The Agilent Technologies SureSelect™ Platform for Target Enrichment

Focus your next-gen sequencing on DNA that matters

Kimberly Troutman
Field Applications Scientist
August 20, 2010

Presentation Agenda

- **Introduction to SureSelect™ target enrichment**
- **eArray and kit production**
- **Current SureSelect™ kit offerings**
- **NGS QPCR kits and automation**

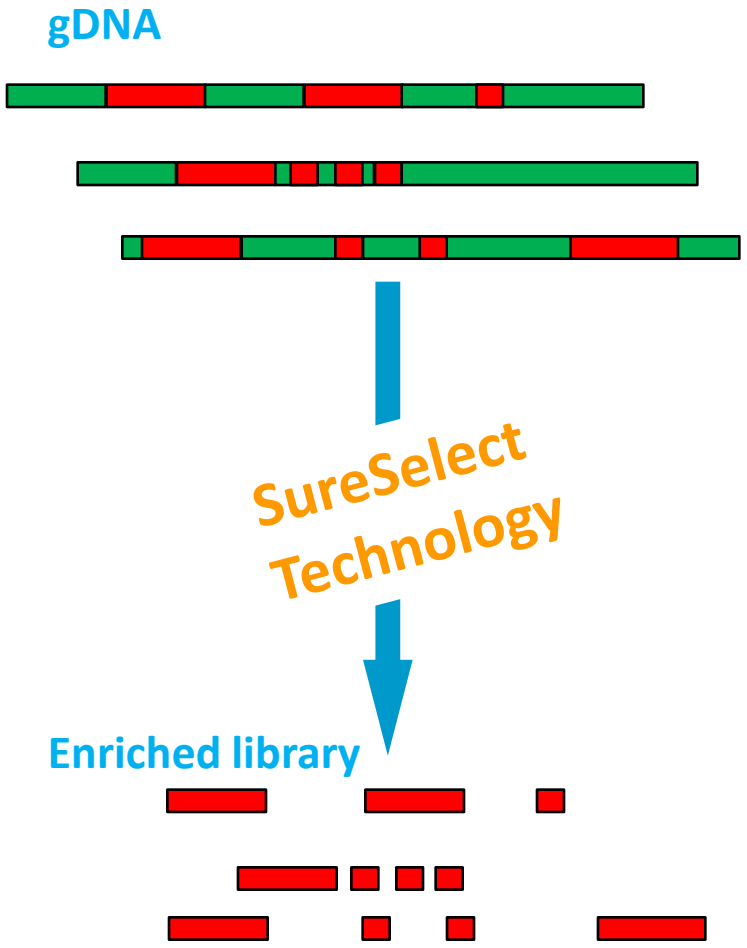
Target Enrichment: A Highly Enabling Process

What?

- Also referred to as genome partitioning, targeted re-sequencing, DNA capture…
- Captures genomic material of interest for next generation sequencer (i.e. Illumina, SOLiD, 454 etc…)

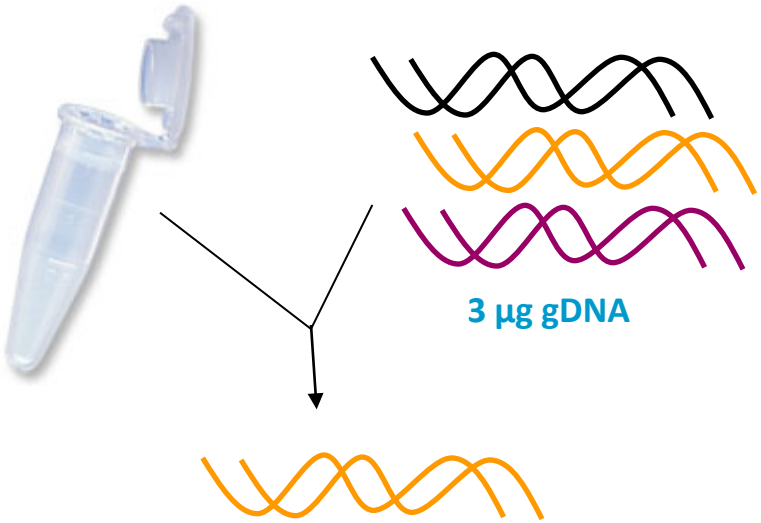
Why?

