Heredity Lab Manual

DNA Extraction from Onion Tissue

Goal: To visualize a large quantity of DNA.

Almost everyone knows what DNA is: the genetic information for all living things. But what does it look like? The purpose of this lab is to give you hands-on experience with DNA by isolating it from onion tissue.

DNA extraction requires 1) breaking open cells and releasing their contents, 2) separating DNA from the other cell contents (mostly proteins and carbohydrates), and 3) precipitating the DNA in order to further purify it and so it can be seen. After precipitation, the DNA is visible as a white-colored, stringy mass. Note: all DNA looks the same, regardless of from what organism it is extracted.

MATERIALS

blender
onion
lysis buffer (for 900mL)
45 mL 1M Tris (pH 8.0)
45 mL 0.5M EDTA
54 mL 5M NaCl

per table:
- ice bath

per groups of three or four:
- 250 ml beaker
- cheese cloth (4 layers)
- about 20 g of onion
- 10% SDS

per pair:
- 100-1000 uL micropipettor
- 50 mL tube
- glass pipette w/ hooked end
- 15 mL ice-cold 95% ethanol (*in ice bath*)

PROCEDURE

Acquire the items above that you'll need per group and per person.

Lysing the cells (*complete the following steps in groups of three or four*)

1. Add about 15 g of diced onion with 75 mL lysis buffer and blend for about 45 seconds. Pour the homogenate into a beaker.

2. Place cheesecloth over another beaker, forming a depression. Hold in place with a rubber band.

3. Pour onion homogenate onto cheesecloth and let it sit for a few moments to filter.

Precipitating DNA (*complete the following steps individually*)

1. Transfer 10 mL of filtered onion homogenate to a 50 mL tube (labeled with your name).

2. Using a micropipetter, add 1 mL of 10% SDS.

3. Mix by swirling and place tube in the ice bath for 5 minutes.

4. Add 15 mL ice-cold ethanol and mix by holding the capped tube horizontally and slowly rocking it back and forth. Watch as the DNA precipitates out of solution and becomes visible. Continue rocking the tube for a couple of minutes.

5. Spool the DNA onto the hooked end of a glass pipette by twirling the pipette in the DNA precipitate.

Since the purpose of this lab is simply to visualize DNA with the naked eye, we will not do any further manipulation of the DNA. We will do that in subsequent labs.

Cleanup:
Rinse off the glass pipettes and return them to their original beaker.
Rinse out other glassware and hang them on the drying rack.
Rinse our 50 mL tubes and place on drying rack (lids on paper towel).
Return micro-pipetters and tip boxes to their proper locations.
Wipe off your benchtop area with damp paper towel.

Manipulation of DNA: Part I
Cutting with Restriction Enzymes

Goal 1: Use restriction enzymes to manipulate DNA molecules so that the sizes of DNA fragments can be determined.

Plasmids are small, circular DNA molecules commonly found inside bacteria. Although plasmids occur naturally in the wild, molecular biologists have found a use for them in the laboratory. Naturally occurring plasmids have been modified by molecular biologists in such a way that they contain certain DNA sequences of that make them more useful. A wide variety of specially modified plasmids can now be purchased from biotechnology supply companies. One application of plasmids is as a 'vehicle' of sorts to carry a DNA fragment of interest into bacteria where it can then be copied as the bacteria replicate.

I have several plasmids into which DNA fragments of varying sizes were inserted by a student of mine for a research project. Each plasmid contains only one fragment of a certain size. You will be manipulating these plasmids using restriction enzymes in order to determine the size (or length, measured in terms of number of base pairs) of the DNA fragments. Restriction enzymes are naturally occurring enzymes produced by many bacteria. These enzymes recognize certain DNA sequences (recognition sequences) and cut the DNA at or near that location. The plasmids that you will be working with have recognition sequences adjacent to each end of the inserted DNA fragment, and thus by treating the plasmid with the restriction enzyme you effectively cut out the inserted fragment. The two resulting DNA fragments, the inserted fragment and the plasmid itself (which is linear at this point—imagine cutting a rubber band at two locations) can be separated based on size using gel electrophoresis. The first part of this lab will focus on digesting the plasmids with enzyme and the second part (next week) will focus on gel electrophoresis and visualization of the fragments.

Protocols

Digestion of plasmid DNA with restriction enzyme

1. To a 1.5 mL tube add 10 μL plasmid DNA
2. Add 1 μL 10X restriction enzyme buffer solution
3. Add 1 μL EcoRI restriction enzyme (1 U/μL)
4. Mix by flicking tube or vortexing, spin down contents by centrifuging briefly, and incubate at 37^{o}C for 30 min.

