	Fosmid Insert Size (bp)	Putative Gene Distr (kb between genes)	
	<i>ab initio</i>	Similarity					<i>ab initio</i>	Similarity-based
38H02	7	6				31,669	4.5	5.3
1			X					
2			transposon protein	+				
3			cytochrome P450	+				
4			cytochrome P450	+				
5			integrase	+				
6			serine/threonine kinase	-				
7			exportin	-				
38H05	11	1				32,050	2.9	32.1
1			X					
2			X					
Not predicted			X		dbj AB2 08565.1			
3			retrotransposon polyprotein	-				
Not predicted			X		dbj AB2 08565.1			
4			X					
5			X					
6			X					
7			X					
8			X					
9			X					
10			X					
11			X					
40B22	9	8				36,230	4.0	4.5
1			unknown	+				
2			cyclin-like F-box	-				
3			X					
4			cyclin-like F-box	+				
5			cyclin-like F-box	-				
6			cyclin-like F-box	-				
7			Arf GTPase activating	-				
8			heavy metal transport/detoxification	+				
9			MuDR family transposase	-				
40M11	9	5				31,718	3.5	6.3
1			X					
2			cyclin 1-like F box	+				
3			X					
4			X					

Fosmid	Predicted Number of Genes		Protein Hit	Orientation	EST Hits (gb no.)	Fosmid Insert Size (bp)	Putative Gene Distr (kb between genes)	
	<i>ab initio</i>	Similarity					<i>ab initio</i>	Similarity-based
5			X					
6			Secretory Protein SEC14	-	DY675 900.1			
Not predicted			X		DY672 841.1			
7			ATPase	-				
8			unknown	+				
9			glycosyl hydrolase	-				
Not predicted			X		CX6621 88.1			
43P07	10	4				43,641	4.4	10.9
1			X					
2			retrotransposon polyprotein	+				
3			X					
4			X					
5			DNA cytosine-5- methyltransferase	-	DY668 476.1			
6			unknown	+	DY668 476.1			
7			methyltransferase small domain	+				
8			X					
9			X					
10			X					
44J07	11	2				29,636	2.7	14.8
1			X					
2			X					
3			X		DY672 792.1			
4			X					
5			X					
6			X					
7			disease resistance	-				
8			unknown	-	DY671 343.1			
9			X					
10			X		DY650 877.1			
11			X					
47H15	9	4				34,817	3.9	8.7
1			X		DY669 025.1			
2			X					
3			polyprotein	-				
4			integrase	-				
5			retrotransposon protein	-				

Fosmid	Predicted Number of Genes		Protein Hit	Orientation	EST Hits (gb no.)	Fosmid Insert Size (bp)	Putative Gene Distr (kb between genes)	
	<i>ab initio</i>	Similarity					<i>ab initio</i>	Similarity-based
6			X					
7			X					
8			X					
9			heat shock	+				
52E09	9	8				36,230	4.0	4.5
1			unknown	+				
2			cyclin-like F-box	-				
3			X					
4	△		cyclin-like F-box	+				
5			cyclin-like F-box	-				
6	△		cyclin-like F-box	-				
7			Arf GTPase activating heavy metal transport/detoxification transposase	-				
8				+				
9				-				
63F17	6	3				28,318	4.7	9.4
1	△△		phospholipase D	+	DY672 511.1			
2			unknown	-				
3			binding	+				
4			X					
5	△		X					
6			X					
72E18	12	11				36,293	3.0	3.3
1			hydrolase	+				
2	△		hydrolase	+				
3			reverse transcriptase	-				
4			hydrolase	+	DV438 212.1			
5	△		X					
6			hydrolase	+	DY669 358.1			
7			unknown	+	DY675 437.1			
8			transferase	+				
9			spliceosome-associated	+				
10			unknown	+				
11			actin 7, actin 11	-				
12			glycoprotein-like	-	DY670 963.1			
84N10	8	2				40,183	5.0	20.1
1	△△		ribosomal L24/L26	+				
2	△△△△△		X					
3			X					

Fosmid	Predicted Number of Genes		Protein Hit	Orientation	EST Hits (gb no.)	Fosmid Insert Size (bp)	Putative Gene Distr (kb between genes)	
	<i>ab initio</i>	Similarity					<i>ab initio</i>	Similarity-based
4			ATP binding	-				
5			X					
6			X					
7			X					
8			X					
Totals	235	129				905,491		
Means	9	5				34,827	4.0	9.1
Sample Standard Deviation	2.1	2.4				4,426	0.9	6.0

APPENDIX C
PCR PRIMERS USED TO AMPLIFY AND SEQUENCE GENE-PAIR HAPLOTYPES

GPH name	Primer name	Primer sequence
GPH4	GPH4a	ACGAGGGCTTGGAAAGAAAGG
	GPH4b	GCCCAACAACAGAAAGACC
GPH5	GPH5#2a	CAATGCCATGGTCTCCGGTC
	GPH5#2b	TGCCGTTGCACACACCTTCC
	GPH5A2	GCTCTTTGGTGTTCAAAGTTGGAT
	GPH5B#2	ATCCAGCCAAACTGAAGGTG
	GPH5A3	CAGCCATGAAGTCAAGGTCA
GPH10	GPH10a	GGCTTCTTCTTGTCCGGCAGC
	GPH10b	GAActCCAGGTCAGATCTTCG
	GPH10c	CTCGCTGCAAATCAGCTACC
	10ABCol7Rev2	GAGTTTGTTCGAGCTGATC
	10ABCol32Rev2	ATAGAGGCGATGTTGTAG
	10AB#3	GGCCCTGATCACTCGACA
	10AB#4	GGTTTGGTTGGTTAAGGTG
	10AB#5	GACAGTACCTGAAAATTTGG
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	PPR1R1	GATCGAACGGCTGATATTTAA
	10PPR1R2	TGTAGCTCATACTTTTGTCTC
GPH23	GPH23F	CTTGAGGGCCATCAGCAC
	GPH23R	TACACCCACGCCTTCATCTC
	GPH23F2	GAActGCGAAGATCTATCTGA
11D02	11D02F	GAGCTGCTGTGTGAACCAA
	11D02R	GTTCAACTCCAGATGAAGTGAGG

GPH name	Primer name	Primer sequence
17O22	17O22F	AAAATGGGTTCACGAGTTC
	17O22Rb	GGGTTTCCTCACAACTTCG
27F10	27F10F	CCTGCAGGGTTTTTCATCAT
	27F10R	TGGAAATGTATTCTGGTTCTCC
29G10	29G10F	TGGCCTTGTTTCCTAAACTCTT
	29G10R	AGAAGAAGGCAGCACCCAAT
32L07	32L07F	GAGTTGAAAAACGGGTCGAA
	32L07Rb	AGGAAAATGCGGGAGAAAGT
34D20	34D20Fb	GCAGAAAGAACTGATGTGCTT
	34D20Rc	CGCAGTCGTAAAAATTCGTCT
	34D20Fb2	TGGGTGTGGATGAACTATACG
40M11	40M11Fd	CAACATTTTGGTGGCCTTCT
	40M11Rd	CGGCCTATGAAACCACAGTT
63F17	63F17F	GCAGAAAGAACTGATGTGCTT
	63F17R	CGCAGTCGTAAAAATTCGTCT
72E18	72E18Fb	GCAGAAAGAACTGATGTGCTT
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Polymorphic segment of GPH10: fragment between primers 10PPR1 and 10AB22

>10PPR1AB22_vesca

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>10PPR1AB22_nubicola

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>10PPR1AB22_viridis

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Clones from unspecific amplification

The size fragments amplified with primers for the vector were not the expected, i.e., they had lower molecular weight than the that had been amplicon cloned.

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APPENDIX E
GENE-PAIR HAPLOTYPE INDIVIDUAL LOCI ALIGNMENTS

To avoid identification of untrue polymorphisms and therefore poor alignments, “N”s were inserted where the chromatogram did not display distinct peaks. EcoRI sites are in green because EcoRI was used to extract inserts from TOPO vectors and detect polymorphic inserts in this vector. Simple Sequence Repeats (SSRs) are magenta-colored.

Gene Pairs Detected by Microcolinearity

GPH5: Single Nucleotide Polymorphisms (SNPs) are in bold. SNPs that occur in more than one clone are likely to reflect real differences (as oppose to amplification or sequencing errors) and are colored red.

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GPH5_nubicola             TGAAGACAAGATCCTAAGGAGGAACTCCGAGTCTGGTTTAGAATTGTTGAGCAAACCTCAA 180
GPH5-vesca                 TGAAGACAAGATCCTAAGGAGGAACTCCGAGTCTGGTTTAGAATTGTTGAGCAAACCTCAA 180
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GPH5_ananassa_clone2      GGAACAAGAAGTAGCACCTCCCAGAAGAAGAAGAAAAATGGGATCTACAGAAAAGAGCT 240
GPH5_ananassa_clone7      GGAACAAGAAGTAGCACCTCCCAGAAGAAGAAGAAAAATGGGATCTACAGAAAAGAGCT 240
GPH5_viridis               GGAACAAGAAGTAGCACCTCCCAGAAGAAGAAGAAAAATGGGATCTACAGAAAAGAGCT 240
GPH5_iinumae              GGAACAAGAAGTAGCACCTCCCAGAAGAAGAAGAAAAATGGGATCTACAGAAAAGAGCT 240
GPH5_nilgerrensis         GGAACAAGAAGTAGCACCTCCCAGAAGAAGAAGAAAAATGGGATCTACAGAAAAGACT 240
GPH5_mandshurica         GGAACAAGAAGTAGCACCTCCCAGAAGAAGAAGAAAAATGGGATCTACAGAAAAGAGCT 240
GPH5_nubicola             GGAACAAGAAGTAGCACCTCCCAGAAGAAGAAGAAAAATGGGATCTACAGAAAAGACT 240
GPH5-vesca                 GGAACAAGAAGTAGCACCTCCCAGAAGAAGAAGAAAAATGGGATCTACAGAAAAGAGCT 240
*****
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GPH5_ananassa_clone2 TGCTCTTGCTTTCTCCTACTCACAGCATCAGCAAGAAGTTTCCTATCAGCTCATGGAGT 300
 GPH5_ananassa_clone7 TGCTCTTGCTTTCCACTACTCACAGCATCAGCAAGAAGTTTCCTATCAGCTCATGGAGT 300
 GPH5_viridis TGCTCTTGCTTTCTCCTACTCACAGCATCAGCAAGAAGTTTCCTATCAGCTCATGGAGT 300
 GPH5_iinumae TGCTCTTGCTTTCTCCTACTCACAGCATCAGCAAGAAGTTTCCTATCAGCTCATGGAGT 300
 GPH5_nilgerrensis TGCTCTTGCTTTCTCCTACTCACAGCATCAGCAAGAAGTTTCCTATCAGCTCATGGAGT 300
 GPH5_mandshurica TGCTCTTGCTTTCTCCTACTCACAGCATCAGCAAGAAGTTTCCTATCAGCTCATGGAGT 300
 GPH5_nubicola TGCTCTTGCTTTCTCCTACTCACAGCATCAGCAAGAAGTTTCCTATCAGCTCATGGAGT 300
 GPH5-vesca TGCTCTTGCTTTCTCCTACTCACAGCATCAGCAAGAAGTTTCCTATCAGCTCATGGAGT 300

GPH5_ananassa_clone2 TCACTTCTATTTCTTGCTTTTCCAAGGCTTGTCTTTCTTGTGTAGGCTTGGACTTAAT 360
 GPH5_ananassa_clone7 TCACTTCTATTTCTTGCTTTTCCAAGGCTTGTCTTTCTTGTGTAGGCTTGGACTTAAT 360
 GPH5_viridis TCACTTCTATTTCTTGCTTTTCCAAGGCTTGTCTTTCTTGTGTAGGCTTGGACTTAAT 360
 GPH5_iinumae TCACTTCTATTTCTTGCTTTTCCAAGGCTTGTCTTTCTTGTGTAGGCTTGGACTTAAT 360
 GPH5_nilgerrensis TCACTTCTATTTCTTGCTTTTCCAAGGCTTGTCTTTCTTGTGTAGGCTTGGACTTAAT 360
 GPH5_mandshurica TCACTTCTATTTCTTGCTTTTCCAAGGCTTGTCTTTCTTGTGTAGGCTTGGACTTAAT 360
 GPH5_nubicola TCACTTCTATTTCTTGCTTTTCCAAGGCTTGTCTTTCTTGTGTAGGCTTGGACTTAAT 360
 GPH5-vesca TCACTTCTATTTCTTGCTTTTCCAAGGCTTGTCTTTCTTGTGTAGGCTTGGACTTAAT 360

GPH5_ananassa_clone2 AGGTGAGCAGGTTAGCTAAAAGCTTCAAACAAAGCGTCAATTGCCACAGTTATTCTTTG 420
 GPH5_ananassa_clone7 AGGTGAGCAGGTTAGCTAAAAGCTTCAAACAAAGCGTCAATTGCCACAGTTATTCTTTG 420
 GPH5_viridis AGGTGAGCAGGTTAGCTAAAAGCTTCAAACAAAGCGTCAATTGCCACAGTTATTCTTTG 420
 GPH5_iinumae AGGTGAGCAGGTTAGCTAAAAGCTTCAAACAAAGCATCAATTGCCACAGTTATTCTTTG 420
 GPH5_nilgerrensis AGGTGAGCAGGTTAGCTAAAAGCTTCAAACAAAGCGTCAATTGCCACAGTTATTCTTTG 420
 GPH5_mandshurica AGG-GAGCAGGTTAGCTAAAAGCTTCAAACAAAGCGTCAATTGCCACAGTTATTCTTTG 419
 GPH5_nubicola AGGTGAGCAGGTTAGCTAAAAGCTTCAAACAAAGCGTCAATTGCCACAGTTATTCTTTG 420
 GPH5-vesca AGGTGAGCAGGTTAGCTAGAAGCTTCAAACAAAGCGTCAATTGCCACAGTTATTCTTTG 420
 *** *****

GPH5_ananassa_clone2 ATAGATATATGCTGAA----CTGTAAGAGACATATTTCAAGCTCTTGGTGTTCAAAGTT 476
 GPH5_ananassa_clone7 ATAGATATATGTTGAA----CTGTAAGAGACATATTTCAAGCTCTTGGTGTTCAAAGTT 476
 GPH5_viridis ATAGATATATGTTGAA----CTGTAAGAGACATATTTCAAGCTC--TGGTGTTCAAAGTT 474
 GPH5_iinumae ATAGATATATGTTGAA----CTGTAAGAGACATATTTCAAGCTCTTGGTGTTCAAAGTT 476
 GPH5_nilgerrensis ATAGATATATGTTGAA----CTGTAAGAGACATATTTCAAGCTCTTGGTGTTCAAAGTC 476
 GPH5_mandshurica ATAGATATATGTTGAA----CTGTAAGAGACATATTTCAAGCTCTTGGTGTTCAAAGTT 475
 GPH5_nubicola ATAGATATATGTTGAATGAAGCTTAAAGAGACATATTTCAAGCTCTTGGTGTTCAAAGTT 480
 GPH5-vesca ATAGATATATGTTGAA----CTGTAAGAGACATATTTCAAGCTCTTGGTGTTCAAAGTT 476

GPH5_ananassa_clone2 GGATTCAATTACATGTAGACACAGTTACCATTTTCCATTTGAAAAGAAGGTAATATGC 536
 GPH5_ananassa_clone7 GGATTCAATTACATGTAGACACAGTTACCATTTTCCATTTGAAAAGAAGGTAATATGC 536
 GPH5_viridis GGATTCAATTACATGT-GACACAGTTACCATTTTCCCATATGAAATAGAAGGTAATATGC 533
 GPH5_iinumae GGATTCAATTACATGTAGACATAGTTACCATTTTCCCATTTGAAATAGAAGGTAATAT-- 534
 GPH5_nilgerrensis GGATTCAATTACATGTAGACACAGTTACCATTTTCCCATTTGAAAAGAAGGTAATATGC 536
 GPH5_mandshurica GGATTCAATTACATGTAGACACAGTTACCATTTTCCCATTTGAAATAGAAGGTAATACGC 535
 GPH5_nubicola GGATTCAATTACATGTAGACACAGTTACCATTTTCCCATTTGAAATAGAAGGTAATACGC 540
 GPH5-vesca GGATTCAATTACATGTAGACACAGTTACCATTTTCCCATATGAAATAGAAGGTAATATGC 536

GPH5_ananassa_clone2 ATGATATAAATATCAAGTTAATTGTACAATGATATTTTGTAAATAGTGAATAATGA 596
 GPH5_ananassa_clone7 ATGATATAAATATCAAGTTAATTGTACAATGATATTTTGTAAATAGTGAATAATGA 596
 GPH5_viridis ATGATATAAATATCAAGTTAATTGTACAGTGATAT---TTGTAACAGTGAAAATAATGA 590
 GPH5_iinumae -----ATCAAGTTAATTGTACAATAATAT---TTGTAATCAGTGAAAATAATGA 580
 GPH5_nilgerrensis ATGATATAAATATCAAGTTAATTGTACAATGATAT---TTGTAATCAGTGAAAATAATGA 593
 GPH5_mandshurica ATGATATAAATATCAAGTTAATTGTACAATGATAT---TTGTAATCAGTGAAAATAATGA 592
 GPH5_nubicola ATGATATAAATATCAAGTTAATTGTACAATGATAT---TTATAATCAGTGAAAATAATGA 597
 GPH5-vesca ATGATATAAATATCTAGTTAATTGTACAATGATAT---TTGTAACAGTGAAAATAATGA 593
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GPH5_ananassa_clone2 CAATCTTCATAACAAAAATTCAGTTATCTTTCCATTGCTGTATGAACTGTTACCATTAGC 656
 GPH5_ananassa_clone7 CAATCTTTTATAACAAAAATTCAGTTATCTTTCCATTGCTGTATGAACTGTTACCATTAGC 656
 GPH5_viridis CAATCTTTTATAACAAAAATTCAGTTATCTTTCCATTGCTGTATGAACTGTTACCATTAGC 650
 GPH5_iinumae CAATCTTTTATAACAAAAATTCAGTTATCTTTTCACCTGCTGTATGAACTGTCACCATTAGC 640
 GPH5_nilgerrensis AAATCTTTTATAACAAAAATTCAGTTATCTTTCCATTGCTGTGAACTGTTACCATTAGC 653
 GPH5_mandshurica CAATCTTTTATAACAAAAATTCAGTATCTTTCCATTGCTGTATGAACTGTTACCATTAGC 652
 GPH5_nubicola CAATCTTTTATAACAAAAATTCAGTATCTTTCCATTGCTGTATGAACTGTTACCATTAGC 657
 GPH5-vesca CAATCTTTTATAACAAAAATTCAGTTATCTTTCCATTGCTGTATGAACTGTTACCATTAGC 653

GPH5_ananassa_clone2 CTCTCACACAAGAACAACCTACACCAAACAAACAGAACCAGACCAAATCACACCAATATAA 716
 GPH5_ananassa_clone7 CTCTCACACAAGAGCAACAACACCAAACAAACAGAACCAGACCAAATCACACCAATATAA 716
 GPH5_viridis CTCTCACACAAGAACAACAACACCAAACAAACAGAACCAGACCAAATCACACCAATATAA 710
 GPH5_iinumae CTCTCACACAAGAACAACAACACCAAACAAACAGAACCAGACCAAATCACACCAATATAA 700
 GPH5_nilgerrensis CTCTCACACAAGAACAACAACACCAAACAAACAGAACCAGACCAAATCACACCAATATAA 713
 GPH5_mandshurica CTCTCACACAAGAACAACAACACCAAACAAACAGAACCAGACCAAATCACACCAATATAA 712
 GPH5_nubicola CTCTCACACAAGACCAACAACACCAAACAAACAGAACCAGACCAAATCACACCAATATAA 717
 GPH5-vesca CTCTCACACAAGAACAACAACACCAAACAAACAGAACCAGACCAAATCACACCAATATAA 713
 *** *****

GPH5_ananassa_clone2 AACAGAATTGGATTTTCATGAAAGGCGGCAAGGCACAATCAATGAAGGAGA--AGACAAA 774
 GPH5_ananassa_clone7 AACAGAATTGGATTTTCATGAAAGGCAGCAAGGCACAATCAATGAAGGAGA--AGACAAA 774
 GPH5_viridis AACATAATTGGATTTTCATGAAAGGCAGCAAGGCATGATCAATGAAGGAGA--AGACAAA 768
 GPH5_iinumae AACAGAATTGGATTTTCATGAAAGGCAGCAAGGCACAATCAATGAAGGAGA--AGACAAA 758
 GPH5_nilgerrensis AACAGAATTGGATTTTCATGAAAGGCAGCAAGGCACAATCAATGAAGGAGAAGACAAA 773
 GPH5_mandshurica A-CAGAATTGGATTTTCATGAAAGGCAGCAAGGCACAATCAATGAAGGAGA--AGACAAA 769
 GPH5_nubicola A-CAGAATTGGATTTTCATGAAAGGCAGCAAGGCACAATCAATGAAGGAGA--AGACAAA 774
 GPH5-vesca AACAGAATTGGATTTTCATGAAAGGCAGCAAGGCACAATCAATGAAGGAGA--AGACAAA 771
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GPH5_ananassa_clone2 GAATCCTTTTGTTCATATGGATTGAATCTGAATTATTTGGAGTGTCTGGCTGTCATATC 834
 GPH5_ananassa_clone7 GAATCCTTTTGTTCATATGGATTGAATCTGAATTATTTGGAGTGTCTGGCTGTCATATC 834
 GPH5_viridis GAATCCTTTTGTTCATATGGATTGAATCTGAATTATTTGGAGTGTCTGGCTGTCATATC 828
 GPH5_iinumae GAAACCTTTTGTTCATATGGATTGAACCTGAATTATCTGGAGTGTCTGGCTGTCATATC 818
 GPH5_nilgerrensis GAATCCTTTTGTTCATATGGATTGAATCTGAATTATTTGGAGTGTCTGGCTGTCATATC 833
 GPH5_mandshurica GAATCCTTTCTGCATATGGATTGAATCTGAATTATTTGGAGTGTCTGGCTGTCATATC 829
 GPH5_nubicola GAATCCTTTCTGCATATGGATTGAATCTGAATTATTTGGAGTGTCTGGCTGTCATATC 834
 GPH5-vesca GAATCCTTTTGTTCATATGGATTGAATCTGAATTATTTGGAGTGTCTGGCTGTCATATC 831
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GPH5_ananassa_clone2 TCATATGCAGGCATGTTACATGTCTG-----CATTTGGTGACAAAAGCTAAATCT 884
 GPH5_ananassa_clone7 TCATATGCAGGCATGTTACATGTCTG-----CATTTGGTGACAAAAGCTAAATCT 884
 GPH5_viridis TCATATGCAGGCATGTTACATGTCTG-----CATTTGGTGACAAAAGCTAAATCT 878
 GPH5_iinumae TCATATGCAGGCATGTTACATGTCTG-----CATTTGGTGACAAAAGCTAAATCT 868
 GPH5_nilgerrensis TCATATGCAGGCATGTTACATGTCTG-----CATTTGGTGACAAAAGCTAAATCT 883
 GPH5_mandshurica TCATATGCAGGCATGTTACATGTCTG-----CATTTGGTGACAAAAGCTAAATCT 879
 GPH5_nubicola TCATATGCAGGCATGTTACATGTCTG-----CATTTGGTGACAAAAGCTAAATCT 884
 GPH5-vesca TCATATGCAGGCATGTTACATGTCTCATGATGCTTTCATTTGGTGACAAAAGCTAAATCT 891
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GPH5_ananassa_clone2 TAACATGACCTAAGAATTAAGACATATTGGACCATTGGGCTTAATCATAGTCTAAGCCCA 944
 GPH5_ananassa_clone7 TAACATGACCTAAGAATTAAGACATATTGGACCATTGGGCTTAATCATAGTCTAAGCCCA 944
 GPH5_viridis TAA-----GAATTAAGACATATTGGACCATTGGGCTTAATCATAGTCTGAGCCCA 928
 GPH5_iinumae TAA-----GAATTAAGACATATTGGACCATTGGGCTTAATCATAGTCTGAGCCCA 918
 GPH5_nilgerrensis TAACATGACCTAAGAATTAAGACATATTGGACCATTGGGCTTAATCATAGTCTAAGCCCA 943
 GPH5_mandshurica TAACATGACCTAAGAATTAAGACATATTGGACCATTGGGCTTAATCATAGTCTAAGCCCA 939
 GPH5_nubicola TAACATGACCTAAGAATTAAGACATATTGGACCATTGGGCTTAATCATAGTCTAAGCCCA 944
 GPH5-vesca TAACCTGACCTAAGTATCAAGACATATTGGACAATTGGGCTTAATCATAGTCTAAGCCCA 951
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GPH5_ananassa_clone2 AATCTGTACTAGCCATAATATGCTTTTTATAGAAA--ATA--CT---GTGATCTTCAC 996
 GPH5_ananassa_clone7 AATCTGTACTAGCCATAATATGCTTTTTGTAGAAA--ATA--CT---GTGATCTTCAC 996
 GPH5_viridis AATCTGTACTAGCCATAATATGCTTTTTATAGAAA--ACA--CTCTCTGTGATCTTCGC 984
 GPH5_iinumae AATCTGCAC TAGCCCGTAATATGCTTTTTATAGAAA--ACAGACTCTCTGTGATCTTCGC 976
 GPH5_nilgerrensis AATCTGTACTAGCCATAATATGCTTTTTATAGAAA--AGAGATTCTCTGTGATCTTCAC 1002
 GPH5_mandshurica AATCTGTACTAGCCATAATATCTTTTTATAGAAAACAGAGTTCTCTGTGATCTTCAC 999
 GPH5_nubicola AATCTGTACTAGCCATAATATCTTTTTATAGAAAACAGAGATTCTCTGTGATCTTCAC 1004
 GPH5-vesca AATCTGTACTAGCCATAATATGCTTTTTATAGAAA--ACA---CTCTGTGATCTTCAC 1005
 ***** * * ***** * * ***** * * ***** * *

GPH5_ananassa_clone2 CATTGAGGAGTCAAGTTACTCAGCCATGAAGTCAAGGTC AAGCCAAGTAGTCAGTTGAG 1056
 GPH5_ananassa_clone7 CATTGAGGAGTCAAGTTACTCAGCCATGAAGTCAAGGTC AAGCCAAGTAGTCAGTTGAG 1056
 GPH5_viridis CATTGAGGAGTCAAGTTACTCAGCCCGTAAGTAAAAGTCCAGTCAAGTAGTCAGTTGAG 1044
 GPH5_iinumae CATTGAGGAGTCAAGTTACTCAGCTCTGAAGTAAAAGTCCAGTCAAGTAGTCAGTTGAG 1036
 GPH5_nilgerrensis CATTGAGGAGTCAAGTTACTCAGCCCTGAAGTAAAAGTCCAGTCAAGTAGTCAGTTGAG 1062
 GPH5_mandshurica CATTGAGGAGTCAAGTTACTCGGCCCTGAAGTAAAAGTCCAGTCAAGTAGTCAGTTGAG 1059
 GPH5_nubicola CATTGAGGAGTCAAGTTACTCGGCCCTGAAGTAAAAGTCCAGTCAAGTAGTCAGTTGAG 1064
 GPH5-vesca CATTGAGGAGTCAAGTTACTCAGCCCTGAAGTAAAAGTCCAGTCAAGTAGTCAGTTGAG 1064
 ***** * * ***** * * ***** * * ***** * *

GPH5_ananassa_clone2 TTCAACTTGTTCTGGGTCTTCAAAGTTCGAAACTTTAAGCTTCAATGGAGGAAGAGAAG 1116
 GPH5_ananassa_clone7 TTCAACTTGTTCTGGGTCTTCAAAGTTCGAAACTTTAAGCTTCAATGGAGGAAGAGAAG 1116
 GPH5_viridis TTCAACTTGTTCTGGGTCTTCAAAGTTTGAAACTTTAAGCTTCAATGGAGGAAGAGAAG 1104
 GPH5_iinumae TTCAACTTGTTCTGGGTCTTCAAAGTTTGAAACTTTAAGCTTCAATGGAGGAAGATAAG 1096
 GPH5_nilgerrensis TTCAACTTGTTCTGGGTCTTCAAAGTTTGAAACTTTAAGCTTCAATGGAGGAAGAGAAG 1122
 GPH5_mandshurica TTCAACTTGTTCTGGGTCTTCAAAGTTTGAAACTTTAAGCTTCAATGGAGGAAGAGAAG 1119
 GPH5_nubicola TTCAACTTGTTCTGGGTCTTCAAAGTTTGAAACTTTAAGCTTCAATGGAGGAAGAGAAG 1124
 GPH5-vesca TTCAACTTGTTCTGGGTCTTCAAAGTTTGAAACTTTAAGCTTCAATGGAGGAAGAGAAG 1124
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GPH5_ananassa_clone2 GATGCCTTTTTATGTTGTTCGAAAGGGAGATGTGGTTGGCATGTATAAAAGCTTGAAG-GA 1175
 GPH5_ananassa_clone7 GATGCCTTTTTATGTTGTTCGAAAGGGAGATGTGGTTGGCATATATAAAAGCTTGAAG-GA 1175
 GPH5_viridis GATGCCTTTTTATGTTGTTCGAAAGGGAGATGTGGTTGGCATATATAAAAGCTTGAAG-GA 1163
 GPH5_iinumae GATGCCTTTTTATGTTGTTCGAAAGGGAGATGTGGTTGGCATATATAAAAGCTTGAAG-GA 1155
 GPH5_nilgerrensis GATGCCTTTTTATGTTGTTCGAAAGGGAGATGTGGTTGGCATATATAAAAGCTTGAAG-GA 1181
 GPH5_mandshurica GATGCCTTTTTATGTTGTTCGAAAGGGAGATGTGGTTGGCATATATAAAAGCTTGAAG-GA 1178
 GPH5_nubicola GATGCCTTTTTATGTTGTTCGAAAGGGAGATGTGGTTGGCATATATAAAAGCTTGAAG-GA 1183
 GPH5-vesca GATGCCTTTTTATGTTGTTCGAAAGGGAGATGTGGTTGGCATATATAAAAGCTTGAAG-GA 1184
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GPH5_ananassa_clone2 TTGCC--AAAACCAAGCTGG-TTCATCGGTAAA-GTTTT--GATCTTTT--AAGCCTTTT 1227
 GPH5_ananassa_clone7 TTGCC--AAAACCAAGCTGG-TTCATCGGTAAA-GTTTT--GATCTTTT--AAGCCTTTT 1227
 GPH5_viridis TTGCC--AAAACCAAGCTGG-TTCATCGGTAAAAGTTTT--GATCTTTT--AAGCCTTTT 1216
 GPH5_iinumae TTGCC--AAAACCAAGCTGG-TTCCTCGGTAAA-GTTTT--GATCTTTT--AAGCCCTTT 1207
 GPH5_nilgerrensis TTGCC--AAA-CCAAGCTGG-TTCCTCGGTAAA-GCTTT--GATCTTTT--AAGCCTTTT 1232
 GPH5_mandshurica TTGCC--AAAACCAAGCTGG-TTCATCGGTAAA-GTTTT--GATCTTTT--AAGCCTTTT 1230
 GPH5_nubicola TTGCC--AAAACCAAGCTGG-TTCATCGGTAAA-GTTTC--GATCTTTT--AAGCCTTTT 1235
 GPH5-vesca TTGCCAAAAACCAAGCTGGTTTCATCGGAAAAAGTTTTGAATCTTTTTAAGCCCTTT 1244
 ***** * * ***** * * ***** * * ***** * *

GPH5_ananassa_clone2 ----GTAATTTG----ATCACTCCATTGTTTTATCAATTTTTGATTCCCATTTGATTA 1279
 GPH5_ananassa_clone7 ----GTAATTTG----ATCACTCCATTGTTTTATCAATTTTTGATTCCCATTTGATTA 1279
 GPH5_viridis ----ATAATTTG----ATCACTCTCATTGTTTTATCAATTTNNNNNNNNNNNNNNNNNNNN 1268
 GPH5_iinumae ----ATAATTTG----ATTACTCTCATTGTTTTATCAATTTTTGATTCCCATTTGATNN 1259
 GPH5_nilgerrensis ----ATAATTTG----ATTACCCTTATTGTTTTATCAANNNNNNNNNNNNNNNNNNNNNN 1284
 GPH5_mandshurica ----GTAATTTG----ATCACTCCATTGTTTTATCAATTTTAGATTCCCATTTGATTA 1282
 GPH5_nubicola ----ATAATTTG----ATCACTCTCATTGTTTTANNNNNNNNNNNNNNNNNNNNNNNNN 1287
 GPH5-vesca TTTAATAATTTGGAATNCCACCTTCCNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 1304
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GPH5_ananassa_clone2 CATTACTGGGCTCTGTTTATTTTGTGAAATAACTATGCCCTTTCGTTCTAGCATGCAAC 1339
 GPH5_ananassa_clone7 CATTACTGGGCTCTGTTTATTTTGTGAAATAACTATGCCCTTTCGTTCTAGCATGCAAC 1339
 GPH5_viridis NNN 1328
 GPH5_iinumae NNN 1319
 GPH5_nilgerrensis NNN 1344
 GPH5_mandshurica CATTACTGGCTCTGTTTATTTTGTGAACTAACTATGCCCTTTCGTTCTAACATGCAAC 1342
 GPH5_nubicola NNN 1347
 GPH5_vesca NNN 1364

GPH5_ananassa_clone2 TGAAATTTACTGCTAGATTGTATTGTTGTGCCGTTATGGTGTTCATTATGTAAAAGAGAA 1399
 GPH5_ananassa_clone7 TGAAATTTACTGCTAGATTGTATTGTTGTGCCGTTATGGTGTTCATTATGTAAAAGAGAA 1399
 GPH5_viridis NNN 1388
 GPH5_iinumae NNN 1379
 GPH5_nilgerrensis NNN 1404
 GPH5_mandshurica TGAAATAACTGCTAGATTGTATAGCTGAGCCTTTATGGTGTTCATTATGTAAAAGAGAA 1402
 GPH5_nubicola NNN 1407
 GPH5_vesca NNN 1424

GPH5_ananassa_clone2 TGAATTCGGTGGTGGGTATAGAGTACCTCCCTGATTTTTTATGAGATACTATGCTTCTG 1459
 GPH5_ananassa_clone7 TGAATTCGGTGGTGGGTATAGAGTACCTCCCTGATTTTTTATGAGATACTATGCTTCTG 1459
 GPH5_viridis NNN 1448
 GPH5_iinumae NNN 1439
 GPH5_nilgerrensis NNN 1464
 GPH5_mandshurica TGAATTCGGTGGTGGGTATAAAGCACCTCCCTGAGATTATATGAGATACTATGCTTCTG 1462
 GPH5_nubicola NNN 1467
 GPH5_vesca NNN 1484

GPH5_ananassa_clone2 GAAAATGTTATAAA-----GATGAAAACCTAGTTTTTCTAAGAACTTGATG-----TTA 1507
 GPH5_ananassa_clone7 GAAAATGTTATAA-----GATGAAAACCTAGTTTTTCTAAGAACTTGATG-----TTA 1506
 GPH5_viridis NNNNNNNNNNNNNN-----NNNNNNNNNNNNNNNNNNNGAACTTGATG-----TTA 1494
 GPH5_iinumae NNNNNNNNNNNNNN-----NNNNNNNACTAGTTTTTCTAAGAACTTGATG-----TCA 1486
 GPH5_nilgerrensis NNNNNNNNNNNNNN-----NNNNNNNNNNNGTTTTTCTAAGAACTTGATG-----TTA 1512
 GPH5_mandshurica GAAAATGTTATAA-----GATGAAAACAACCTTTTTCTAACAACCTTGATG-----TTA 1509
 GPH5_nubicola NNTAAGAACTTGATG-----TTA 1522
 GPH5_vesca NNNNNNNNNNNNNN-----NNNNNNNNNNNNNNNNNNNGTATTAGATGAAAACCTAG 1536
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GPH5_ananassa_clone2 TTT-----ACTTGATGAGTTGATGGAGGATTACGATATGTGGTTTGGTTTTGTTTTTA 1559
 GPH5_ananassa_clone7 TTT-----ACTTGATGAGTTGATGGAGGATTACGATATGTGGTTTGGTTTTGTTTTTA 1558
 GPH5_viridis TTT-----ACTTGATGAGTTGACGGAGGATTACATATAAGGCTTGGTTATGTTTTTA 1546
 GPH5_iinumae TTT-----ACTTGATGAGTTGATGGAGGATTACACATGTGGTTTGGTTTTGTTTTTA 1538
 GPH5_nilgerrensis TTT-----ACTTGATGAGTTGATGGAGGATTACATATGTGGTTTGGTTTTGTTTTTA 1564
 GPH5_mandshurica TTT-----ACTTGATGAGTTGATGGAAGATTACGTATGTGGTTTGGTTTTGTTTTTA 1561
 GPH5_nubicola TTT-----GCTTGATGAGTTGATGGAGGATTACATATGAGGTTTGGTTATGTTTTTA 1574
 GPH5_vesca TTTTCTAAGAACTTGATGAGTTGATGGAGGATTACATATGAGGTTTGGTTATGTTTTTA 1596
 *** ***** ** * ***** *****

GPH5_ananassa_clone2 GGTATCAATCCTTCTGTAAGTGTGTTTAAAGGGTATGGTTTGCCTAAGGAGGCCGAGGA 1619
 GPH5_ananassa_clone7 GGTATCAATCCTTCTGTAAGTGTGTTTAAAGGGTATGGTTTGCCTAAGGAGGCCGAGGA 1618
 GPH5_viridis GGTATGCAATCCTTCTGTAAGTGTGTTTAAAGGGTATGGTTTGCCTAAGGAGGCCGAGGA 1606
 GPH5_iinumae GGTATGCAATCCTTCTGTAAGTGTGTTTAAAGGGTATGGTTTGCCTAAGGAGGCCGAGGA 1598
 GPH5_nilgerrensis GGTATGCAATCCTTCTGTAAGTGTGTTTAAAGGGTATGGTTTGCCTAAGGAGGCCGAGGA 1624
 GPH5_mandshurica GGTATCAATCCTTCTGAAATTGTGTTTAAAGGGTATGGTTTGCCTAAGGAGGCCGAGGA 1621
 GPH5_nubicola GGTATGCAATCCTTCTGTAAGTGTGTTTAAAGGGTATGGTTTGCCTAAGGAGGCCGAGGA 1634
 GPH5_vesca GGTATGCAATCCTTCTGTAAGTGTGTTTAAAGGGTATGGTTTGCCTAAGGAGGCCGAGGA 1656
 ***** ***** ** ***** *****