- Sequence your regions of interest!
- Enables focus on a subset of the genome
- Saves both time and money for downstream sequencing
- Identify homozygous and heterozygous variants in targets relative to the reference genome

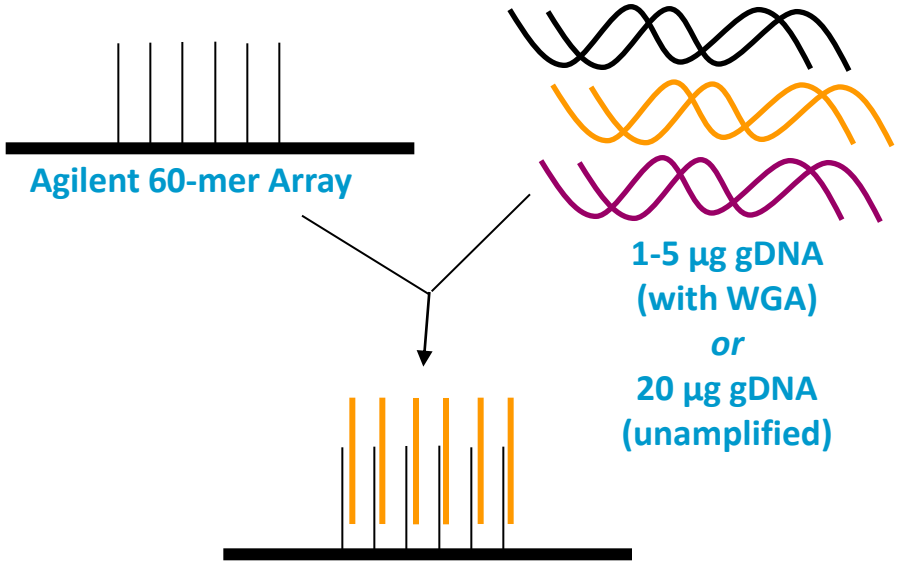


Agilent's SureSelect™ Platform: Two Options

SureSelect Target Enrichment System*
Developed in collaboration with the Broad Institute
Dr. Chad Nusbaum *et al.*



SureSelect DNA Capture Array
Developed in collaboration with Cold Spring Harbor
Dr. Greg Hannon *et al.*



*Flagship Method Released February 2009

Released July 2009

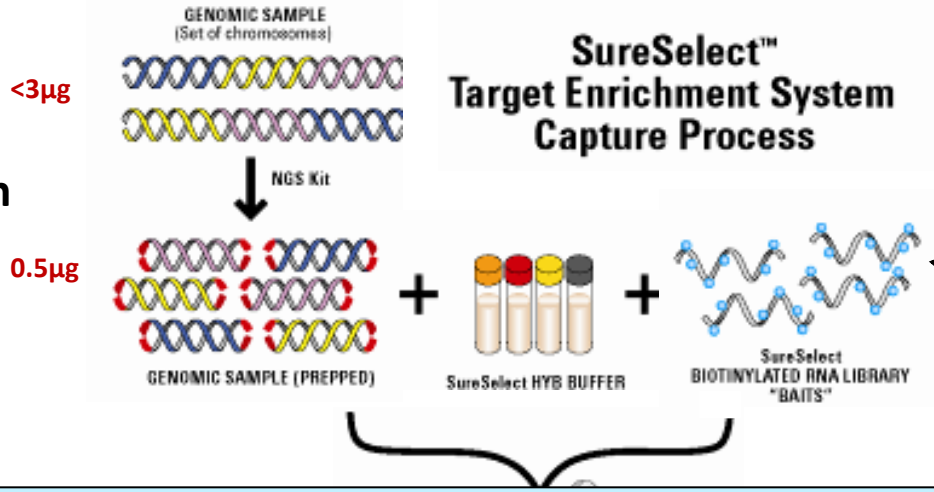
Distinct Target Enrichment Products for Distinct Project Needs

or

	SureSelect Target Enrichment System	SureSelect DNA Capture Array
Throughput	High	Low
Study Sizes	10-1,000's samples	1-10 samples
DNA Input	3 µg	3 µg
Amplified Library	500 ng	20 µg
Captured DNA	Up to 6.9 Mb	Up to 1 Mb
Baits	120-mers cRNA	60-mers DNA