Cleanup:
Return the micro-pipetters and tip boxes to their proper locations.
Wipe off your benchtop area with damp paper towel.

Manipulation of DNA: Part II
Gel Electrophoresis

Goal: To perform gel electrophoresis of the plasmids digested with restriction enzymes last week in order to identify the size of DNA that was inserted into the plasmid.

Protocols

Sample preparation

- To your sample, add 1 μL of gel loading dye.
- Mix by vortexing.
- Place in centrifuge and spin briefly to bring contents to bottom of tube.

Gel electrophoresis

- When you load your sample, be sure to note in which well you place your sample. Wells are numbered 1-10 from left to right.
- I will load each gel with DNA size standard in the first well (starting at the left).
- Load 10 μL of your sample into the appropriate well.
- Record in your notebook which well your sample is in.
- Electrophoresis will be performed at 150 volts for about 15 minutes.
- Gels will be stained, visualized, and photographed.

Identification of fragments and size determination

- After you have a photograph of your gel, examine the fragments and determine which fragments are the plasmid and which is the inserted DNA.
- Use the size standard to determine the length of the inserted DNA of your sample and one from each of the other 3 gels (4 total). Record that on your gel photograph by circling the band representing the inserted DNA and indicating its length.
- Additionally, on your gel photograph, label one instance of each of the following:
    - well, plasmid DNA.

Cleanup:
Return the micro-pipetters and tip boxes to their proper locations.
Wipe off your benchtop area with damp paper towel.

Cell division and mitosis

Goal: To understand the process of mitosis and its role in cell division.

We will start by working in collaborative groups to draw the process of mitosis. After you are familiar with mitosis, we will examine prepared slides of developing fish embryos to visualize real cells caught in the act of mitosis.

PROCEDURE

Part I: the process of mitosis

Chromosome 7 in humans contains the gene responsible for cystic fibrosis. Since humans are diploid, an individual can have one of three genotypes: homozygous normal (both copies of the gene normal; cf^+/cf^+), homozygous mutant (both copies of the gene with a mutation; *cf/cf*) or heterozygous (one normal copy, one mutant copy; cf^+/cf) . Only homozygous mutant individuals will have cystic fibrosis.

Work together in groups to draw the process of asexual cell division (mitosis) in humans, tracking two chromosomes: chromosome 7 and an additional, non-homologous chromosome (remember, humans typically have 23 homologous pairs of chromosomes, you're only drawing 2 pairs for simplicity. You will be sketching the cell of an individual that is heterozygous (cf^+/cf). Start with the cell in pre-DNA replication interphase, then sketch it as it would appear in post-DNA replication interphase. Now sketch it as it would appear in each of the four phases of mitosis (prophase, metaphase, anaphase, and telophase.

Circle and label the following:

- *sister chromatids*
- a *pair of homologous chromosomes*
- two *non-homologous chromosomes*

Label each stage of the process:

- *Pre-DNA replication interphase*
- *Post-DNA replication interphase*
- *prophase*
- *metaphase*
- *anaphase*
- *telophase/cytokinesis*

Label arrows between stages with the following:

- *DNA replicates*
- *Chromosomes condense, spindle fibers form and attach to kinetochores*
- *Kinetochores pull against spindle fibers causing chromosomes to align*
- *Sister chromatids separate*
- *Spindle fibers break down and cell divides*

Part II: prepared slides of fish embryos

Sketch a cell that best represents each of the four stages of mitosis and label the following:

- *chromosomes*
- *centrosomes*
- *spindle fibers*
- *cell membrane*
- *cleavage furrow*

Draw what you actually see, not what you *think* you should see!!!!!!!!

Meiosis & sexual reproduction

Goal: To understand the process of meiosis and its role in sexual reproduction.

You will be working in collaborative groups to draw the process of meiosis and union of gametes. Be sure to focus on both the processes and the end results.

PROCEDURE

Chromosome 7 in humans contains the gene responsible for cystic fibrosis. Since humans are diploid, an individual can have one of three genotypes: homozygous normal (both copies of the gene normal; cf^+/cf^+), homozygous mutant (both copies of the gene with a mutation; *cf/cf*) or heterozygous (one normal copy, one mutant copy; cf^+/cf) . Only homozygous mutant individuals will have cystic fibrosis.