GPH5_ananassa_clone2 GTACCTTGTCTCACATGGGCTTAAGAATGCTTCATGTACTATCAGTGCCAGTGATGTGAA 1679
GPH5_ananassa_clone7 GTACCTTGTCTCACATGGGCTTAAGAATGCTTCATGTACTATCAGTGCCAGTGATGTGAA 1678
GPH5_viridis GTACCTTGTCTCGCATGGGCTTAAGAATGCTTCATATACTATCAGTGCCAGTGATGTGAA 1666
GPH5_iinumae GTACCTTGTCTCACATGGGCTTAAGAATGCTTCATATACTATCAGTGCCAGTGATGTGAA 1658
GPH5_nilgerrensis GTACCTTGTCTCACATGGGCTTAAGAATGCTTCATATACTATCAGTGCCAGTGATGTGAA 1684
GPH5_mandshurica GTACCTTGTCTCGCATGGGCTTAAGAATGCTTCATATACTATCAGTGCCAGTGATGTGAA 1681
GPH5_nubicola GTACCTTGTCTCGCATGGGCTTAAGAATGCTTCATATACTATCAGTGCCAGTGATGTGAA 1694
GPH5-vesca GTACCTTGTCTCGCATGGGCTTAAGAATGCTTCATATACTATCAGTGCCAGTGATGTGAA 1716

GPH5_ananassa_clone2 AGATGGTCTGTTTGAAGCCTTGTGCTTGTCCCTACCAGGTTTGAATTGAT---TTCAT 1736
GPH5_ananassa_clone7 AGATGGTCTGTTTGAAGCCTTGTGCTTGTCCCTACCAGGTTTGAATTGAT---TTCAT 1735
GPH5_viridis AGATGGTCTGTTTGAAGCCTTGTGCTTGTCCCTACCAGGTTTGAATTGATGATTTTCAT 1726
GPH5_iinumae AGATGGTCTGTTTGAAGCCTTGTGCTTGTCCCTACCAGGTTTGAATTGAT---TTCAT 1715
GPH5_nilgerrensis AGATGGTCTGTTTGAAGCCTTGTGCTTGTCCCTACCAGGTTTGAATTGAT---TTCAT 1741
GPH5_mandshurica AGATGGTCTGTTTGAAGCCTTGTGCTTGTCCCTACCAGGTTTGAATTGATGATTTTCAT 1741
GPH5_nubicola AGATGGTCTGTTTGAAGCCTTGTGCTTGTCCCTACCAGGTTTGAATTGATGATTTTCAT 1754
GPH5-vesca AGATGGTCTGTTTGAAGCCTTGTGCTTGTCCCTACCAGGTTTGAATTGATGATTTTCAT 1776

GPH5_ananassa_clone2 GTGTTCTAGTTTCTGTTTGGGCATCTGTTATTTTCATGGCATGTGGCGTGGAGCTAGTTG 1796
GPH5_ananassa_clone7 GTGTTCTAGTTTCTGTTTGGGCATCTGTTATTTTCATGGCATGTGGCGTGAAGCTAGTTG 1795
GPH5_viridis GTGTTCTAGTTTCTGTTTGGGTATCTGTTATTTTCATGGCATGTGGCGTGGAGCTAGTTG 1786
GPH5_iinumae GTGTTCTAGTTTCTGTTTGGGTATCTGTTATTTTCATGGCATGTGGCGTGGAGCTAGTTG 1775
GPH5_nilgerrensis GTGTTCTAGTTTCTGTTTGGGTATCTGTTATTTTCATGGCATGTGGCGTGGAGCTAGTTG 1801
GPH5_mandshurica GTGTTCTAGTTTCTGTTTGGGTATCTGTTATTTTCATGGCATGTGGCGTGGAACTAGTTG 1801
GPH5_nubicola GTGTTCTAGTTTCTGTTTGGGTATCTGTTATTTTCATGGCATGTGGCGTGGAACTAGTTG 1814
GPH5-vesca GTGTTCTAGTTTCTGTTTGGGTATCTGTTATTTTCATGGCATGTGGCGTGGAACTAGTTG 1836

GPH5_ananassa_clone2 CATATGATATTAATCTTTTGGTTCCCTCATTGTATAAATTCAGTTTTTTACTATAAAT 1856
GPH5_ananassa_clone7 CATATGATATTAATCTTTTGGTTCCCTCATTGTATAAATTCAGTTTTTTATTATAAAT 1855
GPH5_viridis CATATGATATTAATCTTTTGGTTCCCTCATTGTATAAATTCAGTTTTTTATTATAAAT 1846
GPH5_iinumae CATATGATATTAATCTTTTGGTTCCCTCATTGTATAAATTCAGTTTTTTATTATAAAT 1835
GPH5_nilgerrensis CATATGGTATTAATCTTTTGGTTCCCTCATTGTATAAATTCAGTTTTTTATTATAAAT 1861
GPH5_mandshurica CATATGATATTAATCTTTTGGTTCCCTCATTGTATAAATTCAGTTTTTTATTATAAAT 1861
GPH5_nubicola CATATGATATTAATCTTTTGGTTCCCTCATTGTATAAATTCAGTTTTTTATTATAAAT 1874
GPH5-vesca CATATGATATTAATCTTTTGGTTCCCTCATTGTATAAATTCAGTTTTTTATTATAAAT 1896

GPH5_ananassa_clone2 CTTTCAGCAGCCAGCATCTTCTATGGTTAATTCAGGGTTCAAATTCAGCCTAATCAGCT 1916
GPH5_ananassa_clone7 CTTTCAGCAGCCAGCATCTTCTATGGTTAATTCAGGGTTCAAATTCAGCCTAATCAGCT 1915
GPH5_viridis CTTTCAGCAGCCAGCATCTTCTATGGTTAATTCAGGGTTCAAATTCAGCCTAATCAGCT 1906
GPH5_iinumae CTTTCAGCAGCCAGCATCTTCTATGGTTAATTCAGGGTTCAAATTCAGCCTAATCAGCT 1895
GPH5_nilgerrensis CTTTCAGCAGCCAGCATCTTCTATGGTTAATTCAGGGTTCAAATTCAGCCTAATCAGCT 1921
GPH5_mandshurica CTTTCAGCAGCCAGCATCTTCTATGGTTAATTCAGGGTTCAAATTCAGCCTGATCAGCT 1921
GPH5_nubicola CTTTCAGCAGCCAGCATCTTCTATGGTTAATTCAGGGTTCAAATTCAGCCTGATCAGCT 1934
GPH5-vesca CTTTCAGCAGCCAGCATCTTCTATGGTTAATTCAGGGTTCAAATTCAGCCTGATCAGCT 1956

GPH5_ananassa_clone2 TACACCAAAGAGAATGCCGGATGTGGTAAATTCAGGCGTCAATGACCCTCCACAAAAGAG 1976
GPH5_ananassa_clone7 TACACCAAAGAGAATGCCGGATGTGGTAAATTCAGGCGTCAATGACCCTCCACAAAAGAG 1975
GPH5_viridis TACACGAAAGAGAATGCCGGATGTGGTCAATTCAGGCGTCAATGACCCTCCACAAAAGAG 1966
GPH5_iinumae TACACGAAAGAGAATGCCGGATGTGGTAAATTCAGGCGTCAATGACCCTCCACAAAAGAG 1955
GPH5_nilgerrensis TACACGAAAGAGAATGCCGGATGTGGTAAATTCAGGCGTCAATGACCCTCCACAAAAGAG 1981
GPH5_mandshurica TACACGAAAGAGAATGCCGGATGTGATAAATTCAGGCGTCAATGACCCTCCACAAAAGAG 1981
GPH5_nubicola TACACGAAAGAGAATGCCGGATGTGATAAATTCAGGCGTCAATGACCCTCCACAAAAGAG 1994
GPH5-vesca TACACGAAAGAGAATGCCGGATGTGATAAATTCAGGCGTCAATGACCCTCCACAAAAGAG 2016

GPH5_ananassa_clone2 ATCGCTGGATGTAAGTATCATATGCTACATGGAACCTTTGTAGTTTGATAGAAGACCTTC 2036
GPH5_ananassa_clone7 ATCGCTGGATGTAAGTATCATATGCTACATGGAACCTTTGTAGTTTGATAGAAGATCTTC 2035
GPH5_viridis ATCGCTGGATGTAAGTATCATATGCTACATGGAACCTTTGTAGTTTGATAGAAGATCTTC 2026
GPH5_iinumae ATCATTTGGATGTAAGTATCATATGCTACATGGAACCTTTGTAGTTTGATAGAAGATCTTC 2015
GPH5_nilgerrensis ATCGCTGGATGTAAGTATCATATGCTACATGGAACCTTTGTAGTTTGATAGAAGATCTTC 2041
GPH5_mandshurica ATCACTGGATGTAAGTATCATATGCTACATGGAACCTTTGTAGTTTGATAGAAGATCTTC 2041
GPH5_nubicola ATCATTTGGATGTAAGTATCATATGCTACATGGAACCTTTGTAGTTTGATAGAAGATCTTC 2054
GPH5-vesca ATCACTGGATGTAAGTATCATATGCTACATGGAACCTTTGTAGTTTGATAGAAGATCTTC 2076
*** *****

GPH5_ananassa_clone2 TATTTGGTTACTCATTGTATGATTCGGGTTTTATTATAATCTTTCAGCAGCCAGCGTCT 2096
GPH5_ananassa_clone7 TATTTGGTTACTCATTGTATGATTCGGGTTTTATTATAATCTTTCAGCAGCCAGCGTCT 2095
GPH5_viridis TATTTGGTTACTCATTGTATGATTCGGGTTTTATTATAATCTTTCAGCAGCCAGCATCT 2086
GPH5_iinumae TATTTGGTTACTCATTGTATGATTCGGGTTTTATTATAATCTTTCAGCAGCCAGCGTCT 2075
GPH5_nilgerrensis TATTTGGTTACTCATTGTATGATTCAGGTTTTATTATAATCTTTCAGCAGCCAGCGTCT 2101
GPH5_mandshurica TATTTGGTTACTCATTGTATGATTCGGGTTTTATTATAATTTTTCAGCAGCCAGGTTCT 2101
GPH5_nubicola TATTTGGTTACTCATTGTATGATTCGGGTTTTATTATAATTTTTCAGCAGCCAAATGTCT 2114
GPH5-vesca TATTTGGTTACTCATTGTATGATTCGGGTTTTATTATAATTTTTCAGCAGCCAGCGTCT 2136

GPH5_ananassa_clone2 TCTATGGTTAATTCAGGCTTCAAAGTTGCACCTGATCAGTTTACACGAATGCGATTGCCG 2156
GPH5_ananassa_clone7 TCTATGGTTAATTCAGGCTTCAAAGTTGCACCTGATCAGTTTACACGAATGCGATTGCCG 2155
GPH5_viridis TCTATGGTTAATTCAGGCTTCAAAGTTGCACCTTACCAGCTTGCACGAATGAGATTGCCG 2146
GPH5_iinumae TCTATGGTTAATTCAGGCTTCAAAGTTGCACCTTATCAGCTTACACGAATGAGATTGCCG 2135
GPH5_nilgerrensis TCTATGGTTAATTCAGGCTTCAAAGTTGCACCTTATCAGCTTACACGAATGAGATTGCCG 2161
GPH5_mandshurica TCTATGGTTAATTCAGGCTTCAAAGTTGCACCTCATCAGCTTACGCGAATGAGATTGCCG 2161
GPH5_nubicola TCTTTGGTTAATTCAGGCTTCAAAGTTGCACCTCATCAGCTTACGCGAATGAGATTGCCG 2174
GPH5-vesca TCTATGGTTAATTCAGGCTTCAAAGTTGCACCTCATCAGCTTACGCGAATGAGATTGCCG 2196
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GPH5_ananassa_clone2 GATGTGGTAAATTCAGG-TGTCAATTTACCCTCCACAGAGGACATTGCCGGATGTAAGTAT 2215
GPH5_ananassa_clone7 GATGTGGTAAATTCAGG-TGTCAATTTACCCTCCACAGAGGACATTGCCGGATGTAAGTAT 2214
GPH5_viridis GATGTGATAAATTCAGG-TGTCAATGACCCTCCACAGAGGACATTGCCAGATGTAAGTAT 2205
GPH5_iinumae GATGTGGTAAATTCAGG-TGTCAATGACCCTCCACAGAGGACATTGCCGGATGTAAGTAT 2194
GPH5_nilgerrensis GATGTGGTAAATTCAGNGTGTCAATGACCCTCCACAGAGGACATTGCCGGATGTAAGTAT 2221
GPH5_mandshurica GATGTGGTAAATTCAGG-TGTCAATGACCCTCCACAGAGGACATTGCCGGATGTAAGTAT 2220
GPH5_nubicola GATGTGGTAAATTCAGG-TGTCAATGACCCTCCACAGAGGACATTGCCGGATGTAAGTAT 2233
GPH5-vesca GATGTGGTAAATTCAGG-TGTCAATGACCCTCCACAGAGGACATTGCCGGATGTAAGTAT 2255

GPH5_ananassa_clone2 CTTATGCTACATGGAAATTTTGTCTGGCCAGATTGGCATGAAATCCAGACACCTTCAG 2275
GPH5_ananassa_clone7 CTTATGCTACATGGAAATTTTGTCTGGCCAGATTGGCATGAAATCCAGACACCTTCAG 2274
GPH5_viridis CTTATGCTACATGGAAATTTTGTCTGGCCAGATTGGCATGAAATCCAGATACCTTCAG 2265
GPH5_iinumae CTTATGCTACATGGAAATTTTGTCTGGCCAGATTGGCATGAAATCCGGATACCTTCAG 2254
GPH5_nilgerrensis CTTATGCTACATGGAAATTTTATTCTGGCCAGATTGGTATGAAATCCAGATACCTTCAG 2281
GPH5_mandshurica CTTATGCTACATGGAAATTTTGTCTGGCCAGATTGGCATGAAATCCAGATACCTTCAG 2280
GPH5_nubicola CTTATGCTACATGGAAATTTTGTCTGGCCAGATTGGCATGAAATCCAGATACCTTCAG 2293
GPH5-vesca CTTATGCTACATGGAAATTTTGTCTGGCCAGATTGGCATGAAATCCAGATACCTTCAG 2315

GPH5_ananassa_clone2 TCTGGCTGGATTATGGAGTTGCGTTGATCACTTGTATTATTGTATTTATTCTGCAAATGAT 2335
GPH5_ananassa_clone7 TTTGGCTGGATTATGGAGTTGCGTTGATCACTTGTATTATTGTATTTATTCTGCAAATGAT 2334
GPH5_viridis TTTGGCTGGATTATGGAGTTGCGTTGATCACTTGTATTATTGTATTTATTCTGCAAATGAT 2325
GPH5_iinumae TTTGGCTGGATTATGGAGTTGCGTTGATCACTTGTATTATTGTATTTATTCTGCAAATGAT 2314
GPH5_nilgerrensis TTTGGCTGGATTATGGAGTTGCGTTGATCACTTGTATTATTGTATTTATTCTGCAAATGAT 2341
GPH5_mandshurica TTTGGCTGGATTATGGAGTTGCGTTGATCACTTGTATTATTGTATTTATTCTGCAAATGAT 2340
GPH5_nubicola TTTGGCTGGATTATGGAGTTGCGTTGATCACTTGTATTATTGTATTTATTCTGCAAATGA- 2352
GPH5-vesca TTTGGCTGGATTATGGAGTTGCGTTGATCACTTGTATTATTGTATTTATTCTGCAAATGAT 2375
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GPH5_ananassa_clone2 GTTTTTCGGCTTCCAGTTTTTCTGCACATAAGCATTTTAAAGCTGATAAATGTAATCGAA 2395
GPH5_ananassa_clone7 GTTTTTCGGCTTCCAGTTTTTCTGCACATAAGCATTTTAAAGCTGATAAATGTAATCGAA 2394
GPH5_viridis GTTTT-CAGCTTCCAGTTTTTCTGCACATAAGCATTTTAAAGCTGATAAATGTAATCGAA 2384
GPH5_iinumae GTTTTTCGGCTTCCAGTTTTTCTGCACATAAGCATTTTAAAGCTGATAAATGTAATCGAA 2374
GPH5_nilgerrensis GTTTTTCGGCTTCCAGTTTTTCTGCACATAAGCATTTTAAAGCTGATAAATGTAATCGAA 2401
GPH5_mandshurica GTTTTTCGGCTTCCAGTTTTTCTTACACATAAGCATTTTAAAGCTGATCATTGTAATCAAA 2400
GPH5_nubicola --TTTTTGGCTTCCAGTTTTTCTTACACATAAGCATTTTAAAGCTGGTCAATTGTAATGAA 2410
GPH5-vesca GTTTTTCGGCTTCCAGTTTTTCTTACACATAAGCATTTTAAAGCTGATCATTGTAATCGAA 2435
*** ***** **

GPH5_ananassa_clone2 CTCAGTAATCTACTACTGGTGTAAAGTTGCCTTGTGTCACCACCCTAAGATCACAATT 2455
GPH5_ananassa_clone7 CTCAGTAATCTACTACTGGTGTAAAGTTGCCTTGTGTCACCAC-CTAAGATCACAATT 2453
GPH5_viridis CTCGAGTAATCTACTACTGGTGTAAAGTTGCCTTGTGTCACCACCCTAAGATCACAATT 2444
GPH5_iinumae CTCGAGTAATCTGCTACTGGTGTAAAGTTGCCTTGTGTCACCACCCTAAGATCACAATT 2434
GPH5_nilgerrensis CTCGAGTAATCTACTACTGGTGTAAAGTTGCCTTGTGTCACCACCCTAAGATCACAATT 2461
GPH5_mandshurica CTCGATAAATCTACTACTGGTGTAAAGTTGCCTTGTGTCACCACCCTAAGATCACAATT 2460
GPH5_nubicola CTCGATAAATCTACTACTGGTGTAAAGTTGCCTTGTGTCACCACCCTAAGATCACAATT 2470
GPH5-vesca CTCGATAATTACTACTACTGGTGTAAAGTTGCCTTGTGTCACCACCCTAAGATCACAATT 2495
*** * ***** **

GPH5_ananassa_clone2 TCGTATTTTATGATCAACACTGAATACCTATGTCTAGTGTATGATTATAGTCATGTGAA 2515
GPH5_ananassa_clone7 TCGTATTTTATGATCAACACTGAATACCTATGTCTAGTGTATGATTATAGTCATGTGAA 2513
GPH5_viridis TCGTATTTTATGATCAACACTGAATACCTATGTCTAGTGTATGATTATAGTCATGTGAA 2504
GPH5_iinumae TCGTATTTTATGATCAACACTGAATACCTATGTCTAGTGTATGATTATAGTCATGTGAA 2494
GPH5_nilgerrensis TCGTATTTTATGATCAACACTGAATACCTATGTCTAGTGTATGATTATAGTCATGTGAA 2521
GPH5_mandshurica TCGTATTTTATGATCAACACTGAATACCTATGTCTAGTGTATGATTATAGTCATGTGAA 2520
GPH5_nubicola TCGTATTTTATGATCAACACTGAATACCTATGTCTAGTGTATGATTATAGTCATGTGAA 2530
GPH5-vesca TCGTATTTTATGATCAACACTGAATACCTATGTCTAGTGTATGATTATAGTCATGTGAA 2555
* ***** **

GPH5_ananassa_clone2 GTGGATTTCTTAATATATGCCTCATCTATGTCTTCATCCAGCAATCCTGCATACTTGAGT 2575
GPH5_ananassa_clone7 GTGGATTTCTTAATATATGCCTCATCTATGTCTTCATCCAGCAATCCTGCATACTTGAGT 2573
GPH5_viridis GTGGATTTCTTAATATATGCCTCATCTATGTCTTCATCCAGCAATCCTGCATACTTGAGT 2564
GPH5_iinumae GTGGATTTCTTAATATATGCCTCATCTATGTCTTCATCCAGCAATCCTGCATACTTGAGT 2554
GPH5_nilgerrensis GTGGATTTCTTAATATATGCCTCATCTATGTCTTCATCCAGCAATCCTGCATACTTGAGT 2581
GPH5_mandshurica GTGGATTTCTTAATATATGCCTCATCTATGTCTTCATCCAGCAATCCTGCATACTTGAGT 2580
GPH5_nubicola GTGGATTTCTTAATATATGCCTTGTCTATGTCTTCATCCAGCAATCCTGCATACTTGAGT 2590
GPH5-vesca GTGGATTTCTTAATATATGCCTCGTCTATGTCTTCATCCAGCAATCCTGCATACTTGAGT 2615
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GPH5_ananassa_clone2 TTGATGGTCTTCAAAGGAAATCCTGGACCATCTGGTGCAGGAGCTGTACTCCGTGCTG 2635
GPH5_ananassa_clone7 TTGATGGTCTTCAAAGGAAATCCTGGACCATCTGGTGCAGGAGCTGTACTCCGTGCTG 2633
GPH5_viridis TTGATGGAGCTTCAAAGGAAATCCTGGATTATCTGGTGCAGGAGCTGTACTCCGTGCTG 2624
GPH5_iinumae TTGATGGAGCTTCAAAGGAAATCCTGGATTATCTGGTGCAGGAGCTGTACTCCGTGCTG 2614
GPH5_nilgerrensis TTGATGGAGCTTCAAAGGAAATCCTGGATTATCTGGTGCAGGAGCTGTACTCCGTGCTG 2641
GPH5_mandshurica TTGATGGAGCTTCAAAGGAAATCCTGGATTATCTGGTGCAGGAGCTGTACTCCGTGCTG 2640
GPH5_nubicola TTGATGGAGCTTCAAAGGAAATCCTGGATTATCTGGTGCAGGAGCTGTACTCCGTGCTG 2650
GPH5-vesca TTGATGGAGCTTCAAAGGAAATCCTGGATTATCTGGTGCAGGAGCTGTACTCCGTGCTG 2675
***** ***** **

GPH5_ananassa_clone2 AAGATGGGAGTGTGTATGTGGAGTTCATGAAAACATTGTGAATCTTTTAGGATATATAT 2695
GPH5_ananassa_clone7 AAGATGGAAGTGTGTATGTGGAGTTCATGAAAACATTGTGAATCTTTTAGGATATATAT 2693
GPH5_viridis AAGATGGGAGTGTGTATGTGGAGTTCATGAAAACATTGTGAATCTTTTAGGATATATAT 2684
GPH5_iinumae AAGATGGGAGTGTGTATGTGGAGTTCATGAAAACATTGTGAATCTTTTAGGATATATAT 2674
GPH5_nilgerrensis AAGATGGGAGTGTGTATGTGGAGTTCATGAAAACATTGTGAATCTTTTAGGATATATAT 2701
GPH5_mandshurica AAGATGGGAGTGTGTATGTGGAGTTCATGAAAACATTGTGAATTTTTTTG-ATATATAT 2699
GPH5_nubicola AAGATGGGAGTGTGTATGTGGAGTTCATGAAAACATTGTGAATTTTTTTATATATAT 2710
GPH5-vesca AAGATGGGAGTGTGTATGTGGAGTTCATGAAAACATTGTGAATTTTTTTG-ATATATAT 2734
***** ***** **


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GPH5_ananassa_clone2      TTTTGT TTTTGTAAAAATGGATCTCTTTATAACATTGGGGCTACTATAGTTGCACCGGCT 2755
GPH5_ananassa_clone7      TTTTGT TTTTGTAAAAATGGATCTCTTTATAACATTGGGGTTACTATAGTTGCACCGGCT 2753
GPH5_viridis              ATTTGT TTTTGTAAAAATGGATCTCTTTATAACATTGGGGTTACTGTAGTTGCACCGGCT 2744
GPH5_iinumae              TTTTGT TTTTGTAAAAAGTGGATCTCTTTATAACATTGGGTTTACTATAGTTGCACCGGCT 2734
GPH5_nilgerrensis         TTTTGT TTTTGTAAAAATGGATCTCTTTATAACATTGGGGTTACTATAGTTGCACCGGCT 2761
GPH5_mandshurica          TTTTGT TTTTGTAAAAATGGATCTCTTTATAACATTGGGGTTGCTATAGTTGCACCGGCT 2759
GPH5_nubicola             TTTTGT TTTTGTAAAAATGG---TTTATAACATTGGGGTTACTATAGTTGCACCGGCT 2766
GPH5-vesca                TTTTGT TTTTGTAAAAATGGATCTCTTCATAACATTGGGGTTACTATAGTTGCACCGGCT 2794
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GPH5_ananassa_clone2      GCGGGAAGGTGTGTGCAACGGCA 2778
GPH5_ananassa_clone7      GCGGGAAGGTGTGTGCAACGGCA 2776
GPH5_viridis              GCGGGAAGGTGTGTGCAACGGCA 2767
GPH5_iinumae              GCGGGAAGGTGTGTGCAACGGCA 2757
GPH5_nilgerrensis         GAGGGAAGGTGTGTGCAACGGCA 2784
GPH5_mandshurica          GCGGGAAGGTGTGTGCAACGGCA 2782
GPH5_nubicola             GCGGGAAGGTGTGTGCAACGGCA 2789
GPH5-vesca                GCGGGAAGGTGTGTGCAACGGCA 2817
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GPH23: SNPs other than introduced by DNA polymerase are true. After preliminary sequence alignment, the putative SNPs were verified by observation of unambiguous peaks in the chromatograms.

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GPH23_iinumae_clone2      CTTGAGGGCCATCAGCACGTCCTTCTGCAATACCATCTTAGTGCTAACG 50
GPH23_iinumae_clone5      CTTGAGGGCCATCAGCACGTCCTTCTGCAATACCATCTTAGTGCTAACG 50
GPH23_mandshurica_clone3  CTTGAGGGCCATCAGCACGTCCTTCTGCAATACCATCTTAGTACTAACG 50
GPH23_ananassa_clone4     CTTGAGGGCCATCAGCACGTCCTTCTGCAATACCATCTTAGTACTAACG 50
GPH23_ananassa_clone3     CTTGAGGGCCATCAGCACGTCCTTCTGCAATACCATCTTAGTACTAACG 50
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GPH23_iinumae_clone2      ACCTTTACAGTGAGAGTGTGACCAGAGGTGCCTGGGCGGAGCTGCCAAC 100
GPH23_iinumae_clone5      ACCTTTACAGTGAGAGTGTGACCAGAGGTGCCTGGGCGGAGCTGCCAAC 100
GPH23_mandshurica_clone3  ACCTTTACAGTGAGAGTGTGACCAGAGGTGCCTGGGCGGAGCTGCCAAC 100
GPH23_ananassa_clone4     ACCTTTACAGTGAGAGTGTGACCAGAGGTGCCTGGGCGGAGCTGCCAAC 100
GPH23_ananassa_clone3     ACCTTTACAGTGAGAGTGTGACCAGAGGTGCCTGGGCGGAGCTGCCAAC 100
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GPH23_iinumae_clone2      CTTTGTGAAGGTTGGTTTCTCAGGGCTTGCTTTGAGTCTGCCATTTGAT 150
GPH23_iinumae_clone5      CTTTGTGAAGGTTGGTTTCTCAGGGCTTGCTTTGAGTCTGCCATTTGAT 150
GPH23_mandshurica_clone3  CTTTGTGAAGGTTGGTTTCTCAGGGCTTGCTTCGTGCTGCCATTTGAT 150
GPH23_ananassa_clone4     CTTTGTGAAGGTTGGTTTCTCAGGGCTTGCTTCGAGTCTGCCATTTGAT 150
GPH23_ananassa_clone3     CTTTGTGAAGGTTGGTTTCTCAGGGCTTGCTTCGAGTCTGCCATTTGAT 150
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GPH23_iinumae_clone2      AAAAGACCTGCCAGAATCCACGCCACCAAAC-TCTTTAGCACTAATCCAA 199
GPH23_iinumae_clone5      AAAAGACCTGCCAGAATCCACGCCACCAAAC-TCTTTAGCACTAATCCAA 199
GPH23_mandshurica_clone3  AAAAGACCTGCCAGAATCCACGCCACCAAAC-TCTTTAGCACTAATCCAA 199
GPH23_ananassa_clone4     AAAAGACCTGCCAGAATCCACGCCACCAAAC-TCTTTAGCACTAATCCAA 199
GPH23_ananassa_clone3     AAAAGACCTGCCAGAATCCACGCCACCAAACATCTTTAGCACTAATCCAA 200
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GPH23_iinumae_clone2      TCCATAACAACCTTCATAAAACACACATAGCATCAACATGCAATAATGTGG 249
GPH23_iinumae_clone5      TCCATAACAACCTTCATAAAACACACATAGCATCAACATGCAATAATGTGG 249
GPH23_mandshurica_clone3  TCCATAACAACCTTCATAAAACACACATAGCATCAACATGCAATAATGTGG 249
GPH23_ananassa_clone4     TCCATAACAACCTTCATAAAACACACATAGCATCAACATGCAATAATGTGG 249
GPH23_ananassa_clone3     TCCATAACAACCTTCATAAAACACACATAGCATCAACATGCAATAATGTGG 250
                          *****

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GPH23_iinumae_clone2 GTCCATAAGAACCATGAGTATGACATA-GAGTCTTCAAGCTTCGATTTC 298
 GPH23_iinumae_clone5 GTCCATAAGAACCATGAGTATGACATA-GAGTCTTCAAGCTTCGATTTC 298
 GPH23_mandshurica_clone3 GTCCACAAGAACCATGAGTATGAC-CA-TAGTCTTCAAGCTTCGATTTC 297
 GPH23_ananassa_clone4 GTCCATAAGAACCATGAGTATGACATA-GAGTCTTCAAGCTTCGATTTC 298
 GPH23_ananassa_clone3 GTCCATAAGAACCATGTGCATGACATAAGATTCTCAAGCTTCGATTTC 300

GPH23_iinumae_clone2 TTATTTGCTTCGAAAGAAGCAAGTTCAGAGTCACACAAACCAGAATATAG 348
 GPH23_iinumae_clone5 TTATTTGCTTCGAAAGAAGCAAGTTCAGAGTCACACAAACCAGAATATAG 348
 GPH23_mandshurica_clone3 TTATTCGCTTCGAAAGAAGCAAGTTCAGAGTCACACAAACCAGAATATAG 347
 GPH23_ananassa_clone4 TTATTTGCTTCGAAAGAAGCAAGTTCAGAGTCACACAAACCAGAATATAG 348
 GPH23_ananassa_clone3 TAATTTGCTTCGAAAGAAGTAAAGTTCAGAGTCACTCAAACCTAATATAG 350
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GPH23_iinumae_clone2 ATCTCAAA--TTTAATGAAACATATTCCTAAGAACCTAAAGCAATATAAA 396
 GPH23_iinumae_clone5 ATCTCAAA--TTTAATGAAACATATTCCTAAGAACCTAAAGCAATATAAA 396
 GPH23_mandshurica_clone3 ATCTCAAAAATTTAATGAAACATATTCCTAAGAACCTAAAGAAATATAAA 397
 GPH23_ananassa_clone4 ATCTCAAA--TTTAATGAAACATATTCCTAAGAGCCTAAAGAAATATAAA 396
 GPH23_ananassa_clone3 ATCTCAAA--TTTAATGAAACATATTCCTAAGAGCCTACACAAATATAAA 398

GPH23_iinumae_clone2 ATCGTAACTGGACTTAATCTGAAATTGTCGTATAAAATTGTAAATCGATCA 446
 GPH23_iinumae_clone5 ATCGTAACTGGACTTAATCTGAAATTGTCGTATAAAATTGTAAATCGATCA 446
 GPH23_mandshurica_clone3 ATCGTAACTGAACTTATTCTGAAATTGTCGTATAAAATTGTAAACCGATCA 447
 GPH23_ananassa_clone4 ATCGTAACTGAACTTAATCTGAAATTGTCGTATAAAATTGTAAATCGATCA 446
 GPH23_ananassa_clone3 ATCGTAACTGAACTTAATCTGAAACTGTCGTATAAAATTGTAAATCGATCA 448

GPH23_iinumae_clone2 AAAACAAACTTCAAGTTCAGATTTCACAGAGCAGATCAGAGATAGCATACA 496
 GPH23_iinumae_clone5 AAAACAAACTTCAAGTTCAGATTTCACAGAGCAGATCAGAGATAGCATACA 496
 GPH23_mandshurica_clone3 ACA-CAAACCTTCAAGTTCAGATTTCACAGAGCAGATCAGAGATAGCATACC 496
 GPH23_ananassa_clone4 AAAACAAACTTCAAGTTCAGATTTCAGAGAGCAGATCAGAGATAGCGTACA 496
 GPH23_ananassa_clone3 AAACCAAACCTTCAATGTTTCAGATTTCACAGACCCTATCAGAGATAGCATACA 498
 * * * * *

GPH23_iinumae_clone2 AGTGACCTAAGAAACAAAACAACATTTCTAACAAGATCGCAAACATTGGAG 546
 GPH23_iinumae_clone5 AGTGACCTAAGAAACAAAACAACATTTCTAACAAGATCGCAAACATTGGAG 546
 GPH23_mandshurica_clone3 AGTGACCTAAGAAACAAAACAACCTTTCTAACAAGATCGCAAACATTGGAG 546
 GPH23_ananassa_clone4 AGTGACCTAAGAAACAAAACAACATTTCCAACAAGATCGCAAACATTTCGAG 546
 GPH23_ananassa_clone3 AGTGACCTCTGAAACAAAACAATAATTTCCAACAAGATCGCAAACATTTCGAG 548

GPH23_iinumae_clone2 ATTAAATACGATGAGCTATGAGACAACCTTTTCCATGCAAATCTAACAAAA 596
 GPH23_iinumae_clone5 ATTAAATACGATGAGCTATGAGACAACCTTTTCCATGCAAATCTAACAAAA 596
 GPH23_mandshurica_clone3 ATTAAATACGATGAGCTATGAGACAACCTTTTCCATGCAAATCTAACAAAA 596
 GPH23_ananassa_clone4 ATTAAATACGATGAGCTATGAGACAACCTTTTCCATGCAAATCTAACAAAA 596
 GPH23_ananassa_clone3 ATTAAATACGATGAGCTATGAGACAACCTTTTCCATGCAAATCTAACAAAA 598

GPH23_iinumae_clone2 GAAACTAAAGGGATCTGGAGAATTAGGGGTTAGAGGTCACCTTAAGAGTT 646
 GPH23_iinumae_clone5 GAGACTAAAGGGATCTGGAGAATTAGGGGTTAGAGGTCACCTTAAGAGTT 646
 GPH23_mandshurica_clone3 GAGAATAAAGGGATCTGGAGAATTAGGGGTTAGAGGTCACCTTAAGAGTT 646
 GPH23_ananassa_clone4 GAGAATAAAGGGATCTGGAGAATTAGGGGTTAGAGGTCACCTTAAGAGTT 646
 GPH23_ananassa_clone3 AAGAATAAAGGGATCTGGAGAATTATGGGTTAGAGGTCACCTTCAGAGTT 648
 * * * * *

GPH23_iinumae_clone2 TCGGTGAAACACAACAACACTGGGGAGACAGAGACAGAGGAGGAACCTGCG 696
 GPH23_iinumae_clone5 TCGGTGAAACACAACAACACTGGGGAGACAGAGACAGAGGAGGAACCTGCG 696
 GPH23_mandshurica_clone3 TGGGTGAAACACAAC----CTGGGGAGACAGAGACAGAGGAGGAACCTGCC 692
 GPH23_ananassa_clone4 TGGGTGAAACACAAC----TGGGGAAGACAGAGACAGAGGAGGAACCTGCC 692
 GPH23_ananassa_clone3 TGGGTGAAACACAAC----TGGGGACGACCTACACCAGGAGGAACCTGCC 694
 * * * * *