Library Preparation

- Illumina SE/PE
- SOLiD



SureSelect™ Target Enrichment System Capture Process

SureSelect

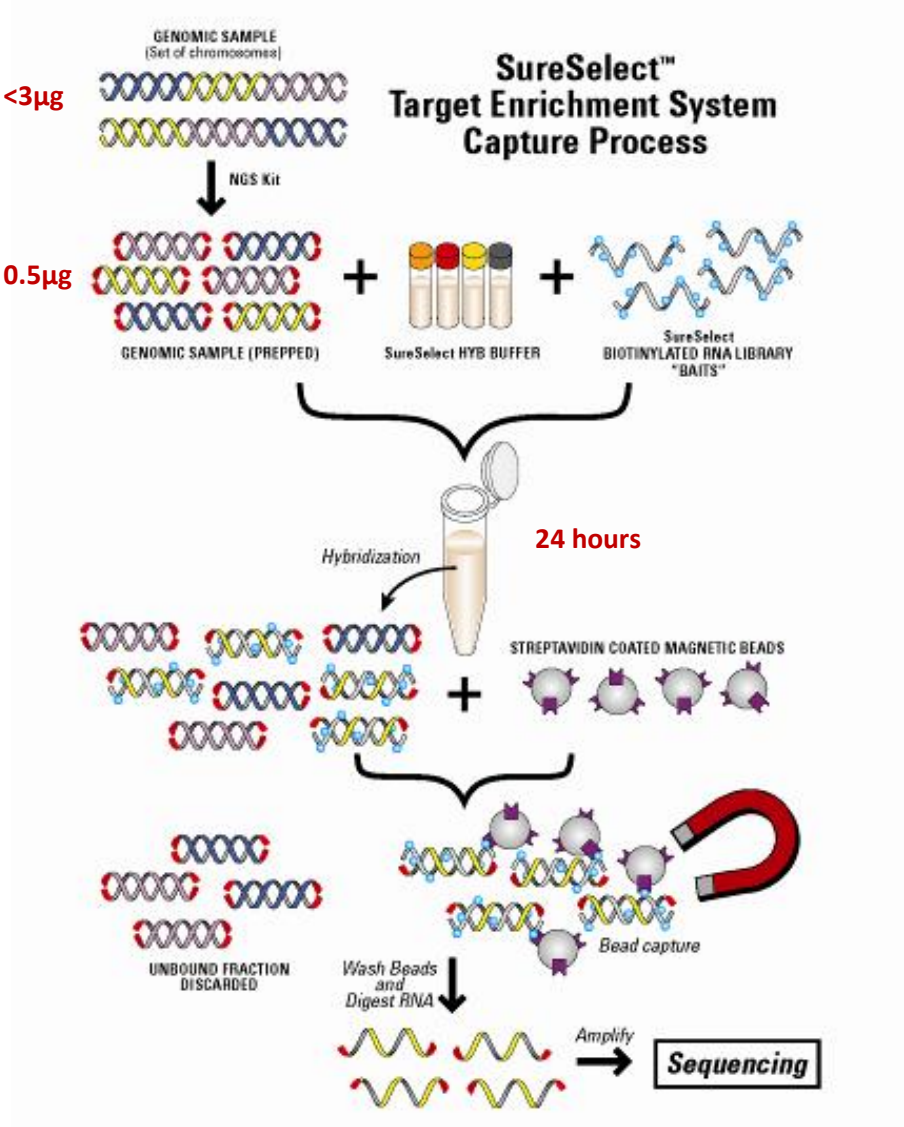
Baits

- cRNA probes
- Long (120 bp)
- Biotin labeled
- User-defined (eArray)
- SurePrint synthesis

Advantages of Agilent Target Enrichment

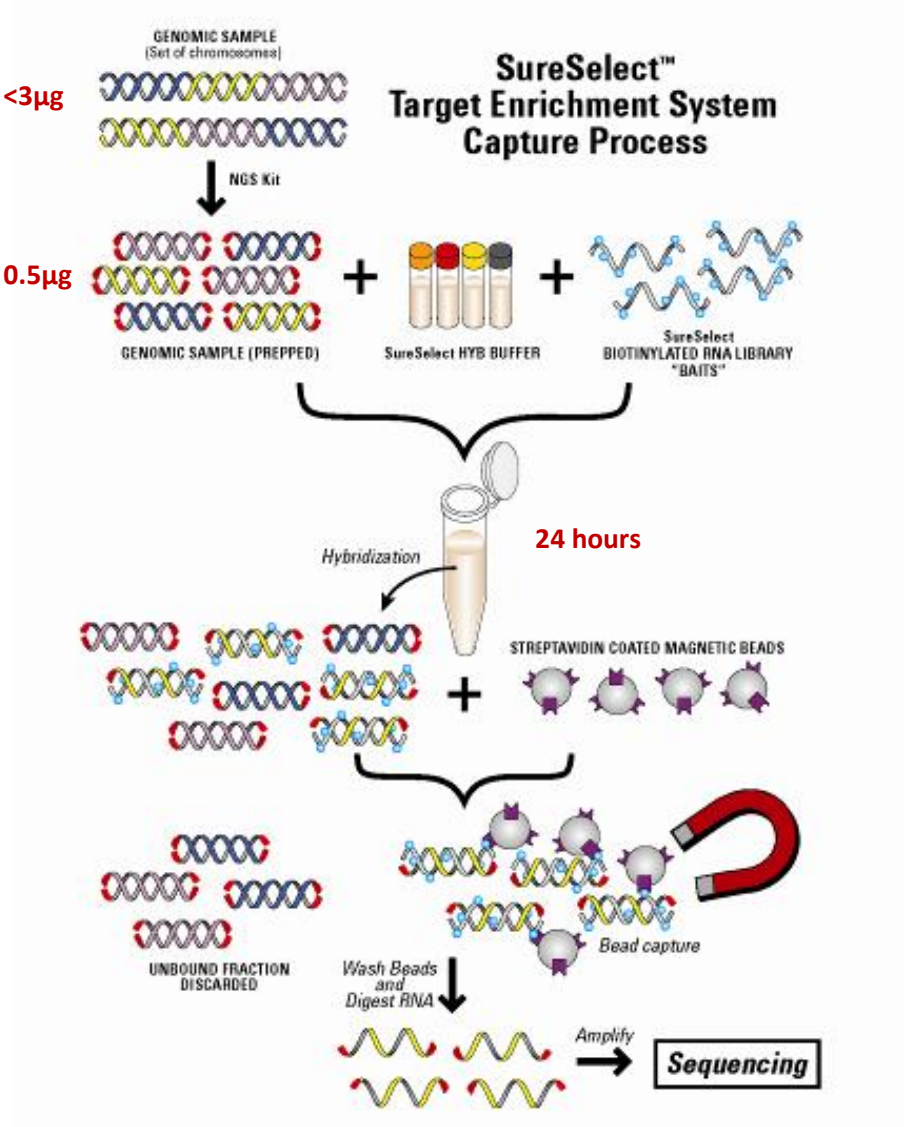
- Long baits tolerate mismatches
- RNA-DNA hybrids stronger than DNA-DNA
- RNA probe is strand-specific:
  - Allows large molar excess of bait
  - Target-limited; improves uniformity
- Easily automated: all steps liquid handling
- 24 hour hybridization
- Low input DNA (< 3 ug)
- Working on Solution Enrichment since 2006, license from Broad