Work together in groups to draw the process of gamete formation (meiosis) and fertilization (union of gametes) beginning with the *gamete-producing, diploid cells* of a male and female (the parents) and ending with all of the *potential, unique diploid cells* of their resulting zygotes (fertilized eggs or offspring). In this scenario, the male parent is heterozygous and the female parent is homozygous normal.

Draw the cells starting in interphase (pre- and post-DNA replication) and continuing on through the eight stages of meiosis. End with the potential, unique zygotes following the union of gametes produced by meiosis. In each drawing, label chromosomes with the designation cf^+ for the normal allele and *cf* for the mutant allele. Label which zygotes (if any) will have *cystic fibrosis*, which are *carriers* (heterozygous) for the mutated allele, and which are *homozygous normal*.

Label your diagram with following stages and processes (use each term at least once):

Circle and label the following:

- *sister chromatids*
- a *pair of homologous chromosomes*
- two *non-homologous chromosomes*

Label each stage of the process:

- *Pre-DNA replication interphase*
- *Post-DNA replication interphase*
- *prophase I*
- *metaphase I*
- *anaphase I*
- *telophase I/cytokinesis*
- *prophase II*
- *metaphase II*
- *anaphase II*
- *telophase II/cytokinesis*
- *gametes*
- *potential zygotes*

Label arrows between stages with the following:

- *DNA replication*
- *Homologous chromosomes pair, crossover, then condense/spindle fibers form*
- *Spindle fibers attach to chromosomes then homologous pairs align along metaphase plate*
- *Homologous pairs separate*
- *Spindle fibers break down and cell divides*
- *Spindle fibers form*
- *Spindle fibers attach and pull sister chromatids to along metaphase plate*
- *Sister chromatids separate*
- *Spindle fibers break down and cell divides*
- *Cells develop into gametes*

Probability, Statistics & hypothesis testing

Goal: To gain a working understanding of probability, statistics, and hypothesis testing.

This lab will focus on using probability, statistics, and hypothesis testing to answer questions regarding the inheritance of genetic traits. These are very important skills that you will be applying in lecture and subsequent labs. Learn them now or pay the price later!

Likelihoods and Single Events

1. Given the flip of a fair coin, list all possible outcomes and the likelihoods of each.

Potential outcomes	Likelihoods
sum of all likelihoods =	

2. Given a single birth, list all possible outcomes (in terms of sex) and the likelihoods of each.

Potential outcomes	Likelihoods
sum of all likelihoods =	

3. Given a heterozygous individual (A/a), list all possible outcomes following meiosis (gamete genotypes) and the likelihoods of each.

Potential outcomes	Likelihoods
sum of all likelihoods =	

4. Given a fair, 6-sided die (die=dice, singular), list all possible outcomes and the likelihoods of each.

Potential outcomes	Likelihoods
sum of all likelihoods =	

Likelihoods and Multiple Events

When combinations of events are considered, one must take into account the likelihoods of *each* event and apply one or both of the two rules of probability, the product rule and the sum rule.

Product rule: If one wants to know the probability of two or more independent events occurring simultaneously or consecutively, use the product rule. For example, if you flip two coins, what is the probability of getting heads for one *and* heads for the other [p (h *and* h)]?

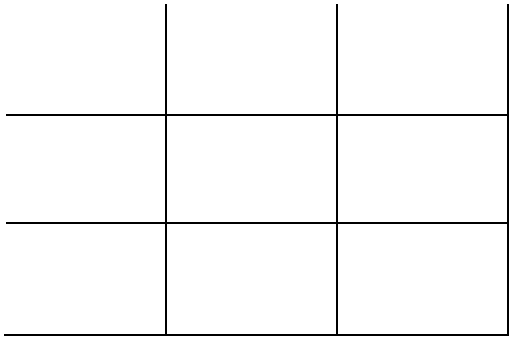
Sum rule: If one wants to know the probability of one event or another occurring, use the sum rule. For example, if you flip a coin, what is the likelihood of getting heads *or* tails [p(h *or* t)]? Note the use of the key words 'and' and 'or' in these two statements.

It is easy to loose track of all potential outcomes (and the multiple ways of getting those outcomes). For example, if one wants to know the likelihood of flipping a fair coin and getting heads *and* tails, you must be aware that there are two ways of getting this outcome: p(h and t) or p (t and h). Again, note the use of the key words 'and' and 'or'. Because of this, it is helpful to use one of the two graphical methods for keeping track of combinations of events: the grid method and the fork method.