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GPH23_iinumae_clone2      AATATCTATCTGAA-ACCAAACAAGTAAAAAGGGTTAGCTGTCAGTAA 745
GPH23_iinumae_clone5      AATATCTATCTGAA-ACCAAACAAGTAAAAAGGGTTAGCTGTCAGTAA 745
GPH23_mandshurica_clone3  AAGATCTATCTGACCACCAACCAAGTAAAAAGGGTTAAGCTATCAGTAA 742
GPH23_ananassa_clone4    AAGATCTATCTGAA-ACCAAACAAGTAAAAAGGGTTAGCTGTCAGTAA 741
GPH23_ananassa_clone3    AAAATCTATCTGAA-ACCTAAC-AAATAAAAAGGGTCTATCTGTCAATAA 742
                          ** ***** ** * ** ***** * ** ** **
GPH23_iinumae_clone2      -CGAGCTCCTAACCGTCCATCTCCAATCTTGTACAGGGGTGATCCT-ACGC 793
GPH23_iinumae_clone5      -CGAGCTCCTAACCGTCCATCTCCAATCTTGTACAGGGGGGATCCT-ACGC 793
GPH23_mandshurica_clone3  -CGAGCTCCTAACCGTCCATCTCCAATCTTGTACAGGGGTGATCCT-ACGC 790
GPH23_ananassa_clone4    -CGAGCTCCTAACCGTCCATCTCCAATCTTGTACAGGGGTGATCCT-ACGC 789
GPH23_ananassa_clone3    ACGAGCTCCCTATCGTCCATCTCCAATCTTGTAAAGGGGTGATCCTTACGC 792
                          ***** * ***** ***** ***** *****
GPH23_iinumae_clone2      GTCCACTTGTCCCTCTCCAGTTCTAACTATGTACACGCTAGCTGCGGATT 843
GPH23_iinumae_clone5      GTCCACTTGTCCCTCTCC-AGTTCTAACTATGTACACGCTAGCTGCGGATT 842
GPH23_mandshurica_clone3  GTCCACTTGTCCCTCTCCAGTTCTAACTGTGTAGACGCTAGCTGCGGATT 840
GPH23_ananassa_clone4    GTCCACTTGTCCCTTCTCCAGTTCTAACTATGTAGACGCTAGCTGCGGATT 839
GPH23_ananassa_clone3    TTCCCTTGTCCCTCTCCCATCTA-CTAAGTAGACGCTAGCTGCGGATT 841
                          *** ***** ** ***** * ** *****
GPH23_iinumae_clone2      GTTATTATGTTTTGGATAGAATAGAATACCTTTGCAAAATAGGAAGCTCC 893
GPH23_iinumae_clone5      GTTATTATGTTTTGGATAGAATAGAATACCTTTGCAAAATAGGAAGCTCC 892
GPH23_mandshurica_clone3  GTTATTATTTTTGGATAG----AATACCTTTGCAAAATAGGAAGCTCC 885
GPH23_ananassa_clone4    GTTATTATGTTTTGGATAG----AATACCTTTGCAAAATAGGAAGCTCC 884
GPH23_ananassa_clone3    GTTATTATGTTTTGGATAG----AATACCTTTGCAAAATGGAAGCTCC 886
                          ***** ***** ***** ***** *****
GPH23_iinumae_clone2      -----TCCTTGTTTTTCGGCAAGAGAAAGGCCAAAATATCTGACCATT 937
GPH23_iinumae_clone5      -----TCCTTGTTTTTCGGCAAGAGAAAGGCCAAAATATCTGACCATT 936
GPH23_mandshurica_clone3  -----TCCTTGTTTTTCGGCAAGAGAAAGGCCAAAATATCTGACCATT 929
GPH23_ananassa_clone4    -----TCCTTGTTTTTCTGCAAGAGAAAGGCCAAAATATCTGACCATT 928
GPH23_ananassa_clone3    AGCTCCTCCTTGTTTTTCGGCAAGAGAAAGGCCAAAATATCAGACCTTC 936
                          ***** ***** ***** *****
GPH23_iinumae_clone2      CGACGCCGGAGCTTCCTCAGAAAGCCGGTTCCGTCCGCAACATCGATGCC 987
GPH23_iinumae_clone5      CGACGCCGGAGCTTCCTCAGAAAGCCGGTTCCGTCCGCAACATCGATGCC 986
GPH23_mandshurica_clone3  CGACGCCGGAGCTTCCTCAGAAAGCCAGTACTATCCGCCACATCGATGCC 979
GPH23_ananassa_clone4    CGACGCCGGAGCTTCCTCAGAAAGCCAGTACCATCCGCAACATCGATGCC 978
GPH23_ananassa_clone3    CGACGCCGGAGCTTCCTCAGAAAGCC--TACCATCCGCAACATCGTTGCC 984
                          ***** ***** ***** *****
GPH23_iinumae_clone2      AGGCCTTGCGAGGTTGCCTCCGCTTCTTTGGATTGTGTTTTTCGTGGTT 1037
GPH23_iinumae_clone5      AGGCCTTGCGAGGTTGCCTCCGCTTCTTTGGATTGTGTTTTTCGTGGTT 1036
GPH23_mandshurica_clone3  AGGCCTTGCGAGGTTGCCTCCGCTTCTTTGGATTGTGTTTTTCGTGGTT 1029
GPH23_ananassa_clone4    AGGCCTTGCGAGGTTGCCTCCGCTTCTTTGGATTGTGTTTTTCGTGGTT 1028
GPH23_ananassa_clone3    AGGCCTTGCGAGGTTGCCTCCGCTTCTTTGGATTGTGTTTTTCGTGGTA 1034
                          *****
GPH23_iinumae_clone2      TAGGAGATTGTTGAACAAAAAGAAAAACATAACATATGATGAATGAATT 1087
GPH23_iinumae_clone5      TAGGAGATTGTTGAACAAAAAGAAAAACATAACATATGATGAATGAATT 1086
GPH23_mandshurica_clone3  TAGGAGATTGCTGAACAAAAAGAAAAACATAACATATGATGAATGAATT 1079
GPH23_ananassa_clone4    TAGGAGATTGTTGAACAAAAAGAAAAACATA---TATGATAAATGAATT 1075
GPH23_ananassa_clone3    TAGGAGATTGTTGAACAAAAAGAAAAACATAACATATGATGAATGAATC 1084
                          ***** ***** *****
GPH23_iinumae_clone2      ATCAAATTAATTAACCAAGGTGACAATACAAGATGAGAACCACCAAGGGTT 1137
GPH23_iinumae_clone5      ATCAAATTAATTAACCAAGGTGACAATACAAGATGAGAACCACCAAGGGTT 1136
GPH23_mandshurica_clone3  ATCAAATTAATTAATCAAGGTGACAATACAAGATGAGAACCACCAAGATT 1129
GPH23_ananassa_clone4    ATCAAATTAATTAATCAAGGTGACGATACAAGATGAGAACCACCAAGGGTT 1125
GPH23_ananassa_clone3    ATCAAATTAATTAATCAAGGTGAC----- 1108
                          ***** *****

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GPH23_iinumae_clone2 CAATAGTGTGTACTCTCAAGCCTAATACTAACAACAACAAAGAAAGATTTC 1187
GPH23_iinumae_clone5 CAATAGTGTGTACTCTCAAGCCTAATACTAACAACAACAAGAAAGATTTC 1186
GPH23_mandshurica_clone3 CAATAGTGTGTACTCTCAAGCCTAATACTAACAACAACAAGAAAGATT-C 1178
GPH23_ananassa_clone4 CAATAGTGTGTACTCTCAAGCCTAATACTAACAACAACAAGAAAGATT-C 1174
GPH23_ananassa_clone3 -----AAAGAAAGATT-A 1120

GPH23_iinumae_clone2 TATCCTTCCATTCCCAAATCAAAAACCACTACAATGTACCGTC----- 1230
GPH23_iinumae_clone5 TATCCTTCCATTCCCAAATCAAAAACCACTACAATGTACCGTC----- 1229
GPH23_mandshurica_clone3 TATCGTTCCATTCCCAAATCAAAAACCACTACAATGTACCGTC----- 1221
GPH23_ananassa_clone4 TATCCTTCCATTCCCAAGATCAAAAACCACTCTAATGTACCGCCGGCAAAG 1224
GPH23_ananassa_clone3 TATCCTTCCATTCCCTAAGTCAAAAACCACTACAATGTACCGCCGGCAAAG 1170
**** *

GPH23_iinumae_clone2 -----TAATTGAATTGACATTGTAAGTGAGAGATAGTGTACAGAGCTGC 1274
GPH23_iinumae_clone5 -----TAATTGAATTGACATTGTAAGTGAGAGATAGTGTACAGAGCTGC 1273
GPH23_mandshurica_clone3 -----GGCA----AAGTG-----CTGC 1234
GPH23_ananassa_clone4 TGCTGCTAATAGAATTGACATTGTAAGTAGGGGATAGTGTACAGAGCAGC 1274
GPH23_ananassa_clone3 TGCTGCTAATAGAATTGACATTGTAAGTGAGGGGATAGTGTACAGAGCTGC 1220
* * * * * * * * * * * * * * * * * * *

GPH23_iinumae_clone2 ATTGGGAGATAGTGTATCGTGACACTCTATGAAGGGG-ATGCTTAAGAG 1323
GPH23_iinumae_clone5 ATTGGGAGATAGTGTATCGTGACACTCTATGAAGGTG-ATGCTTAAGAG 1322
GPH23_mandshurica_clone3 --TAGGAGGTAGTGTATCGTGACACTCTATGAAGGTG-ATGCTTAAGAG 1281
GPH23_ananassa_clone4 TCTAGGAGGTAGTGTATCGTGACACTTTATTGGGGTGGATGCTAAGGGG 1324
GPH23_ananassa_clone3 AATAGGAGGTAGTGTATCGTGACACTCTA--GAGGTG-ATGCTTAAGAG 1267
* *

GPH23_iinumae_clone2 GGTTCG---ATCAATGACAA-ACATGAGGGCAAATA-GAAGGTCTACTGG 1368
GPH23_iinumae_clone5 GGTTCG---ATCAATGACAA-ACATGAGGGCAAATA-GAATGTCTACTGG 1367
GPH23_mandshurica_clone3 GGACAC---GTCAATAGCAA-GTATGAGGGGAAAAAAGGATGTTTACTGG 1327
GPH23_ananassa_clone4 GTTCAG---GTTATGAGCAA-GCATGGGGGTAAAGGGGATATCTACTGG 1370
GPH23_ananassa_clone3 GGTCAAGGTCAATGGCAAAGCATGAGGGTTAAAGAGGATGTTTACTGA 1317
* *

GPH23_iinumae_clone2 CATGTCGAATGACAATGTCGTAATTAGTTAAGTGAAACTTATATTTCAAG 1418
GPH23_iinumae_clone5 CATGTCGAATGACAATGTCGTAATTAGTTAAGTGAAACTTATATTTCAAG 1417
GPH23_mandshurica_clone3 CATGTCGAATGATAATTTCTGTAATTAGTTAAGTGAAACTTATATTTCAAG 1377
GPH23_ananassa_clone4 CATTTTGAATGACAATGTTGTAATGA-----AACTTATATTTCAAG 1413
GPH23_ananassa_clone3 CATGTTGAAGACAATGTCGTAATTAGTT-----AAAGT-----GAA 1354
*** *

GPH23_iinumae_clone2 GTACTTTGAC-TTAGTATTTAGAAAACTGTAACATCGAAAGGAGTTCAA 1467
GPH23_iinumae_clone5 GTACTTTGAC-TTAGTATTTAGAAAACTGTAACATCGAAAGGAGTTCAA 1466
GPH23_mandshurica_clone3 GTACTTTGAC-CTAGTATTTGGAAAAACCGTAACATCTAAA----- 1417
GPH23_ananassa_clone4 GTATTTTGAT-TTAATATTTAGAAAACTGTAACATCAAAGGGGTTCAA 1462
GPH23_ananassa_clone3 GTACTGTGAAGTTAGTATTTGAAAACTGTAACATCGGAAGGGGTTCAA 1404
*** *

GPH23_iinumae_clone2 TACATTTGACGACATATTTTATGAGGTTTCTATAAATTAGTTATGAGAG 1517
GPH23_iinumae_clone5 TACATTTGACGACATATTTTATGAGGTTTCTATGAATTAGTTATGAGAG 1516
GPH23_mandshurica_clone3 TACACTTGACGACATATTTTATGAGATTTCTATGAATTAGTTATGAGAG 1467
GPH23_ananassa_clone4 TACATTTGCCGACATATTTTATGAGGTTTTTATGAATTAGTTATGAGAG 1512
GPH23_ananassa_clone3 TACATTTGACGACATATTTTATGAAGTTATTAGGAATTAGTTACGAGAG 1454
**** *

GPH23_iinumae_clone2 ATGGTTTTTC-CTTGGACTATTTTGATTTTG-ATGTTTCCTTAACACACAT 1565
GPH23_iinumae_clone5 ATGGTTTTTC-CTTGGACTATTTTGATTTTG-ATGTTTCCTTAACACACAT 1564
GPH23_mandshurica_clone3 ATGGTTTTTC-TTTAGACTATTTTGATTTTGATGTTTCCTTAACACACAT 1516
GPH23_ananassa_clone4 ATGGTTTTTC-CTTGGACTATTTAGATTTTG-ATGTTTCCTTAACACACAT 1560
GPH23_ananassa_clone3 ATGGTTTTTTCCTTAGAATATTTTGATTTTG-ATGTTTCCTTGACACACAT 1503
***** *

GPH23_iinumae_clone2 TATATTTA-----AAGTAATTTTCCGTATCACTTA 1595
GPH23_iinumae_clone5 TATATTTA-----AAGTAATTTTCTGTATCACTTA 1594
GPH23_mandshurica_clone3 TATATTTCTCCATTTTCTTTTATGTATAAGTAATTTTCTGTATCACTTA 1566
GPH23_ananassa_clone4 TATATTTCTCCATTTCTT-----GTAAGTAATTTTCTGTATCACTTA 1603
GPH23_ananassa_clone3 TATATTTCTCCATGTTCTTCT-ATGTATAAGTAATTTTCTGTATCACTTA 1552

GPH23_iinumae_clone2 AAAACATTTCTTACTCTTTCCAGAAGCATCTCCAAACATCTCC----- 1638
GPH23_iinumae_clone5 AAAACATTTCTTACTCTTTTCCAAACCATCTCCAAACATCTCC----- 1637
GPH23_mandshurica_clone3 AAAACATTTCTTACTCTTTCCAGAAGCATCTCTA---TCCCC----- 1605
GPH23_ananassa_clone4 AAAACATTTCTTACTCTTCCAGAAACATCTCCAAACATCCCTA--AACC 1651
GPH23_ananassa_clone3 GAAACATTTCTTACTCTTTCCAGAAGCATCTCCAAACATCCCCCTAAACC 1602
***** * ** ***** * ** *

GPH23_iinumae_clone2 -----CTAA-ATGTCAATGTC-----AATAGATGAAAGATCAACCT 1673
GPH23_iinumae_clone5 -----CCAA-ATGTCCATGGCCA---TAATAGATGAAAGATCAACCT 1675
GPH23_mandshurica_clone3 -----CTAA-A-----CC 1612
GPH23_ananassa_clone4 GATAGCTCTAACATGTCAATGTC-----AATAGATGAAAGATCAACCT 1694
GPH23_ananassa_clone3 AATAGCCCTAACATGTCAATGTCACATGTCAATAGATGAAAGATCAACCT 1652
* * * *

GPH23_iinumae_clone2 AAATGGTACCATATGTCCATACATAAAAAAGACCCAAAAAGA----- 1714
GPH23_iinumae_clone5 AAATGGTACCATATGTCCATACATAAAAAAGACCCAAAAAGA----- 1716
GPH23_mandshurica_clone3 AAATACACTAACATGTCAATGTCCA-----CCAAAAAGA----- 1646
GPH23_ananassa_clone4 AAATGGTACCATATGTCCATACATAAAAAAGACCCAAAAAGA----- 1735
GPH23_ananassa_clone3 AAATGGTACCATATGTCCATACATAAAAAAGACCCAAAAATAAATAAG 1702
**** * ***** * ** ***** *

GPH23_iinumae_clone2 AA-TAAATAAGCACCTTCATTTTAAAGCGCCATAAAAAAGTAGAGAAGAAT 1763
GPH23_iinumae_clone5 AA-TAAATAAGCACCTTCATTTTAAAGCGCCATAAAAAAGTAGAGAAGAAT 1765
GPH23_mandshurica_clone3 AA-TAAATAAGCACCTTCATTTTAAAGCGCCATAAAAAATAGAGAAGAAT 1695
GPH23_ananassa_clone4 AAATAAATAAGCACCTTCATTTTAAAGCGCCATAAAAAAGTAGAGAAGAAT 1785
GPH23_ananassa_clone3 AAATAAATATGCACCTTCATTTTAAAGCGCCAGAAAAAGTAGAGAAGAAT 1752
** ***** *****

GPH23_iinumae_clone2 ATAAGGTTTGAAGTGATCAAGGGGATAAGCAGTTTAAAGGTCGACTTGTTT 1813
GPH23_iinumae_clone5 ATAAGGTTTGAAGTGATCAAGGGGATAAGCAGTTTAAAGGTCGACTTGTTT 1815
GPH23_mandshurica_clone3 ATAAGGTTTGAAGTGAACAAGGGGATAAGCAGTTTAAAGGTCGACTTGTTT 1745
GPH23_ananassa_clone4 ACAAGGTTTGAAGTGATCAAGGGGATAAGCAGTTTAAAGGTCGACTTGTTT 1835
GPH23_ananassa_clone3 ATAAGGTTTGAAGTGATCAAGGGGATAAGCAGTTTAAAGGTCGACTTGTTT 1802
* *****

GPH23_iinumae_clone2 GGAAACAATGCTAACCACCACCCTGCCAGTCTCAGCT---G**CTCCTCCT** 1860
GPH23_iinumae_clone5 GGAAACAATGCTAACCACCACCCTGCCAGTCTCAGCT---G**CTCCTCCT** 1862
GPH23_mandshurica_clone3 GGAAACAATGCTGACCACCACCCTGCCACTCTCAGTCTCAG**CTCCTCCT** 1795
GPH23_ananassa_clone4 GGAAACAATGCTAACCACCACCCTGCCACTCTCAGCT---G**CTCCTCCT** 1882
GPH23_ananassa_clone3 GGAAACAATGCTAACCACCACCCTGCCACTCAGTCTCAG**CTCCTCCT** 1852
***** *****

GPH23_iinumae_clone2 **CCT**CTGCTTCCCAACTCCCACCCTCTTCCACTCTCTATCACCAAACCCA 1910
GPH23_iinumae_clone5 **CCT**CTGCTTCCCAACTCCCACCCTCTTCCACTCTCTATCACCAAACCCA 1912
GPH23_mandshurica_clone3 **CCT**CTACTTCCCAACTCCCACCCTCTTCCACTCTCTAACCAAACCCA 1845
GPH23_ananassa_clone4 **CCT**CTGCTTCCCAACTCCCACCCTCTTCCACTCTCTATCACCAAACCCA 1932
GPH23_ananassa_clone3 **CCT**CTGCTTCCCAACTCCCATCGCTCTTCCACTCTCTATCACCAAACCCA 1902
***** ***** * *****

GPH23_iinumae_clone2 ATCTCCCTCAGATTCTCCTCCACATTACAGCTAACCAAAACCAGAACCAG 1960
GPH23_iinumae_clone5 ATCTCCCTCAGATTCTCCTCCACATTACAGCTAACCAAAACCAGAACCAG 1962
GPH23_mandshurica_clone3 ATCTCCCTCAGATTCTCCTCCACATTACAGCTAACTAAAACCAGAAC--- 1892
GPH23_ananassa_clone4 ATCTCCCTCAGATTCTCCTCCACATTACAGCTAACCAAAACCAGAAC--- 1979
GPH23_ananassa_clone3 ATCTCCCTCAGATTCTCCTCCACATTAAAGCTAACCAAAACCAGAAC--- 1949
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GPH23_iinumae_clone2      AACCAGACCAACCCTTAAAACTCTCACTCGCCAAAAATGCCAGCTCCCTG 2010
GPH23_iinumae_clone5      AACCAGACCAACCCTTAAAACTCTCACTCGCCAAAAATGCCAGCTCCCTG 2012
GPH23_mandshurica_clone3  ---CAGACCAACCCTTAAAACTCTCACTCGCCAAAAATGCCAGCTCCCTG 1939
GPH23_ananassa_clone4     ---CAGACCAACCCTTAAAACTCTCACTCGCCAAAAATGCCAGCTCCCTG 2026
GPH23_ananassa_clone3     ---CAGACCAACCCTTAAAACTCTCACTCGCCAAAAATGCCAGCTCCCTG 1996
                        *****

GPH23_iinumae_clone2      CTCTGAGAGTGTCTGCTAATTACGAAGCTGCCCTGCCACGGCTGAGGCC 2060
GPH23_iinumae_clone5      CTCTGAGAGTGTCTGCTAATTACGAAGCTGCCCTGCCACGGCTGAGGCC 2062
GPH23_mandshurica_clone3  CTCTGAGAGTGTCTGCTAATAACGAAGCTTCCCTGCCACAGCTGAGGCC 1989
GPH23_ananassa_clone4     CTCTGAGAGTGTCTGCTAATTACGAAGCTGCCCTGCCACGGCTGAGGCC 2076
GPH23_ananassa_clone3     CTCTGAGAGTGTCTGCTAATTACGAAGCTGCCCTGCCACGGCTGAGGCC 2046
                        *****

GPH23_iinumae_clone2      TCCACGGTGCCGTGGAGATGAAGGCGTGGGTGTA 2095
GPH23_iinumae_clone5      TCCACGGTGCCGTGGAGATGAAGGCGTGGGTGTA 2097
GPH23_mandshurica_clone3  TCCACGGTGCCGTGGAGATGAAGGCGTGGGTGTA 2024
GPH23_ananassa_clone4     TCCACGGTGCCGTGGAGATGAAGGCGTGGGTGTA 2111
GPH23_ananassa_clone3     TCCACAGTGCCGTGGAGATGAAGGCGTGGGTGTA 2081
                        *****

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Gene Pairs Detected Through Prediction from Genomic Sequence

GPH10

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GPH10_ananassa_clone2      GGCTTCTTCTGTCCGGCAGCCTCTTCAGCCACTCGTCCTCCGGCGCCGC 50
GPH10_ananassa_clone20     GGCTTCTTCTGTCCGGCAGCCTCTTCAGCCACTCGTCCTCCGGCGCCGC 50
GPH10_ananassa_clone18     GGCTTCTTCTGTCCGGCAGCCTCTTCAGCCACTCGTCCTCCGGCGCCGC 50
GPH10_ananassa_clone19     GGCTTCTTCTGTCCGGCAGCCTCTTCAGCCACTCGTCCTCCGGCGCCGC 50
                        *****

GPH10_ananassa_clone2      CGATACCTCCTCCGCTCCGACGACTTCGAACACAGCGGAATCGCTAGCC 100
GPH10_ananassa_clone20     CGATACCTCCTCCGCTCCGACGACTTCGAACACAGCGGAATCGCTAGCC 100
GPH10_ananassa_clone18     CGATACCTCCTCCGCTCCGACGACTTCGAACACAGCGGAATCGCTAGCC 100
GPH10_ananassa_clone19     CGATACCTCCTCCGCTCCGACGACTTCGAACACAGCGGAATCGCTAGCC 100
                        *****

GPH10_ananassa_clone2      TCCTTATCGGAGACCGAACGAGCCGAAACGGCGTCGCTTTAGGCGAGAGT 150
GPH10_ananassa_clone20     TCCTTATCGGAGACCGAACGAGCCGAAACGGCGTCGCTTTAGGCGAGAGT 150
GPH10_ananassa_clone18     TCCTTATCGGAGACCGAACGAGCCGAAACGGCGTCGCTTTAGGCGAGAGT 150
GPH10_ananassa_clone19     TCCTTATCGGAGACCGAACGAGCCGAAACGGCGTCGCTTTAGGCGAGAGT 150
                        *****

GPH10_ananassa_clone2      GAATAGCGAACTGAGTAGTTTGATTTGAGAAGAGGATGTAATTGGTAAC 200
GPH10_ananassa_clone20     GAATAGCGAACTGAGTAGTTTGATTTGAGAAGAGGATGTAATTGGTAAC 200
GPH10_ananassa_clone18     GAATAGCGAACTGAGTAGTTTGATTTGAGAAGAGGATGAAATTGGTAAC 200
GPH10_ananassa_clone19     GAATAGCGAACTGAGTAGTTTGATTTGAGAAGAGGATGAAATTGGTAAC 200
                        *****

GPH10_ananassa_clone2      GGAGAAGAAGACTGTCGACATTTTTGGAGAAAGCTTTCATCTTTGAAGTG 250
GPH10_ananassa_clone20     GGAGAAGAAGACTGTCGACATTTTTGGAGAAAGCTTTCGCTTTGAAGTG 250
GPH10_ananassa_clone18     GGAGAAGAAGACTGTCGACATTTTTAGAGAAAGCTTTCAGCTTTGAAGTG 250
GPH10_ananassa_clone19     GGAGAAGAAGACTGTCGACATTTTTAGAGAAAGCTTTCAGCTTTGAAGTG 250
                        *****

GPH10_ananassa_clone2      GAGTGTAGGATAATAACAACTCGTTATC----- 279
GPH10_ananassa_clone20     GAGTGTAGGATAATAACAACTCGTGATTAAGACAGGATTAATGTGAC 300
GPH10_ananassa_clone18     GAGTGTAGGATAATAACAACTCGTTATC----- 279
GPH10_ananassa_clone19     GAGTGTAGGATAATAACAACTCGTTATC----- 279
                        ***** **

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GPH10_ananassa_clone2 -----
GPH10_ananassa_clone20 TGAGGTTTGGTTGGTTAAGGTGTTAACTGATAAAATTTAAGGTCATAGGTT 350
GPH10_ananassa_clone18 -----
GPH10_ananassa_clone19 -----

GPH10_ananassa_clone2 -----TAAAAGGCAGGT 291
GPH10_ananassa_clone20 CAAACCTCACGACATATGTAGGGTGTATGAATTATTAATAAAAAGACAAAT 400
GPH10_ananassa_clone18 -----TAAAAGGCAGGT 291
GPH10_ananassa_clone19 -----TAAAAGGCAGGT 291
***** ** *

GPH10_ananassa_clone2 TTAATATCAGCCGTTAGATCATATTACGGCCCTGATCACTCGACATATGT 341
GPH10_ananassa_clone20 TTAATATCAGCCGTTAGATCATATTACGGCC-TGATCACTCGACATATGT 449
GPH10_ananassa_clone18 TTAATATCAGCCGTTAGATCACATTACGGCCCTGATCACTCGACATATGT 341
GPH10_ananassa_clone19 TTAATATCAGCCGTTAGATCATATTACGGCCCTGATCACTCGACATATGT 341
*****

GPH10_ananassa_clone2 TGATATACGCCAACTCAAATTCGATATATATTTTCGATATACAT----- 386
GPH10_ananassa_clone20 TGATATACGCCAACTCAAATTCGATATATATTTTCGATATACGT----- 494
GPH10_ananassa_clone18 TGATATACGCCAACTCAAATTCGATATATATTTTCGATATACATTTT 391
GPH10_ananassa_clone19 TGATATACGCCAACTCAAATTCGATATATATTTTCGATATACATTTT 391
*****

GPH10_ananassa_clone2 -----ATATTTTAT 396
GPH10_ananassa_clone20 -----ATATTTTAT 504
GPH10_ananassa_clone18 TTTTAAGTAACTAAATGACTATTCGATATATATTTTCGATATACATTTT 441
GPH10_ananassa_clone19 TTTTAAGTAACTAAATGACTATTCGATATATATTTTCGATATACATTTT 441
*** **

GPH10_ananassa_clone2 TTTTAAAGTAACTAAATGACTATGTACATCGTTTAAACAAAAGAAACAAT 446
GPH10_ananassa_clone20 TTTTAAATAAATTAATAACTATTTACGTTGTTTAAACAAAAGAAACAAT 554
GPH10_ananassa_clone18 TTTTAAAGTAACTAAATGACTATTTACGTCGGTTAATAAAAAGAAACAAT 491
GPH10_ananassa_clone19 TTTTAAAGTAACTAAATGACTATTTACGTCGGTTAATAAAAAGAAACAAT 491
*****

GPH10_ananassa_clone2 TGAAGTTAAATTAAGAGCACCATAACAGCTGAGAAAGAGTACGAGAACAA 496
GPH10_ananassa_clone20 TGAAGTTAAATTAAGAGCACCATAACAGCTGAGCAAGAGTACGAGAACAA 604
GPH10_ananassa_clone18 TGAAGTTAAATTAAGAGCACCATGACAG-----AGTACGAGAACAA 532
GPH10_ananassa_clone19 TGAAGTTAAATTAAGAGCACCATGACAG-----AGTACGAGAACAA 532
*****

GPH10_ananassa_clone2 AAGTATGAGCTAAAACAA-----ATAGAGAAAATA 526
GPH10_ananassa_clone20 AAGTATGAGCTACATCATTTG-----TTCATATAGAGAAAATA 642
GPH10_ananassa_clone18 AAGTATGAGCTACATTGTTTGTCTCGTCGGTTTGTTCATATGGAGAAAATG 582
GPH10_ananassa_clone19 AAGTATGAGCTACATTGTTTGTCTCGTCGGTTTGTTCATATGGAGAAAATA 582
*****

GPH10_ananassa_clone2 TAGAGGCGATGTTGTAGAAATAATTGAACATTAGAAAATTAATTACCTA 576
GPH10_ananassa_clone20 TAGAGGCGATGTTGTAGAAATAATTGAACATTAGAAAATTAATTACCTA 692
GPH10_ananassa_clone18 TAGAGGCGATGTTGTAGAAATAATTGAACATTAGAAAATTAATTACCTA 632
GPH10_ananassa_clone19 TAGAGGCGATGTTGTAGAAATAATAGAACATTAGAAAATTAATTACCTA 632
*****

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GPH10_ananassa_clone20 AAAGCCGATGAGTAAAATAATAACGAACCTCGTAACCTAAAAGCGGCTTCA 742
GPH10_ananassa_clone18 AAAGCCGATGAGTAAAATAATAACAAACTCGTAACCTAAAAGCGGCTTCA 682
GPH10_ananassa_clone19 AAAGCCGATGAGTAAAATAATAACAAACTCGTAACCTAAAAGCGGCTTCA 682
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GPH10_ananassa_clone2 TATCATCCGTTGATCATATATGCGGGTGTGATTGAAAACCAAAGTTAA 676
GPH10_ananassa_clone20 TATCATCCGTTGATCATATATGCGGGTGTGATTGAAAACCAAAGTTAA 792
GPH10_ananassa_clone18 TATCATCCACTGGATCATATATGCGGGTGTGATTGAAAACCAAAGTTAA 732
GPH10_ananassa_clone19 TATCATCCACTGGATCATATATGCGGGTGTGATTGAAAACCAAAGTTAA 732
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 GPH10_ananassa_clone19 CCCGCCAAAGCCTAATTCCAATTTTCATTTCCACCAAAAACAAAACCC 782

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 GPH10_ananassa_clone18 ACACGACGCCGTTTTGCTCCAATCCCCCCCCCTTCTTCTTCAACCCCA 832
 GPH10_ananassa_clone19 ACACGACGCCGTTTTGCTCCAATCCCCCCCC--TTTCTTCTTCAACCCCA 831

GPH10_ananassa_clone2 TAGTCGCCTC--AGCTCAGTTCATTTGTCTCAGATGCGATGGCCTCCG 818
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 GPH10_ananassa_clone18 TAGTCGCCTCCTCAGCTCAGTTCATTTGTCTCA--TGCGATGGCTTCCG 880
 GPH10_ananassa_clone19 TAGTCGCCTCCTCAGCTCAGTTCATTTGTCTCA--TGCGATGGCTTCCG 879

GPH10_ananassa_clone2 -----GCGACCCAATCTCCGACTACACCCAAACACATCGCATT 856
 GPH10_ananassa_clone20 -----GCGACCCAATCTCCGACTACACCCAAACACATCGCATT 972
 GPH10_ananassa_clone18 ACTCGAATTCGCGGACCCAATCTCCTCTACACCCAAACCCATCGCATC 930
 GPH10_ananassa_clone19 ACTCAAATTCGCGGACCCAATCTCCTCTACACCCAAACCCATCGCATC 929

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 GPH10_ananassa_clone19 GTCCTTCTAATCGACCTCAACCCACTCCTCAATCTCCAAGATCCAACCCA 979

GPH10_ananassa_clone2 ATTCTCACCTCTGTCTCTCCTCAATCAAACCCCTAACCTCCTTCCCTT 956
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 GPH10_ananassa_clone18 ATTCTCACCCCTGTCTCTCCTCAATCAAACCCCTAACCTCCTTCCCTT 1030
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GPH10_ananassa_clone19 CTGTGGTTTGAGGTCTAATTTGGCTGTTGTGTTTTTACCAGCGTGTCAAT 1374
* * * * *

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GPH10_ananassa_clone20 TTGTGAATGAGTTCCTGAATTGTGAGTTGAATTGTGAGGGTTTGGAGGAT 1472
GPH10_ananassa_clone18 TTGTGAATGAGTTCCTGAATTGTGAG-----GGTTTGGAGGAT 1413
GPH10_ananassa_clone19 TTGTGAATGAGTTCCTGAATTGTGAG-----GGTTTGGAGGAT 1412
* * * * *

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GPH10_ananassa_clone19 TTCAATGTGTTTTGTGAGAGGTTTCGAGGGTTTTTCGAGAATGTGGATGA 1462
* * * * *

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GPH10_ananassa_clone18 GGCATTTGTGTGTAGAGATATTC AATTGAGTTGGGTTGATGTGAGGTATG 1513
GPH10_ananassa_clone19 GGCATTTGTGTGTAGAGATATTC AATTGAGTTGGGTTGATGTGAGGTATG 1512
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GPH10_ananassa_clone2 GATTCGATAGCGGTGAGGATGAGGTAGTTGGATTGAAATGTGGTGTTTTC 1479
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GPH10_ananassa_clone18 GATTCGATAGCGGTGAGGATGAGGTAGTTGGATTGAAATGTGGTGTTTTC 1563
GPH10_ananassa_clone19 GATTCGATAGCGGTGAGGATGAGGTAGTTGGATTGAAATGTGGTGTTTTC 1562
* * * * *

GPH10_ananassa_clone2 GAGAGGGGGGTTAGGAGTTTAGGGTGGGGGTTTTGCTCATCTGATTCCGAT 1529
GPH10_ananassa_clone20 GAGAGGGGGGTTAGGAGTTTAGGGTGGGGGTTTTGCTCATCTGATTCCGAT 1672
GPH10_ananassa_clone18 GAGAGGGGGGTTAGGAGTTTAGGGTGGGGGTTTTGCTCATCTGATTCCGAT 1613
GPH10_ananassa_clone19 GAGAGGGGGGTTAGGAGTTTAGGGTGGGGGTTTTGCTCATCTGATTCCGAT 1612
* * * * *

GPH10_ananassa_clone2 TGTGCTTGGTTTCGGCTCTTGTTC AATTTGGTTTGATTTATCCAGAGATTG 1579
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GPH10_ananassa_clone19 TGTGCTTGGTTTCGGCTCTTGTTC AATTTGGTTTGATTTATCCAGAGATTG 1662
* * * * *

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GPH10_ananassa_clone18 GGGTGTCACTAGGATTTTCGGGTGTAATGATCGATATAAGAAGGTTAGA 1713
GPH10_ananassa_clone19 GGGTGTCACTAGGATTTTCGGGTGTAATGATCGATATAAGAAGGTTAGA 1712
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GPH10_ananassa_clone18 CAAGTTTTGTGATCTTGAGTTGGCTGATTGAAAATGTTGTGTAGGAGTA 1813
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* * * * *

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GPH10_ananassa_clone18 TATTTCAAATGATAGTGGTGTATCACATACTGGAATCCTTAAGCCTTTTA 2213
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GPH10_ananassa_clone18 GATGAAGAATGAGATGTGCAAACCTGATGCTGATTTGAACGACTTTTGTG 2363
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GPH10_ananassa_clone20 GGTCGCAAACCTGGGCCTTCACCATCTAATAAGCATTCTGCTGAGATTGAT 2472
GPH10_ananassa_clone18 GGTCGCAAACCTGGGCCTTCACCATCTAATAAGCATTCTGCTGAGATTGAT 2413
GPH10_ananassa_clone19 GGTCGCAAACCTGGGCCTTCACCATCTAATAAGCATTCTGCTGAGATTGAT 2411

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* *****

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GPH10_ananassa_clone18 AAGAGGCTTACTTTTGCCAGGCAACGTAGCAGCTCAAAAAGTTGAAATTT 2563
GPH10_ananassa_clone19 AAGAGTTTACTTTTGCCAGGCAACGTAGCAGCTCAAAAAGTTGAAATTT 2561

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GPH10_ananassa_clone18 CTAAAATGCTGGATGAAACAGATTAAAAAAGTGAAGTATCCAATAACGGA 2613
GPH10_ananassa_clone19 CTAAAATGCTGGATGAAACAGATTAAAAAAGTGAAGTATCCAATAACGGA 2611

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GPH10_ananassa_clone18 GGAGTCTAAGGTGCACCAGGAAAAACAAAAGGAGATGAGCAATAGGTTGG 2663
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 GPH10_ananassa_clone19 GAGGGAGGCTTCTTTGGTGACTGGACCCTAGAAAATTTATGCTGGAAAGAT 3409

GPH10_ananassa_clone2 TATAAAAAGCAGGTAGATGAGTCACATGTATAAATCTAATTACCCATAAC 3377
 GPH10_ananassa_clone20 TATAAAAAGCAGGTAGATGAGTCACATGTATAAATCTAATTACCCATAAC 3521
 GPH10_ananassa_clone18 TATAAAAAGCAGGTAGATGAGTCACATGTATAAATCTAATTACCCATAAC 3459
 GPH10_ananassa_clone19 TATAAAAAGCAGGTAGATGAGTCACATGTATAAATCTAATTACCCATAAC 3459

GPH10_ananassa_clone2 TATTATTTTCTAATGAAATT-GTATTCATGAACACTGAAATGGTAGATAC 3426
 GPH10_ananassa_clone20 TATTATTTTCTAATGAAATTTGTATTCATGAACACTGAAATGGTAGATAC 3571
 GPH10_ananassa_clone18 TATTATTTTCTAATGAAATTTGTATTCATGAACACTGAAATGGTAGATAC 3509
 GPH10_ananassa_clone19 TATTATTTTCTAATGAAATTTGTATTCATGAACACTGAAATGGTAGATAC 3509

GPH10_ananassa_clone2 TCAGTTATTTACAATGAAACTCCAATATATGTTTATGGTTTGCCTGTTAA 3476
 GPH10_ananassa_clone20 TCAGTTATTTACAATGAAACTCCAATATATGTTTATGGTTTGCCTGTTAA 3621
 GPH10_ananassa_clone18 TCAGTTATTTACAATGAAACTCCAGTATATGTTTATGTTTTGCCTGTTAA 3559
 GPH10_ananassa_clone19 TCAGTTATTTACAATGAAACTCCAATATATGTTTATGTTTTGCCTGTTAA 3559

GPH10_ananassa_clone2 TGATACTTTTATCAGTACTTCGATGAAACATATAGTGTGAAACAATTAT 3526
 GPH10_ananassa_clone20 TGATACTTTTATCAGTACTTCGATGAAACATATAGTGTGAAACAATTAT 3671
 GPH10_ananassa_clone18 TGATACTTTTATCAGTACTTCGATGAAACATATAGTGTGAAACAATTAT 3609
 GPH10_ananassa_clone19 TGATACTTTTATCAGTACTTCGATGAAACATATAGTGTGAAACAATTAT 3609

GPH10_ananassa_clone2 GTGATTGATTTGTATGCCCTCCCAAAGGCCTTTGGGGGTAGTATGAAGA 3576
 GPH10_ananassa_clone20 GTGATTGATTTGTATGCCCTCCCAAAGGCCTTTGGGGGTAGTATGAAGA 3721
 GPH10_ananassa_clone18 GTGATTGATTTGTATGCCCTCCCAAATGGCCTTTGGGGGTAGTAAAGAAGA 3659
 GPH10_ananassa_clone19 GTGATTGATTTGTATGCCCTCCCAAATGGCCTTTGGGGGTAGTAAAGAAGA 3659

GPH10_ananassa_clone2 AGGGAGACATTGACAGTCAAAAATATTATCTCCTTATTTTACGTACAAAA 3626
 GPH10_ananassa_clone20 AGGGAGACATTGACCGTCAAAACTATTATCTCCTTATTTTACGTACAAAA 3771
 GPH10_ananassa_clone18 AGGGAGACATTGACAGTCAAAAATATTATCTCCTTATTTTACGTACAAAA 3709
 GPH10_ananassa_clone19 AGGGAGACATTGACAGTCAAAAATATTATCTCCTTATTTTACGTACAAAA 3709