SureSelect™ Target Enrichment System: Workflow



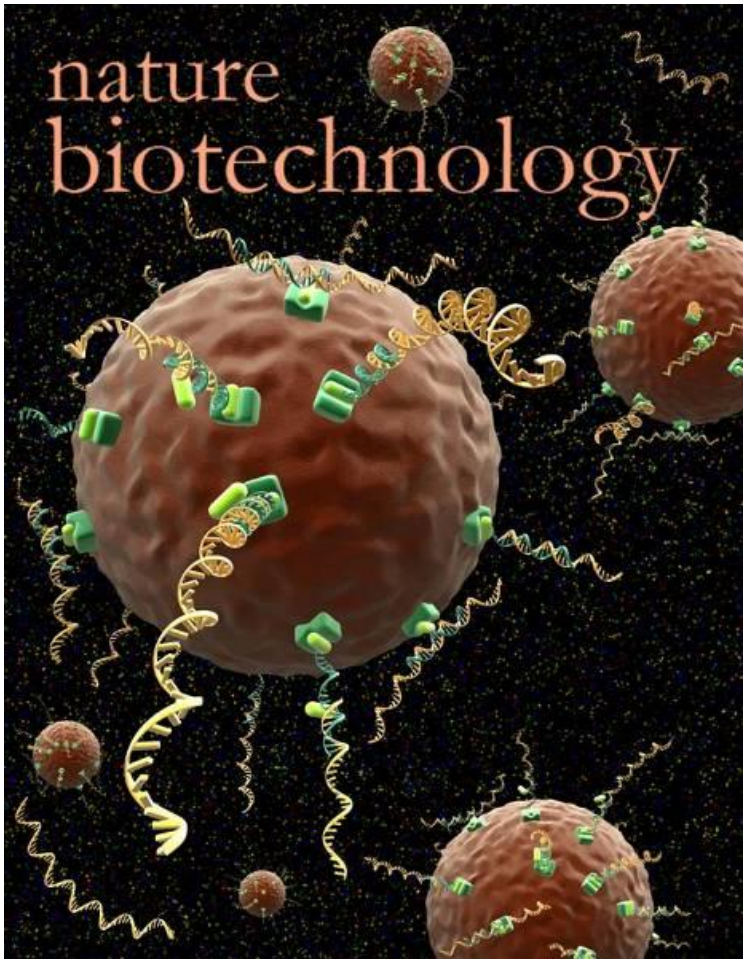
HiSeq 2000 & Illumina GAII$_x$

SureSelect™ Target Enrichment System: Workflow



SOLiD 3 & SOLiD 4

Broad Paper on Cover of February, 2009 Nature Biotechnology Underlying Technology of SureSelect™ Target Enrichment System



ARTICLES

nature biotechnology

Solution hybrid selection with ultra-long oligonucleotides for massively parallel targeted sequencing

Andreas Gnirke[1], Alexandre Melnikov[1], Jared Maguire[1], Peter Rogov[1], Emily M LeProust[2], William Brockman[1,5], Timothy Fennell[1], Georgia Giannoukos[1], Sheila Fisher[1], Carsten Russ[1], Stacey Gabriel[1], David B Jaffe[1], Eric S Lander[1,3,4] & Chad Nusbaum[1]

Targeting genomic loci by massively parallel sequencing requires new methods to enrich templates to be sequenced. We developed a capture method that uses biotinylated RNA 'baits' to fish targets out of a 'pond' of DNA fragments. The RNA is transcribed from PCR-amplified oligodeoxynucleotides originally synthesized on a microarray, generating sufficient bait for multiple captures at concentrations high enough to drive the hybridization. We tested this method with 170-mer baits that target >15,000 coding exons (2.5 Mb) and four regions (1.7 Mb total) using Illumina sequencing as read-out. About 90% of uniquely aligning bases fell on or near bait sequence; up to 50% lay on exons proper. The uniformity was such that ~60% of target bases in the exonic 'catch', and ~80% in the regional catch, had at least half the mean coverage. One lane of Illumina sequence was sufficient to call high-confidence genotypes for 89% of the targeted exon space.