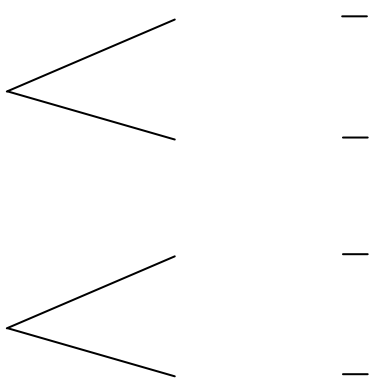
Using both the grid and the fork method, list all possible combinations of events and the likelihoods of each for the three following scenarios.

1. Two fair coins are flipped:

Grid Method



Fork Method



Potential outcomes	Likelihoods
Sum of all likelihoods =	

2. Two births (list all possible combinations of sex and the likelihoods of each):

Grid Method

Fork Method

Potential outcomes	Likelihoods
Sum of all likelihoods =	

3. Two heterozygous individuals mate and produce offspring (A/a x A/a). The potential outcomes to be considered here are offspring genotypes--the diploid genotypes resulting from union of sperm (one of two types) and ova (one of two types).

Grid Method

Fork Method

Potential outcomes	Likelihoods
Sum of all likelihoods =	

Hypothesis testing

... a method of determining whether to accept or reject an hypothesis based on the *probability* that the hypothesis is correct *given a particular data set*.

For example, we expect (hypothesize) that it is equally probable to get heads as tails when flipping a coin. Do you know for sure? Maybe a particular coin is weighted. How can you tell if your hypothesis is valid? Collect data (flip a coin) and conduct a statistical test on that data.

A chi-square (X^2) analysis measures 'goodness of fit' by measuring how similar *observed results* are relative to the *results expected* based on a particular hypothesis. It is based on a test statistic that measures the divergence of the observed data from the data expected given the null hypothesis of no difference. Before conducting a chi-square analysis, you must calculate the expected values given the null hypothesis and total number of observations.

Forget statistics for a moment and just use your common sense. If you flipped four different coins 100 times each and got the following results, how confident would you be as to the "fairness" of the coins (that it is equally likely to get heads as tails)? Rate your confidence from 1 to 4 (1 = very confident, 4 = not confident at all).

Coin	Results	Confidence
A	48 heads, 52 tails	
B	44 heads, 56 tails	
C	40 heads, 60 tails	
D	10 heads, 90 tails	

Question: Explain in your own words why you ranked your level of confidence as you did.

Chi-square analysis is based on the same way of thinking. It is simply a more precise way of determining your confidence by comparing *observations* with *expectations*.

Question: In the above example, what would you *expect* to be the results of tossing a "fair" coin 100 times?

This is what you expect, given your null hypothesis that the coin is fair.

Question: Would you be surprised if actual results were slightly different from your expectation? What would cause such a deviation?

Now use a chi-square analysis to more accurately rank the "fairness" of the four coins, or in other words, *test the null hypothesis that each coin is fair*. The following example shows you how.

The example below takes you step-by-step through a chi-square analysis using the results of coin A.

Step 1. State your null (N_0) and alternative (N_{alt}) hypotheses:

- *N_0, = the coin is fair and thus it is equally likely to get heads as tails*
- *N_{alt} = the coin is not fair and thus it is more likely to get tails than heads (or vice versa)*

Step 2. Calculate the chi-square ($\mathbf{X^2}$) value using the following formula:

$\boldsymbol{X^2}$ *= sum of* $(O - E)^2/E$

where O = observed and E = expected numbers (numbers expected based on the null hypothesis)

Events	**Observed numbers**	**Expected numbers**	$\boldsymbol{(O - E)^2/E}$
Heads	48	1/2 (100) = 50	$(48\text{-}50)^2/50 = 0.08$
Tails	52	1/2 (100) = 50	$(52\text{-}50)^2/50 = 0.08$
Total	100	100	$\boldsymbol{X^2}$ *= sum of* $(O - E)^2/E = 0.08 + 0.08 =$ **0.16**

Step 3. Determine the degrees of freedom (df):

df = number of categories – 1 (heads is a category, tails is a category)

Degrees of freedom takes into account the number of independent values used in the test and is usually one fewer than the sample size (or one fewer than the number of categories).

Step 4. Use a $\boldsymbol{X^2}$ table to convert your $\boldsymbol{X^2}$ value into an approximate *probability value*. Or better yet, use a computer to calculate the exact *probability value*.

IMPORTANT! What does this probability value (*p-value*) mean?