GPH10_ananassa_clone2 TTGATGACTCCTCATCAGGCTGTTGAAGGCAGGGTTGACAGAGAACAGAA 3676
 GPH10_ananassa_clone20 TTGATGACTCCTCATCAGGCTGTTGAAGGCAGGGTTGACAGAGAACAGAA 3821
 GPH10_ananassa_clone18 TTGATGACTCCTCATCAGGCTGTTGAAGGCAGGGTTGACAGAGAACAGAA 3759
 GPH10_ananassa_clone19 TTGATGACTCCTCATCAGGCTGTTGAAGGCAGGGTTGACAGAGAACAGAA 3759

GPH10_ananassa_clone2 AAGCTAAATACCTCCTTTGCATAATTTTCATATGACTTAAGTGACTTTCCT 3726
 GPH10_ananassa_clone20 AAGCTAAATACCTCCTTTGCATAATTTTCATATGACTTAAGTGACTTTCCT 3871
 GPH10_ananassa_clone18 AAGCTAAATACCTCCTTTGCATAATTTTCATATGACTTAAGTGACTTTCCT 3809
 GPH10_ananassa_clone19 AAGCTAAATACCTCCTTTGCATAATTTTCATATGACTTAAGTGACTTTCCT 3809

GPH10_ananassa_clone2 TATTAATCTAGATTTGCAACCTTGTTTTTCTGACACTATGTATGCATACA 3776
 GPH10_ananassa_clone20 TATTAATCTAGATTTGCAACCTTGTTTTTCTGACACTATGTATGCATACA 3921
 GPH10_ananassa_clone18 TATTAATCTAGATTTGCAACCTTGTTTTTCTGACACTATGTATTCATACA 3859
 GPH10_ananassa_clone19 TATTAATCTAGATTTGCAACCTTGTTTTTCTGACACTATGTATTCATACA 3859

GPH10_ananassa_clone2 ACTTTTGCAATTGTATTCTGTATGTTGCAATAGTTCATTCCCTTTGTTTTC 3826
 GPH10_ananassa_clone20 ACTTTTGCAATTGTATTCTGTATGTTGCAATAGTTCATTCCCTTTGTTTTC 3971
 GPH10_ananassa_clone18 ACTTTTGCAATTGTATTCTGTATGTTGCAATAGTTCATTCCCTTTGTTTTC 3909
 GPH10_ananassa_clone19 ACTTTTGCAATTGTATTCTGTATGTTGCAATAGTTCATTCCCTTTGTTTTC 3909

GPH10_ananassa_clone2 CAGACCCAAAAAAACTGCCCAAATTTATGTGAAAACACTGCATTTATGT 3876
 GPH10_ananassa_clone20 CAGACCCAAAAAAACTGCCCAAATTTATGTGAAACACTGCATTTATGT 4021
 GPH10_ananassa_clone18 CAGACCCAAAAAAACGGCCCAAATTTATGTGAAAACACTGCATTTATGT 3959
 GPH10_ananassa_clone19 CAGACCCAAAAAAACGGCCCAAATTTATGTGAAAACACTGCATTTATGT 3959

GPH10_ananassa_clone2 TTGAAGAAGTAGGATTAGGCAGGTAGACTGATGATTCAATTCCTAAATTT 3926
 GPH10_ananassa_clone20 TTGAAG---TAGGATTAGGCAGGTAGACTGATGATTCAATTCCTAAATTT 4068
 GPH10_ananassa_clone18 TTGAAGAAGTAGGATTAGGCAGGTAGACTGATGATTCAATTCCTAAATTT 4009
 GPH10_ananassa_clone19 TTGAAGAAGTAGGATTAGGCAGGTAGACTGATGATTCAATTCCTAAATTT 4009

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GPH10_ananassa_clone2      TCAGGTACTGTCAGACTCTTGAAGACGTGGTTCATAAAAATCTACACAAA 3976
GPH10_ananassa_clone20     TCAGGTACTGTCAGACTCTTGAAGACGTGGTTCATAAAAATCTACACAAA 4118
GPH10_ananassa_clone18     TCAGGTACTGTCAGACTCTTGAAGACGTGGTTCATAAAAATCTACACAAA 4059
GPH10_ananassa_clone19     TCAGGTACTGTCAGACTCTTGAAGACGTGGTTCATAAAAATCTACACAAA 4059
*****

GPH10_ananassa_clone2      ATGGATTTGTTACTGTTTGATGATGAGGAAGAACTCCCTAATAATTTATF 4026
GPH10_ananassa_clone20     ATGGATTTGTTACTGTTTGATGATGAGGAAGAACTCCCTAATAATTTATC 4168
GPH10_ananassa_clone18     ATGGATTTGTTACTGTTTGATGATGAGGAAGAACTCCCTAATAATGTATF 4109
GPH10_ananassa_clone19     ATGGATTTGTTACTGTTTGATGATGAGGAAGAACTCCCTAATAATGTATF 4109
*****

GPH10_ananassa_clone2      CAACAGCGAGGATAGCAGTCATTCATACAAAGAAAAACCAGGGAAAGATG 4076
GPH10_ananassa_clone20     CAACAGCGAGGATAGCAGTCATTCATACAAAGAAAAACCAGGGAAAGATG 4218
GPH10_ananassa_clone18     CAACAGCGAGGATAGCAGTCATTCATACAAAGAAAAACCAGGGAAAGATG 4159
GPH10_ananassa_clone19     CAACAGCGAGGATAGCAGTCATTCATACAAAGAAAAACCAGGGAAAGATG 4159
*****

GPH10_ananassa_clone2      AGGTGGGTGAAAATAGTAGAATGAAGAAATTGGTATCAGCAGAAGATGAA 4126
GPH10_ananassa_clone20     AGGTGGGTGAAAATAGTAGAATGAAGAAATTGGTATCAGCAGAAGATGAA 4268
GPH10_ananassa_clone18     AGGTGGGTGAAAATAGTAGAATGAAGAAATTGGTATCAGCAGAAGATGAA 4209
GPH10_ananassa_clone19     AGGTGGGTGAAAATAGTAGAATGAAGAAATTGGTATCAGCAGAAGATGAA 4209
*****

GPH10_ananassa_clone2      TCCCCTGATCCACAGAAACATTACAATGGAAGGCCAAGTGCTCAAGTACT 4176
GPH10_ananassa_clone20     TCCCCTGATCCACAGAAACATTACAATGGAAGGCCAAGTGCTCAAGTAGT 4318
GPH10_ananassa_clone18     TCCCCTGATCCACAGAAACATTACAATGGAAGGCCAAGTGCTCAAGTAGT 4259
GPH10_ananassa_clone19     TCCCCTGATCCACAGAAACATTACAATGGAAGGCCAAGTGCTCAAGTAGT 4259
*****

GPH10_ananassa_clone2      TAAACAAGAAGAGCATGCTCGCAAGTTGATGAAAGCTCAAGAGAGTAGAG 4226
GPH10_ananassa_clone20     TAAACAAGAAGAGCATGCTCGCAAGTTGATGAAAGCTCAAGAGAGTAGAG 4368
GPH10_ananassa_clone18     TAAACAAGAAGAGCATGCTCGCAAGTTGATGAAAGCTCAAGAGAGTAGAG 4309
GPH10_ananassa_clone19     TAAACAAGAAGAGCATGCTCGCAAGTTGATGAAAGCTCAAGAGAGTAGAG 4309
*****

GPH10_ananassa_clone2      AGAGGGCTTGAGAAATTGCTTCTTTTACCAAGTCGGGTAGCTGATTTGCAG 4276
GPH10_ananassa_clone20     AGAGGGCTTAGGAGAATTGCTTCTTTTACCAAGTCGGGTAGCTGATTTGCAG 4418
GPH10_ananassa_clone18     AGAGAGCTAGGAGAATTGCTTCTTTTACCAAGTCGGGTAGCTGATTTGCAG 4359
GPH10_ananassa_clone19     AGAGAGCTTAGGAGAATTGCTTCTTTTACCAAGTCGGGTAGCTGATTTGCAG 4359
**** *

GPH10_ananassa_clone2      CGAG 4280
GPH10_ananassa_clone20     CGAG 4422
GPH10_ananassa_clone18     CGAG 4363
GPH10_ananassa_clone19     CGAG 4363
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Polymorphic fragments of GPH10: clones from octoploid *versus* clones from diploids. SSRs detected are magenta-colored.

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10PPR1AB22_nubicola      AACGGAGAAGAAGACTGTCGACATTTTTAGAGAAAGCTTTCAGCTTTGAA 50
10PPR1AB22_mandshurica   AACGGAGAAGAAGACTGTCGACATTTTTAGAGAAAGCTTTCAGCTTTGAA 50
10PPR1AB22-vesca         AACGGAGAAGAAGACTGTCGACATTTAAGAGAAAGCTTTCAGCTTTGAA 50
10PPR1AB22_viridis       AACGGAGAAGAAGACTGTCGACATTTCTAGAGAAAGCTTTCAGCTTTGAA 50
10PPR1AB22_nilgerrensis  AACGGAGAAGAAGACTGTCGACATATCTAGAGAAAGCTTTCAGCTTTGAA 50
10PPR1AB22_ananassa_clone18 AACGGAGAAGAAGACTGTCGACATTTTTAGAGAAAGCTTTCAGCTTTGAA 50
10PPR1AB22_ananassa_clone20 AACGGAGAAGAAGACTGTCGACATTTTTGGAGAAAGCTTTCGGCTTTGAA 50
10PPR1AB22_ananassa_clone19 AACGGAGAAGAAGACTGTCGACATTTTTAGAGAAAGCTTTTAGCTTTGAA 50
10PPR1AB22_ananassa_clone2 AACGGAGAAGAAGACTGTCGACATTTTTGGAGAAAGCTTTCATCTTTGAA 50
10PPR1AB22_iinumae      AACGGAGAAGAAGACTGTCGACATTTTTAGAGAAAGCTTTCAGCTTTGAA 50
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10PPR1AB22_nubicola      GT-----G-----TAGGATAATAACAAAGAAACTCGTTATCTGAAAGACA 90
10PPR1AB22_mandshurica  GT-----G-----TAGGATAATAACAAAGAAACTCGTTATCTGAAAGACA 90
10PPR1AB22-vesca        GT-----G-----TAGGATAATAACAAAGAAACTCGTTATCTGAAAGACA 90
10PPR1AB22_viridis      GT-----G-----TAGGATAATAACAAAGAAACTCGTTATCTGAAAGACA 90
10PPR1AB22_nilgerrensis GT-----GGAGTGTAGGATAATAAC----AAACTCGTTATCTGAAAGACA 91
10PPR1AB22_ananassa_clone18 GT-----GGAGTGTAGGATAATAAC----AAACTCGTTATCTAAAAGACA 91
10PPR1AB22_ananassa_clone20 GT-----GGAGTGTAGGATAATAAC----AAACTCGTGAT-TAAAAGACA 90
10PPR1AB22_ananassa_clone19 GT-----GGAGTGTAGGATAATAAC----AAACTCGTTATCTAAAAGACA 91
10PPR1AB22_ananassa_clone2 GT-----GGAGTGTAGGATAATAAC----AAACTCGTTATCTAAAAGGCA 91
10PPR1AB22_iinumae      CTTTGAAGTAGTGTAGGATAATAAC----AAACTCGTTATCTAAAAGACA 96
                          *      *      *****      *****  ** * **** **

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10PPR1AB22_nubicola      GGTTTAATATCAGC-----CGTTGGATCATA---TT 118
10PPR1AB22_mandshurica  GGTTTAATATCAGC-----CGTTGGATCATA---TT 118
10PPR1AB22-vesca        AGTTTAATATCAGC-----CGTTGGATCATA---TT 118
10PPR1AB22_viridis      AGTTTAATACCAGC-----CGTTGGATCATA---TT 118
10PPR1AB22_nilgerrensis GGTTTAATATCAGC-----CGTTGGATTATA---TT 119
10PPR1AB22_ananassa_clone18 GGTTTAATATCGGC-----CGTTAGATCACA---TT 119
10PPR1AB22_ananassa_clone20 GGATTAATGTCAGTGAGGTTTGGTTGGTTAAGGTGTTAACTGATAAATTT 140
10PPR1AB22_ananassa_clone19 GGTTTAATATCAGC-----CGTTAGATCATA---TT 119
10PPR1AB22_ananassa_clone2 GGTTTAATATCAGC-----CGTTAGATCATA---TT 119
10PPR1AB22_iinumae      GGTTTAATATCAGC-----CGTTAGATCCTA---TT 124
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10PPR1AB22_nubicola      ACGGCCCTG-----ATCGCTCGACATA----- 140
10PPR1AB22_mandshurica  ACGGCCCTG-----ATCGCTCGACATA----- 140
10PPR1AB22-vesca        ACGGCCCTG-----ATCGCTCGACATA----- 140
10PPR1AB22_viridis      ACTGCCCTG-----ATCGCTCGACATA----- 140
10PPR1AB22_nilgerrensis CCGGCCCTG-----ATCTCTCGACATA----- 141
10PPR1AB22_ananassa_clone18 ACGGCCCTG-----ATCACTCGACATATGTTGA-TATACGCCTAACT- 160
10PPR1AB22_ananassa_clone20 AAGGTCATAGGTTCAAACCTCACGACATATGTAGGGTGTATGAATTATTA 190
10PPR1AB22_ananassa_clone19 ACGGCCCTG-----ATCACTCGACATATGTTGA-TATACGCCTAACT- 160
10PPR1AB22_ananassa_clone2 ACGGCCCTG-----ATCACT----- 134
10PPR1AB22_iinumae      -----

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10PPR1AB22_nubicola      -----A 141
10PPR1AB22_mandshurica  -----A 141
10PPR1AB22-vesca        -----A 141
10PPR1AB22_viridis      -----A 141
10PPR1AB22_nilgerrensis -----T 142
10PPR1AB22_ananassa_clone18 -----CAAATTCGATAT-----ATATT----- 177
10PPR1AB22_ananassa_clone20 ATAAAAGACAAATTTAATATCAGCCGTTAGATCATATTACGGCCTGATCA 240
10PPR1AB22_ananassa_clone19 -----CAAATTCGATAT-----ATATT----- 177
10PPR1AB22_ananassa_clone2 -----
10PPR1AB22_iinumae      -----

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10PPR1AB22_nubicola      TTCGATATATATATATA-----TTATTTTTTTTCTAAA-----AA 175
10PPR1AB22_mandshurica  TTCGATATATATATATA-----TTATTTTTTTTCTAAA-----AA 173
10PPR1AB22-vesca        TTCGATATATATATATATATATTTTTTTTTTTTCTAAA-----AA 181
10PPR1AB22_viridis      TTCGATATATATATATATA-----TTATTTTTTTTCTAAA-----AA 177
10PPR1AB22_nilgerrensis GTTGATATACGC-----CTGAC-----TC 161
10PPR1AB22_ananassa_clone18 TTCGATATACA-----TTTTTTTTTTAAGTAACTAAATGACTATTTCGAT 221
10PPR1AB22_ananassa_clone20 CTCGACATATGTTGATATAC-----GCCCAACTCAA-----ATTTCGAT 278
10PPR1AB22_ananassa_clone19 TTCGATATACA-----TTTTTTTTTTAAGTAACTAAATGACTATTTCGAT 221
10PPR1AB22_ananassa_clone2 --CGACATATGTTGATATAC-----GCCCAACTCAA-----ATTTCGAT 170
10PPR1AB22_iinumae      -----

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10PPR1AB22_nubicola AAAAAA--TCGATATACAGTATATT---TTTTTTGAATTAATTAATAA 219
10PPR1AB22_mandshurica AAAAAAATCGATATACAGTATATT---TTTTTTGAATTAATTAATGA 220
10PPR1AB22-vesca AAAAAA--TCGATATACAGTATATT---TTTTTTGAATTAATTAATGA 225
10PPR1AB22_viridis AAATAAA--TCGATATACAGTATATT---TTTTTTGAAGTAATTAATGA 223
10PPR1AB22_nilgerrensis AAAT---TCTATATACA-----TTTTCGAAAGAATT--TTTG 193
10PPR1AB22_ananassa_clone18 ATATATTTTCGATATACA-TTT-----TTTTTTTAAAGTAACTAAATGA 264
10PPR1AB22_ananassa_clone20 ATATATTTTCGATATACG-TATATTTTATTTTTTTAAATAATTAATAA 327
10PPR1AB22_ananassa_clone19 ATATATTTTCGATATACA-TTT-----TTTTTTTAAAGTAACTAAATGA 264
10PPR1AB22_ananassa_clone2 ATATATTTTCGATATACA-TATATTTTATTTTTTTAAAGTAACTAAATGA 219
10PPR1AB22_iinumae -----

10PPR1AB22_nubicola GTATTTAGATCGCTTAAAAAGA-TAAACAATTGAAGTTGGTTTAGAAGCA 268
10PPR1AB22_mandshurica GTATTTAGATCGCTTAAAAAATAAACAATCGAAGTTGAATTAGGAGCA 270
10PPR1AB22-vesca GTATTTAGATCGCTTAAAAAGA-TAAACAATCGAAATTTGGTTTAAAGAACA 274
10PPR1AB22_viridis TTATTTAAATCGCTTAAAAAGA-TAAACAA--GAAGTTGGTTTAGGAGCA 270
10PPR1AB22_nilgerrensis TTGTTGAAGTAACT--AAATGACTATACGATTGAAGATAGATTAAGAGAA 241
10PPR1AB22_ananassa_clone18 CTATTTACGTCGGTTAATAAAA-GAAACAATTGAAGTTAAATTAAGAGCA 313
10PPR1AB22_ananassa_clone20 CTATTTACGTTGTTTAAACAAA-GAAACAATTGAAGTTAAATTAAGAGCA 376
10PPR1AB22_ananassa_clone19 CTATTTACGTCGGTTAATAAAA-GAAACAATTGAAGTTAAATTAAGAGCA 313
10PPR1AB22_ananassa_clone2 CTATGTACATCGTTTAAACAAA-GAAACAATTGAAGTTAAATTAAGAGCA 268
10PPR1AB22_iinumae -----

10PPR1AB22_nubicola TCATAGGAGC-----AAGAGTACGAGAACAAAAGTATGAGCTTACTGT 312
10PPR1AB22_mandshurica CCATAGGAGC-----AAGAGTATGAGAACAAAAGTATGAGCTACATTGT 314
10PPR1AB22-vesca CCATAGGAGC-----AAGAGTATGAGAACAAAAGTATGAGCTTACTGT 318
10PPR1AB22_viridis CCATAGGAGC-----AAGAGTATGAGAACAAAAGTATGAGCCACTGT 314
10PPR1AB22_nilgerrensis ACATAGCAACTGAGTAAAAAGTATGAGAACAAAAGTATGAGCTTACTGT 291
10PPR1AB22_ananassa_clone18 CCATGACAG-----AGTACGAGAACAAAAGTATGAGCTTACTGT 353
10PPR1AB22_ananassa_clone20 CCGTAAACAGTGGAGC-AAGAGTACGAGAACAAAAGTATGAGCTTACTGT 425
10PPR1AB22_ananassa_clone19 CCATGACAG-----AGTACGAGAACAAAAGTATGAGCTTACTGT 353
10PPR1AB22_ananassa_clone2 CCATAACAGCTGAGA-AAGAGTACGAGAACAAAAGTATGAGCTTAAACAA 317
10PPR1AB22_iinumae -----

10PPR1AB22_nubicola TTGCTCGTGGTTTATTTATATGGAGAAAATATCAAGGTGATGTTGTATA 362
10PPR1AB22_mandshurica TTGCTCGTGGTTTATTTATATGGAGAAAATATCAAGGTGATGTTGTATA 364
10PPR1AB22-vesca TTGCTCCTCGGTTTATTTATATGGAGAAAATATCAAGGTGATGTTGTATA 368
10PPR1AB22_viridis TTGCTCTTCGGTTTGTTTATACAGAGAAAATATAAAAGTATGTTGTAGA 364
10PPR1AB22_nilgerrensis TTGCTCCTCGGTCGTTTATATGGAGAAAATATAAATGTGATGTTGTAAA 341
10PPR1AB22_ananassa_clone18 TTGCTCGTGGTTTGTTCATATGGAGAAAATGTAGAGGCGATGTTGTAGA 403
10PPR1AB22_ananassa_clone20 -----TTGTTTATATAGAGAAAATATAGAGGCGATGTTGTAGA 463
10PPR1AB22_ananassa_clone19 TTGCTCGTGGTTTGTTCATATGGAGAAAATATAGAGGCGATGTTGTAGA 403
10PPR1AB22_ananassa_clone2 -----ATAGAGAAAATATAGAGGCGATGTTGTAGA 347
10PPR1AB22_iinumae -----

10PPR1AB22_nubicola AACAAATTAACATTACAAAA--TCAAATTACTTAACAATGAACCATCTTC 410
10PPR1AB22_mandshurica AACAAATTAACATTACAAAA--TCAAATTACTTAACAATAAACCATCTTC 412
10PPR1AB22-vesca AACAAATTAACATTACAAAA--TCAAATTACTTAACAATGAACCATTTTC 416
10PPR1AB22_viridis AACAAATTAACACTAAAAAA--TCAAATTACTTAACAACGAACCATCTTC 412
10PPR1AB22_nilgerrensis AATAATTGAACATTAATAAAAAATTAATTTATCTAATAACGAATCATCTTT 391
10PPR1AB22_ananassa_clone18 AATAATTGAACATTAGAAAA--TTAAATTACCTAA----- 436
10PPR1AB22_ananassa_clone20 AATAATTGAACATTAGAAAA--TTAAATTACCTAA----- 496
10PPR1AB22_ananassa_clone19 AATAATAGAACATTAGAAAA--TTAAATTACCTAA----- 436
10PPR1AB22_ananassa_clone2 AATAATTGAACATTAGAAAA--TTAAATTACCTAA----- 380
10PPR1AB22_iinumae -----

10PPR1AB22_nubicola	AGACATGT----	AAAATCAGAAAGTTAAAAGGTTTCGAGTCGCATATGAGT	456
10PPR1AB22_mandshurica	AGACATGT----	AAAATCAAAAAGTTAAAAGGTTTCGAGTCGCATATGAGT	458
10PPR1AB22-vesca	AGACATGT----	AAAATCATAAAATTTAAAAGGTTTCGAGTCGCATATGAGT	462
10PPR1AB22_viridis	AGACATAC----	AAGATCAGAAAGTTAAGAGGTTTCGAGTCGCACATGAGT	458
10PPR1AB22_nilgerrensis	AGACGTACGTACAAAATCAGAGAGTTAAGAGATTTCGAGTTGC-----		433
10PPR1AB22_ananassa_clone18	-----		
10PPR1AB22_ananassa_clone20	-----		
10PPR1AB22_ananassa_clone19	-----		
10PPR1AB22_ananassa_clone2	-----		
10PPR1AB22_iinumae	-----		

10PPR1AB22_nubicola	TTGTCGAGCTGATCAAATACCACAGTTTACTTGACTGAACAAACTTACGT	506
10PPR1AB22_mandshurica	TTGTCGAGCTGATCAAATACCACAGTTTACTTGACTGAACAAACTTACGT	508
10PPR1AB22-vesca	TTGTCGAGCTGATCAAATACCACAGTTTACTTGACTGAACAAACTTACGT	512
10PPR1AB22_viridis	TTCTTGAGGCGATCAAATACCACAGTTTACTTGACTCAACAACTTTACGC	508
10PPR1AB22_nilgerrensis	-----TCAAATACCATATTTTACTTGACTTAACAAT-----	464
10PPR1AB22_ananassa_clone18	-----	
10PPR1AB22_ananassa_clone20	-----	
10PPR1AB22_ananassa_clone19	-----	
10PPR1AB22_ananassa_clone2	-----	
10PPR1AB22_iinumae	-----	

10PPR1AB22_nubicola	A-ACGAGTCAAACGAGCTAAAAACGAGTCGAATAAAAAATCGGGCACCATC	555
10PPR1AB22_mandshurica	A-ACGAGTCAAACGAGCTAAAAACGAGTCGAATAAAAAATCGGGCACCATC	557
10PPR1AB22-vesca	A-ACGAGTCAAACGAGCTAAAAACGAGTCGAATAAAAAATCGGGCACCATC	561
10PPR1AB22_viridis	ATACGAGTCAAACGAGCTAAAAACGAGTCGAATAAAAAATCGGGCACCATC	558
10PPR1AB22_nilgerrensis	--ACGAGTCAAACGAGCTAAAAACGAGTCGATTAAA-----	498
10PPR1AB22_ananassa_clone18	-----	
10PPR1AB22_ananassa_clone20	-----	
10PPR1AB22_ananassa_clone19	-----	
10PPR1AB22_ananassa_clone2	-----	
10PPR1AB22_iinumae	-----	

10PPR1AB22_nubicola	TATATCGAGACTATGTAAGAGCCGAGGAGTAAAA--TAATAACAAACTCG	603
10PPR1AB22_mandshurica	AATATCGAGACTATGTAAGAGCCGAGGAGTAAAA--TAATAACAAACTCG	605
10PPR1AB22-vesca	AATATCGAGACTATGTAAGAGCCGAGGAGTAAAA--TAATAACAAACTCG	609
10PPR1AB22_viridis	AATATCGAGACTATGTAAGAGCCGAGGAGTAAAAATAATAACAAACTCG	608
10PPR1AB22_nilgerrensis	-----TCTAAGAGCCGAGGAGTAAAG--TAATAACAAACTCG	533
10PPR1AB22_ananassa_clone18	-----AAGCCGATGAGTAAAA--TAATAACAAACTCG	466
10PPR1AB22_ananassa_clone20	-----AAGCCGATGAGTAAAA--TAATAACGAACTCG	526
10PPR1AB22_ananassa_clone19	-----AAGCCGATGAGTAAAA--TAATAACAAACTCG	466
10PPR1AB22_ananassa_clone2	-----AAGCCGATGAGTAAAA--TAATAACGAACTCG	410
10PPR1AB22_iinumae	-----AAGAGCCGAGGAGTAAAA--TAATAACAAAGTCG	156

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10PPR1AB22_nubicola	TTATCTAAAAGACAGGTTTAAATATCAGCCCTTGACCATATGTACAGGTTG	653
10PPR1AB22_mandshurica	TTATCTAAAAGACAGGTTTAAATATCAGCCGTTGGACCATATGTACAGGTTG	655
10PPR1AB22-vesca	TTATCTAAAAGACAGGTTTAAATATCAGCCGTTGGACCATATGTACAGGTTG	659
10PPR1AB22_viridis	TTATCTAAAAGACAGGTTTAAATATCAGCCGTTGGACCATATGTACAGGTTG	658
10PPR1AB22_nilgerrensis	TTATCTAAAATACAGGTTTAAATATCAGCCGTTGGATCATATATACAGGTTG	583
10PPR1AB22_ananassa_clone18	TAACCTAAAAGC--GGCTTCATATCATCCACTGGATCATATATGCGGGTG	514
10PPR1AB22_ananassa_clone20	TAACCTAAAAGC--GGCTTCATATCATCCGCTTGATCATATATGCGGGTG	574
10PPR1AB22_ananassa_clone19	TAACCTAAAAGC--GGCTTCATATCATCCACTGGATCATATATGCGGGTG	514
10PPR1AB22_ananassa_clone2	TAACCTAAAAGC--GGCTTCATATCATCCGCTTGATCATATATGCGGGTG	458
10PPR1AB22_iinumae	TAACCTAAAAGC--GGCTTCATATCATCTACTGGATCATATATGCGGGTG	204

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10PPR1AB22_nubicola TGATTCGAAAACCGAAGTTAACCCGCCAAACCCTCCTTCC-AATTTTCAT 702
 10PPR1AB22_mandshurica TGATTCGAAAACCGAAGTTAACCCGCCAAACCCTCATTTCCCAATTTTCAT 705
 10PPR1AB22-vesca TGATTCGAAAACCGAAGTTAACCCGCCAAACCCTCATTCCCAATTTTCAT 709
 10PPR1AB22_viridis TGATTCGAAAACCGA-GTTAACCCGCCAAACCCTCCTTCCCAATTTT-AT 706
 10PPR1AB22_nilgerrensis TGATTCGAAAACCGAAGTTAACCCGCCAAACCCTCATTCCCAATTTTCAT 633
 10PPR1AB22_ananassa_clone18 TGATTCGAAAACCAAAGTTAACCCGCCAAAGCCTAATTCCCAATTTTCAT 564
 10PPR1AB22_ananassa_clone20 TGATTCGAAAACCAAAGTTAACCCGCCAAAGCCTAATTCCCAATTTTCAT 624
 10PPR1AB22_ananassa_clone19 TGATTCGAAAACCAAAGTTAACCCGCCAAAGCCTAATTCCCAATTTTCAT 564
 10PPR1AB22_ananassa_clone2 TGATTCGAAAACCAAAGTTAACCCGCCAAAGCCTAATTCCCAATTTTCAT 508
 10PPR1AB22_iinumae TGATTCGAAAACCAAAGTTAACCCGCCAAA-----CCCAATTTTCAT 246
 ***** * ***** ** ***** *

10PPR1AB22_nubicola TTCCACCAAAAACAAAACC 722
 10PPR1AB22_mandshurica T-CCACCAAAAACAAAACC 724
 10PPR1AB22-vesca T-CCACCAAAAACAAAACC 728
 10PPR1AB22_viridis TTCCACCAAAAACAAAACC 726
 10PPR1AB22_nilgerrensis T-CCACCAAAAACAAAACC 652
 10PPR1AB22_ananassa_clone18 TTCCACCAAAAACAAAACC 584
 10PPR1AB22_ananassa_clone20 TTCCACCAAAAATAAAAC- 643
 10PPR1AB22_ananassa_clone19 TTCCACCAAAAACAAAACC 584
 10PPR1AB22_ananassa_clone2 TTCCACCAAAAACAAAACC 528
 10PPR1AB22_iinumae TTCCACCAAAAACAAAACC 266
 * ***** *****

 11D02

11D02_viridis GAGCTGCTGTGTGA-CCAAATGGGTACAGAAGAAGCCNGTTTGCCAAACCTACCCATGAT 59
 11D02_nubicola GAGCTGCTGTGTGA-CCAAATGGG-TACAGA-GAAGCCNGTTTGCCAAACCTACCCATGAT 57
 11D02-vesca GAGCTGCTGTGTGAACCAAATGG-TACAGA-GAAGCCG-TTTGCCAAACCTACCCATGAT 57
 11D02_iinumae GAGCTGCTGTGTGAACCAAATGG-TACAGA-GAAGCCG-TTTGCCAAACCTACCCATGAT 57
 ***** ***** ***** ***** *****

11D02_viridis CCAATCAA-TGCATAAACTTTAAGAACTCAAATACCAAACGATCAAACATAATGACTGAA 118
 11D02_nubicola CCAATCAAATGCATAAACTTTAAGAACTCAAATACCAAACGATCAAACATAATGACTGAA 117
 11D02-vesca CCAATCAAATGCATAAACTTTAAGAACTCAAATACCAAACGATCAAACATAATGACTGAA 117
 11D02_iinumae CCAATCAAATGCATAAACTTTAAGAACTCAAATACCAAACGATCAAACATAATGACTGCA 117
 ***** *****

11D02_viridis ATGAACAAAATCAAATGAGCAAAGACTAAATGAGAAAACAGACCTCTTTGTAAACTGGG 178
 11D02_nubicola ATGAACAAAATCAAATGGGCAAAGACTAAATGAGAAAACAGACCTCTTTGTAAACTGGG 177
 11D02-vesca ATGAACAAAATCAAATGGGCAAAGACTAAATGAGAAAACAGACCTCTTTGTAAACTGGG 177
 11D02_iinumae ATGAACAAAATCAAATGGGCAAAGACTAAATGAGAAAACAGACCTCTTTGTAAACTGGG 177
 ***** *****

11D02_viridis TTTTGGGGTTTAAAGCCATGGGCACCATATGAGCCCAACCAAGAGCTGCCATTCCCTGTT 238
 11D02_nubicola TTTTGGGGTTTAAAGCCATGGGCACCATATGAGCCCAACCAAGAGCAGCCATTCCCTGTT 237
 11D02-vesca TTTTGGGGTTTAAAGCCATGGGCACCATATGAGCCCAACCAAGAGCAGCCATTCCCTGTT 237
 11D02_iinumae TTTTGGGGTTTAAAGCCATGGGCACCATATGAGCCCAACCAAGAGCTGCCATTCCCTGTT 237
 ***** *****

11D02_viridis TATCACAAAATCACAATTTGGGTCTCTCAGATTGATGCAAAAATCACCA---AACACAA 294
 11D02_nubicola TATCACAAAATCACAATTTGGGTCTCTCAGATTGATGCAAAAATCACAGACAAAACACAA 297
 11D02-vesca TATCACAAAATCACAATTTGGGTCTCTCAGATTGATGCAAAAATCACAGACAAAACACAA 297
 11D02_iinumae TATCACAAAATCACAATTTGGGTCTCTCAGATTGATGCAAAAATCACAGACAAAACACAA 297
 ***** *****

11D02_viridis TTTGATACAGAAGTCTTCACACAGAGAATATGACATTTGTAATTA AACACAGAATAAAAA 354
 11D02_nubicola TTTGATACAGAAGTCTTCACACAGAGAATATGACATTTGTAATTA AACACAGAATAAAAA 357
 11D02-vesca TTTGATACAGAAGTCTTCACACAGAGAATATGACATTTGTAATTA AACACAGAATAAAAA 357
 11D02_iinumae TTTGATACAGAAGTCTTCACACAGAGAATATGACATTTGTAATTA AACACAGAATAAAAA 357
 *** ***** *****

11D02_viridis TGATAACTTTTCAATAGTATAAGGAGAAGATGAGGACAGTACCAGAGACTGCAGCTACTT 414
11D02_nubicola TGATAACTTTTCAATAGTATAAGAAGGAGATGAGGACAGTACCAGAGACTGCAGCTACTT 417
11D02-vesca TGATAACTTTTCAATAGTATAAGAAGGAGATGAGGACAGTACCAGAGACTGCAGCTACTT 417
11D02_iinumae TGATAACTTTTCAATACTATAAGAAGGAGATGAGGACAGTACCAGAGACTGCAGCTACTT 417
***** ** *****

11D02_viridis TGTGCCATAGCATAGGATTCATTGCTGTATTCTTCCCTTAACCTGGTTTCCTTTCAGTGT 474
11D02_nubicola TGTGCCACAGCATAGGATTCATTGCTGTATTCTTCCCTTTGCTTGGTTTCCTTTCAGCCT 477
11D02-vesca TGTGCCACAGCATAGGATTCATTGCTGTATTCTTCCCTTTGCTTGGTTTCCTTTCAGTCT 477
11D02_iinumae TGTGCCATAGCATAGGATTCATTGCTGTATTCTTCCCTTAACCTGGTTTC----- 468
***** * *****

11D02_viridis CTTGACCTTCTTCTAAAACGACGGAGTCGGTGAAACTGTGCAAGTCTTCTGTGA---- 530
11D02_nubicola CTTGACTTCTTCTAAAACGACGTAGTCGGTGCAACTGTGCAAGTCTTCTGTGATGCA 537
11D02-vesca CTTGACTTCTTCTAAAACGACGTAGTCGGTGCAACTGTGCAAGTCTTCTGTGATGCA 537
11D02_iinumae -----GACGGAGTCGGTGCAACTGTGCAAGTCTTCTGTGATGCA 508
**** *****

11D02_viridis ATTTTCTTTTCTAGGTGATTTTTTTTTTCTTTTATAATTAATTTGGTTTTATTTTTCCAA 590
11D02_nubicola ATTTTCTTTTCTAGGTGATTTTTTTT--CTTTTATAATTAATTTGGTTTTATTTTTCCAA 595
11D02-vesca ATTTTCTTTTCTAGGTGATTTTTTTT--CTTTTATAATTAATTTGGTTTTATTTTTCCAA 595
11D02_iinumae ATTTTCTTTTCTAGGTGTTTTTTTTT--CTTTTATAATTAATTTGGTTTTATTTTTCCAA 566
***** *****

11D02_viridis ATAATACCTGAAAGACTTTTTTTTTTTTTTTTGGATAGAAATACCTAAAAGACTTCATAA 650
11D02_nubicola ATAATACCTGAAAGACTTTTTTTTC-----GATAG----- 625
11D02-vesca ATAATACCTGAAAGACTTTTTTTTTT-----CGATAGGA----- 628
11D02_iinumae ATAATACCTGAAAGACTTTTTTTTTT-----CGATAGAAATACCTGTTAAGACT----- 616
***** *****

11D02_viridis AAGCTGTTAAGGCTTCATTTAGGATTCAGTAATTTTTTTTGGACAGTATTACGGGACAC 710
11D02_nubicola -----GATTGCAGTAATTTTTTTTGGACAGTATTACGGGACAC 663
11D02-vesca -----TTGCAGTAATTTTTTTTGGACAGTATTACGGGACAC 664
11D02_iinumae -----TAAGACTTCATTTAGTATTGCAGTAATTTTTTTT--GGACAGTATTACGGGACAC 668
***** *****

11D02_viridis TGTGACAGCTT--GAGTTTGAATCTTAGGTGGGATGATTTAAGTATCTTAGTTGAATGGA 768
11D02_nubicola TGTGACAGCTTTAGAGTTTGAATCTTAGGTGGGATGATTTAAGTATCTTAGTTGAATGGA 723
11D02-vesca TGTGACAGCTTTAGAGTTTGAATCTTAGGTGGGATGATTTAAGTATCTTAGTTGAATGGA 724
11D02_iinumae TG--ACAGCTTTAGAGTTTGAATCTTAGGTGGGATGATTTAAGTATCTTAGTTGAACGGA 726
** *****

11D02_viridis TGTATGACATATTGGTGATTAGTATTAGAGTTATGAGA-----AAATAAAATGAAAAT 822
11D02_nubicola TGTATGACATATTGGTCATTAGTATTAGAGTTATGAGAAAGAGAAAATAAAATGAAAAT 783
11D02-vesca TGTATGACATATTGGTGATTAGTATTAGAGTAATGAGAAAGAGAAAATAAAATGAAAAT 784
11D02_iinumae TGTATGACATATTGGTACTTAGTATTAGAGTTATGAGAA---AAGAAAAATGAAAAT 782
***** *****