The development and commercialization of a new generation of increasingly powerful sequencing methodologies and instruments[1–4] have lowered the cost per nucleotide of sequencing data by several orders of magnitude. Within a short time, several individual human

have been tested on target sets complex enough to match the scale of current next-generation sequencing instruments.

The first method, microarray capture[9,12,13], uses hybridization to arrays containing synthetic oligonucleotides that match the target

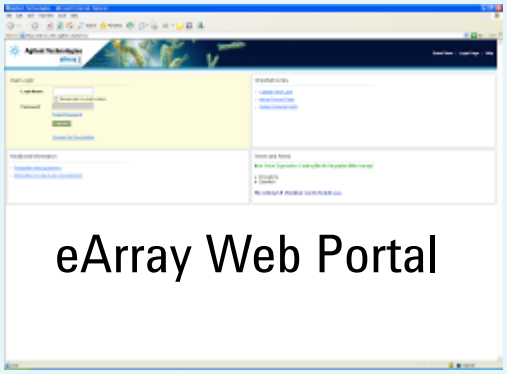
Presentation Agenda

- Introduction to SureSelect™ target enrichment
- eArray and kit production
- Current SureSelect™ kit offerings
- NGS QPCR kits and automation

SureSelect™ Target Enrichment System

1. Design & Order

Select custom Target Enrichment baits using eArray



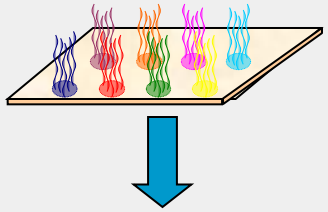