The *p-value* is the likelihood that *chance alone* caused the *observed* value to differ from the *expected* value rather than the null hypothesis not being an accurate statement. Stated differently, it is the *probability* that your null hypothesis is correct (based on your sample).

For example, if the *p*-value is 0.99, there is a 99% probability that chance alone caused the 48:52 ratio (versus 50:50 which is what you expected). In other words, there is a 99% probability that the coin is fair! This provides support for your null hypothesis.

For example, if the p-value is 0.02, there is a 2% probability that chance alone caused the deviation. In other words, there is only a 2% probability that the coin is fair and thus a 98% probability that the coin is NOT fair! This does not provide much support for your null hypothesis and you should consider rejecting it.

Probabilities of *less than 0.05* (5%) are usually considered significant enough to reject the null hypothesis. We will use 5% as our rejection level (known as the alpha value).

IMPORTANT! It is critical to understand that you can never prove or disprove a hypothesis. After all, you've only calculated the *probability* that it is correct! There is always a chance, no matter how small, that it is incorrect! It is also critical to understand the effect of sample size. The larger your sample, the more confident you can be in your conclusion.

On your own: Complete the above example by filling in the blanks below.

- Restate the null hypothesis.

- X^2 = _____ , df = _____ (*copy these values from above*)

- Based on a chi-square table, the probability (*p*) of the observed 48:52 heads:tails ratio being due to chance and sampling error rather than an *actual* difference from 50:50 is _____ %.

- Based on the computer calculation, the exact probability is _____%.

- In your own words, what does this particular *p*-value mean stated *in terms of* the null hypothesis?

- Based on 5% as the rejection level, do you *reject* the null hypothesis? _____

- Does this prove that the coin is fair? _____ Why or why not?

On your own: Do a chi-square analysis for each of the other three coins and record the results below.

	Observed		Expected					
Coin	H	T	H	T	X^2	df	*p(table)*	*p(computer)*
A	48	52	50	50	0.16	1	between 50% and 90%	
B								
C								
D								

Question: As the observed deviation from expected increases, what happens to the chi-square value?

Question: As the observed deviation from expected increases, what happens to the p-value?

Question: Do the four p-values above agree with your initial rankings?

Question: What is in the chi-square formula that allows it to come to the same conclusion that you did using your common sense?

Gene Expression and Inheritance in Corn

Goal: To understand how gene expression leads to an observed phenotype and to use chi-square analysis to test a hypothesis.

Part I

Given the following parental genotypes and cross...

Male		Female
T/t ; B/b	x	T/t ; B/b

a) based on the genotype, what is the phenotype of the parents? _____

----------*Complete the following four problems (neatly presented) on a separate sheet*----------

b) sketch cells representing both parents (illustrating their chromosomal and allelic makeup)

c) sketch cells representing each genetically-unique gamete produced by both parents

d) using your knowledge of probabilities, predict the phenotypic ratios of their progeny

e) for each progeny phenotype, sketch all unique cells (genotypes) that can produce this phenotype

Part II

We think that the ear of corn you will be examining includes progeny of the cross shown above. Use a chi-square analysis to test this hypothesis.

a) State the null hypothesis in terms of the expected phenotypic ratio.

b) Count the number of kernels exhibiting each phenotype.

phenotype	*observed number*	*total observed* _____
Yellow	_____	
Purple	_____	
Red	_____	

c) Perform the chi-square analysis.

phenotype	*observed*	*expected*	$(O-E)^2/E$
Yellow	_____	_____	_____
Purple	_____	_____	_____
Red	_____	_____	_____

X^2 = sum of $(O-E)^2/E$ = _____, df = _____

Using a X^2 table, the probability that the null hypothesis is correct is _____ %

Using the computer, the probability that the null hypothesis is correct is _____ %

Do you *reject* the null hypothesis? _____

What does the *p*-value mean stated in terms of the null hypothesis?

Pedigree Analysis

Goal: To infer the underlying mechanisms of gene expression and inheritance by examining pedigrees.

The patterns of inheritance recorded in pedigrees can be used to make inferences about the underlying mechanisms of gene expression and inheritance. Analyzing the results of specific genetic crosses is more informative in this regard, but performing such crosses is often not possible or practical--for example, humans or other animals with long generation times.

The objective of this lab is to familiarize you with the methods of analyzing pedigrees in order to address the following questions regarding the traits under consideration.