11D02_viridis ACAGTACTGGCAATAAACACAATACGGTGGAGCAATCAACAAGCAATAGATTGACAA-G 881
11D02_nubicola ACAGTACTGGCAATAAACACAATACGGTGGAGCAATCAACAATGCAATAGATTGACAAAG 843
11D02-vesca ACAGTACTGGCAATAAACACAATACGGTGGAGCAATCAACAATGCAATAGATTGACAAAG 844
11D02_iinumae ACAGTACTGGCAATAAACACAATTCGGAGGAGCAATCAACAATGCAATAGATTGGCAA-G 841
***** *****

11D02_viridis AAATGAAGACCTAAAAAAACCATTGCATTAATGCAATAGTGTGATTTTCCAATCTCTC 941
11D02_nubicola AAATGAAGACCTAAAAAAA--CCATTGCATTAATGCAATAGTGTGATATTCCAATCTCTC 902
11D02-vesca AAATGAAGACCTAAAAAAA--CCATTGCATTAATGCAATAGTGTGATATTCCAATCTCTC 903
11D02_iinumae AAATGAAGACCTAAAAAAA--CCATTGCATTAATGCAATAGTGTGATTTTCCAATCTCTC 900
***** *****

11D02_viridis CTGAATAGTATTACAACCTCTCCTGGACAAGTCATAACTGTGGGGGGTAATGGTGTAAACA 1001
11D02_nubicola CTGAATAGTATTACAACCTCTCCTGGACAAGTCATAACTGTGGGGGGTAATGGTGTAAACA 962
11D02-vesca CTGAATAGTATTACAACCTCTCCTGGACAAGTCATAACTGTGGGGGGTAATGGTGTAAACA 963
11D02_iinumae CTGAATAGTATTACAACCTCTCCTGGACAAGTCATAACTGTGGGGGGTAATGGTGTAAACA 960
***** ** *****

11D02_viridis	AACAGTCACTAGAATCGAAATTGTTTGTGTCACAAGTTTGTGCTGGGCAGACATAGCACCCCA	1061
11D02_nubicola	AACAGTCACTAGAATCGAAATTGTTTGTGTCACAAGTTTGTGCTGGGCAGACATAGCACCCCA	1022
11D02-vesca	AACAGTCACTAGAATCGAAATTGTTTGTGTCACAAGTTTGTGCTGGGCAGACATAGCACCCCA	1023
11D02_iinumae	AACAGTCACTAGAATCGAAATTGTTTGTGTCACAAGTTTGTGCTGGGCAGACGTAGCACCCCC	1020

11D02_viridis	TAAATCATATCAGATGGGGTTAATGCTACCCAGGTGTGACATATTTGTACAGTTAAACCT	1121
11D02_nubicola	TAAATCATATCAGATGGGGTTAATGCTACCCAGGTGTGACATATTTGTACAGTTAAACCT	1082
11D02-vesca	TATATCATATCAGATGGGGTTAATGCTACCCAGGTGTGACATATTTGTACAGTTAAACCT	1083
11D02_iinumae	TAAATCATATCAGATGGGGTTAATACTACCCAGGTGTGACATATTTGTACAGTTAAACCT	1080
	** *****	
11D02_viridis	AATTTTGTCTAAAGAATGCTAAAATCGAA--CTCCCAAGCAACCAAATCTTCTGTTCCCC	1179
11D02_nubicola	AATTTTGTCTAAAGAATGCTAAAATCGAACTCCCAAGCAACCGAATCTTCTGTTCCCC	1142
11D02-vesca	AATTTTGTCTAAAGAATGCTAAAATCGAA--CTCCCAAGCAACCGAATCTTCTGTTCCCC	1141
11D02_iinumae	AATTTTGTCTAAAGAATGCTAAAATCGAA--CTCCCAAGCAACCGAATCTTCTGTTCCCC	1138

11D02_viridis	TGCTTTAGTATGTTGTGATTATGCCTCTGCTTCCCCAGCAGCATGAATCCGCTCGTCTGG	1239
11D02_nubicola	TGCTTTAGTATGTTGTGGTTATGCCTCAGCTTCCCCAGCAGCATGAATCCGCTCGTCTGG	1202
11D02-vesca	TGCTTTAGTATGTTGTGGTTATGCCTCAGCTTCCCCAGCAGCATGAATCCGCTCGTCTGG	1201
11D02_iinumae	TGCTTTAGTATGTTGTGGTTATGCCTCAGCTTCCCCAGCAGCATGAATCCGCTCGTCTGG	1198

11D02_viridis	AGTTACAGCATGAAGCAGTTCGTCTCT-----TGTTGC	1272
11D02_nubicola	AGTTACAGCATGAAGCAGTTCGTCTCT-----TGTTGC	1235
11D02-vesca	AGTTACAGCATGAAGCAGTTCATCTCT-----TGTTGC	1234
11D02_iinumae	AGTTACAGCATGAAGCAGTTCGTCTCTAGTTGCGCATGAGGTAGCTCGTCTCTTGTGTC	1258
	***** ** *****	
11D02_viridis	AGCATGAGGTAGCTCGTCTCTTGTGTCAGTTTGAGGTAGCTCGTCTGGCATTGCAGCATG	1332
11D02_nubicola	AGCATGAGGTAGCTCGTCTCTTGTGTCAGTTTGAGGTAGCTCATCTGGCATTGCAGCATG	1295
11D02-vesca	AGCATGAGGTAGCTCGTCTCTTGTGTCAGTTTGAGGTAGCTCATCTGGCATTGCAGCATG	1294
11D02_iinumae	AGCATGAGGTAGCTCGTCTCTTGTGTCAGTTTGAGGTAGCTCGTCTGGCATTGCAAGATG	1318

11D02_viridis	AAGCTGCTCGTCTGGAGTTGCAGCATTAAAGTAGTCCTTCTGGAGTTGCAGCAGGATCTAG	1392
11D02_nubicola	AAGCNN	1355
11D02-vesca	AAGCTGCTCGTCTGGAGTTGCAGCATTAAAGTAGTCCTTCTGGAGTTGCAGCAGGATCCAG	1354
11D02_iinumae	AAGCTGCTCGTCTGGAGTTGCAGCATTAAAGTAGTCCTTCTGGAGTTGCAGCAGGATCCAG	1378

11D02_viridis	GTCCCAACACTNN	1452
11D02_nubicola	NN	1415
11D02-vesca	GTCCCAACACTTACCAGGTAGGTTAGTCTCTTCTGCGTCGAGTAACCATGCGGGCACCTG	1414
11D02_iinumae	GTCCCAACACTTACCAGGTAGGTTAGTCTCTTCTGCGTCGAGTAACCATGCGGGTACCTG	1438
11D02_viridis	NN	1512
11D02_nubicola	NN	1475
11D02-vesca	GTGAGAAAAGCGTAACATCTCTCTTCTCGGAATCCATAGAATGGCGCTTCTGTCCGTATC	1474
11D02_iinumae	GTGGGAAAAGCGTAACATCTCTCTTCTCGGAATCCATAGAATGGCGCTTCTGTCCGTATC	1498
11D02_viridis	NN	1572
11D02_nubicola	NN	1535
11D02-vesca	AGTCCGGTATACTGACCTAAATCCAGCCAACCTTACAAGGGGTGAGACACAAACCAAT	1534
11D02_iinumae	AGTCCGGTATACTGACCTAAATCCAGCCAACCTTACAAGGGGTGAGACACAA-CACCAAT	1557
11D02_viridis	NN	1632
11D02_nubicola	NN	1595
11D02-vesca	CTCTCAGAGTAATCATCAAGAACCTCCACCATTGATATTGGTGCCTCACTTCATCTGG	1594
11D02_iinumae	CTCTCAGAGTAATCATCAAGAACCTCCACCATTGATAT-GGTGCCTCACTTCATCTGG	1616

11D02_viridis NNNNNNNN 1640
 11D02_nubicola NNNNNNNN 1603
 11D02-vesca AGTTGAAC 1602
 11D02_iinumae AGTNNNNN 1624

17022

17022-vesca AAAATGGGTTGCACGAGTTCGTGAACGTACAATTTACGACCCAAAGCGTCCAATACTGCT 60
 17022-mandshurica -AAATGGGTTGCACGAGTTCGTGAACGTACAATTTACGACCCAAAGCGTCCAATACTGCT 59
 17022-viridis TAAATGGGTTGCACGAGTTCGTGAACGTACAATTTACGACCCAAAGCGTCCAATACTGCT 60
 17022-nubicola -AAATGGGTTGCACGAGTTCGTGAACGTACAATTTACGACCCAAAGCGTCCAATACTGCT 59
 17022-iinumae AAAATGGGTTG-ACGAGTTCGTGAACA-ACACTTTACGACCCAAAGCGTCCAATACTTCT 58
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17022-vesca TAATTTGACAACAGACATAGTAGAGGAAAACAGGTACCTCCAATGCAAGGAATCGGCACT 120
 17022-mandshurica TAATTTGACAACAGACATAGTAGAGGAAAACAGGTACCTCCAATGCAAGGAATCGGCACT 119
 17022-viridis TAATCTGACAACGGACATAGTAGAGGAAAACAGGTACCTCCAATGCAAGGAATCGGCACT 120
 17022-nubicola TAATTTGACAACGGACATAGTAGAGGAAAACAGGTACCTCCAATGCAAGGAATCGGCACT 119
 17022-iinumae TAATTTGACAACGGACATAGTAGAGGAAAACAGGTACCTCCAATGCAAGGAATCGGCACT 118
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17022-vesca AGAGACTGCATTTCTTATAAAGGCAATGGAATCGTAGAGACTGCATTTCTTACTCAGTAC 180
 17022-mandshurica AGAGACTGCATTTCTTATAAAGGCAATGGAATCGTAGAGACTGCATTTCTTACTCAGTAC 179
 17022-viridis AGAGACTGCATTTCTTATAAAGGCAATGGAATCGTAGAGACTGCATTTCTTACTCAGTAC 180
 17022-nubicola AGAGACTGCATTTCTTATAAAGGCAATGGAATCGTAGAGACTGCATTTCTTACTCAGTAC 179
 17022-iinumae AGAGACTGCATTTCTTATAAAGCAATGGAATCGTAGAGACTGCATATCTTACTCAGCAC 178
 ***** **

17022-vesca TGAATCTGTTGGTCAGCAACACAGAACTAGCTGTGGGCAATGTTTAACTTCCCGAAATT 240
 17022-mandshurica TGAATCTGTTGGTCAGCAACACAGAACTAGCTGTGGGCAATGTTTAACTTCCCGAAATT 239
 17022-viridis TGAATCTGTTGGTCAGCAACACAGAACTAGCTGTGGGCAATGTTTAACTTCCCGAAATT 240
 17022-nubicola TGAATCTGTTGGTCAGCAACACAGAACTAGCTGTGGGCAATGTTTAACTTCCCGAAATT 239
 17022-iinumae TGAATCTGTTGGTCAGCAACACAGAACTAGCTGTGGGCAATGATAAATCTCCCGAAATT 238
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17022-vesca CAACAGCCATCAGAGTTCATCTGCCAATCAGGGCAAATATGACTCTACATTCATTCGATC 300
 17022-mandshurica CAACAGCCATCAGAGTTCATCTGCCAATCAGGGCAAATATGACC--ACATTCATTCGATC 297
 17022-viridis CAACAGCCATCAGAGTTCATCTGCCAATCAGGGCAAATATGACTCTACATTCATTCGATC 300
 17022-nubicola CAACAGCCATCAGAGTTCATCTGCCAATCAGGGCAAATATGACTCTACATTCATTCGATC 299
 17022-iinumae CAACAGCCATCAGAGTTCATCTGCCAATCAGGGCAAATATGACTCTACATTCATTCGATC 298
 ***** **

17022-vesca CCCTTATCACTGTAGGGCTTCATTGGAAACGCTTTGGTCAGCGCAAGACTGATGTTGATA 360
 17022-mandshurica CCCTTATCACTGTAGGGCTTCATTGGAAACGCTTTGGTCAGCGCAAGACTGATGTTGATT 357
 17022-viridis CCCTTATCACTGTAGGGCTCCATTGGAAACGCTTTGGTCAGCGCAAGACTGATGTTGATT 360
 17022-nubicola CCCTTATCACTGTAGGGCTCCATTGGAAACGCTTTGGTCAGCGCAAGACTGATGTTGATT 359
 17022-iinumae CCCTTATCACTGTAGGGCTCCATTGGAAACGCTTTGGTCAGCGCAAGACTGATGTTGATT 358
 ***** **

17022-vesca GTAGCCTAGTTTGTCTTCTTATGCTGAAGCAAAATATGTAATCACCTACGCTACAGAATA 420
 17022-mandshurica GTAGCCTAGTTTGTCTTCTTATGCTGAAGCAAAATATGTAATCACCTAGGCTACAGAATA 417
 17022-viridis GTAGCCTAGTCTAGTTTCTTATGCTGAAGCAAAATATGTAATCACCTAGGCTACAGAATA 420
 17022-nubicola GTAGCCTAGTTTGTCTTCTTATGCTGAAGCAAAATATGTAATCACCTAGGCTACAGAATA 419
 17022-iinumae GTAGCCTAGTTTGTCTTCTTATGCTGAAGCAAAATATGTAATCACCTAGGCTACAGAAGA 418
 ***** *

17022-vesca GTGTT-ACTTGTTACCGGACATGTTTACAATCTTTGAAGATGAAGAACGGTACCAGTTAC 479
 17022-mandshurica GTGTT-ACTTGTTACCGGACATGTTTACAATCTTTGAAGATGAAGAACGGTACCAGTTAC 476
 17022-viridis GTGTT-ACTTGTTACCGGACATGTTTACAATCTTTGAAGATAAAGAACGGTACCAGTTAC 479
 17022-nubicola GTGTTTACTTGTTACCGGACATGTTTACAATCTTTGAAGATGAAGAACGGTACCAGTTAC 479
 17022-iinumae GTGTT-ACTTATTACCGGACATGTTTACAATCTTTGAAGATGAAGAACGGTACCAGTTAC 477
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17022_vesca CCAACATAATCATAGTTATTTTGGCCATTGATATTTTGATTAACGTGTAATTGATCGCT 539
17022_mandshurica CCAACATAATCATAGTTATTTTGGCCATTGATATTTTGATTAATGTGTAATTGATCGCT 536
17022_viridis CCAACATAATCATACTTGTTTTGGCCATTGATATTTTGATTAATATGTAATTGATCGCT 539
17022_nubicola CCAACATAATCATAGTTATTTTGGCCATA-----ATTTTGATTAATATGTAATTGATCGCT 534
17022_iinumae CCAACATAATCATAGTTATTTTGGGAATATTGATATTTTGATTAATATGTAATTGATCGCT 537
***** ** ** * * * *****

17022_vesca ACTTGAATGATGTATATTAT-----GAATGGCACTATTTAATATTTTGGGCTGCTACCTA 594
17022_mandshurica ACTTGAATGATGTATATTAT-----GAATGGCACTATTTAATATTTTGTGCTGCTACCTA 591
17022_viridis ACTGGAATGATGTATATTATATTATGATGGAATGGCACTATTTAATATTTTGGGCTGCTACCTA 599
17022_nubicola ACTTGAATGATGTATATTAT-----GAATGGCACTATTTAATATTTTGGGCTGCTACCTA 589
17022_iinumae ACTTGACTGATGTATATTAT-----GAATGTCACCTATTTAATATTTTGGGCTGCTACCTA 592
*** ** *****

17022_vesca CTCTTCAACAAACTCTAATTAATTAACCAAACATCAGTGTCAAGTCACACCAACCTAG 654
17022_mandshurica CTCTTCAACAAACTCTAATTAATTAACCAAACATCAGTGTCAAGTCACACCAACCTAG 651
17022_viridis CTCTTCAACAAACTCTAATTAATTAACCAAACATCAGTGTCAAGTCACACCAACCTAG 659
17022_nubicola CTCTACAACAAACTCTAATTAATTAACCAAACATCAGTGTCAAGTCACACCAACCTAA 649
17022_iinumae CTCTTCAACAAACTTTAATTAATTAACCAAACATCAGTGTCAAGTCACACCAACCTAG 652
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17022_vesca TTAAACTTTCCATTATAAGTAGCTTTCCCAATAACCTACCTCCCAAAAATAGTTACTTTA 714
17022_mandshurica TTAAACTTTCCATTATAAGTAGCTTTCCCAATAACCTACCTCCCAAAAATAGTTACTTTA 711
17022_viridis TTAAACTTTCCATTATAAGTAGCTTTCCCAATAACCTACCTCCCAAAAATAGTTACTTTA 719
17022_nubicola TTAAACTTTCCATTACAAGTAGCTTTCCCAATAACCTACCTCCCAAAAATAGTTACTTTA 709
17022_iinumae TTAAACTTTCCATTATAAGTAGCTTTCCCAATAACATACCTCCCAAAAATAGTTACTTTA 712

17022_vesca AA-GCTAGTTCCTGTCAAATAGTGAACCACCATCAACTCTTCCCTATAATTCTGGATTTG 773
17022_mandshurica AA-GCTAGTTCCTGTCAAATAGTGAACCACCATCAACTCTTCCCTATAATTCTGGATTTG 770
17022_viridis AAAGCTAGTTCCTGTCAAATAGTGAACCACCATCAACTCTTCCCTATAATTCTGGATTTG 779
17022_nubicola AA-GCTAGTTCCTGTCAAATAGTGAACCACCATCAACTCTTGCCTATAATTCTGGATTTG 768
17022_iinumae AA-GCTGGTTCCTGTCAAATAGTGAACCACCATCAACTCTTCCCTATAATTCTGGATTTG 771
** ** *****

17022_vesca TTAAGTTCGCTAGTATGTGTTGAACTTTGTTTCTTTTACAAAGACAAAAGGACTCTGGTCAT 833
17022_mandshurica TTAAGTTCGCTAGTATGTGTTGAACTTTGTTTCTTTTACAAAGACAAAAGGACTTTGGTCAT 830
17022_viridis TTAAGTTCGCTAGTATGTGTTGAACTTTGTTTCTTTTACAAAGACAAAAGGACTTTGGTCAT 839
17022_nubicola TTAAGTTCGCTAGTATGTGTTGAACTTTGTTTCTTTTACAAAGACAAAAGGACTTTGGTCAT 828
17022_iinumae TTAAGTTCGCTAGTATGTGTTGAACTTTGTTTCTTTTACAAAGACAAAAGGACTTCGGTCAT 831

17022_vesca CAGTGTCAAACCTAGAAGAACCGTGAATTGCGAC----- 866
17022_mandshurica CAGTGTCAAACCTAGAAGAACCGTGAATTGCGACTATACCAGGATGCCTTTGGTCACTTAC 890
17022_viridis CAGTGTCAAACCTAGAAGAACCTGTGAATTGCGACTACACCAGGATGCCTTTGGTCACTTAC 899
17022_nubicola CAGTGTCAAACCTAGAAGAACCGTGAATTGCGACTATACCAGGATGCCTTTGGTCACTTAC 888
17022_iinumae CAGTGTCAAACAAGAAGAACCGTGAATTGCGACTATACCAGGATGCCTTTGGCCACTTGC 891

17022_vesca -----CCCTCAGAATGTCAAATGAGATCACTGTGATTCTTTTAA 907
17022_mandshurica CAACCTCAAGAAAAGGAC-CCCTCAGAATGTCAATGAGATCACTGTGATTCTTTTAA 949
17022_viridis CAACCTCAAGAAAAGGACCCCTCAGAATGTCAAATGAGATCACTGTGATTCTTTTAA 959
17022_nubicola CAACCTCAAGAA-----TGTCAAAATGAGATCTCTGTAATTCTTTTAA 932
17022_iinumae CAACCTCAAGAAAAGGAC-CCCTCAGAATGTCAAGATGAGATCACTGTGATTCTTTTAA 950

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17022_vesca      AATTTTAAACAGCGATTCTTCTACAAAAGATG-GACTAAATTCACCTTGTACTGTACAAA 966
17022_mandshurica AATTTTAAACAGTGATTCTTCTACAAAAGATAAGACTAAATTCACCTTGTACTGTACAAA 1009
17022_viridis    AATTTTAAACAGTGATTCTTCTACAAAAGA-----CTAAATTCACCTTGTACTGTACAAA 1014
17022_nubicola  AATTTTAAACAGTGATTCTTCTACAAAAGATG-GACTAAATTCACCTTGTACTGTACAAA 991
17022_iinumae   AATTTTGACAGTGATTCTTCTACAAAAGATG-GACCAAATTCACCTTGTACTGTACAAA 1009
*****  ****  *****
* *****

17022_vesca      AAACGAGTTTGAGTAGTGGGAATCGTTCCAATAT-ATTTCTGCTCTGTTTACCAATTGCC 1025
17022_mandshurica AAACGAGTTTGAGTAGTGGGAATCGTTCCAATAT-ATTTCTGCTCTGTTTACCAAGTGCC 1068
17022_viridis    AAACGAGTTTGAGTAGTGGGAATCGTTCCAATAT-ATTTCTGCTCTGTTTACCAATTGCC 1073
17022_nubicola  AAACGAGTTTGAGTAGTGGGAATCGTTCCAATATATTTTCTGCTCTGTTTACCAATTGCC 1051
17022_iinumae   AAACGAGTTTGAGTAGTGGGAATCGTTCCAATAT-ATTTCTGCTCTGTTTACCAATTGCC 1068
*****  *****

17022_vesca      AGGATGATACAAACATCTAAACTCTACAGGAACCCCTTTTCTAGCAAAAAGAA-TGAGAAGA 1084
17022_mandshurica AGGATGATACAAACATCTAAACTCTACAGGAACCATCTTCTAGCAAAAAAA-TGAGAAGA 1127
17022_viridis    AGGATGATACAAACATCTAAACTCTACAGGAACCCCTTTTCTAGCAAAAAAA-TGAGAAGA 1132
17022_nubicola  AGGATGATTCAAACATCTAAACTCTACAGGAACCCCTTTTCTAGCAAAAAAA-TGAGAAGA 1110
17022_iinumae   AGGATGATACAAACATCTAAACTCTACAGGAACCCCTTTTCTAGCAAAAAAAATGAGAAGA 1128
*****  *****
* *****

17022_vesca      AAGAACTCTACAAGAATCCAAAGCGCGAAAACAAAATCAGAACTAAGACTAGACATGAAC 1144
17022_mandshurica AAGAACTCTACAAGAATC-AAAGCGCGAAAACAAAATCAGAACTAAGACTAGACATGAAC 1186
17022_viridis    AAGAACTCTACAAGAATCCAAAGTGCGAAAACAAAATCAGAACTAAGACTAGACATGAAC 1192
17022_nubicola  AGGAACTCTACAAGAATCCAAAGCGCGAAAACAAAATCAGAACTAAGACTAGACATGAAC 1170
17022_iinumae   AAGAACTCTACAAGAATCCAAAGCGCGAAAACAAAATCAGAACTAAGACTAGACATGAAC 1188
* *****

17022_vesca      AAATTTGCTGCAGCCTCCACTGATGAGCTTCTCCAGCAAGAACAAAAGAATCAAACCAGA 1204
17022_mandshurica AAATTTGCTGCAGCCTCCACTGAGGAGCATCTCCAGCAAGAACAAAAGAATCAAACCAGA 1246
17022_viridis    AAATTTGCTGCAGCCTCCACTGATGAGCTTCTCCAGCAAGAACAAAAGAATCAAACCAGA 1252
17022_nubicola  AAATTTGCTGCAGCCTCCACTGATGAGCTTCTCCAGCAAGTACAAAAGAATCAAACCAGA 1230
17022_iinumae   AAATTTGCTGCAGCCTCCACTGATGAGCTTCTCCAGCAAGAACAAAAGAATCAAACCAGA 1248
*****  *****

17022_vesca      TAAAATGGAAAATCTCCTCTCACGTTGGAACAATATCATTGATTTTCAGATTTTGTCTCAG 1264
17022_mandshurica TAAAATGGAAAATCTCCTCTCACGTTGGAACAATATCATTGATTTTCAGATTTTGTCTCAG 1306
17022_viridis    TAAAATGGAAAATCTCCTCTCACGTTGGAACAATATCATTGATTTTCAGATTTTGTCTCAG 1312
17022_nubicola  TAAAATGGAAAATCTCCTCTCACGTTGGAACAATATCATTGATTTTCAGATTTTGTCTCAG 1290
17022_iinumae   TAAAATGGAAAATCTCCTCTCACGTCGGAACAATATCATTGATTTTCAGATTTTGTCTCAG 1308
*****  *****

17022_vesca      ATTCTTCGTCAACAGTAGATAGTCCGCCTTCTCTGATGAAGGATGGATTTCAGAAAATTTG 1324
17022_mandshurica ATTCTTCGTCAACAGTAGATAGTCCGCCTTCTCTGATGAAGGATGGATTTCAGAAAATTTG 1366
17022_viridis    ATTCTTCGTCAACAGTAGATAGTCCGCCTTCTCTGATGAAGGATGGATTTCAGAAAATTTG 1372
17022_nubicola  ATTCTTCGTCAACAGTAGATAGTCCGCCTTCTCTGATGAAGGAAGGATTTCAGAAAATTTG 1350
17022_iinumae   ATTCTTCGTCAACAGTAGATAGTCCGCCTTCTCTGATGAAGGATGGATTTCAGAAAATTTG 1368
*****  *****

17022_vesca      CTACAAAAGCCCATAACTTGTAATA-CATCATCGAAGT-TTGTGAGGAAACCC 1374
17022_mandshurica CTACAAAAGCCCATAACTTGTAAGGCATCATCGAAGTATTGTGAGGAAACCC 1418
17022_viridis    CTACAAAAGCCCATAACTTGTAATA-CATCATCGAAGT-TTGTGAGGAAACCC 1422
17022_nubicola  CTACAAAAGCCCATAACTTGTAATA-CATCATCGAAGT-TTGTGAGGAAACCC 1400
17022_iinumae   CTACAAAAGCCCATAACTTGTAATA-CATCATCGAAGT-TTGTGAGGAAACCC 1418
*****  *****

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27F10

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27F10_vesca      CCTGCAGGGTTTTTCATCATGTAAGGACCTCCATTGT-CAGTAGCTTTATGCATATCATC 59
27F10_mandshurica CCTGCAGGGTTTTTCATCATGTAAGGACCTCCATTGT-CAGTAGCTTTATGCATATCATC 59
27F10_nubicola  CCTGCAGG--TTTTTATCATGTAAGGACCTCCATTGT-CAGTAGCTTTATGCATATCATC 57
27F10_iinumae   CCTGCAGG-TTTTTATCATGTAAGGACCTCCATTGT-CAGTAGCTTTATGCATATCATC 58
27F10_ananassa  CCTGCAGG-TTTTT-ATCATGTAAGGACCTCCATTGT-CAGTAGCTTTATGCATATCATC 57
27F10_viridis   --TGCGGG-TTTTTATCATGTAAGGACCTCCATTGTTTCGGTAGCTTTATGCATATCATC 57
*** **  ****  *****
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27F10_vesca TTCATCACAACAGCTGAAGCAGCTCATG-ATTCCCTTTAAACACACACAAAAAAA--CC 115
27F10_mandshurica TTCATCACAACAGCTGAAGCAGCTCATG-ATTCCCTTTAAACACACACAAAAAAA--CC 115
27F10_nubicola TTCATCACAACAGCTGAAGCAGCTCATG-ATTCCCTTTAAACACACACAAAAAAA--CC 114
27F10_iinumae TTCATCACAACAGCTGAAGCAGCTCATG-ATTCCCTTTAAACACACA--AAAAAAA--CC 113
27F10_ananassa TTCATCACAACAGCGGAAGCAGCTCATGGACTCCTTTAAACACACA--AAAAAAA--CC 113
27F10_viridis TTCATCACAACAGCTGAAGCAGCTCATG-ATTCCCTTTAAACACACAAAAAAAACACC 116
***** * ***** * ***** *

27F10_vesca ACAGTCAAATGAGGAAATGAACAATACCCAAGTCATGAACACACAAAATTCAGTAAAA 175
27F10_mandshurica ACAATCAAATGAGGAAATGAACAATACCCAAGTCATGAACACACAAAATTCAGTAAAA 175
27F10_nubicola ACAATCAAATGAGGAAATGAACAATACCCAAGTCATGAACACACAAAATTCAGTAAAA 174
27F10_iinumae ACAATCAAATGAGGAAATGAACAATACCCAAGTCATGAACACACAAAATTCAGTAAAA 173
27F10_ananassa ACGATCAAATGAGGAAATGAACAATACCTAAGTCATGAACACACAAAATTCAGTAAAA 173
27F10_viridis ATAATCAAATGAGGAAATGAACAATACCTTAGTCATGAACACACAAAATTCAGTAAAA 176
* ***** * ***** * ***** * ***** *

27F10_vesca AGTAAAAAGGGATCCGCTTCAATCCAATCCCATCAAACCTTGACAGACCTTTGGAGACAAAT 235
27F10_mandshurica AGTAAAAAGGGATCCGCTTCAATCCAATCCCATCAAACCTTGACAGACCTTTGGAGACAAAT 235
27F10_nubicola AG-AAAAAGGGATCCGCTTCAATCCAATCCCATCAAACCTTGACAGACCTTTGGAGACAAAT 233
27F10_iinumae AGAAAAAGGGATCCGCTTCAATCCAATCCCATCAAACCTTGACAGACCTTTGGAGACAAAT 233
27F10_ananassa AGAAAAAGGGATCCGCTTCAATCCAATCCCATCAAACCTTGACACCCCTTTGGAGACAAAT 233
27F10_viridis AGAAAAAGGGATCCGCTTCAATCCAATCCCATCAAACCTTGACAGACCTTTGGAGACAAAT 236
** ***** * ***** * ***** *

27F10_vesca TTCGTTGCTTAATGTAATAAGCAACAAAAAATTCAGCTCAGCTGGATCAAAGCCAGATG 295
27F10_mandshurica TTCGTTGCTTAATGTAATAAGCAACAAAAAATTCAGCTCAGCTGGATCAAAGCCAGATG 295
27F10_nubicola TTCGTTGCTTAATGTAATAAGCAACAAAAAATTCAGCTCAGCTGGATCAAAGCCAGATG 293
27F10_iinumae TTCGTTGCTTAATGTAATAAGCAACAAAAA-TCCAGCTCAGCTGGATCAAAGCCAGATG 292
27F10_ananassa TTCGTTGCTTAATGTAATAAGCAACAAAAA-TTCAGCTCAGCTGGATCAAAGCCAGATG 292
27F10_viridis TTCGTTGCTTAATGTAATAAGCAACAAAAAATTCAGCTCAGCTGGATCAAAGCCAGATG 296
***** * ***** * ***** *

27F10_vesca AAAAAGATTTAAACTTTAAACAAGAAAATAAAGATCAGAGAAAGAAAATATGATGGGTAG 355
27F10_mandshurica AAAAAGATTTAAACTTTAAACAAGAAAATAAAGATCAGAGAAAGAAAATATGATGGGTAG 355
27F10_nubicola AAAAAGATTTAAACTTTCAACAAGAAAATAAAGATCAGAGAAAGAAAATATGATGGGTAG 353
27F10_iinumae AAAAAGATTTAAACTTTACCAAGAAAATAAAGGTTCAGAGAAAGAAAATATGATGGGTAG 352
27F10_ananassa AAAAAGATTTAAACTTTACCAAGAAAATAAAGGTTCAGAGAAAGAAAATATGATGGGTAG 352
27F10_viridis AAAAAGATTTAAACTTTAAACAAGAAAATAAAGATCAGAGAAAGAAAATATGATGGGNAG 356
***** * ***** * ***** * ***** *

27F10_vesca ATCGGGAGAGATAAAAATTACCTGAATCTGAAGTGGGGGAAGTGAGTCAGTGAAGGACTGA 415
27F10_mandshurica ATCGGGAGAGATAAAAATTACCTGAATCTGAAGTGGGGGAAGTGAGTCAGTGAAGGACTGA 415
27F10_nubicola ATCGGGAGAGATAAAAATTACCTGAATCTGAAGTGGGGGAAGTGAGTCAGTGAAGGACTGA 413
27F10_iinumae ATCGGGAGAGATAAAAATTACCAAGATCTGAAGTGGGGGAAGTGAGTCAGTGAAGGACTGA 412
27F10_ananassa ATCGGGAGAGATAAAAATTACCAAGATCTGAAGTGGGGGAAGTGAGTCAGTGAAGGACTGA 412
27F10_viridis ATCGGGAGAGATAAAAATTACCTGAATCTGAAGTGGGGGAAGTGAGTCAGTGAAGGACTGA 416
***** * ***** * ***** *

27F10_vesca GTTGGTGGAGTCTTGGGAGATCTGAGATATGAGCTCTAAAGCCGGCG-AAGGATGCGCGG 474
27F10_mandshurica GTTGGTGGAGTCTTGGGAGATCTGAGATATGAGCTCTAAAGCCGGCG-AAGGATGCGCGG 474
27F10_nubicola GTTGGTGGAGTCTTGGGAGATCTGAGATATGAGCTCTAAAGCCGGCG-AAGGATGCGCGG 472
27F10_iinumae GTTGTGGAGTCTTGGGAGATCTGAG-----CTCTAAAGCCGGCG-AAGGATGCGCGG 464
27F10_ananassa GTTGTGGAGTCTGGAAGATCTGAG-----C-----CCGGCG-AAGGATGCGCGG 457
27F10_viridis GTTGGTGGANTCTTGGGAGATCTGAGATATGAGCTCTAAAGCCGGCGCAAGGATGCCCGG 476
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27F10_vesca CGCAGGATAGGAGGAAAAGGGTGCCTAGGATAACCCAATCAATGAACCAATGAGAATA 534
27F10_mandshurica CGCAGGATAGGAGGAAAAGGGTGCCTAGGATAACCCAATCAATGAACCAATGAGAATA 534
27F10_nubicola CGCAGGATAGGAGGAAAAGGGTGCCTAGGATAACCCAATCAATGAACCAATGAGAATA 532
27F10_iinumae CGCAGGATAGGAGGAAAAGGGTGCCTAGGATAACCCAATCAATGAACCAATGAGAATA 524
27F10_ananassa CGCAGGATCGGAGGAAAAGGGTGCCTAGGATAACCCAATCAATGAACCAATGAGAACA 517
27F10_viridis CGCAGGATAGGAGGAAAAGGGTGCCTAGGATAACCCACTCCANGAACANATGACAAATG 536
***** * ***** * ***** * ***** *

27F10_vesca	CGCTAGTGATTTTGATTATGAATTCTATAAATCTATAAAAA-TTTATTTTCATTTCTTAA	593
27F10_mandshurica	CGCTAGTGATTTTGATTATGAATTCTATAAATCTATAAAAA-TTTATTTTCATTTCTTAA	593
27F10_nubicola	CGCTAGTGATTTTGATTATGAATTCTATAAATCTACAAAAAATTTATTTTCATTTCTTAA	592
27F10_iinumae	CGCTAGTGATTTTGATTATGAATTCTATAAATCTACAAAAA-TTTATTTTCATTTCTTAA	583
27F10_ananassa	CGCTAGTGATTTTGATTATGAATTCTATAAATCTACAAAAA-TTTATTTTCATTTCTTAA	576
27F10_viridis	CNN	596
	*	
27F10_vesca	TTCTTACTCTGTTTCGGTGTGGCCAGATTTGACTCTTCTGTGCTTCAGT-----TTTG	647
27F10_mandshurica	TTCTTACTCTGTTTCGGTGTGGCCAGATTTGACTCTTCTGTGCTTCAGT-----TTTG	647
27F10_nubicola	TTCTTACTCTGTTTCGGTGTGGCCAGATTTGACTCTTCTGTGCTTCAGT-----TTTG	646
27F10_iinumae	TTCTTACTCTGTTTCGGTGTGGCCAGATTTGACTCTTCTGTGCTTCAGT-----TTTG	643
27F10_ananassa	TTCTTACTCTGTTTCGGTGTGGCCAGATTTGACTCTTCTGTGCTTCAGT-----TTTG	636
27F10_viridis	NN	656
27F10_vesca	ACCATTTACTTTTATAACCTCAGGAAGGGTTCAAGCGCGGCCTGCCACGTGGTGAATTC-	706
27F10_mandshurica	ACCATTTACTTTTATAACCTCAGGAAGGGTTCAAGCGCGGCCTGCCACGTGGTGAATTC-	706
27F10_nubicola	ACCATTTACTTTTATAACCCCGGAAGGGTTCAAGCGCGGCCTGCCACGTGGTGAATTC-	705
27F10_iinumae	ACCATTTACTTTTATAACCCCGGAAGGGTTCAAGCGCGGCCTGCCACGTGGTGAATTC-	703
27F10_ananassa	GCCATTTACTTTTATAACCCCGGAAGGGTTCAAGCGCGGCCTGCCACGTGGTGAATTC-	696
27F10_viridis	NN	716
27F10_vesca	-----AAAAGAGTCTGGAAGCAAAGCCTTGACCTCGTGGAATTCGTCTCTCCCC	755
27F10_mandshurica	-----AAAAGAGTCTGGAAGCAAAGCCTTGACCTCGTGGAATTCGTCTCTCCCC	755
27F10_nubicola	-----AAAAGAGTCTGGAAGCAAAGCCTTGACCTCGTGGAATTCGTCTCTCCCC	754
27F10_iinumae	GGTTCGTCCGGAAGAGTCTGGAAGCAAAGCCTTGACCTCGTGGAATTCGTCTCTCCCC	763
27F10_ananassa	GGTTCGTCCGGAAGAGTCTGGAAGCAAAGCCTTGACCTCGTGGAATTCGTCTCTCCCC	756
27F10_viridis	NN	776
27F10_vesca	TCCCGGTAACAGTAACTTTATCGACAAAACGCTTCTTATTTTATTTTATTTTGGCG	815
27F10_mandshurica	TCCCGGTA-CAGTAACTTTATCGTTTTACCGCTAGTATGTCTCTGTCTCTCTCT--GTC	812
27F10_nubicola	TCCCGGTAACAGTAACTTTATCGTTTTACCGCTAGTATGTCTCTCTCTCTCTCTCT--GTC	805
27F10_iinumae	TCCCGGTAACAGTAACTTTATCGTTTTACCGCTAGTATGTCTCTCTCTCTCTCTCTCT--GACA	818
27F10_ananassa	TCCCGGTAACAGTAACTTTATCGTTTTACCGCTAGTATGTCTCTCTCTCTCTCTCTCT--GACA	804
27F10_viridis	NN	836
27F10_vesca	AGCAAAACGCTTCTTATTTGTTTTGGGTCTGTACGCTTTTGGGTCTTATTTGTCAAGTTT	875
27F10_mandshurica	GACATAACGCTTCTTATTTGTTTTGGGTCTGTACGCTTTTGGGTCTTATTTGTCAAGTTT	872
27F10_nubicola	GACATGACGCTTCTTATTTCTTTGGGTCTGTACGCTTTTGGGTCTTATTTGTCAAGTTT	865
27F10_iinumae	TATATAACGCTTCTTATTTGTTTTGGGTCTGTACGCTTTTGGGTCTTATTTGTCAAGTTT	878
27F10_ananassa	-----TATTTGTTTTGGGTCTGTACGCTTTTGGGTCTTATTTGTCAAGTTT	850
27F10_viridis	NN	896
27F10_vesca	CAATCACTAGCAGGA-----AGACTTGCGTAT-----	902
27F10_mandshurica	CAATCACTAGCAGGT-----AGACTTGCGTAT-----	899
27F10_nubicola	CAATCACTAGCAGGA-----AGACTTGCGTAT-----	892
27F10_iinumae	CAATCACTTGAAACT-----	893
27F10_ananassa	CAATCACTTGAAACATAGCAGGAAGACTTGCATAT-----	885
27F10_viridis	NNNNNNNNNNNNNNNN-----NNNNNNNNNTATGCAAAAATACACTCATATTTATGTA	948
27F10_vesca	-----	
27F10_mandshurica	-----	
27F10_nubicola	-----	
27F10_iinumae	-----	
27F10_ananassa	-----	
27F10_viridis	GAAAACGAGAATTGAACCTTAACCTCTTACAAACAACCTATGAAATGTATAATATATGT	1008