eArray Web Portal

or

select catalog oligo set

2. Kit Production

55K unique 120 mer oligos synthesized on one wafer



Oligos released

Oligo IVT to RNA-biotin

3. Single Tube Workflow

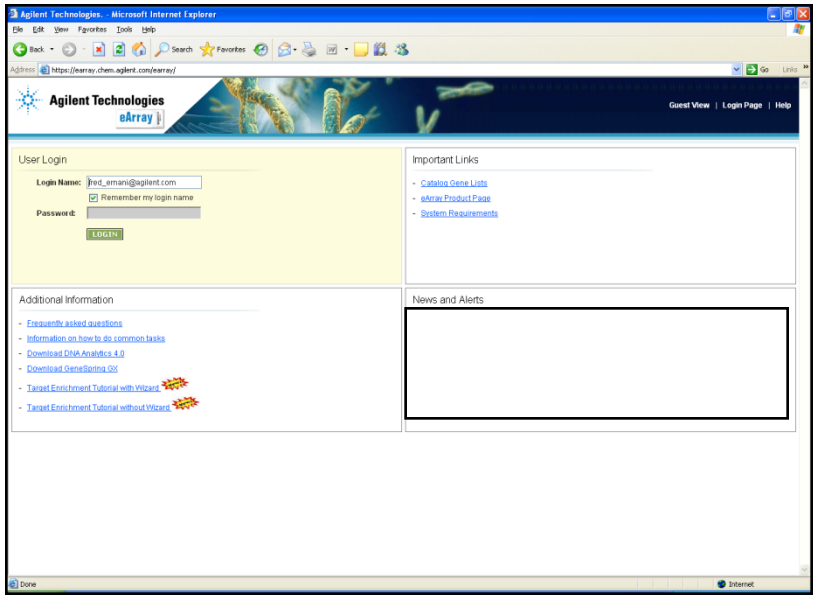
SureSelect™ Kit shipped to customer

Kit Includes

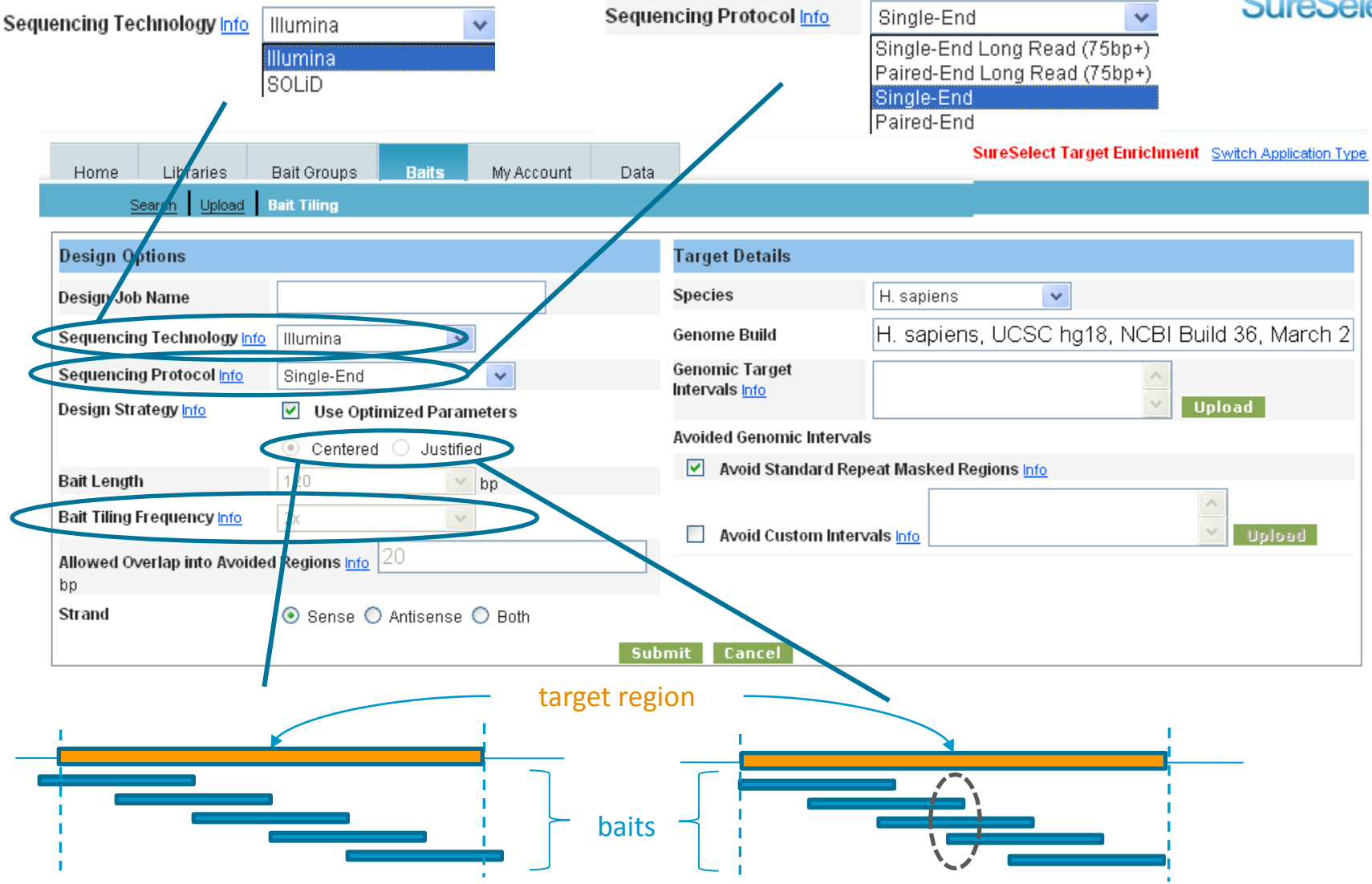
1. Biotinylated-cRNA
2. Reagents
3. Protocol

Target Enrichment Design Application in eArray

- **eArray is a tool to design and order custom microarrays, qPCR primers and SureSelect products (and it is free!!)**
- **eArray is divided into "Application Spaces"**
  - Allows for application specific functionality
- **Target Enrichment application space features:**
  - Create custom baits and bait libraries
  - Search existing designs/baits
    - Catalog and custom
  - Upload custom bait designs
  - Download design files
  - Share designs
  - Get quotes