- Is the gene influencing the trait autosomal or X-linked?
- Is the 'mutant' phenotype dominant or recessive?
- What are the genotypes of parents and potential offspring?
- What is the likelihood of an unborn offspring being affected?

Proceedure

<u>For each of the four genetic diseases described below, address the above questions.</u>

Phenylketonuria (PKU)

- Appears in progeny of unaffected parents
- Sexes affected equally

Marfan Syndrome

- Phenotype appears in every generation
- Parent of affected progeny always affected

Duchene muscular dystrophy

- More males affected than females
- No progeny of an affected male are affected
- All of affected male's daughters will have half of their sons affected

Hypophosphatemia (a type of vitamin D-resistant rickets)

- Affected males pass the condition to all their female offspring
- Affected females pass condition to half their sons and daughters

Additional questions:

1. Will rare, recessive alleles usually be found in heterozygotes or homozygotes? Why?

2. What impact does inbreeding have on the expression of recessive alleles? Why?

Evolutionary Analysis of Genetic Data

Goal: To use genetic data to infer evolutionary relationships among organisms.

We've all seen evolutionary trees depicting hypothetical evolutionary relationships among various groups of taxa. But do you know how those trees were derived? This exercise focuses on making an evolutionary tree based on genetic data.

Evolution can be defined as descent with modification, and genetic data provide the strongest supporting evidence of evolution. Genetic data can be used to explore the evolutionary relationships among individual organisms, populations, species, and all higher taxonomic levels, including Kingdoms. How are genetic data interpreted in order to draw these conclusions? The following analogy is useful.

Imagine a class assignment where 10 students write a 10,000-word essay on a particular topic. The teacher gets back 10 identical essays. What is the chance that all 10 students came up with an identical essay? The most likely scenario is that someone must have written the original paper and the others must have copied. Now imagine that one of the papers (John's) is perfect but the others contain several errors. David's paper is identical to John's with one exception: word #300 is different. Mike's paper is identical to John's except that there are two mistakes, the same one at word #300 and another at word #1500. Susan's paper has those two mistakes and another at word #214. This pattern continues for the remaining students. How would you interpret this? The same logic involved in interpreting this example is applied when analyzing genetic data. DNA is passed on from one individual to another (just like the essays), accumulating changes and errors each time it is copied.

The amino acid sequences provided were obtained from a database available through the internet using the publicly-accessible Entrez search and retrieval system at http://www.ncbi.nlm.nih.gov/Entrez/. This database contains DNA and amino acid sequences obtained by researchers from around the world to be shared with the scientific community.

In this lab, you will be using amino acid and DNA sequences to explore the evolutionary relationships among vertebrates and among whales and their closest living relatives.

Q1 *Draw a tree describing the relationships among the essays described above.*

<u>Part I: Vertebrate evolution</u>

Using the myoglobin amino acid sequences provided:

1. Determine the number of amino acid positions that differ relative to the first sequence (in italics). Amino acids that are identical with the first sequence are indicated by a dot. Deletions are indicated by a dash. Write these values in the table provided.
2. Calculate the percent difference by dividing the number of *different* amino acid positions by the total number of positions.
3. Using the scale provided, draw an evolutionary tree based on the percent difference values calculated above. Draw lightly in pencil because you will need to erase and redraw.

4. You can fine-tune this tree by searching for 'shared-derived characters'. A shared-derived character is any character that is shared by a group of individuals but that is unique (derived) relative to all other individuals. It is likely that these individuals share a common ancestor that had this character.

5. Join the branches of individuals having 'shared-derived characters'. The more 'SDC's a group of individuals have, the closer you can join the branches relative to the tips of the branches.

6. The last step is to identify the species and interpret your results. When everyone is finished, I will provide you with a key identifying the species from which each sequence was derived.

Q2 *Does this evolutionary tree fit well with what you know about other types of evidence such as the fossil record and comparative anatomy?*

Q3 *Do you think that this gene would be useful for determining evolutionary relationships of human populations? Why or why not?*