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27F10_vesca -----
27F10_mandshurica -----
27F10_nubicola -----
27F10_iinumae -----
27F10_ananassa -----
27F10_viridis AAAGACGATTAAATATATGTATAATATAATATAATATATGTATTGTTTTATATTTATAACAT 1068

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27F10_vesca -----
27F10_mandshurica -----
27F10_nubicola -----
27F10_iinumae -----
27F10_ananassa -----
27F10_viridis ACTATAGATATAAATACAATTAAATATAAATGTTCAAATTTTAGCAAGAGGTATGTTT 1128

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27F10_vesca -----
27F10_mandshurica -----
27F10_nubicola -----
27F10_iinumae -----
27F10_ananassa -----
27F10_viridis CGAAACCATGACTGCTCTGATGGAAAATATGACCACTTACGATCAAAAACAAGCTATCAT 1188

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27F10_vesca -----
27F10_mandshurica -----
27F10_nubicola -----
27F10_iinumae -----
27F10_ananassa -----
27F10_viridis TGCATTATATTTGTGAAAAAATTATATTTATCACTTCATTTTTTGGGCCACAATCTAAG 1248

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27F10_vesca -----
27F10_mandshurica -----
27F10_nubicola -----
27F10_iinumae -----
27F10_ananassa -----
27F10_viridis TTTAGTAGAGGCCTATTACCAACCGTACCAACTAAGTCGGTATACCAACATCGATGGTTG 1308

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27F10_vesca -----
27F10_mandshurica -----
27F10_nubicola -----
27F10_iinumae -----
27F10_ananassa -----
27F10_viridis GTTTTGATAGAGGATTTTGCTACCAATCATAAGTTGGTTGGTACATGATATTGGTAAAT 1368

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27F10_vesca -----A 903
27F10_mandshurica -----A 900
27F10_nubicola -----A 893
27F10_iinumae -----
27F10_ananassa -----A 886
27F10_viridis AAAGTCGGTATATCTACCAATGCCAGCCCTACTTGAACCTAGCCGGAAGACTTCATATA 1428

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27F10_vesca ATTGAAATAGCCACTATCTATACT--CTATATGCAAACACAA--GAGTAGAGAAGGAGA 958
27F10_mandshurica ATTGAAATAGCCACTATCTATACT--CTATATGCAAACACAA--GAG-AGAGAAGGAGA 954
27F10_nubicola ATTGAAATAGCCATTATCTATACT--CTATATGCAAACACAA--GAG-AGAGAAGGAGA 947
27F10_iinumae ATTGAAATAGCTGAAATACACACTTACTATATGCAAACACAA--GGG-AGAG-AGGAGA 948
27F10_ananassa ATTGAAATAGCTGCAATACACACTTGCTATATGCAAACACAACAAGAG-AGAGGAGGAGA 945
27F10_viridis ATTGAAATAGCTGAGATACACACTTGCTATATGCAAACACAA--GAGTAGAGAAGGAGA 1485
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27F10_vesca          ACCAGAATACATTCCA 975
27F10_mandshurica   ACCAGAATACATTCCA 971
27F10_nubicola      ACCAGAAT--CATCCA 961
27F10_iinumae       ACCAGATC---ATCTA 962
27F10_ananassa      ACCAGAAT--CATCCA 959
27F10_viridis       ACCAGAATACATTCCA 1502
*****              ** *

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29G10

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29G10_vesca          TGGCCTTGTTTCCTAAACTCTTCAGGGTCTAGAGCTTTGGAGAGGTAGGAAGAGTTTATT 60
29G10_mandshurica   TGGCCTTGTTTCCTAAACTCTTCAGGGTCTAGAGCTTTGGAGAGGTAGGAAGAGTTTATT 60
29G10_nubicola      TGGCCTTGTTTCCTAAACTCTTCAGGGTCTAGGGCTTTGGAGAGGTAGGAAGAGTTGATT 60
29G10_nilgerrensis  TGGCCTTGTTTCCTAAACTCTTCAGGGTCTAGGGCTTTGGAGAGTTAGGAAGAGTTGATT 60
*****              *****

29G10_vesca          TCTAGAGGGAGGCTACCCATTTGAAGTAGAGATTTGGACTAAAAACAACCTTGAAAG--GAA 118
29G10_mandshurica   TCTAGAGGGAGGCTACCCATTTGAAGTAGAGATTTGGACTAAAAACAACCTTGAAAG--GAA 118
29G10_nubicola      TCTAGAGGGAGGCTACCCATTTGAAGTAGAGATTG-ACTAAAAACAACCTTGAAAGAGGAA 119
29G10_nilgerrensis  TCTAGTGGGAGGCTACCCATTTGAAGTAGAGATTTGGACTAAAAACAACCTTGAAAG--GAA 118
*****              *****

29G10_vesca          GATGGGGAGGATAAATAAAAAGGATAGAAACTGCTCAAGTGCTTAACAATGGTTGTAGAC 178
29G10_mandshurica   GATGGGGAGGATAAATAAAAAGGATAGAAACTGCTCAAGTGCTTAACAATGGTTGTAGAC 178
29G10_nubicola      GATGGGG-----AGGATAGAAACTGCTCAAGTGCTTAACAATGGTTGTAGAC 166
29G10_nilgerrensis  GATGGGGAGGATAAATAAAAAGGATAGAAACTGCTCAAGTGCTTAACAATGGTTGTGGAC 178
*****              *****

29G10_vesca          GAGTTGTGTCTTGTCTGCATATATTGAAGAGATTATATAGAGGTGCATGTAGGATGAAGAC 238
29G10_mandshurica   GAGTTGTGTCTTGTCTGCATATATTGAAGAGATCATATAGAGGTGCATGAAGGATGAAGAC 238
29G10_nubicola      GAGTTGTGTCTTGTCTGCATATATTGAAGAGATTATATAGAGGTGCATGTAGGATGAAGAC 226
29G10_nilgerrensis  GAGTTGTGTCTTGTCTGCATATATTGAAGAGATTATATAGAGGTGCATGTAGGATGAAGAC 238
*****              *****

29G10_vesca          GCCGTATCTTAAATTTTGATTTGGTTCTTCTCA-----CACACCAGAGATTGAGTTCG 291
29G10_mandshurica   ACCGTATCTTAAATTTTGATTTGGTTCTTCTCAGTTCTCACACACCAGAGATTGAGTTCG 298
29G10_nubicola      ACCGTATCTTAAATTTTGATTTGGTTCTTCTCA-----CACACCAGAGATTGAGTTCG 279
29G10_nilgerrensis  ACCGTATCTTAAATTTTGATTTGGTTCTTCTCA-----CACACCAGAGATTGAGTTCG 291
*****              *****

29G10_vesca          GATCATCGGATCCGAAAAATCAAGTCCTTGTGTATAAAAAGCACGTTACGGAGTGATCCCA 351
29G10_mandshurica   GATCATCGGATCCGAAAAATCAAGTCCTTGTGTATAAAAAGCACGTTACGGAGTGATCCCA 358
29G10_nubicola      GATCATCGGATTGAAAAATCAAGTCCTTGTGTATAAAAAGCACGTTACGGAGTGATCCCA 339
29G10_nilgerrensis  GATAATCGGATTCGAAAAATCAAGTCCTTGTGTGTA AAAAGCACGTTGCGGAGTGATCCCA 351
*** *****

29G10_vesca          CTCATCAATAAGTTATCGGACTTAATTATTGTACGGTGGACCACGTCAGTCTGGCATAT 411
29G10_mandshurica   CTCATCAATAAGTTATCGGACTTAATTATTGTACGGTGGACCACGTCAGTCTGGCATAT 418
29G10_nubicola      CTCATCAATAAGTTATCGGACTTAATTATTGTACGGTGGACCATGTCAGTCTGGCATAT 399
29G10_nilgerrensis  CTCATCAATAAGTTATCGGACTTAATTATTGTACGGTGGACCACGTCAGTCTGGCATAT 411
*****              *****

29G10_vesca          CGATCATCACTCCCAATCTTGTGCGATCATCAATTTGGCATGCATATCAGACCCAAGCCAT 471
29G10_mandshurica   CGATCATCACTCCCAATCTTGTGCGATCATCAATTTGGCATGCATATCAGACCCAAGCCAT 478
29G10_nubicola      CGATCATCACTCCCAATCTTGTGCGATCATCAATTTGGCATGCATATCAGACCCAAGCCAT 459
29G10_nilgerrensis  CTATCATCACTCTCAATCTTGTGCGATCATCAATTTGGC-TACATATCAGACCCAAGCCAT 470
* *****

29G10_vesca          TACTTGCTTCTATGAACGTATTTATATCATTCTAATCACCAGAAATATGGATAATATT 531
29G10_mandshurica   TACTTGCTTCTATGAACGTATTTATATCATTCTAATCACCAGAAATATGGATAATATT 538
29G10_nubicola      TACTTGCTTCTATGAATGATTTATATCATTCTAATCACCAGAAATATGGATAATATT 519
29G10_nilgerrensis  TACTTGCTTCTATGAATGATTTATATCATTCTAATCACCAGAAATATGGATAATATT 530
*****              *****

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29G10_vesca TCTTATTCACAACGACGATTGGCTTCTTGGTGTGTTGCGCTTTGTTAGGAC-AGTTCATT 590
 29G10_mandshurica TCTTATTCACAACGACGATTGGCTTCTTGGTGTGTTGCGCTTTGTTAGGAC-AGTTCATT 597
 29G10_nubicola TCTTATTCACAACGACGATTGGCTTCTTGGTGTGTTGCGCTTTGTTAGGAC-AGTTCATT 578
 29G10_nilgerrensis TCTTATTCACAACGACGATTGGCTTCTTGGTGTGTTGCGCTTTGTTAGGACCAGTTCATT 590

29G10_vesca GAATTCAGGAATCCACAATTGGGTGCTGCCTTCTTCT 628
 29G10_mandshurica GAATTCAGGAATCCACAATTGGGTGCTGCCTTCTTCT 635
 29G10_nubicola GAATTCAGGAATCCACAATTGGGTGCTGCCTTCT--- 612
 29G10_nilgerrensis GAATTCAGGAATCCACAATTGGGTGCTGCCTTCTTCT 628

32L07

32L07_vesca GAGTTGAAAAACGGGTCTGAATCCCGGCACCACCGTCCCGTCCGCTAGGACTTGAATCCT 60
 32L07_viridis GAGTTGAAAAACGGGTCTGAATCCCGGCACCACCGTCCCGTCCGCTAGGACTTGAATCCT 60

32L07_vesca TCCAAGGTCACCTCCTTGATGTACATAGCTGCCCTCGCCGGAGAGGTGCGGACGCTAATC 120
 32L07_viridis TCCAAGGTCACCTCCTTGATGTACATAGCTGCCCTCGCCGGAGAGGTGTTGGACGCTAATC 120

32L07_vesca GGAAGCCGATTTTGGAGAGATTTAGTGTGCGGTGATAGATCGGAACCCCTAGAAATCTGAGC 180
 32L07_viridis GGTAGCCGATTTTGAAGAGATTTAGGGTCCGGTGTAGATCGGAACCCCTAGAAA----- 173
 ** *****

32L07_vesca TTCTGGTTTTTGCTTTCGGAAGTTGAGAGTCTGAAATGACATGGTTCGAATTTCTTTTTTG 240
 32L07_viridis -----

32L07_vesca TTGTTTTCCGCTTTTTTGGTGGGTTCGAATTTTTAGACCAAGGCGGGAGATATTTGGGCC 300
 32L07_viridis -----

32L07_vesca AGTGATTTATATCTTGGGCTCACTCTGGGACTCATGTCTTTGGGCTCGTCGACCTCGAG 360
 32L07_viridis -----

32L07_vesca GTGCTCATGAAGTCCGGCCGCTCCTCAGGGTCGAAACACCGCGTACTACTGACTACTGT 420
 32L07_viridis -----

32L07_vesca GTCATCGCTTTAGAATTCATTAATTGGCTTTGCGAGCTATAAATAATTGTGATTTGGTT 480
 32L07_viridis -----

32L07_vesca TGAATTTAGGTAAGTTTTAGTATTAGTATTTATCAACGGGAATTGCGGAGATGAGAAAAG 540
 32L07_viridis -----

32L07_vesca TTGAGGTTGATTTGGGGGAGTGTGGTGTGTTAGTTAGTTGAATTATTAGAAAACGAAAAA 600
 32L07_viridis -----

32L07_vesca ATAACAGAAGAATATAAATGTGGATGGATTATTGGATTAAGATTTGATTCAACGGAAGAA 660
 32L07_viridis -----

32L07_vesca GGAGGCGTGGTGTGTGTTTTGATAGTCTAATTTGAACTGTTTTGCTTCTGACAGCTAAAA 720
 32L07_viridis -----

32L07_vesca TCTATCCGGTGGTGAAAAATCAGCATCGGCTACTATGTACTTTTAATCGCAACGCAT 780
 32L07_viridis -----

32L07_vesca	TAGCGATGGAGGTGACTTGTCTAATTTACTAAGTTTATTTAGGTTGTTACTGGTACATTT	840
32L07_viridis	-----	
32L07_vesca	TATGTGTTTATTGCCGTGGATGTAGTTTGTATGGGCCAGTTGACCAGCAGTTTCAAATGG	900
32L07_viridis	-----	
32L07_vesca	CAGGCCAATAGGGCCAACCTAGATGTAGTTGAATTTTGGGAAGGAAAAAAAAAAGCAA	960
32L07_viridis	-----	
32L07_vesca	CCAAAAGACATCACCACGAGCCACTTTGGCCTATCTATATATATTACTTCCTTGCTTAAT	1020
32L07_viridis	-----ATACGTCACCACGAGCCACTTTGGCCTATCTATGT----TACTTCCTTGCTTAAT	224
	* * * * *	
32L07_vesca	GTGTTGCTCAATTGCTAAACAATATCATCAATGTCTAAAAAACGCGCCTCAAGGCTAAG	1080
32L07_viridis	GTGTTGCTCAATTGCTCAACAATATCATCAATGTCTAAAAAACGCGCCTCGAGGCTAAG	284

32L07_vesca	GCAAGGAAGGCGT-GCCTTAGGACGACCTCTGAAAGACATTTGATATCAAAGGTGTGAT	1139
32L07_viridis	GCAAGGAAGGCGTCACCTTAGGACGACCTCTGAAAGACATTTGATATCAAAGGTGTGAT	344

32L07_vesca	TGAGGCGCGCATCAAGACGACGAGGTCAAGGTGCCAATACAACATCAGGTTATAGGTT	1199
32L07_viridis	TGAGGCGCGCATCAAGACGACGAGGTCAAGGTGCCAATACAACATCAGGTTATAGATT	404

32L07_vesca	TGAATCTCACTTTGAGAAATGTGATGGTTTGAACGGTTAAATCTATTGTCTTTTATATT	1259
32L07_viridis	TGAATCTCACTTTGAGAAATGTGATGGTTTGAACGGTTAAATCTATTGTCTCTTTATATT	464

32L07_vesca	GTATGGGCGGTAAAATTAATGTTAAACTTCGGTAAATTTGTCAAATGTTTAAATAGTATAA	1319
32L07_viridis	GTATGGGTGGTAAAATTAATGTTAAATTTTCGGTAAATTTGTCAAATGTTTAAATAGTATAA	524

32L07_vesca	GAATCTACATATAGTAGGTGTAATAATAGATACCGAAATGATAATATTTTGTGAATAACGT	1379
32L07_viridis	GAATCTACATATAGTAGGTGTAATAATAGATACCGAAATGATAATATGTTGTGAATAATAT	584

32L07_vesca	ACGTCATATGATTTAATATTAAGACTTTGTACGATTTAACGTTACACATTAATAATGTTAG	1439
32L07_viridis	ACGTCATATGATTTAATATTAAGACTTTGTACGATTTAATGCTACACATTAATAATGTTAG	644

32L07_vesca	ATAAAAAGTTTATATCATCATCAACATCGATGTTTCAATAAAATTTTATAACGTTCAATGC	1499
32L07_viridis	ATAAAAAGTTTATATCATCATCATCATCGATGTTTCAATAAAATTTTATAACGTTCAATGC	704

32L07_vesca	GGTACAAATCTCCCAATGACTATTATCGAGTACAACGTCCATATCCGACACATGATATAG	1559
32L07_viridis	GGTACAAATCTCCCAATGACTATAATCGAGTACAACGTCCATATCCGACACATGATATAG	764

32L07_vesca	GCTATCAAATTATCAAACCCTTTGATCCGATTCGTAGCTTTGACGACTATAAGCTTAG	1619
32L07_viridis	GCTATCAAATTATCAAACCCTTTGATCCGATTCGTAGCTTTGACGACTATAAGCTTAG	824

32L07_vesca	TTAAGTTTAGTAGGACTCACCGAATTTTCGCACTAGTAGGACAAAAGATGGTAAGATTC	1679
32L07_viridis	TTAAGTTTAGTAGGACTCACCGAATTTTCGCACTAGTAGGACGAAAAGATGGTAAGATTC	884

32L07_vesca	CTTTCATTTTCTTCTTTACTATCCTTCTTTTCTCAATTTTCCCTAGAATCCTACAAC	1739
32L07_viridis	CTTTTATTTTCTTCTTTACTATCCTTCTTTTCTCAATTTTCCCTAGAATCCTACAAC	944

32L07_vesca	AAGAAAGGACTTTGGCCCTTGTGCTCCTTTATCATCTTAAAAGCATCACCACCATCCCC	1799
32L07_viridis	AAGAAAGGACTTTGGCCCTTGTGCTCCTTTATCATCTTAAAAGCATCACCACCATCCCC	1004

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32L07_vesca      TATATAGATGCATATTTCACTATCAAGCTACCCAAGTATGCAAATTTATAGCATCTCATT 1859
32L07_viridis    TATTTAGATGCATATTTCACTATCAAGCTACCCAAGTATGCAAATTAATAGCATCTCATT 1064
*** *****

32L07_vesca      TCTTGTTCCTCTAGCTATTCTACTCAATGCATATCAACAACCTGACCCAGTTCTCCTAT 1919
32L07_viridis    TCTTGTTCCTCTAGCTATTCTACTCAATGCATATCAACAACCTGACCCAGTTCTCCTAT 1124
*** *****

32L07_vesca      AATTGCTGGCAGATAGTAATACCAATTACTCCAGAATCTTCACACCCAGAACTTGAAATT 1979
32L07_viridis    AATTGCTGGCAGATAGTAATACCAATTACTCCAGAATCTTCACACCCAGAACTTGAAATT 1184
*** *****

32L07_vesca      ACACGACCTCAATACTCCAACAGTAC-----AAAACAACCCAGATGATCAAAACA 2030
32L07_viridis    ACACGACCTCAATACTCCAACAGTACTGTGTCAGTACAAAACAACCCAGATGATCAAAACA 1244
*** *****

32L07_vesca      CATAACATTCCTTTATTTTCATCTTATTGGGAAAATCTCTATATCTATTATCTTCATTATTC 2090
32L07_viridis    CTTAAAATTCCTTTATTTTCATCTTATTG-----CTATCTCTATCATCTTCATTATTC 1295
* * * * * *****

32L07_vesca      AATTTTCTACTGCTATGCTATACATGTTACAAAAGAGAAAAGAAAGACACTAGTCCAT 2150
32L07_viridis    AATTTTCTACTGCTATGCTATACATGTTACAAAAGAGAAAAGAAAGACACTAGCCAT 1355
*** *****

32L07_vesca      ATCACATAGGCCATGTCCTTCCCAATTCTAACCCAACAATTCAAGGACCACACCCATGAG 2210
32L07_viridis    ATCACATAGGCCATGTCCTTCCCAATTCTAACCCAACAATTCAAGGACCACACCCATGA- 1414
*** *****

32L07_vesca      TAGTGGCACTGAATCACTGAATCGTCGCCTTCACAACTACACTACCTATCCAACCCAGAC 2270
32L07_viridis    --GTGGCACTGAATCACTGAATCGTCACCTTCACAACCACACTACCTATCCAACCCAGAC 1472
*** *****

32L07_vesca      TCAACACAGATGAAAATTCACAGCAGCTAAGAATATAGTACTAGTTTGTCTATCTTTT 2330
32L07_viridis    -----ACAGATGAAAATTCACAGCAGCTAAGAATATAGTACCAATTTTGTCTATCTTTT 1527
*** *****

32L07_vesca      TTCTTTACCAAAACAAAAAACCCCTGTAGTAACCAATATAACCGCTAACAGCTTTTCCC 2390
32L07_viridis    TTCTTTACCAAAACAAAAAGATCCTGTAGTAACCAATATAACAGCTAACAGCTTTTCCC 1587
*** *****

32L07_vesca      ATCCTGCCATAACAGCTTTTCCCCTGCAGTATGGGAAACCCTTATCTAAAACCCCCGA 2450
32L07_viridis    ATCCTGCCATAACAGCTTTTCCCCTGCAGTATGGGAAACCCTGATCTAAA-TCCCCGA 1646
*** *****

32L07_vesca      TTTATAGTAACAAAAAATAAATAAAATAATTTACTTTCCTCATTACCATTTTACCCTC 2510
32L07_viridis    TTTATAGTAACAAAAAATAAATAAAATAATTTGCTTTCCTCATTACCATTTTACCCTC 1706
*** *****

32L07_vesca      ATCTTCTCCTCATTGCCACTTGAACCCCACTCTCCATGCTCCTGAACCTTCTCAACA 2570
32L07_viridis    ATCTTCTCCTCATTGCCACTTGAACCCCACTCTCCATGCTCCTGAACCTTCTCAACA 1766
*** *****

32L07_vesca      CCCTTCTAGGGCAATGTCAAAGCGTCTTTTACCGTCTCCAACCCCTCCTGCGGTTTCG 2630
32L07_viridis    CCCTTCTAGGGCAATGTCAAAGCGTCTTTTACCGTCTCCAACCCCTCCTGCGGTTTCG 1826
*** *****

32L07_vesca      CGTACAGAAAATTCGGTATGTAATCGATAACTTTCTCCCGCATTTTCCT 2679
32L07_viridis    CGTACAGAAAATTCGGTATGTAATCGATAACTTTCTCCCGCATTTTCCT 1875
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34D20

34D20_vesca GCAGAAAAGAACTGATGTGCTTTCCGGAGGGACTGACAGTGAAAAAGGACAGTGCAGTTC 60
34D20_mandshurica NNNNNGAAGAACTGATGTGCTTTCCGGAGGGACTGACAGTGAAAAAGGACAGAGCAGTTC 60
34D20_nilgerrensis NGCAGAAAAGAACTGATGTGCTTTCCGGAGGGACTGACAGTGAAAAAGGACAGTGCAGTTC 60
34D20_iinumae NNNNNNNNNACTGATGTGCTTTCCGGAGGGACTGACAGTAGAAAAGGACAGTGCAGTTC 60
34D20_ananassa NNNNNNNNNACTGATGCGCTTTCCGGAGGGACTGACAGTGAAAAAGGACAGTGCAGTTC 60
34D20_viridis NNNGAGAAAGAACTGATGTGCTTTCCGGAGGGACTGACAGTGAAAAAGGACAGTGCAGTTC 60
34D20_nubicola NNN 60

34D20_vesca AGGGGATAAAGGAAGTATTAATGTTAGGCATCCAAGACGGCATCTGGTTTTGGAGTCCCT 120
34D20_mandshurica AGGGGATAAAGGAAGTATTAATGTTAGGCATCCAAGACGGCATCTGGTTTTGGAGTCCCT 120
34D20_nilgerrensis AGGGGATAAAGGAAGTATTA-TGTTAGGCATCCAAGACGGCATCTGGTTTTGGAGTCTCT 119
34D20_iinumae AGGGGATAAAGGAAGTATTAATGTTAGGCATCCAAGACGGCATCTGGTTTTGGAGTCCCT 120
34D20_ananassa ATGGGATAAAGGAAGTATTAATGTTAGGCATCCTAGACGGCATCTGGTTTTGGAGTCCCT 120
34D20_viridis AGGAGATAAAGGAAGTATTAATGTTAGGCATCCAAGACGGCATCTGGTTTTGGAGTCCCT 120
34D20_nubicola NNNNNNNNNNNNNNNNNNNNATGTT-GGCATCC-AGACGGCATCTGGTTTTGGAGTCCCT 118
**** * 60

34D20_vesca CTCCAAGAAATGGAGCAAGTCCTACTTCCTACGCGAATTTGATTTCTACAAGGTGAGCAA 180
34D20_mandshurica CTCCAAGAAATGGAGCAAGTCCTACTTCCTACGCGAATTTGATTTCTACAAGGTGAGCAA 180
34D20_nilgerrensis CTCCAAGAAATGGAGCAAGTCCTACTTCCTACGCGAATTTGATTTCTACAAGGTGAGCAA 179
34D20_iinumae CTCCAAGAAATGGAGCAAGTCCTACTTCCTACGCGAATTTGATTTCTACAAGGTGAGCAA 180
34D20_ananassa CTCCAAGAAATGGAGCAAGTCCTACTTCCTACGCGAATTTGATTTCTACAAGGTGATCAA 180
34D20_viridis CTCCAAGAAATGGAGCAAGTCCTACTTCCTACGCGAATTTGATTTCTACAAGGTGAGCAA 180
34D20_nubicola CTCCAAGAAATGGAGCAAGTCCTACTTCCTACGCGAATTTGATTTCTACAAGGTGAGCAA 178
***** * 60

34D20_vesca CATGCCTGCAAACCTAGATATATTTTGTTTTTCTTACTATTACAGTGTGTGTTATGTGAAT 240
34D20_mandshurica CATGCCTGCAAACCTAGATATATTTTGTTTTTCTTACTATTACAGTGTGTGTTATGTGAAT 240
34D20_nilgerrensis CATGCCGGCAAACCTAGTTATATTTTGTTTTTCTTACTATTACAGTGTGTGTTATGTGAAT 239
34D20_iinumae CATGCCTGCAAACCTAGATATATTTTGTTTTTCTTACTATTACAGTGTGTGTTATGTGAAT 240
34D20_ananassa CATGCCTGCACACTAGATATATTTTGTTTTTCTTACTATTACAGTGTGTGTTATGTGAAT 240
34D20_viridis CATGCCTGCAAACCTAGATATATTTTGTTTTTCTTACTATTACAGTGTGTGTTATGTGAAT 240
34D20_nubicola CA-GCCTGCAAACCTAGATATATTTTGTTTTTCTTACTATTACAGTGTGTGTTATGTGAAT 237
** * 60

34D20_vesca CATCTGCATATTATCTATATCTAACTCTATGGTATAATCATTTCAGAACTACAAGGAAAGA 300
34D20_mandshurica CATCTGCAAATTTATCTATATCTAACTCTATGGTATAATCATTTCAGAACTACAAGGAAAGA 300
34D20_nilgerrensis CATCTGCATATTATCTATATCTAACTCTATGGTATAATCATTTCAGAACTACAAGGAAAGA 299
34D20_iinumae CATCTGCATATTATCTATATCTAACTCTATGGTATAATCATTTCAGAACTACAAGGAAAGA 300
34D20_ananassa CATCTGCATATTATCTATATCTAACTCTATGGTATAATCATTTCAGAACTACCAGGAAAGA 300
34D20_viridis CATCTGCATATTATCTATATCTAACTCTATGGTATAATCATTTCAGAACTACAAGGAAAGA 300
34D20_nubicola CATCTGCATATTATCTATATCTAACTCTATGGTATAATGATTTCAGAACTACAAGGAAAGA 297
***** * 60

34D20_vesca TTATCGGCGAGAAGGTGTTTTGCATGCAAGCAGCAGAAAATGCTATGGGCCAATTTCCCT 360
34D20_mandshurica TTATCGGCGAGAAGGTGTTTTGCATGCAAGCAGCAGAAAATGCTATGGGCCAATTTCCCT 360
34D20_nilgerrensis TTATCGGCGAGAAGGTGTTTTGCATGCAAGCAGCAGAAAATGCTATGGGCCAATTTCCCT 359
34D20_iinumae TTATCGGCGAGAAGGTGTTTTGCATGCAAGCAGCAGAAAATGCTATGGGCCAATTTCCCT 360
34D20_ananassa TTATCGGCAAGAAGGTGTTTTGCATGCATGCAGCACAAAATGCTATGGGCCAATTTCCCT 360
34D20_viridis TTATCGGCGAGAAGGTGTTTTGCATGCAAGCAGCAGAAAATGCTATGGGCCAATTTCCCT 360
34D20_nubicola TTATCGGCGAGAAGGTGTTTTGCATGCAAGCAGCAGAAAATGCTATGGGCCAATTTCCCT 357
***** * 60

34D20_vesca TGCAAACACTTGCTATGGTGAATGACTGCAAGTTGCGGGCAATAGGGGCTTCGGGTCTT 420
34D20_mandshurica TGCAAACACTTGCTATGGTGAATGACTGCAAGTTGCGGGCAATAGGGGCTTCGGGTCTT 420
34D20_nilgerrensis TGCAAACACTTGCTATGGTGAATGACTGCAAGTTGCGGGCAATAGGGGCTTCGGGTCTT 419
34D20_iinumae TGCAAACACTTGCTATGGTGAATGACTGCAAGTTGCGGGCAATAGGGGCTTCGGGTCTT 420
34D20_ananassa TGCAAACACTTGCTATGGTGAATGACTGCAAGTTGCGGGCAATAGGGGCTTCGGGTCTT 420
34D20_viridis TGCAAACACTTGCTATGGTGAATGACTGCAAGTTACGGGCAATAGGGGCTTCGGGTCTT 420
34D20_nubicola TGCAAACACTTGCTATGGTGAATGACTGCAAGTTGCGGGCAATAGGGGCTTCAGGCCCTT 417
***** * 60

34D20_vesca TTCCCTCACA... 480
34D20_mandshurica TTCCCTCACA... 480
34D20_nilgerrensis TTCCCTCACA... 479
34D20_iinumae TTCCCTCACA... 480
34D20_ananassa TTCCCTCACA... 480
34D20_viridis TTCCCTCACA... 480
34D20_nubicola TTCCCTCACA... 477

34D20_vesca TTAAGTAGTAACTGAGGAATCCAATTGCAATCTGTGCTTTCCATGCACAGGAGA... 540
34D20_mandshurica TTAAGTAGTAACTGAGGAATCCAATTGCAATCTGTGCTTTCCATGCACAGGAGA... 540
34D20_nilgerrensis TTAAGTAGTAACTGAGGAATCCAATTGCAATCTGTGCTTTCCATGCACAGGAGA... 539
34D20_iinumae TTAAGTAGTAACTGAGGAATCCAATTGCAATCTGTGCTTTCCATGCACAGGAGA... 539
34D20_ananassa TTAAGTAGTAACTGAGGAATCCAATTGCAATCTGTGCTTTCCATGCACAGGAGA... 540
34D20_viridis TTAAGTAGTAACTGAGGAATCCAATTGCAATCTGTGCTTTCCATGCACAGGAGA... 540
34D20_nubicola TTAAGTAGTAACTGAGGAATCCAATTGCAATCTGTGCTTTCCATGCACAGGAGA... 537
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34D20_vesca GGTGAACCGTATGTCTATATAGATATGTCGTATGTTAGATAGGATACATAGTATGTGGGT 600
34D20_mandshurica GGTGAACCGTATGTCTATATAGATATGTCGTATGTTAGATAGGATACATAGTATGTGGGT 600
34D20_nilgerrensis GGTGAACCGTATGTCTATATAGATATGTCGTATGTTAGATAGGATACATAGTATGTGGGT 599
34D20_iinumae GGTGAACCGTATGTCTATATAGATATGTCGTATGTTAGATAGGATACATAGTATGTGGGT 599
34D20_ananassa GGTGAACCGTATGTCTATATAGATATGTCGTATGTTAGATAGGATACATAGTATGTGGGT 600
34D20_viridis GGTGAACCGTATGTCTATATAGATATGTCGTATGTTAGATAGGATACATAGTATGTGGGT 600
34D20_nubicola GGTGAACCGTATGTCTATATAGATATGTCGTATGTTAGATAGGATACATAGTATGTGGGT 597

34D20_vesca GTGGATGA... 660
34D20_mandshurica GTGGATGA... 660
34D20_nilgerrensis GTGGATGA... 659
34D20_iinumae GTGGATGA... 659
34D20_ananassa GTGGATGA... 660
34D20_viridis GTGGATGA... 660
34D20_nubicola GTGGATGA... 657
*** *****

34D20_vesca TGGGTATGAATCTCCCTCCCGGCCACACTAGACCACACTTTTGA... 720
34D20_mandshurica TGGGTATGAATCTCCCTCCCGGCCACACTAGACCACACTTTTGA... 720
34D20_nilgerrensis TGGGTATGAATCTCCCTCCCGGCCACACTAGACCACACTTTTGA... 719
34D20_iinumae TGGGTATGAATCTCCCTCCCGGCCACACTAGACCACACTTTTGA... 695
34D20_ananassa TGGGTATGAATCTCCCTCCCGGCCACACTAGACCACACTTTTGA... 720
34D20_viridis TGGGTATGAATCTCCCTCCCGGCCACACTAGACCACACTTTTGA... 720
34D20_nubicola TGGGTATGAATCTCCCTCCCGGCCACACTAGACCACACTTTTGA... 717
**** *****

34D20_vesca CGTCCTAGATTTTGTGCCGACTATCACAATAGTGAATTAAGTTGGTCTCCTAGCCATA 780
34D20_mandshurica CGTCCTAGATTTTGTGCCGACTATCACAATAGTGAATTAAGTTGGTCTCCTAGCCATA 780
34D20_nilgerrensis CGGCCTAGATTTTGTGCCGACTATCACAATAGTGAATTAAGTTGGTCTCCTAGCTATA 775
34D20_iinumae ---CTAGATTTTGTGCCGACTATCACAATAGTGAATTAAGTTGGTCTCCTAGCTATA 747
34D20_ananassa CGGCCTAGATTTTGTGCCGACTATCACAATAGTGAATTAAGTTGGTCTCCTAGCTATA 776
34D20_viridis CGGCCTAGATTTTGTGCCGACTATCACAATAGTGAATTAAGTTGGTCTCCTAGCTATA 776
34D20_nubicola CGGCCTAGATTTTGTGCCGACTATCACAATAGTGAATTAAGTTGGTCTCCTAGCTATA 773

34D20_vesca GTTTCCTAGTACTATTCTACTGATATCATGTATTGCCTCAGCTTTTGGACAATGGAATATGA 840
34D20_mandshurica GTTTCCTAGTACTATTCTACTGATATCATGTATTGCCTCAGCTTTTGGACAATGGAATATGA 840
34D20_nilgerrensis GTTTCCTAGTACTATTCTACTGATATCATGTATTGCCTCAGCTTTTGGACAATGGAATATGA 835
34D20_iinumae GTTTCCTAGTACTATTCTACTGATATCATGTATTGCCTCAGCTTTTGGACAATGGAATATGA 807
34D20_ananassa GTTTCCTAGTACTATTCTACTGATATCATGTATTGCCTCAGCTTTTGGACAATGGAATATGA 836
34D20_viridis GTTTCCTAGTACTATTCTACTGATATCATGTATTGCCTCAGCTTTTGGACAATGGAATATGA 836
34D20_nubicola GTTTCCTAGTACTATTCTACTGATATCATGTATTGCCTCAGCTTTTGGACAATGGAATATGA 833

34D20_vesca TGAATTTGGAATGAATACAAAACCTGCTTTGTCCATCTATTAGCATTTTCTGAAACCCAA 900
34D20_mandshurica TGAATTTGGAATGAATACAAAAGACTGCTTTGTCCATCTATTAGCATTTTCTGAAACCCAA 900
34D20_nilgerrensis TGAATATGGAAC-----AAAGCTGCTTTGTCCATCTATTAGCATTTTCTGAAACCCAA 888
34D20_iinumae TGAATATGGAATGA--ACAAAACCTGCTTTGTCCATCTATTAGCATTTTCTGAAACCCAA 865
34D20_ananassa TGGATATGGAATGA--ACAAAACCTGCTTTGTCCATCTATTAGCATTTTCTGAAACCCAA 894
34D20_viridis TGAATATGGAATGA--ACAAAAGCTGCTTTGTCCATCTTTTAGCATTTTCTGAAACCCAA 894
34D20_nubicola TGAATATGGAATGA--ACAAAAGCTGCTTTGTCCATCTGTTAGCATTTTCTGAAACCCAA 891
** ** ***** ** *****
34D20_vesca AAGATGGGTACATGTTTGCTTATTCTCTTTATCTAGTGCATCATGTGAGTTATCAAGTTC 960
34D20_mandshurica AAGATGGGTACATGTTTGCTTATTCTCTTTATCTAGTGCATCATGTGAGTTATCAAGTTC 960
34D20_nilgerrensis AAGATGGGTACATGTTTGCTTATTCTCTTTATCTAGTGCATCATGTGAGTTATCAAGTTC 948
34D20_iinumae AAGATGGGTACATGTTTGCTTATTCTCTTTATCTAGTGCATCATGTGAGTTATCAAGTTC 925
34D20_ananassa AAGATGGGTACATGTTTGCTTATTTCTTTATCTAGTGCATCATGTGAGTTATCAAGTTC 954
34D20_viridis AAGATGGGTACATGTTTGCTTATTCTCTTTATCTAGTGCATCATGTGAGTTATCAAGTTC 954
34D20_nubicola AAGATGGGTACATGTTTGCTTATTCTCTTTATCTAGTGCATCATGTGAGTTATCAAGTTC 951