Target Enrichment Design in eArray



Bait Design is Dependent on Read Length Output of Sequencer

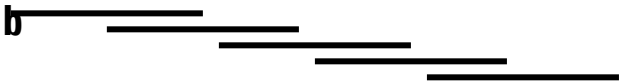
SureSelect

1x-tiled for 76 bp PE sequencing

55k baits @120bp → 6.9 Mb

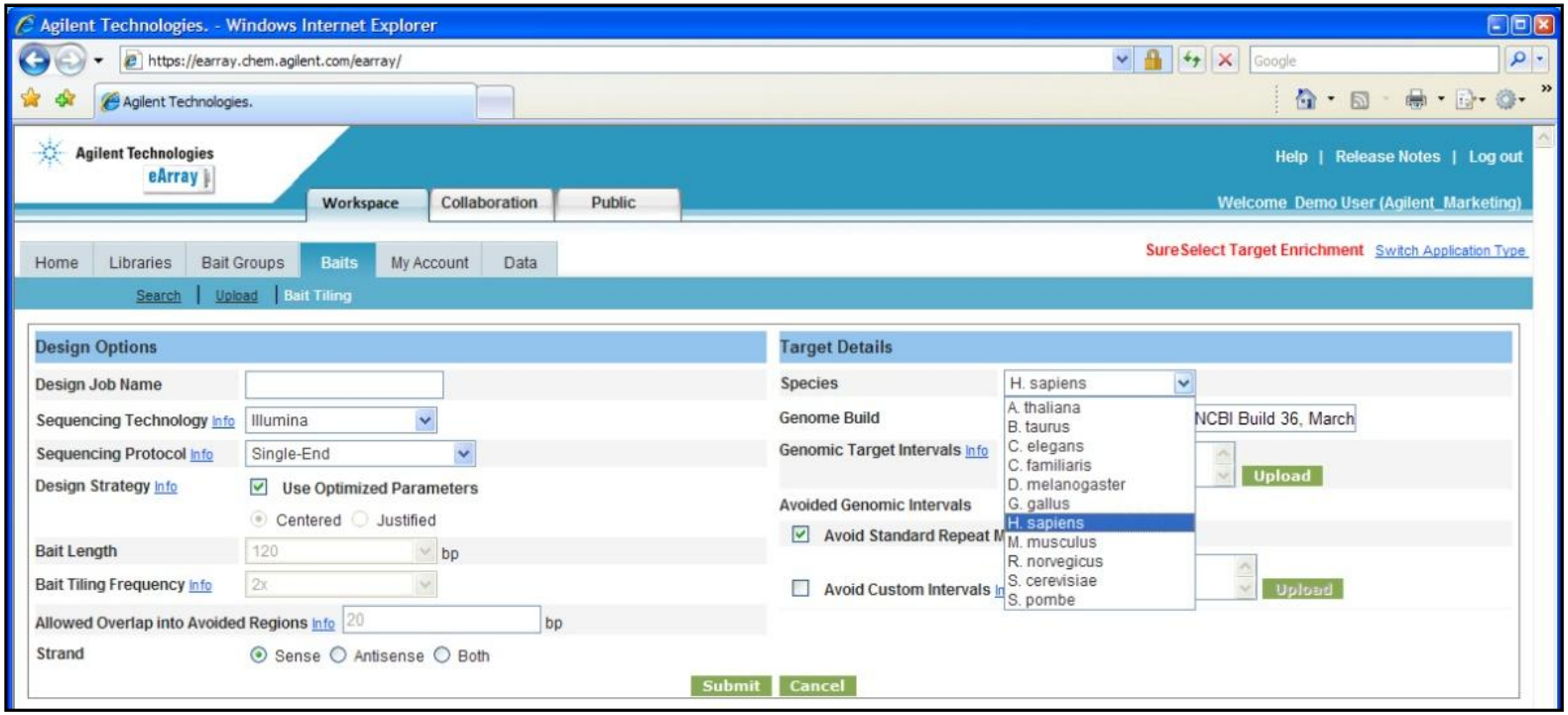
2x-tiled for 36 bp PE & SE sequencing

55k baits @ 120bp → 3.45 Mb



- eArray currently restricts to 2x to 5x tiling for 36bp PE & SE sequencing
- End to end tiling enabled (1x) for 76 bp PE kit and human exome capture
  - Human exome target enrichment kit contains baits designed by end to end tiling
  - Optimal for 76 bp paired-end sequencing on the Illumina GA

eArray – Supported Species



H. sapiens, M. musculus, R. norvegicus, D. melanogaster, C. elegans, C. familiaris, S. cerviseae, S. pombe, G. gallus, B. taurus, A. thaliana