Part II: The whale's closest living relative

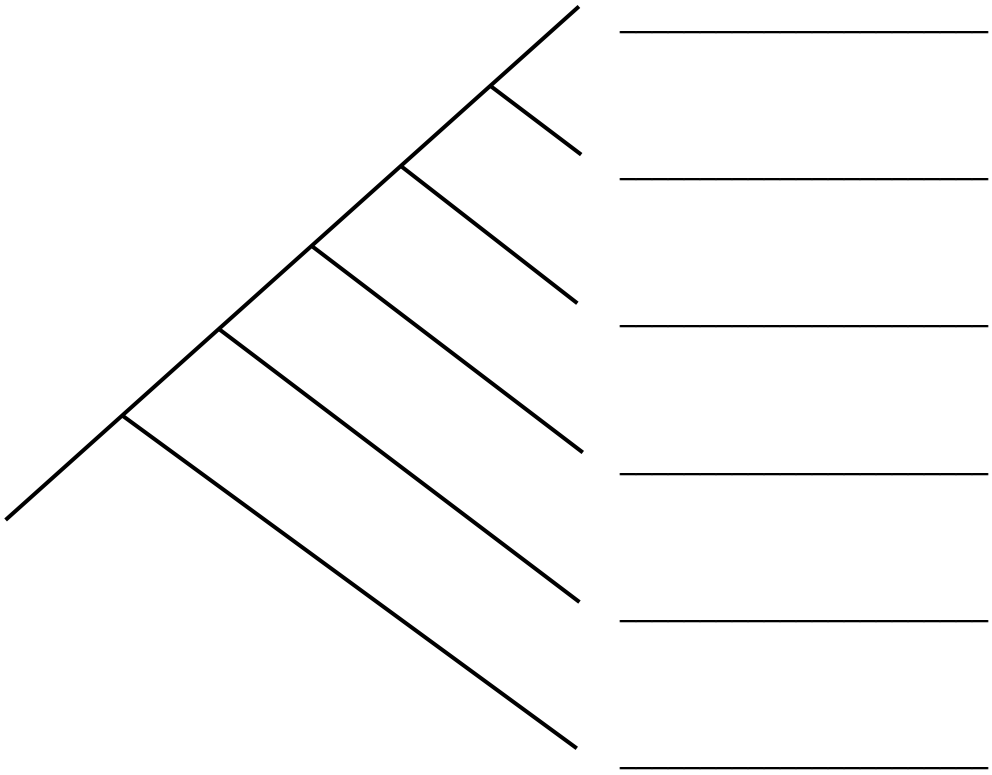
For over 100 years, scientists have thought that whales may have evolved from an ungulate ancestor. Morphology and fossil data support this inference. But which living ungulate is most closely related to whales? Deer, pigs, hippos, or camels? Fossil evidence is not conclusive at the moment, but genetic data provide a convincing answer to this question.

Alu elements are not the only DNA sequences that can copy and paste themselves randomly in the genome. SINES and LINES (short and long interspersed elements) are two additional examples of this unusual type of DNA sequence. The insertion of these DNA sequences is a random event and, thus, if two individuals have a SINE or LINE at a particular location in their genome, they must have inherited that DNA sequence from a common ancestor. This DNA insert would be a 'shared-derived character' and can be used to reconstruct evolutionary relationships.

The following table shows the presence or absence of 18 different SINES and LINES in 4 groups of ungulates and two whales. Each number represents a taxa and each letter represents a particular SINE or LINE insert. 'x's indicate taxa that have a particular DNA insert at a particular location in their genome. These data are from: *Nikaido, Rooney, and Okada. 1999. Phylogenetic relationships among cetartiodactyls based on insertions of short and long interspersed elements: _____ are the closest extant relatives of whales. Proceedings of the National Academy of Sciences, 96:10261.*

Taxa	SINE or LINE insert																	
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*	*M*	*N*	*O*	*P*	*Q*	*R*
20																		
21	x			x	x	x	x			x	x	x	x	x				
22	x			x	x	x	x			x	x	x	x		x	x	x	
23	x	x	x															
24	x			x	x	x	x			x	x	x	x		x	x	x	x
25	x			x	x	x	x	x	x									

Identify groups of taxa that share SINE or LINE inserts (shared-derived characters) and place them on the tree (provided below) in a way that best fits the data. At the appropriate location on the tree, indicate which taxa share which inserts. Here's the general procedure: Decide which taxon is most dissimilar and place it on the longest branch. All other taxa share which character? Indicate that on the tree. Of the remaining taxa, which is the most dissimilar? Place it on the next longest branch. Does it have any unique characters? If so, indicate that on the tree. All remaining taxa share which character(s)? And so on, and so on...



Q4 According to the tree you just constructed, which ungulate is the closest living relative of the whales?

Population Genetics of Domestic Cats in Texas

Goal: To determine if a population is being influenced by evolutionary forces.