34D20_vesca ATGTTTATGCATCTCTGCTGATTTAGGAATTAGGATTGCAGTACTTGTATAGTTGTATTGA 1020
34D20_mandshurica ATGTTTATGCATCTCTGCTGATTTAGGAATTAGGATTGCAGTACTTGTATAGTTGTATTGA 1020
34D20_nilgerrensis ATGCTATGCATCTCTGCTGATTTAGGAATAAGGATTGCAGTACTTGTATAGTTGTATTGA 1008
34D20_iinumae ATGTTTATGCATCTCTGCTGATTTAGGAATTAGGATTGCAGTACTTGTATAGTTGTATTGA 985
34D20_ananassa ATGTTTATGCATCTCTGCTGATTTAGGATTAGGATTGCAGTACTTGTATAGTTGTATTGA 1014
34D20_viridis ATGTTTATGCATCTCTGCTGATTTAGGAATTAGGATTGCAGTACTTGTATAGTTGTATTGA 1014
34D20_nubicola ATGTTTATGCATCTCTGCTGATTTAGGAATTAGGATTGCAGTACTTGTATAGTT--ATTGA 1009
**** *
34D20_vesca TCTGATATAACATAAAATTTAATGAATCTAATAGACATTTT-CCTAGTTAACAGAGGATA 1079
34D20_mandshurica TCTGATATAACATAAAATTTAATGAATCTAATAGACATTTT-CCTAGTTAACAGAGGATA 1079
34D20_nilgerrensis TCTGATATAACATAAAATTTAATGAATCTAATATAAAATTTT-CCTAGTTAAG----- 1059
34D20_iinumae TCT-----AAATTTT-CCTAGTTAACAGAGGATA 1014
34D20_ananassa TCT-----AAATTTT-CCTAGTTAACAGAGGATA 1043
34D20_viridis TCTGATATAACTCCATT-AATGAATCTAATATAAAATTTTTCCTAGTTAACAGAGGATA 1073
34D20_nubicola TCTAATATAACATAAAATTTAATGAATCTAATATAAAATTTT-CCTAGTTAAG----- 1060
*** *
34D20_vesca GGTCTCCGGCTGACCTTATCCTACAAGGAAATAGAAACGTACAATTAACGCATTATACAC 1139
34D20_mandshurica GGCTCCGGCTGACCTTATCCTACAAGGAAATAGAAACGTACAATTAACGCATTATACAC 1139
34D20_nilgerrensis -----CCAAAAGACCTTATCCTACAAGGAAACAGAAACGTACAATTAACGCATTATACAC 1114
34D20_iinumae GGTCTCCGGCTGACCTTATCCTACAAGGAAACAGAAACGTACAATTAACGCATTATACAC 1071
34D20_ananassa GGACTCCGGCTGACCTTATCCTACAAGGAAACAGAAACGTACAATTAACGCATTATACAC 1100
34D20_viridis GGTCTCCGGCTGACCTTATCCAACAAGGAAACAGAAACATAACAATTAACGCATTATACAC 1133
34D20_nubicola -----TCAAAAGACCTTATCCTACAAGGAAACAGAAACGTACAATTAACGCATTATACAC 1115
* **
34D20_vesca AAGACTGGTCTATATAAGGCATCAAATCTCTTTATCTGTTTCATTGATCATATTGTCCT 1199
34D20_mandshurica AAGACTGGTCTATATAAGGCATCAAATCTCTTTATCTGTTTCATTGATCATATTGTCCT 1199
34D20_nilgerrensis AAGACTGGTCTATATAAGGCATCAAATCTCTTTATCTGTTTCATTGATCATATTGTCCT 1154
34D20_iinumae AAGACTGGTCTATATAAGGCATCAAATCTCTTTATCTGTTTCATTGATCATATTGTCCT 1111
34D20_ananassa AAGACTGGTCTATATAAGGCATCAAATCTCTTTATCTGTTTCATTGATCATATTGTCCT 1140
34D20_viridis AAGACTGGTCTATATAAGGCATCAAATCTCTTTATCTGTTTCATTGATCATATTGTCCT 1173
34D20_nubicola AAGACTGGTCTATATAAGGCATCAAATCTCTTTATCTGTTTCATTGATCATATTGTCCT 1155

34D20_vesca CTTTATCTGTTTTCATACTTTCAT--TGATCATATTGTCCTAGTACTGGAAGAGCTATATTT 1257
34D20_mandshurica CTTTATCTGTTTTCATACTTTCAT--TGATCATATTGTCCTAGTACTGGAAGAGCTATATTT 1257
34D20_nilgerrensis -----TTCAT--TGATCATATTGTCCTAGTACTGGAAGAGCTATATTT 1194
34D20_iinumae -----TTCAT--TGATCATATTGTCCTAGTACTGGAAGAGCTATATTT 1151
34D20_ananassa -----TTCAT--TGATCATATTGTCCTAGTACTGGAAGAGCTATATTT 1180
34D20_viridis -----TTCAT--TGATCATATTGTCCTAGTACTGGAAGAGCTATATTT 1213
34D20_nubicola -----TTCATATTGTCCTAGTACTGGAAGAGCTATATTT 1197

34D20_vesca ATCAGATAACAGAAAGTGCTTACTTGCTGGTTCATACTCAATATGGATCCGAAGGTCCTT 1317
34D20_mandshurica ATCAGATAACAGAAAGTGCTTACTTGCTGGTTCATACTCAATATGGATCCGAAGGTCCTT 1317
34D20_nilgerrensis ATCAGATAACAGAAAGTGCTTACTTGCTGGTTCATACTCAATATGGATCCGAAGGTCCTT 1254
34D20_iinumae ATCTGATAACAGAAAGTGCTTACTTGCTGGTTCCTACTCATTATGGATCCGAAGGTCCTT 1211
34D20_ananassa ATCTGATAACAGAAAGTGCTTACTTGCTGGTTCATACTCAATATGGATCCGAAGGTCCTT 1240
34D20_viridis ATCTGATAACAGAAAGTGCTTACTTGCTGGTTCATACTCAATATGGATCCGAAGGTCCTT 1273
34D20_nubicola ATCTGATAACAGAAAGTGCTTACTTGCTGGTTCATACTCAATATGGATCCGAAGGTCCTT 1257
*** ***** **

34D20_vesca AGTTACAATGGTGTGACCTGAGCATGAGCGACTTGGATCTTCTTAGAGGCCCTTGTTAC 1377
34D20_mandshurica AGTTACAATGGTGTGACTTGAGCATGAGCGACTTGGATCTTCTTAGAGTCCCTTGTTAC 1377
34D20_nilgerrensis AGTTACAATGGTGTGACCTGAGCATGAGCGACTTGGATCTTCTTAGAGGCCCTTGTTAC 1314
34D20_iinumae AGTTACAAGGTGTGACCTGAGCATGAGCGACTAGGATATTCTTAGAGGACCTTATTAC 1271
34D20_ananassa AGTTCCAAGGTGTGACCTGAGAATGAGCGACTTGGATCTTCTTAGAGGCCCTTATTAC 1300
34D20_viridis AGTTACAATGGTGTGACCTGAGCATGAGCGACTTGGATCTTCTTAGAGGCCCTTATTAC 1333
34D20_nubicola AGTTACAAGGTGTAGACCTGAGCATGAGCGACTTGGATCTTCTTAGAGGCCCTTGTTAC 1317
**** * * ***** ** ***** **

34D20_vesca TTAACCGATAGCATCATTGATTCTATTTCACTCATCTTACTTCCCATTATGATGATGAT 1437
34D20_mandshurica TTAACCGATAGCATCATTGATTCTATTTCACTCATCTTACTTCCCATTATGATGATGAT 1437
34D20_nilgerrensis TTAACCGATAGCATCATT-----CACTTATCTTACTTCCCATTATGATGATGAT 1363
34D20_iinumae TTAACCGATAGCATCATTGATTCTATTTCACTTATCTTACTTCCCATTATGATGATGAT 1331
34D20_ananassa TTAACCGATAGCATCATTCAATTTCTATTTCACTTATCTTACTTCCCATTATGATGATGAT 1360
34D20_viridis TTAACCGATAGCATCATTGATTCTATTTCACTTATCTTACTTCCCATTATGATGATGAT 1393
34D20_nubicola TTAACCGATAGCATCATTGATTCTATTTCACTCATCTTACTTCCCATTATGATGATGAT 1377
***** *****

34D20_vesca ATCCTTCTGGTTTCCCCTAATATCTCTGATCTTCTGGTAAATTCTCCGGATCCCGAGGAT 1497
34D20_mandshurica ATCCTTCTGGTTTCCCCTAATATCTCTGATCTTCTGGTAAATTCTCCGGATCCCGAGGAT 1497
34D20_nilgerrensis ATCCTTCTGGTTTCCCCTAATATCTCTGATCTTCTGGTAAATTCTTGGATCCTGAGGAT 1423
34D20_iinumae ATCCTTCTGGTTTCCCCTAATATCTCTGATCTTCTGGTAAATTCTCCGGATCCCGAGGAT 1391
34D20_ananassa ATCCTTCTGGTTTCCCCTAATATCTCTGATTTTCTGGTAAATTCTCCGGATCCCGAGGAT 1420
34D20_viridis ATCCTTCTGGTTTCCCCTAATATCTCTGATCTTCTGGTAAATTCTCCGGATCCCGAGGAT 1453
34D20_nubicola ATCCTTCTGGTTTCCCCTAATATCTCTGATCTTCTGGTAAATTCTCCGGATCCCGAGGAT 1437
***** * ***** **

34D20_vesca GAGCTTAGAGCCTTTGCGGAGTCTGACCAACTTGGTAAAAGGAAAGTTGTGATCTTCGCA 1557
34D20_mandshurica GAGCTTAGAGCCTTTGCGGAGTCTGACCAACTTGGTAAAAGGAAAGTTGTGATCTTCGCA 1557
34D20_nilgerrensis GAGCTTAGAGCCTTTGCGGAGTCTGACCAACTTGGTAAAAGGAAAGTTGTGATCTTCGCA 1483
34D20_iinumae GAGCTTAGAGCCTTTGCGGAGTCTGACCAACTTGGTAAAAGGAAAGTTGTGATCTTCGCA 1451
34D20_ananassa GAGCTTAGAGCCTTTGCGGAGTCTGACCAACTTGGTAAAAGGAAAGTTGTGATCTTCGCA 1480
34D20_viridis GAGCTTAGAGCCTTTGCGGAGTCTGACCAACTTGGTAAAAGGAAAGTTGTGATTTGCA 1513
34D20_nubicola GAGCTTAGAGCCTTTGCGGAGTCTGACCAACTTGGTAAAAGGAAAGTTGTGATCTTCGCA 1497
***** **

34D20_vesca GTGAATGATAACAAAGATCCGAGTCTGAAAGCGACGGCGGAAACCATTGGAGCTTGCTGGTG 1617
34D20_mandshurica GTGAATGATAACAAAGATCCGAGTCTGAAAGCGACGGCGGAAACCATTGGAGCTTGCTGGTG 1617
34D20_nilgerrensis GTGAATGATAACAAAGATCCGAGTCTGAAAGCGACGGCGGAAACCATTGGAGCTTGCTGGTG 1543
34D20_iinumae GTGAATGATAACAAAGATCCGAGTCTGAAAGCGACGGCGGAAACCATTGGAGCTTGCTGGTG 1511
34D20_ananassa GTGAATGATAACAAAGATCCGAGTCTGAAAGCGACGGCGGAAACCATTGGAGCTTGCTGGTG 1540
34D20_viridis GTGAATGATAACGAAGATCCGAGTCTGAAAGCGACGGCGGAAACCATTGGAGCTTGCTGGTG 1573
34D20_nubicola GTGAATGATAACGAAGATCCGAGTCTGAAAGCGACGGTGGAAACCATTGGAGCTTGCTGGTG 1557

34D20_vesca TATTTTCAGAAAATCAAACGCATTTCGTACATTACGACAGCTTGGGGGTAACAATAGTTTG 1677
34D20_mandshurica TATTTTCAGAAAATCAAACGCATTTCGTACATTACGACAGCTTGGGGGTAACAATAGTTTG 1677
34D20_nilgerrensis TATTTTCAGAAAATCAAACGCATTTCGTACACTACGACAGCTTGGGGGTAACAATAGTTTG 1603
34D20_iinumae TATTTTCAGAAAATCAAACGCATTTCGTACATTACGACAGCTTGGGGGTAACAATAGTTTG 1571
34D20_ananassa TATTTTCAGAAAATCAAACGCATTTCGTACATTACGACAGCTTGGGGGTAACAATAGTTTG 1600
34D20_viridis TATTTTCAGAAAATCAAACGCATTTCGTACATTACGACAGCTTGGGGGTAACAATAGTTTG 1633
34D20_nubicola TATTTTCAGAAAATCAAACGCATTTCGTACATTACGACAGCTTGGGGGTAACAATAGTTTG 1617

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34D20_vesca GAAGCTAGGAAAATGTATACAGTATTCAAGAAACTTGTGGCTGCTCCAGCAACACAAGCA 1737
34D20_mandshurica GAAGCTAGGAAAATGTATACAGTATTCAAGAAACTTGTGGCTGCTCCAGCAACACAAGCA 1737
34D20_nilgerrensis GATGCTAGGAAAATGTATACAGTATTCAAGAAACTTGTGGCAGCTCCAGCAACACAAGCA 1663
34D20_iinumae GAAGCTAGGAAAATGTATACAGTATTCAAGAAACTTGTGGCTGCTCCAGCAACACAAGCA 1631
34D20_ananassa GATGCTAGGAAAATGTATACAGCATTCAAGAAACTTGTGGCAGTTCAGCAACACAAGCA 1660
34D20_viridis GAAGCTAGGAAAATGTATACAGTATTCAAGAAACTTGTGGCTGCTCCAGCAACACAAGCA 1693
34D20_nubicola GAAGCTAGGAAAATGTATACAGTATTCAAGAAACTTGTGGCTGCTCCAGCAACACAAGCA 1677
** *****

34D20_vesca CCAATAACTCCAGCTGGGACTAGTAGTTTGGCTACCAACAACAGTTCTACAATGAGACAC 1797
34D20_mandshurica CCAATAACTCCAGCTGGGACTAGTAGTTTGGCTACCAACAACAGTTCTACAATGAGACAC 1797
34D20_nilgerrensis CCAA---CTCCATCTGGGACTAGTAGTTTGGTTACCAACAACAGTTCTACAATGCGACAC 1720
34D20_iinumae CCAATAACTCCAGCTGGGACTAGTAGTTTGGCTACCAACAACAGTTCTACAATGAGACAC 1691
34D20_ananassa CCAA---CTCCAGCTGGGACTAGTAGTTTGGTTACCAACAACAGTTCTACAATGGGACAC 1717
34D20_viridis CCAATAACTCCAGCTGGGACTAGTAGTTTGGTTACCAACAACAGTTCTACAATGAGACAC 1753
34D20_nubicola CCAATAACTCCAGCTGGGACTAGTAGTTTGGTTACCGACAACAGTTCTACAATGAGACAC 1737
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34D20_vesca GAGTGCCACTCTACGCAGTCGCGCGGATTTATAGACTATACCAAGACAATGCTTGGGGTT 1857
34D20_mandshurica GAGTGCCACTCTACGCAGTCGCGCGGATTTATAGACTATACCAAGACAATGCTTGGGGTT 1857
34D20_nilgerrensis GAGTGCTACTCTACGCGGTCGCGCGGATTTATAGACTATACCAAGACAATGCTTGGGGTT 1780
34D20_iinumae GAGTGCCACTCTACGCAGTCGCGCGGATTTATAGACTATACCAAGACAATGCTTGGGGTT 1751
34D20_ananassa GAGTGCTACTCTACGCAGTCGCGCGGTTTATAGACCATAACCAAGATAATGCTTCGGGTT 1777
34D20_viridis GAGTGCCACTCTACGCAGTCGCGCGGATTTATAGACTATACCAAGACAATGCTTGGGGTT 1813
34D20_nubicola GAGTGCCACTCTACGCAGTCGCGCGGATTTATAGACTATACCAAGACAATGCTTGGGGTT 1797
*****

34D20_vesca TGGGGTTTTGTTGTCAACTACATTTTGTCAAAGTACTTGCGTCTGTTTGGAAATTATCAT 1917
34D20_mandshurica TGGGGTTTTGTTGTCAACTACATTTTGTCAAAGTACTTGCGTCTGTTTGGAAATTATCAT 1917
34D20_nilgerrensis TGGGGTTTTGTTGTCAATTACATTTTGTCAAAGTACTTGCGTCTGTTTGGAAATTATCAT 1840
34D20_iinumae TGGGGTTTTGTTGTCAACTACATTTTGTCAAAGTACTTGCGTCTGTTTGGAAATTATCAT 1811
34D20_ananassa GGGGGTTTTGTTGTCAAATACATTTTGTCAAAGTACTTGCGTCTGTTTGGAAATTATCAT 1837
34D20_viridis TGGGGTTTTGTTGTCAACTACATTTTGTCAAAGTACTTGCGTCTGTTTGGAAATTATCAT 1873
34D20_nubicola TGGGGTTTTGTTGTCAACTACATTTTGTCAAAGTACTTGCGTCTGTTTGGAAATTATCAT 1857
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34D20_vesca TATCATCCTTCGGAAGTGTGCTATCCCATGCAAAAAATCACCAATAGTA---ATCATGGA 1974
34D20_mandshurica TATCATCCTTCGGAAGTGTGCTATCCCATGCAAAAAATCACCAATAGTA---ATCATGGA 1974
34D20_nilgerrensis TATC---CTTCGAAAGTGTGTTATCCCATGCAAAAAGATCACCAATAGTAGTAATCATGGA 1897
34D20_iinumae TATCATCCTTCGGAAGTGTGCTATCCCATGCAAAAAATCACCAATAGTA---ATCATGGA 1868
34D20_ananassa CATCATCCTTCGGAAGTGTGTTATCCCATGCAAAAAGATCACCAATAGTA---ATCATGGA 1894
34D20_viridis TATCATCCTTCGGAAGTGTGTTATCCCATGCAAAAAATCACGAATGGTA---ATCATGGA 1930
34D20_nubicola TATCATCCTTCGGAAGTGTGTTATCCCATGCAAAAAATCACCAATAGTA---ATCATGGA 1914
*** *****

34D20_vesca GATGATGA-----TGATGTTAATGAACCTTGGTATAGAGAAG-AGACTCT 2018
34D20_mandshurica GATGATGA-----TGATGTTAATGAACCTTGGTATAGAGAAG-AGACTCT 2018
34D20_nilgerrensis GATGATGAAGAGCGTAATGATGATGTTAATGAACCTTGGTATAGAGAAG-AGACCGT 1956
34D20_iinumae GATGATGA-----TGATGTTAATGAACCTTGGTATAGAGAAG-AGACTCT 1912
34D20_ananassa GATGATGAAGAGCTTAATGATGATGTTAATGAACCTTGGTATAGAGAAG-AGACTCT 1953
34D20_viridis GGTGATGA-----TGATGTTAATGAACCTTGGTATAGAGAAG-AGACTCT 1974
34D20_nubicola GATGATGA-----TGATGTTAATGAACCTTGGTATAGAGAAGGAGACTCT 1959
* *****

34D20_vesca TATGCCTCAGCAGACGAATTTTACGACTGCG 2050
34D20_mandshurica TATGCCTCAGCAGACGAATTTT-ACGAGTGCG 2049
34D20_nilgerrensis TATGCCTCAGCAGACGAATTT--ACGACTNNN 1986
34D20_iinumae T-TGCCTCAGCAGACGAATTT--ACGACTNNN 1941
34D20_ananassa TATGCCTCGGCAGACGAATTTT-ACGACTGCG 1984
34D20_viridis TATGCCTCAGCAGACGAATTT--ACGAC-GCG 2003
34D20_nubicola TATGCCTCAGCAGACGAATTT--ACGAC-GCA 1987
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40M11

40M11_vesca	CAACATTTGGTGGCCTTCTTGACATTCCAGTTTCTGGCCCTCAGATGCCTTGCAATGGA	60
40M11_mandshurica	CAACATTTGGTGGCCTTCTTGACATTCCAGTTTCTGGCCCTCAGATGCCTTGCAATGGA *****	60
40M11_vesca	TGCATCAGAACAGTATGTGGACAGCTTCTCGGGTACTGCCTTTAACAAATTTCTCACCTC	120
40M11_mandshurica	TGCATCAGAACAGTATGTGGACAGCTTCTCGGGTACTGCCTTTAACAAATTTCTCACCTC *****	120
40M11_vesca	ATTAATCTGCAAACAATAAGATTTTTTAGGCAAAGCAGAACTATGAGTTCCCCAAACTAA	180
40M11_mandshurica	ATTAATCTGCAAACAATAAGATTTTTTAGGCAAAGCGGAAGTATGAGTTCCCCAAACTAA *****	180
40M11_vesca	TAGCTTTCAAACAAGTAGAGGAGCACATTTACTAAAGATACCTTTGCCTGCTGCTCTTCA	240
40M11_mandshurica	TAGCTTTCAAACAAGTAGAGGAGCACATTTACTAAAGATACCTTTGCCTGCTGCTCTTCA *****	240
40M11_vesca	CTTGTTAAAATACTCTCAGAGCCATTTGAGGAAGATTTTTTTATTCCCGCACTCATAGTT	300
40M11_mandshurica	CTTGTTAAAATACTCTCAGAGCCATTTGAGGAAGATTTTTTTATTCCCGCACTCATAGTT *****	300
40M11_vesca	TTGAGGGGAAACTCTGCAAATCAACAATGGAGATTTCAAACCTTATGTCCTAGTTTCACA	360
40M11_mandshurica	TTGAGGGGAAACTCTGCAAATCAACAATGGAGATTTCAAACCTTATGTCCTAGTTTCACA *****	360
40M11_vesca	GTTCCCTTCGGTCTCCCATCACCATCAAATACAATAAATTTCAATATATTTAACAAAAAA	420
40M11_mandshurica	GTTCCCTTCGGTCTCCCATCACCATCAAATACAATAAATTTCAATATATTTAACAAAAAA *****	420
40M11_vesca	ATTGCTCTTCATCCACAAAACACAGAGTCTCATCTTCATTGTTCAATATATCATTTGA	480
40M11_mandshurica	ATTGCTCTTCATCCACAAAACACAGAGTCTCATCTTCATTGTTCAATATATCATTTGA *****	480
40M11_vesca	AATTAACAACCTTTTATTCTTCTAGTCAACCACATTTGCGAGCTACTTGTTAACTCATAA	540
40M11_mandshurica	AATTAACAACCTTTTATTCTTCTAGTCAACCACATTTGCGAGCTACTTGTTAACTCATAA *****	540
40M11_vesca	ACCCTTTCTTCCGATCCATAGCTATCAAATATCCAATCTAAACGAGACTACTACTTTGTT	600
40M11_mandshurica	ACCCTTTCTTCCGATCCATAGCTATCAAATATCCAATCTAAACGAGACTACTACTTTGTT *****	600
40M11_vesca	CACAACGAATCCAACACAAAAGGATCAAAAAACCATCCAAAACCTCATGCACAACATAAT	660
40M11_mandshurica	CACAACGAATCCAACACAAAAGGATCAAAAAACCATCCAAAACCTCATGCACAACATAAT *****	660
40M11_vesca	CAACCAAATATTTAACCACAAAAACAAGCACAAATTCTCCAAGTACAAAAAGAAATGGG	720
40M11_mandshurica	CAACCAAATATTTAACCACAAAAACAAGCACAAATTCTCCAAGTACAAAAAGAAATGGG *****	720
40M11_vesca	CTTTAGACACCAGGAAGGCATATCAAACCGGCCACACACGTTAAAGGGATACAAAGATC	780
40M11_mandshurica	CTTTAGACACCAGGAAGGCATATCAAACCGGCCACACACGTTAAAGGGATACAAAGATC *****	780
40M11_vesca	TCACCTGGACCAAAGACAGAACTGGGTGGTTGCTGACTGAGCAAAGCCAATATCTCGGAG	840
40M11_mandshurica	TCACCTGGACCAAAGACAGAACTGGGTGGTTGCTGACTGAGCAAAGCCAATATCTCGGAG *****	840
40M11_vesca	CTCCTCAGATGTCGGAGAGACCCATCTGAACCCAAGTCACTGACTGTTACAGCAACTA	900
40M11_mandshurica	CTCCTCAGATGTCGGAGAGACCCATCTGAACCCAAGTCACTGACTGTTACAGCAACTA *****	900
40M11_vesca	CAAACGCAAAGATA GAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAAG ATGAG	960
40M11_mandshurica	NN -----	924

40M11_vesca TCACCCGACTGATCAAAGTCGTCCAAAGAATTCTCAAACGTGGTTTCATAGGCCG 3116
40M11_mandshurica NNN ~3000

63F17

63F17_vesca CGCTCTATGGAAGGGACAAGAGACTGAAATAGCAATGGGG-TCCTACCAACCTCATCA 59
63F17_mandshurica CGCTCTATGGAAGGGACAAGAGACTGAAATAGCAATGGGGTCTACCAACCTCATCA 60
63F17_viridis CGCTCTATGGAAGGGACAAGAGACTGAAATAGCAATGGGGTCTACCAACCTCATCA 60
63F17Rrc_ananassa NNN 60

63F17_vesca TACATGGGCAAGAAATCATTCTAGTCCTCTCGGACAGGTAATCACAGAATCCAGATTAGA 119
63F17_mandshurica TACATGGGCAAGAAATCATTCTAGTCCTCTCGGACAGGTAATCACAGAATCCAGATTAGA 120
63F17_viridis TACATGGGCAAGAAATCATTCTAGTCCTCTCGGACAGGTAATCACAGAATCCAGATTATA 120
63F17Rrc_ananassa NNN 120

63F17_vesca TGCAGGTTTGAATTATTAGAGTCTATAAAGGGACATAGTTACAACGTGTTGTATGCTTT 179
63F17_mandshurica TGCAGGTTTGAATTATTAGAGTCTATAAAGGGACATAGTTACAACGTGTTGTATGCTTT 180
63F17_viridis TGCCGGTTTGAATTATTAGAGTCTATAAAGGGAC-TAGTTACAACGTGTT-----TTT 173
63F17Rrc_ananassa NNN 180

63F17_vesca TCCA--TTTTTTTTTATTTTTTTATTTTTTTGAGAATGTATGCTTTTTCACTTGTATGGCCT 237
63F17_mandshurica TCCA--TTTTTTTT-ATTTTTTTATTTTTTTGAGAATGTATGCTTTTTCACTTATATGGCCT 237
63F17_viridis TCCACTTTTTTTTTTTTTTTTTTTTTTTTGGAGAATGTATGCTTTT-CACTTATATGGCCT 232
63F17Rrc_ananassa NNN 240

63F17_vesca GAAGTTGCGAATGTTTGGTTGATAGATATTTGGATATAGAATGTCACCTATGGGCAGAGC 297
63F17_mandshurica GAAGTTGCGAATGTTTGGTTGATAGATATTTGGATATAGAATGTCACCTATGGGCAGAGC 297
63F17_viridis GAAGTTGCGAATGTTTGGTTGATAGATATTTGGATATAGAATGTCACCTATGGGCAGAGC 292
63F17Rrc_ananassa NNN 300

63F17_vesca ACACAGGAACCGTTGAGGACTGTTTTAGAGAACCAGAGAGTCTTGAATGTGTTAGGAGAG 357
63F17_mandshurica ACACAGGAACCGTTGAGGACTGTTTTAGAGAACCAGAGAGTCTTGAATGTGTTAGGAGAG 357
63F17_viridis ACACAGGAACCGTTGAGGACTGTTTTAGAGAACCAGAGAGTCTTGAATGTGTTAGGAGAG 352
63F17Rrc_ananassa NNN 360

63F17_vesca TTAGAGCAATGGGTGAGATGAACTGGAAACAATTTGCTGCTGAGGAGGTTACAGAGATGA 417
63F17_mandshurica TTAGAGCAATGGGTGAGATGAACTGGAAACAATTTGCTGCTGAGGAGGTTACAGAGATGA 417
63F17_viridis TTAGAGCAATGGGTGAGATGAACTGGAGACAATTTGCTGCTGAGGAGGTTACAGAGATGA 412
63F17Rrc_ananassa NNN 420

63F17_vesca GGGGTCATCTATTGAAGTATCCAGTTGAAATTGATCGAAAAGGCAAAGTCACATCCCTTC 477
63F17_mandshurica GGGGTCATCTATTGAAGTATCCAGTTGAAATTGATCGAAAAGGCAAAGTCACATCCCTTC 477
63F17_viridis GGGGTCATCTATTGAAGTATCCAGTTGAAATTGATCGAAAAGGCAAAGTCACATCCCTTC 472
63F17Rrc_ananassa NNN 480

63F17_vesca CTGGATGTGAGAGTTTCCCCGATGCAGGAGGAAATATAACCGGTTCTTTCTTGGCATTTC 537
63F17_mandshurica CTGGATGTGAGAGTTTCCCCGATGCAGGAGGAAATATAACCGGTTCTTTCTTGGCATTTC 537
63F17_viridis CTGGATGTGAGAGTTTCCCCGATGCAGGAGGAAATATAACCGGTTCTTTCTTGGCATTTC 532
63F17Rrc_ananassa NNN 540

63F17_vesca AAGAAAATTTGACAATTTGATCACCAGTTCAGTTTTATAGAAGAAGTACAGTTAGTACAGT 597
63F17_mandshurica AAGAAAATTTGACAATTTGATCACCAGTTCAGTTTTATAGAAGAAGTACAGTTAGTACAGT 597
63F17_viridis AAGAAAATTTGACAATTTGATCACCAGTTCAGTTTTATTGAAGAAGTACAGTTCAGT---ACAGT 588
63F17Rrc_ananassa NNN 600

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63F17_vesca          TTTGAAACGTTTTTTTGTGTATTTAGCAAACCCATAGGAGGATAGGGTTTTCTTTTATT 657
63F17_mandshurica   ATTGAAACGTTTTTTTCGTTGTATTTAGCAAACCCATAGGAGGATAGGGTTTTCTTTTATT 657
63F17_viridis       TTTGAAACGTTTTTTTGTGTATTTAGCAAACGCATAGGAGGATAGGGTTTTCTTTTATT 648
63F17Rrc_ananassa   NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 660

63F17_vesca          CAACAGGGATATAGGCGCTTTTAGGGTTTCTTTTCCTATTCAATTTTCGTTCTTTGGTAGA 717
63F17_mandshurica   CAACAGGGATATAGGCGCTTTTAGGGTTTCTTTTCCTATTCAATTTTCGTTCTTTGGTAGA 717
63F17_viridis       CAACAGGGATATAGGCGCTTTTAGGGTTTCTTTTCCTATTGAATTTTCGTTCTTTGGTATA 708
63F17Rrc_ananassa   NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNTTCAATTTTCATTCTGGGATAGA 720
                      ** *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *

63F17_vesca          CCAAGTCGCTTCTTTGGCATTCAAGGAAACCTGAGCATTGATCTGCCTGTGCATCACATC 777
63F17_mandshurica   CCAAGTCGCTTCTTTGGCATTCAAGGAAACCTGAGCATTGATCTGCCTGTGCATCACATC 777
63F17_viridis       CCAAGTCCCTTCTTTGGCATTCAAAGAAACCTAAGCATTGATCTGCCTGTGCATCACGTC 768
63F17Rrc_ananassa   CCAAGTCACTTTTTTGGCGTTCAAGGAAACCTGAGCATTGATCTGCCGGTGCATCACATC 780
                      ***** ** *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *

63F17_vesca          CAGAGTTGCAGATTGTTTAGAGAAGAATTCCAATAAATTCCTTTTGTACAGTTTGGTTAA 837
63F17_mandshurica   CAGAGTTGCAGATTGTTTAGAGAAGAATTCCAAT---TCCTTTTGTACAGTTTGGTTAA 833
63F17_viridis       TAGAGTTGCAGATTGTTTAGAGAAGAATTCCAATAAATTCCTTTTGTACAGTTTGGTTAA 828
63F17Rrc_ananassa   CAGAGTTGCAGATTGTTTATAGAAGAATTCCAATAAATTCCTTTTGTACAGTTTGGTTAA 840
                      ***** *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *

63F17_vesca          CTTTTGGTATTCAACAACGCATTGTACAACCTCTGCCAATTTGGCACATTATAATGTTGAT 897
63F17_mandshurica   CTTTTGGTGTTCACAACGCATTGTACAACCTCTGCCAATTTGGCACATTATAATGTTGAT 893
63F17_viridis       CTTCTGGTGTTCACAACGCATTGTACAACCTCTGCCAATTTGGCACATTATAATGTTGAT 888
63F17Rrc_ananassa   CTTTTGGTGTTCACAACGCATTGTACAACCTCTGCCAATTTGGCACATTATAATGTTGAT 900
                      *** *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *

63F17_vesca          ATGCAGGTAACATCTCTGACTATGCATCTTTGCTTTTTCTCTTTTTTTGAGAACAAGGC 957
63F17_mandshurica   ATGCAGGTAACATCTCTGACTATGCATCTTTGCTTTTTCTCTATTTTTGAGAACAAGGC 953
63F17_viridis       ATGCAGGTAACATCTCTGACTATGCATCTTTGCTTTTTCTCTGTTTTGAGAACAAGGC 948
63F17Rrc_ananassa   ATGCAGGTAACATCTCTGACTATGCATCTTTGCTTTTTCTCTTTTTTTGAGAATAAGGC 960
                      ***** *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *

63F17_vesca          ATCTTGTTTATGTGTAGCCAACCTGAAGCACTGTATTTAAATAATGCTAAAACAGTGTTA 1017
63F17_mandshurica   ATCTTGTTTATGTGTAGCCAACCTGAAGCACTGTATTTAAATAATGCTAAAACAGTGTTA 1013
63F17_viridis       ATCTTGTTTATTTGTGGCCAACCTGAAGCACTGTATTTAAATAATGCTAAGACCGTGTCA 1008
63F17Rrc_ananassa   ATCTTGTTTATGTGTAGCCAACCTGAAGCACTGTATTTAAATAATGCTAAAACAGTGTTA 1020
                      ***** ** *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *

63F17_vesca          ATTTTGTATATAAAAGTGTAGGCAACAATGAACCTGAACTTGAACCTGAACTTGATAAGAA 1077
63F17_mandshurica   ATTTTGTATATAAAAGTGTAGGCAACAATGAACCTGAACTTGAAC-----TTGATAAGAA 1067
63F17_viridis       ATTTTGTATACAAAAGTCTAGGCAACAATGAACCTGAACTT-----GATAAGAA 1056
63F17Rrc_ananassa   ATTTTGTATATAAAAGTGTAGGCAACAATGAACCTGAACTTGAACCTGAACTTGATAAGAA 1080
                      ***** *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *

63F17_vesca          AATAGATCCCAGAGATGGTCTACTACTACCCTTGACTACACAAGTTACTCATTCTTTAC 1137
63F17_mandshurica   AATAGATCCTAGAGATGGTCTACTACTACTCTTGACTACACAAGTTACTCATTCTTTAC 1127
63F17_viridis       AATAGATCCTAGAGATGGTCTACTACTACCCTTGACTACACAAGTTGCTCATTCTTTAC 1116
63F17Rrc_ananassa   AATAGATCCCAGAGATGGTCAATCTACTACCCTTGACTACACAAGTTACTCATTCTTTAC 1140
                      ***** *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *

63F17_vesca          ATGTGAAATGGCTATCCAGAGCAGTCGTA--TTTCATGAGATATTAACAAGCTTTGGACG 1195
63F17_mandshurica   ATGTGAAATGGCTATCCAGAGCAGTCGTAATTTTCATGAGATATTAACAAGCTTTGGACG 1187
63F17_viridis       ATGTGAAATGGCTATCCAGAGCAGTCGTAATTTTCATGAGATATTAACAAGCTTTGGACG 1176
63F17Rrc_ananassa   ATGTGAAATGGCTATCCAGAGCAGTCGTA--TTTCATGAGATATTAACAAGCTTTGGACG 1198
                      ***** *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *

63F17_vesca          TCCAGTTGCCAGGTTTCATCTCTACTCGGAGGCCAAGTCGAGCAAGGGCAGGCACATCAAC 1255
63F17_mandshurica   TCCAGTTGCCACGTTTCATCTCTACTCGGAGGCCAAGTCGAGCAAGGGCAGGCACATCAAC 1247
63F17_viridis       TCCAGTTGCCAGGTTTCATGTCTACTCGGAGGCCAAGT-GAGCAAGGGCAGGCACATCAAC 1235
63F17Rrc_ananassa   TCCAGTTGCCAGGTTTCATCTCTACTCGGAGGCCAAGTCGAGCAAGGGCAGGCACATCAAC 1258
                      ***** *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *

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63F17_vesca AGACCCCTTAA 1266
63F17_mandshurica AGACCC----- 1253
63F17_viridis AGACCCCTAAA 1246
63F17Rrc_ananassa AGACCCCTCAA- 1268

72E18

72E18_vesca GCTAGGGAAAACAGCTCGTGGAGCATCATCTCCAGCAAATCCGGCCTAAGCATTAATATC 60
72E18_mandshurica GCTAGGGAAAACAGCTCGTGGAGCATCATCTCCAGCAGACCCGGCCTAAACATTAACATC 60
72E18_nilgerrensis NNN 60
72E18_viridis NNTTAATATC 60
72E18_iinumae GCTAGGGAAAACAGCTCGTGGAGCATCATCTCCAGCAAACCCGGCCTAAACATTAACATC 60
72E18_ananassa GCTAGGGAAAACAGCTCGTGGAGCATCATCTCCAGCAAACCCGGCCTAAACATTAACATC 60

72E18_vesca AAA-TCAGTCCTTGAGATTGACATGCATAAAAAAGACAATAAAGGGTACAAAAACAACC 119
72E18_mandshurica AAAATCAGTCCTTGAGATTCAACATGCATAACAAAGACAATAAAGGGTACAAAAACAACC 120
72E18_nilgerrensis NNN 120
72E18_viridis AAAATCAGTCCTTGAGATTCAACATGCATAACAAAGACAATAAAGGGTACAAAAACAACC 120
72E18_iinumae AAAATCAGTCCTTGAGATTGACATGCATAAAAAAGACAATAAAGGGTACAAAAACAACC 120
72E18_ananassa AAAATCAGTCCTTGAGATTCAACATGCATAAAAAAGACAATAAAGGGTACAAAAACAACC 120

72E18_vesca ACTCAAACAATCACAACATAATATCATTCAATACCTTGACCATTCCGGTTCCATTATCAC 179
72E18_mandshurica ACTCAAACAATCACAACATAATATCATTCAATACCTTGACCATTCCGGTTCCATTATCAC 180
72E18_nilgerrensis NNN 180
72E18_viridis ACTCAAACAATCACAACATAATATCATTCAATACCTTGACCATTCCGGTTCCATTATCAC 180
72E18_iinumae ACTCAAACAATCACAACATAATAGCATCCAATACCTTGACCATTCCGGTTCCATTATCAC 180
72E18_ananassa ACTCAAACAATCACAACATAATATCATTCAATACCTTGACCATTCCGGTTCCATTATCAC 180

72E18_vesca ACACAAGCGGCTGAATGTCTCGGTTTCTGCCATCTTCTTCTACCTGCAACATACACCAC 239
72E18_mandshurica ACACGAGCGGCTGAATGTCTCGGTTTCTGCCATCTTCTTCTACCTGCAACATACACCAC 240
72E18_nilgerrensis NNN 240
72E18_viridis ACACGAGCGGCTGAATGTCTCGGTTTCTGCCATCTTCTTCTACCTGCAACATACACCAC 240
72E18_iinumae ACACGAGCGGCTGAATGTCTCGGTTTCTGCCATCTTCTTCTACCTGCTACATACACCAC 240
72E18_ananassa ACACGAGCGGCTGAATGTCTCGGTTTCTGCCATCTTCTTCTACCTGCAACATACATCAC 240

72E18_vesca AATCAATGACAACAATGCCTCATTACACAACAAGAAATAGACATTCAAAAACAAAACA 299
72E18_mandshurica AATCAA-----ATGCTACATTACACAACAAGAAATAGACATTCAAAGACAAAACA 292
72E18_nilgerrensis NNNNNN-----NN 292
72E18_viridis AATCAATGACAACAATGCCTCATTACACAACAAGAAATAGACATTCAAAAACAAAACA 300
72E18_iinumae AATCAATGACACCAATGCCTCATCGACACAACAACAAGAAATAGACATTCAAAAACAAAACA 300
72E18_ananassa AATCAATGACAACAATGCCTCA-----AACAAATAGACATTCAAAAACAAAACA 289

72E18_vesca CAATACACACTACTAATGTG----- 319
72E18_mandshurica CAA-ACACACTACTAACGTG----- 311
72E18_nilgerrensis NNNNNNNNNNNNNNNNNNNNNNN----- 312
72E18_viridis CAATACACACTACTAATGTG----- 320
72E18_iinumae CAATACACGATGCTAACATTTCCCTAATCTCTCCTCCATCAACTAAAATCTCCATTCCAA 360
72E18_ananassa CAATACACAATGCTAACATTTCCCTAATATCTCCTCCATCAACTAAAATCTCCATGCCAG 349

72E18_vesca -----GCACAGAAACCAAAGCATGATTCAAAAACAAAACCTAGAACATCTACAT 366
72E18_mandshurica -----GCACGAAACCAAAGCATGATTCAAAAACAAAACCTAGAACATCTACAT 358
72E18_nilgerrensis -----NN 359
72E18_viridis -----GCACAGAAACCAAAGCATGATTCAAAAACAAAACCTAGAACATCTACAT 367
72E18_iinumae ATCACACACTACTACACAGAAACCAAAGCATGATTCAAAAACAAAACCAAGAACATCTACAT 420
72E18_ananassa ATCAC-----CACAGAAACCAAAGCATGATTCAAAAACAAAACCAAGAACATCTACAT 400

72E18_vesca	AGTTCTCTCACAAATAGTAAAGAAACGATCTTTGACAATCAAAGGCATCGAAAGCTAGTA	426
72E18_mandshurica	AGTTCTCTCACAAATAGTAAAGAAACGATCGTTGACAATCAAAGGCATCGAAAGCTAGTA	418
72E18_nilgerrensis	NN	419
72E18_viridis	AGTTCTCTCACAAATAGTAAAGAAACGATCTTTGACAATCAAAGGCATCGAAAGCTAGTA	427
72E18_iinumae	AGTCCTCTCACAAATAGTAAAGAAACGATCTTTGACAATCAAAGGCATCGAAAGCTAGTA	480
72E18_ananassa	AGTTCTCTCACAAATAGTAAAGAAACGATCTTTGACAATCAAAGGCATCGAAAGCTAGTA	460
72E18_vesca	AAGAAACGATCTTTCAGATGGGAAATACCCAAATTTGATTGCTACATGCATAAAAACCCTC	486
72E18_mandshurica	AAGAAACGATCTTTCAGATGGGAAATACCCAAATTTGATTGCTATATACATAAAAACCCTC	478
72E18_nilgerrensis	NN	479
72E18_viridis	AAGAAACGATCTTTCAGATGGGAAATACCCAAATTTGATTGCTACATGCATAAAAACCCTC	487
72E18_iinumae	AAGAAACGATCTTTCAGATGGGAAATACCCAAATTTGATTGCTATATACATAAAAACCCTC	540
72E18_ananassa	AAGAAACGATCTTTCAGATGGGAAATGCCCAAATTTGATTACTATATACATAAAAACCCTC	520
72E18_vesca	AAATTGATACGAAATCAAACAATGCAGCAATCAAATCATTCACATAAAAAA-TTCAA	545
72E18_mandshurica	AAATTGATACGAAATCAAACAATGCAGCAATCAAATCATTCACATAAAAAAATTCAA	538
72E18_nilgerrensis	NN	539
72E18_viridis	AAATTGATACGAAATCAAACAATGCAGCAATCAAATCATTCACATAAAAAAATTCAA	547
72E18_iinumae	AAATTGATACGAAATCAAACAATGCAGCAATCAAATCATTCACATAAAAAAATTCAA	600
72E18_ananassa	AAATTGATACGAAATCAAACAATGCAGCAATCAAATCATTCACAG-AAAAAATTCAA	579
72E18_vesca	GAAA-AAA GAGAGAGA --AAATTACAGATTTAAAGCGACGAACAA-TGAAAAGGAATGA	601
72E18_mandshurica	GAAA-AAA GAGAGAGA --AAATTACAGATCTAAAGCGACGAACAG-TGAGAAGGAATGA	594
72E18_nilgerrensis	NNAAA-AAA GAGAGAGAGA ---TTACAGATCTAN-GCGACGAACAA-TGAGAAGGAATGA	593
72E18_viridis	AGAAATAAA GAGAGAGA --AAATTACAGATCTAAAGTGACGAACAA-TGAGAAGGAATGA	604
72E18_iinumae	GAAAAAAGAA GAGAGA --AAATTACAGATCTAAAGCGACGAACAAATGAGAAGGAATGA	658
72E18_ananassa	GAAAAAAGAA GAGAGAGA AAATTACAGATCTAAAGCGACGAACAA-TGAGAAGGAATGA * * * * *	638
72E18_vesca	GAGGCAAAGAGAAGAGATGAGGAAGTTGACCTTTGTGAATGAGAGTGAGTGAGG GAGAGA	661
72E18_mandshurica	GAGGCAGAGAGAAGAGATGAGGAAGTTGACCTTTGTGAATGAGAGTGAGTGAGG GAGAGA	654
72E18_nilgerrensis	GAGGCAGAGAGAAGAGATGAGGAAGTTGACCTTTGTGAATGAGAGTGAGT----- GA	645
72E18_viridis	GAGGCAGAGAGAAGAGATGAGGAAGTTGACCTTTGTGAATGAGAGTGAGT GAGAGA	662
72E18_iinumae	GAGACAGAGAGAAGAGATGAGGAAGTTGACCTTTGTGAATGAGAGT----- GAGAGA	710
72E18_ananassa	GAGGCAGAGAGAAGAGATGAGGAAGTTGACCTTTGTGA-----GTGAGG GAGAGA * * * * *	688
72E18_vesca	GAGAGAGAGATCGACGACGAAGCAGAGCGAAAGAGACGAGTGTGGTGTTTGTGAGTTGAG	721
72E18_mandshurica	GAGAGAGAGATCGACGACGAAGCAGAGCGAAAGAGACGAGTGTGGTGTTTGTGAGTTGAG	714
72E18_nilgerrensis	GAGAGAGAGATCGAAGACGAAGCAGAGCGAAAGAGACGAGTGTGGTGTTTGTGAGTTGAG	706
72E18_viridis	GAGAGAGAGATCGAAGACGAAGCAGAGCGAAAGAGACGAGTGTGGTGTTTGTGAGTTGAG	722
72E18_iinumae	GAGAGAGAGATCGAAGACGAGGCGAGAGCGAAAGAGACGAGTGTGGTGTTTGTGAGTTGAG	771
72E18_ananassa	GAGAGAGAGATCGAAGACGAAGCTGAGCGAAAGAGACGAGTGTGGTGTTTGTGAGTTGAG * * * * *	749
72E18_vesca	GCGAAAGAATTGGAGCAAATAAAGGAGTGGGATTGACGAGTAATCTCAGCCGTTTGATT	781
72E18_mandshurica	GCGAAAGAATTGGAGCAAATAAAGGAGTGGGATTGACGAGTAATCTCAGCCGTTTGATT	774
72E18_nilgerrensis	GCGAAAGAATTGGAGCAAATAAAGGAGTGGGATTGACGAGTAATCTCAGCCGTTTGATT	765
72E18_viridis	GCGAA- GAATTGNACCNNNATANAGGAGTGNGATTGACNAGTTATCTCNGCNGNTTGATT	781
72E18_iinumae	GCGAAAGAATTGGAGCAAATAAAGGAGTGGGATTGACGAGTAATCTCAGCCGTTTGATT	830
72E18_ananassa	GCGAAAGAATTGGAGCAAATAAAGGAGTGGGATTGACGAGTAATCTCAGCCGTTTGATT * * * * *	808
72E18_vesca	TATGGACCGCTTATTGAGCCCTTTGTGGG-CCATTACAGCTCCTTCCGCTGTTCCAGT	840
72E18_mandshurica	TATGGACCGCTTATTGAGCCCTTTGTGGG-CCATTACAGCTCCTTCCGCTGTTCCAGT	833
72E18_nilgerrensis	TATGGACCGCTTATTGAGCCCTTTGTGGG-CCATTACAGCTCCTTCCGCTGTTCCAGT	824
72E18_viridis	TATGGACCGCTTATTGAGCCCTTTGTGGG-CCATNACNGCTCCTNCCNGTGTNCCNGC	840
72E18_iinumae	TATGGACCGCTTATTGAGCCCTTTGTGGG-CCATTACAGCTCCTTCCGCTGTTCCAGT	890
72E18_ananassa	TATGGACCGCTTATTGAGCCCTTTGTGGG-CCATTGAGCTCCTTCCGCTGTTCCAGT * * * * *	867

72E18_vesca CATT TTT TTT TCTCCACCTTCTTTACCTTTTGGCCCTCAGTCCCTT-CCCTTTTCTCCCAA 899
72E18_mandshurica CATT TTT TTT TCTCCACCTTCTTACCTTTTGGCCCTCAGTCCCTT-CCCTTTTCTCCCAA 892
72E18_nilgerrensis CATT TTT TTT TCTCCACCTTCTG-----CCCCATCCCTT-CCCTTTTCTCCCAA 871
72E18_viridis CATNTTTATCTCCACCTTCTNCACNTTNTGCCNCNCNTGNCNT-CCCTTTTCTCCCN 899
72E18_iinumae CATT TTT T--CTCCACCTTCTTACCTTTCTGCCCTCGTTCCTTCCCTCTTCTCCCAA 948
72E18_ananassa CATT TTT T--CTCCACCTT----- 884
*** **

72E18_vesca TTCTT-----TCTCAACTCT 914
72E18_mandshurica TTCTT-----TCTCAACTCT 907
72E18_nilgerrensis TTCTT-----TCTCAACTCT 886
72E18_viridis TTCTN-----NCTCANATCT 914
72E18_iinumae CTCTTCTTAGCCTAATTGCATTTTCATTTTAGTGCTTAGATCAATATGATNNNNNNNNN 1008
72E18_ananassa -----

72E18_vesca TCTTAAACCTAATTGCATTTCCCTAATTGCATTTTCATTTTAGTGCTTAGATCAATATGA 974
72E18_mandshurica TCTTAAACCTAATTGCATTTCCCTAATTGCATTTTCATTTTAGTGCTGAGATCAATATGA 967
72E18_nilgerrensis TCTTAAACCTAAT-----TGCATTTTCATTTTANTGCTTANATCAATATGA 932
72E18_viridis TCTNAANGCTNATNGCNTTTNCCNNANNGCATTTGNATTTNNGNGCTTCAATCAATNCGN 974
72E18_iinumae NNNNNNNNNNNNNNN-----NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 1056
72E18_ananassa ACCTTTTGGCCCT-----CATTCCCTTCCCTCTTCTCCGATCCCAACTTTC 932

72E18_vesca TTAAGAAGCTTCATTTTGTCAACACAAGGCAACAAGGACA--CAGGGGAGCATTTTCGATC 1032
72E18_mandshurica TTAAGAAGCTTCATTTTGTCAACACAAGGCAACAAGGACA--CAAGGGAGCATGTCGATC 1025
72E18_nilgerrensis TTAAGAAGCTCCATTTTGTCAACACAAGGCAACAAGGACN--TANGGGAGCATGTCGATC 990
72E18_viridis NNNNAANGCTNCGTNTTGTCTNNCANGGNTNCAAGGANCTNNGGGANGCNGTTGATC 1034
72E18_iinumae NNN 1116
72E18_ananassa TCAACTCTTCTTAAACCCACCCAGTTGCATTTTCATTTAGTGCTTAAATCAATATGGTTA 992

72E18_vesca ATCGTTCCAGTCATTTTCGTATATAAT-TTGGGCTTGAAATGGTTGATC-GGTCGTAAAA 1090
72E18_mandshurica ATCGTTCCAGTCATTTTCGTATATAAT-TTGGGCTTGAAATGGTTAATC-AATCGTAAAA 1083
72E18_nilgerrensis ATCGTTCCGGTCACTTTTCGTATATAAT-TTGGACTTAAATGGTTGATC-GATCGTAAAA 1048
72E18_viridis GTC AANTNCGGCTGGTNATANANAATATNNGACNTNAAATGGTTGATTGNNTCGTTAAA 1094
72E18_iinumae NNN 1176
72E18_ananassa AGAAGCTTCATTTTGTCAACACAAGGCAACAAGGACATAGGGGAGCATGTGGATGATCGT 1052

72E18_vesca TTTAAAATGACGTTTGTATGATATCTATAAAGA-GGACATAATTTACTTTGTATGTCA 1149
72E18_mandshurica TTTAAAATGACGTTTGTATGATATCTATAAAGA-GGACATAATTTACTTTTATATGTCA 1142
72E18_nilgerrensis TTTGAAATGACGTTTGGTATGATATTTGTAAAGA-GGACATAATTTACT----- 1097
72E18_viridis TTNGAAATAANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNAAAAGGNCNTAATTANTNNGTANNTCA 1154
72E18_iinumae NNN 1231
72E18_ananassa TTTTAAACTACGTTTGGTATGATATCGTAAGGAGGACATAATTTACTTTGTATG----- 1107

72E18_vesca GGT TTTAATATGGAA---CGAAGAGTATGGGTGAAA-GTGTC-ATCCCACACATTTTAA 1203
72E18_mandshurica GGT TTTAATACGGAA---GGAAGAGTATGGGTGAAA-GTGTC-ATCCCACACATTTTAA 1196
72E18_nilgerrensis -----ATGGAA---CGAAGAGTATAGGTGAAA-GTGTC-ATCCCACACATTTTAA 1142
72E18_viridis AGGTTTAAANTAGGGAAATGGAGGAGTGNGGGTGAAAAGTGTCCATCCCACAGCTTTTAA 1214
72E18_iinumae -----NN 1282
72E18_ananassa -----TCNGGTTTTTAAACGGAATGAAGAGTGTGGGAGAAAGTGTCCATCCCACAC 1158

72E18_vesca AAGAGCTGTAATGTAGGG-TAATGAGCA-CAACTGCAAGCTGCATCC-TATAA-TGGATC 1259
72E18_mandshurica AAGAGCTGTAATGTAGGG-TAATGAGCA-CAACTGCAAGCTGCATCC-TATAA-TGGATC 1252
72E18_nilgerrensis AAGAGCTTAAATGTAGGGTAATGAGCA-CAACTACAAGCTGCATCC-TATAA-GGGATC 1199
72E18_viridis ANGAGCNTTAANGNAGGG-NAANGNCAGNAACNGCAAGCTGCATNCTNTAATNGGATC 1273
72E18_iinumae NNN 1342
72E18_ananassa ATTCA TTTTAAANN 1218

72E18_vesca AATCAGAACATTAA-ACAACGTAAAGAGGAAGGTATTTGCTTTACACAACCTTATAAAAAT 1318
72E18_mandshurica AATCAGAACAAATA-ACAACGTAAAGAGGAAGGTATTTGCTTTACACAACCTTATAAAAAT 1311
72E18_nilgerrensis AATCAGAACATTAA-ACAACGTAAAGAGGAAGGTATTTGCTTTACACAACCTTATAAAAAT 1258
72E18_viridis AATCAGAACATTAAAGNCAACGTAAAGAGGAAGGTATTTGCTTTACACAACCTTATAAAAAT 1333
72E18_iinumae NNN 1402
72E18_ananassa NNN 1278

72E18_vesca GATGAGGATCTACTC-AAAATCCAGACTACCATGGTTGGCAAATTAGATCCTCACTGTA 1377
72E18_mandshurica GATGAGGATCTACTC-AAAATCCAGACTACCATGGTTGGCAAATTAGATCCTCACTGTA 1370
72E18_nilgerrensis GATGAGGATCTACTC-AAAATCCAGACTACCATGGTTGGCAAATTAGATCCTGCCTGTA 1317
72E18_viridis GATGNGGATNTACTCCAAAAGTGAGGACTACCATGGTCGGCAAATTAGTTCCTGACTGTA 1393
72E18_iinumae NNN 1462
72E18_ananassa NNN 1338

72E18_vesca ACCAGCTAGGCATTGGTAATGCATAATAGCTATAGCTAACTATAGGTGGGAGACTCATCA 1437
72E18_mandshurica ACCAGCTAGGCATTGGTAATGCATAATAGCTATAGCTAACTATAGGTGGGAGACTCATCA 1430
72E18_nilgerrensis ACCAGCTAGGCATTGGCAATGCATAATAGCTAGAGCTAACCATAGGTGGGAGACTCATCA 1377
72E18_viridis ACCAGCTAGGCATNGGCAATGCATAATAGCTATAGCTGACTATAGGTGGGAGACTCATCA 1453
72E18_iinumae NNN 1522
72E18_ananassa GTGGTGGCAAATTAGATCCTCACCTATAGTAGGGTAACTATAGGTGGGAGACTNGTCA 1398

72E18_vesca TTGAGATCATAGAAAAACAAAGATGAAAGAAAGAAATGAAGAAACAAGCAACAGCTATTC 1497
72E18_mandshurica TTGAGATCATAGAAAAACAGAGATGAAAGAAAGAAATGATGAAACAAGCAACAGCTATTC 1490
72E18_nilgerrensis TTGAGATCATAGAAAA-----AAAATGATGA--AAACAAGCAACAGTTATTC 1423
72E18_viridis TTGAGATCAGAGAAAAACNAAGATGAAAGAAAGAAATGATGAAACAAG--AATAGTTATTC 1512
72E18_iinumae NNNNNNNNNNNNNNNNNNN-----NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 1567
72E18_ananassa TTGTGATCATAGAAAA-----GAAATGATGAAACAAGCAACAGTTACTC 1443

72E18_vesca GAAAGCAAGTACAGAAGGGATTGTTTCATGAAGTGTTCACCAAGTCCACAGCTTAGGGCATT 1557
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72E18_nilgerrensis GAAAGCAAGTACAGAAGGGATTGTTTCATGAAGTGTTCACCAAGTCCACAGCTTAGGGCATT 1483
72E18_viridis GAAAGCAAGTACAGAAGGGATTGTTTCATGAAGTGTTCACCAAGTCCACAGCTTAGGGCATT 1572
72E18_iinumae NNN 1627
72E18_ananassa GAAAGCAGGTACAGAAGGGATTGTTTCATGAAGTGTTCACCAAGTCCACAGCTTAGGGCATT 1503

72E18_vesca CTTAGAAGTAACAAGCTTACCAACTTCCATTTACTTGTTCAGTTCATGATGATATTAA 1617
72E18_mandshurica CTTAGAAGCAACAAGCTTACCAACTTCCATTTACTTGTTCAGTTCATGATGATATTAA 1610
72E18_nilgerrensis CTTAGAAGCAACAAGCTTACCAACTTCCATTTACTTGTTCAGTTCATGATGATATTAA 1543
72E18_viridis CTTAGAAGCAACAAGCTTGCACACTTCCATTTACTTGTTCAGTTCATGNTGATATTAN 1632
72E18_iinumae NNN 1687
72E18_ananassa CTTAGAAGCAACAAGCTTACCAACTTCCATTTACTTGTTCAGTTCAGGGTGACATTAC 1563

72E18_vesca CCATCCAACGAAAAATCCAAAGGTACTGTGACAGAAAGCTCAAGGGGATATCTGTGTTTT 1677
72E18_mandshurica CCATCCAACGAAAAATCCAAAGGTACTGTGACAGAAAGCTCAAGGGGATATCTGTGTTTT 1670
72E18_nilgerrensis -----GTCATGT-----TGTTTT 1555
72E18_viridis CCATCCAACGAAAAATCCAAAGGTACTGTGACTGAAAGCTCAAGGGGATATCNGTGTTTT 1692
72E18_iinumae NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCCGAAAGCTCAATGGGATATCTGTGTTTT 1747
72E18_ananassa CCNTCCAACGAAAAATCCAAAGGTACTGTGACTGAAAGCCCAAGGGGATATCCGTGTTTT 1623

72E18_vesca AAAGCCACAACATGACTAAATATAAATGCTTCCAATTTCTAAAGTTACATTCGTTTTGTG 1737
72E18_mandshurica AAAGCCACAACATGACTAAATATAAATGCTTCCAATTTCTAAAGTTACATTCGTTTTGTG 1730
72E18_nilgerrensis AAAGCCACAACATGACTAAATATAAATGCTTCCAATTTCTAAAGTTACATTCGTTTTGTG 1615
72E18_viridis AAAGCCACTACATGACTAAATATAAATGCTTCCAATTTCTAAAGTTACATTCGTTTTGTG 1752
72E18_iinumae AAAGCCACAACATGACTAAATATAAATGCTTCCAATTTCTAAAGTTACATTCGTTTTGTG 1807
72E18_ananassa AAAGCCACAACATGACTAAATATAAATGCTTCCAATTTCTAAAGTTACATTCGTTTTGTG 1683

72E18_vesca CAAATGACAAAACAGTTCAAATTGACTGCATAA-ATAGATTACTCTTGTATAGATCAACA 1796
72E18_mandshurica CAAATGACAAAACAGTTCAAATTGACTGCATAA-ATAGAT-ACTCTTGTATAGATCAACA 1788
72E18_nilgerrensis CAGATGACAAAACAGTTCAAATTGACTGCATAAGATAGATTACTCTTGTATAGATCAACA 1675
72E18_viridis CAAATGACAAAACAGTTCTAAATTGACTGCATAAGATAGATTACTCTTGTATAGATCAACA 1812
72E18_iinumae CAAATGACAAAACAGTTCAAATTGACTGCATAA-ATAGATTACTCTTGTATAGATCAACA 1866
72E18_ananassa CAAATGACAAAACAGTTCAAATTGACTGCATAAGATAGATTACTCTTGTATAGATCAACA 1743
** *****

72E18_vesca AGCAAATCTCCAAGTTCTTATTACAAAGTCTAAGCAGAATACTAACATCAATATTGAAAT 1856
72E18_mandshurica AGCAAATCTCCAAGTTCTTATTACAAAGTCTAAGCAGAATACTAACATCAATATTGAAAT 1848
72E18_nilgerrensis AGCAAATCTCCAAGTTCTTATTACAAAGTCTAAGCAGAATACTAACATCAATATTGAAAT 1735
72E18_viridis AGCAAATCTCCAAGTTCTTATTACAAAGTCTAAGCAGAATACTAACATCAATATTGAAAT 1872
72E18_iinumae AGCAAATCTCCAAGTTCTTATTACAAAGTCTAAGCAGAATACTAACATCAATATTGAAAC 1926
72E18_ananassa AGCAAATCTCCAATTTCTTATTACAAAGTCTAAGCAGAATACTAACATCAATATTGAAAT 1803

72E18_vesca TGGATAAATATGCGATCTGAACTTCTTCACGTTGATGACCTATCGTAGGAAATGGAATTG 1916
72E18_mandshurica TGGATAAATATGCGATCTGAACTTCTTCACGTTGATGACCTATCGTAGGAAATGGAATTG 1908
72E18_nilgerrensis TGGATAAATATGCGATCTGAACTTCTTCACGTTGATGACCTATCATAGGAAATGGAATTG 1795
72E18_viridis TGGATAAATATGCGATCTGAACTTCTTCACGTTGATGAGCTATCGTAGGAAATGGAATTG 1932
72E18_iinumae TGGATAAATATGCGATCTGAACTTCTTCACGTTGATGACCTATCGTAGGAAATGGAATTG 1986
72E18_ananassa TGGATAAATATGCGATCTGAACTTCTTCACGTTGATGACCTATCGTAGGAAATGGAATTG 1863

72E18_vesca AACACTTGACACCAAAGAGAACAATGAAGGTAGCCTCGCCAATCACTTCTACAAGAATGG 1976
72E18_mandshurica AACACTTGACACCAAAGAGAACAATGAAGGTAGCCTCGCCAATCACTTCTACAAGAATGG 1968
72E18_nilgerrensis AACACTTGACACCAAAGAGAACAACGAAGGTAGCCTCGCCAATCACTTCTACAAGAATGG 1855
72E18_viridis AACACTTGACACCAAAGAGAACAACGAAGGTAGCCTCGCCAATCACTTCTACAAGAATGG 1992
72E18_iinumae AACACTTGACACCAAAGAGAACAATGAAGGTAGCCTCGCCAATCACTTCTACAAGAATGG 2046
72E18_ananassa AACACTCGACACCAAAGAGAACAACAAGGTAGCCTCACCATCACTTCTACAAGAATGG 1923

72E18_vesca GGGTAGAATCACCATCGACGTGGATACTTGGGTCTTCCGTCCTTCCCATCAAATAGCTG 2036
72E18_mandshurica GGGTAGAATCACCATCGACGTGGATACTTGGGTCTTCCGTCCTTCCCATCAAATAGCTG 2028
72E18_nilgerrensis GGGTAGAATCACCATCTACGTGGATACTTGGGTCTTCCGTCCTTCCCATCAAATAGCTG 1915
72E18_viridis AGGTAGAATCACCATCGACGTGGATACTTGGGTCTTCCGTCCTTCCCATCAAATAGCTG 2052
72E18_iinumae GGGTAGAATCACCATCGACGTGGATACTTGGGTCTTCCGTCCTTCCCATCAAATAGCTG 2106
72E18_ananassa GGGTAGAATCACCATCGACGTGGATACTTGGGTCTTCCGTCCTTCCCATCAAATAGCTG 1983

72E18_vesca GACATGGCAGGGTGTACAAAAGATCAATATTGCATGTAAGAGCTTCTACATACAACT 2096
72E18_mandshurica GACATGGCAGGGTGTACAAAAGATCAATATTGCATGTAAGAGCTTCTACATACAACT 2088
72E18_nilgerrensis GACATGGCAGGGTGTACAAAAGATCAATATTGCATGTAAGAGCTTCTACATACAACT 1975
72E18_viridis GACATGGCAGGGTGTACAAAAGATCAATATTGCATGTAAGAGCTTCTACATACAACT 2112
72E18_iinumae GACATGGCAGGGTGTACAAAAGATCAATATTGCATGTAAGAGCTTCTACATACAACT 2166
72E18_ananassa GACATGGCAGGGTGTACAAAAGATCAATATTGCATGTAAGAGCTTCTACATACAACT 2043

72E18_vesca CAAATGGATATGTTCTGGCGCTTGTAGAATATAATTATGTATACAAATATGCATGTACAG 2156
72E18_mandshurica CAAATGGATATGTTCTGGCGCTTGTAGAATATAATTATGTATACAAATATGCATGTACAG 2148
72E18_nilgerrensis CATATGGATATGTTCTGGCGATTGCAGAATATAATTATGTATACAAATATGCATGTACAG 2035
72E18_viridis CAAATGGAGATGTTCTGGCGCTTGTAGAATATAATTATGTATACAAATATGCATGTACAG 2172
72E18_iinumae CAAATGGATATGTTCTGGCGCTTGTAGAATATAATTATGTATACAAATATGCATGTACAG 2226
72E18_ananassa CAAATGGATATGTTCTGGCGCTCGTAGAATATAATTATGTATACAAATATGCATGTACAG 2103
** *****

72E18_vesca AGCTTCCACATACAACTCATATGAATACTTGTAAATTTATGCAATTTAATTTCAATAAAA 2216
72E18_mandshurica AGCTTCCACATACAACTCATATGTATACTTGTAAATTTATGCAATTTAATTTCAATAAAA 2208
72E18_nilgerrensis AGCTTCTACATACAACTCATACGAATACTTGTAAATTTAGGCAATTTAATTTCAATAAAA 2095
72E18_viridis AGCTTCCACATACAACTCATATGAATACTTGTAAATTTATGCAATTTAATTTCAATAAAA 2232
72E18_iinumae AGCTTCCACATACAACTCATATGAATACTTGTAAATTTATGCAATTTAATTTCAATAAAA 2286
72E18_ananassa AGCTTCCACATACAACTCATATGAATACTTGTAAATTTATGCAATTTAATTTCAATAAAA 2163

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72E18_vesca      GGTGAGTTTAAATAGACCAAGATGTTAGCTAAAAAAA---CAGACAAAACATTTAAGCA 2273
72E18_mandshurica GGTGAGTTTAAATAGACCAAGATGTTAGCTAAAAAAA--CAGACAAAACATTTAAGCA 2266
72E18_nilgerrensis GGTGAGTTTAAATAGACCAAGATGTTAGCTAAAAAAA----GACAAAACATTTAAGCA 2150
72E18_viridis     GGTGAGTTTAAATAGACCAAGATGTTAGCTAAAAAAAAGACAAAACATTTAAGCA 2292
72E18_iinumae     GGTGAGTTTAAATAGACCAAGATGTTAGCTAAAAAAAAC---AGACAAAACATTTAAGCA 2343
72E18_ananassa    GGTGAGTTTAAATAGACCAAGATGTTAGCTAAAAA--AC---AGACAAAACATTTAAGCA 2218
***** * *****

72E18_vesca      AAAGAAGAGCAGTAGAAGGTATTAAGATACCAAACAACATATTTGGGTTGGAGGACAAAG 2333
72E18_mandshurica AAAGAAGAGCAGTAGAAGGTATTAAGATACCAAACAACATATTTGGGTTGGAGGACAAAG 2326
72E18_nilgerrensis AAAGAAGAGCAGTAGAAGGTATTAAGATACCAAACAACATATTTGGGTTGGAGGACAATG 2210
72E18_viridis     AAAGAAGAGCAGTAGAAGGTATTAAGATACCAAACAACATATTTGGGTTGGAGGACAAAG 2352
72E18_iinumae     AAAGAAGAGCAGTAGAAGGTATTAAGATACCAAACAACATATTTGGGTTGGAGGACAAAG 2403
72E18_ananassa    AAAGAAGAGCAGTAGAAGGTATTAAGATACCAAACAACATATTTGGGTTGGAGGACAAAG 2278
***** * *****

72E18_vesca      TAGTATAGAGGAGTGACCTTCTTTAAACGGCGGTGTTTTCTTAGGGCCCAATTGGTCAT 2393
72E18_mandshurica TAGTATAGAGGAGTGTTCCCTTCTTTAAACGGCGGTGTTTTCTTAGGGCCCAATTGGTCAT 2386
72E18_nilgerrensis TAGCATAGAGGAGTGACCTTCTTTAAACGGCGGTGCTTTCTTAGGGCCCAAGTTGGTCAT 2270
72E18_viridis     TCGTATAGAGGAGTGACCTTCTTTAAACGGCGGTGTTTTCTTAGGGCCCAATTGGTCAT 2412
72E18_iinumae     TAGTATAGAGGAGTGACCTTCTTTAAACGGCGGTGTTTTCTTAGGGCCCAATTGGTCAT 2463
72E18_ananassa    TAGTATAGAGGAGTGACCTTCTTTAAACGGCGGTGTTTTCTTAGGGCCCAATTGGTCAT 2338
* * *****

72E18_vesca      GATAGAAGCAGCAACTACAGCAAAAAGATAACCAGCAACCGTCTGTGTTGCAATATTTAAA 2453
72E18_mandshurica GATAGAAGCAGCAACTACAGCAAAAAGATAACCAGCAACCGTCTGTGTTGCAATATTTAAA 2446
72E18_nilgerrensis TATAGAAGCAGCAACTGCAGCAAAAAGATAACCAGCAACCGTCTGTGTTGCAATATTTAAA 2330
72E18_viridis     GATAGAAGCAGCAACTACAGCAAAAAGATAACCAGCAACTGTCTGTGTTGCAATATTTAAA 2472
72E18_iinumae     GATAGAAGCAGCAACTACAGCAAAAAGATAACCAGCAACCGTCTGTGTTGCAATATTTAAA 2523
72E18_ananassa    GATAGAAGCAGCAACTACAGCAAAAAGATAACCAGCAACCGTCTGTGTTGCAATATTTAAA 2398
***** * *****

72E18_vesca      ACCCAACCCTGATAAATCTCAGTCGTGTAATTTGCACATGTCACAATATTGAATAGAAA 2513
72E18_mandshurica ACCCAACCCTGATAAATCTCAGTCGTGTAATTTGCACATGTCACAATATTGAATAGAAA 2506
72E18_nilgerrensis ACCCAACCCTGATAAATCTCAGTCGTGTAATTTGCACATGTCACAATATTGAATAGAAA 2390
72E18_viridis     ACCCAACCCTGATAAATCTCAGTCGTGTAATTTGCACATGTCACAATATTGAATAGAAA 2532
72E18_iinumae     ACCCAACCCTGATAAATCTCAGTCGTGTAATTTGCACATGTCACAATATTGAATAGAAA 2583
72E18_ananassa    ACCCAACCCTGATAAATCTCAGTCGTGTAATTTGCACATGTCACAATATTGAATAGAAA 2458
***** * *****

72E18_vesca      ACCACGTGGTATTTGATAGCCTCCACTTCCATCAGGACTTCGCAGATTCCTCAGTAGAAT 2573
72E18_mandshurica ACCACGTGGTATTTGATAGCCTCCACTTCCATCAAGACTTCTCAGATTCCTCAGTAGAAT 2566
72E18_nilgerrensis ACCACGAGGTATTTGATAGCCTCCACTTCCATCAGGACTTCGCAGATTCCTCAGTAGAAT 2450
72E18_viridis     ACCACGTGGTATTTGATAGCCTCCACTTCCATCAGGACTTCGCAGATTCCTCAGTAGAAT 2592
72E18_iinumae     ACCACGTGGTATTTGATAGCCTCCACTTCCATCAGGACTTCGCAGATTCCTCAGTAGAAT 2643
72E18_ananassa    ACCACGTGGTATTTGATAGCCTCCGCTTCCATCAGGACTTCGCAGATTCCTCAGTAGAAT 2518
***** * *****

72E18_vesca      ATGGCAATAGAAGTTTCGCAATTTGATTTATTATCCCAAACCAAACCCA 2622
72E18_mandshurica ATGGCAATAGAAGTTTCGCAATTTGATTTATTATCCCAAACCAAACCCA 2615
72E18_nilgerrensis ATGGCAATACAAGTTTCGCGATTTGATTTATTATCCCAAACCAAACCCA 2499
72E18_viridis     ATGGCAATAGAAGTTTCGCAATTTGATTTATTATCCCAAACCAAACCCA 2641
72E18_iinumae     ATGGCAATAGAAGTTTCGCAATTTGATTTATTATCCCAAACCAAACCCA 2692
72E18_ananassa    ATGGCAATAGAAGTTTCGCAATTTGATTTATTATCCCAAACCAAACCCA 2567
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BIOGRAPHICAL SKETCH

Denise Cristina Manfrim Tombolato was born to Vadir and Marlene Tombolato on March 20, 1976, in Campinas, São Paulo, Brazil. She received her bachelor's degree in agronomic engineering from the Escola Superior de Agricultura "Luiz de Queiroz," at the University of São Paulo in 1998. While an undergraduate student in Brazil, Denise was granted the FAPESP and CNPq fellowships to investigate molecular markers for disease resistance in maize, working under the supervision of Dr. Luiz Eduardo Aranha Camargo at the Plant Pathology Department. During the last year of her undergraduate studies, she was introduced to University of Florida's professor Dr. Richard D. Berger, who was on sabbatical studies at ESALQ. Denise was invited to spend one semester in Dr. Berger's laboratory, carrying out investigation on plant disease epidemiology for the completion of her degree.

Dr. David Pete Weingartner granted her a research assistantship from 1999 to 2002, when she earned her master's degree from the Plant Pathology Department at the University of Florida. In 2002, she started her doctorate program at the Horticultural Sciences Department, where she attended the majority of the courses offered to the Plant Molecular and Cellular Biology program. Upon graduation, she will work as a genetic technologist for Ball Helix, the biotechnology research branch of Ball Horticultural Company in West Chicago. She is very excited about the upcoming changes in her life!