The frequencies of alleles and genotypes in populations do not change from one generation to the next *unless* evolutionary forces are influencing the population. Such forces include non-random mating, migration, selection, small population size, and/or mutation. If *observed* number of each *genotype* matchs what we would *expect* to see based on observed allelic frequencies, it is likely that none of the above forces are acting on that population. In this case, the population is said to be in *Hardy-Weinberg equilibrium*--the population is not evolving at a rate that is detectable.

We will be analyzing the data gathered by everyone in the lab regarding phenotypes of domestic cats in Texas. Our data represents a small *sample* of the total cat population. It would not be practical (or possible) to sample every single cat. Like all samples, ours will serve as a *random representation* of the total population. We will be using numbers of observed phenotypes and knowledge of the underlying genetics of each character to calculate 1) observed genotypic (or phenotypic) frequencies, 2) allelic frequencies, and 3) the expected number of each genotype (or phenotype) assuming Hardy-Weinberg equilibrium. *Using this information we will then determine if this population is in Hardy-Weinberg equilibrium for two of the genes (X^O and S).*

Keep in mind that the number of animals is of no real importance, think of them as *containers* for holding *alleles*. In population genetics, it is the number of *alleles* that's important.

Genotype (phenotype)	Observed no. of each phenotype	Total no. of individuals observed	Allelic frequencies (assume equal sex ratio)		Expected genotypic frequencies	Expected no. of each genotype
$H^L/-$						
H^S/H^S						
$P^W/-$						
P^C/P^C						
$A^+/-$						
A^-/A^-						
$C^I/-$						
C^D/C^D						
S^P/S^P			S^P =		S^P/S^P	S^P/S^P
S^N/S^P			S^N =		S^N/S^P	S^N/S^P
S^N/S^N					S^N/S^N	S^N/S^N
X^{O+}/Y			Females	Males	X^{O+}/Y	X^{O+}/Y
X^{O-}/Y			X^{O+} =	X^{O+} =	X^{O-}/Y	X^{O-}/Y
X^{O+}/X^{O+}				X^{O-} =	X^{O+}/X^{O+}	X^{O+}/X^{O+}
X^{O+}/X^{O-}			X^{O-} =	Y =	X^{O+}/X^{O-}	X^{O+}/X^{O-}
X^{O-}/X^{O-}					X^{O-}/X^{O-}	X^{O-}/X^{O-}

1. *State your null hypothesis.*

2. *Record the observed and expected genotypic frequencies and calculate the* X^2 value for both genes.

Spot locus					
Genotype	**Observed #**	**Expected #**	**O - E**	**$(O - E)^2$**	**$(O - E)^2 / E$**
S^P/S^P					
S^P/S^N					
S^N/S^N					
				$X^2 =$	

Orange locus					
Genotype	**Observed #**	**Expected #**	**O - E**	**$(O - E)^2$**	**$(O - E)^2 / E$**
X^{O+}/Y					
X^{O-}/Y					
X^{O+}/X^{O+}					
X^{O+}/X^{O-}					
X^{O-}/X^{O-}					
				$X^2 =$	

3. Determine the degrees of freedom.
   df = [number of categories (genotypes) - 1] - 1 *{yes, subtract 1 twice in this case}*

4. Convert your X^2 value into a probability (*p*) using a X^2 table. *p* is the *probability* that your null hypothesis is correct. Probabilities less than 5% are usually considered *significant* enough to reject the null hypothesis. Remember, you can never prove or disprove a hypothesis. After all, you've only calculated the *probability* that it is correct!

For the piebald spotting locus:

- $X^2 =$ _____ , df = _____
- The probability (*p*) that the null hypothesis is correct is _____
- Do you reject the null hypothesis? _____

For the orange locus:

- $X^2 =$ _____ , df = _____
- The probability (*p*) that the null hypothesis is correct is _____
- Do you reject the null hypothesis? _____

Q1 *Based on our sample, do you conclude that the local population of cats is in Hardy-Weinberg equilibrium for the orange (X^O) locus? The spot (S) locus?*

Q2 *In your own words, what do these results mean in terms that* anyone *could understand?*

Population Genetics of Humans

Using the Alu data for the entire lab group, perform a chi-square analysis to test the null hypothesis of Hardy-Weinberg equilibrium. Keep in mind that the lab group does not represent a true interbreeding population so don't be surprised at the results—it could be in equilibrium or it could be out of equilibrium, depending on the genetic ancestry of the class members.

Genotype	Observed Numbers	Expected Numbers
Alu+/Alu+		
Alu+/Alu-		
Alu-/Alu-		