Sequence Any Genome- eArray XD

NEWSFOCUS

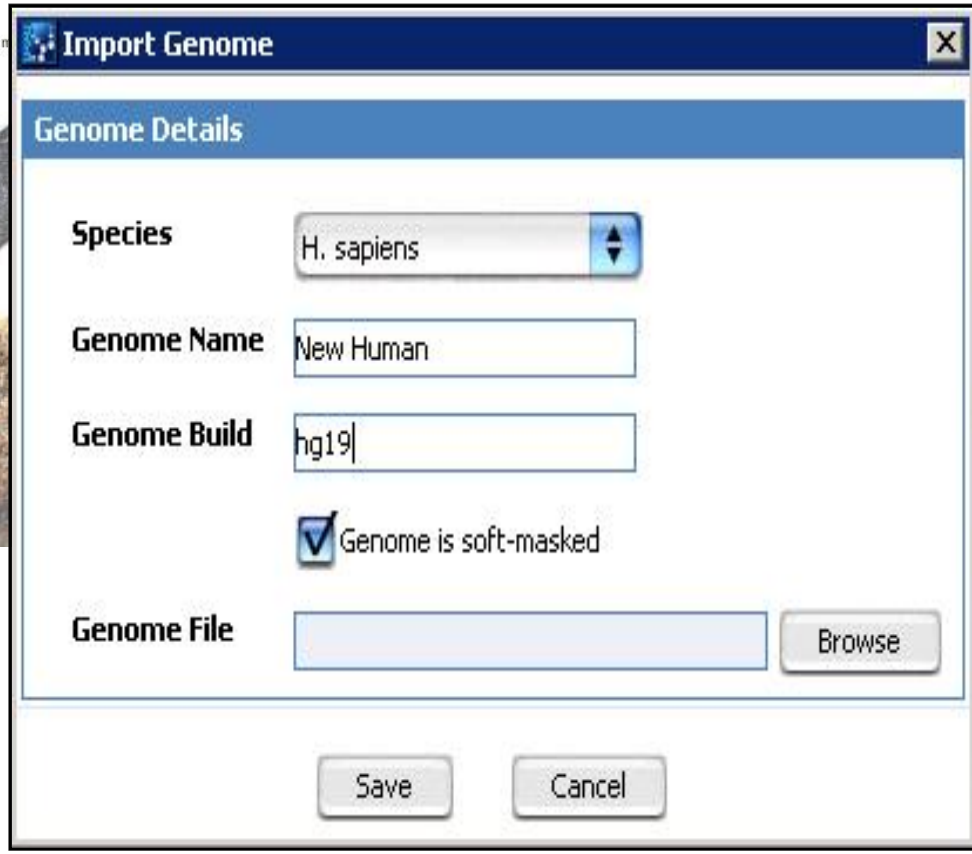
DNA SEQUENCING

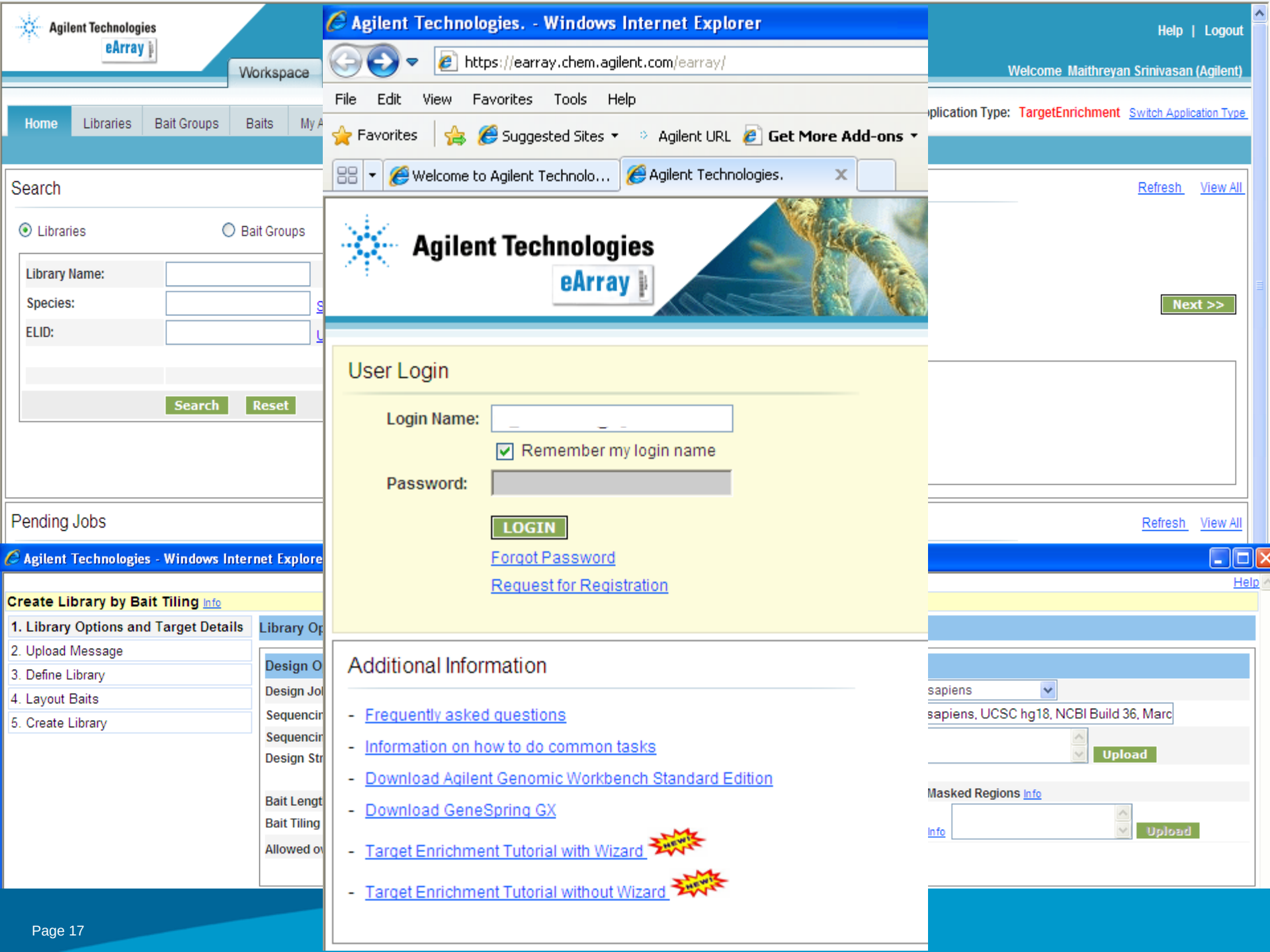
No Genome Left Behind

A project to sequence 10,000 vertebrates has just been launchednds of other vertebrates

one day have their genomes sequenced.

Published by AAAS

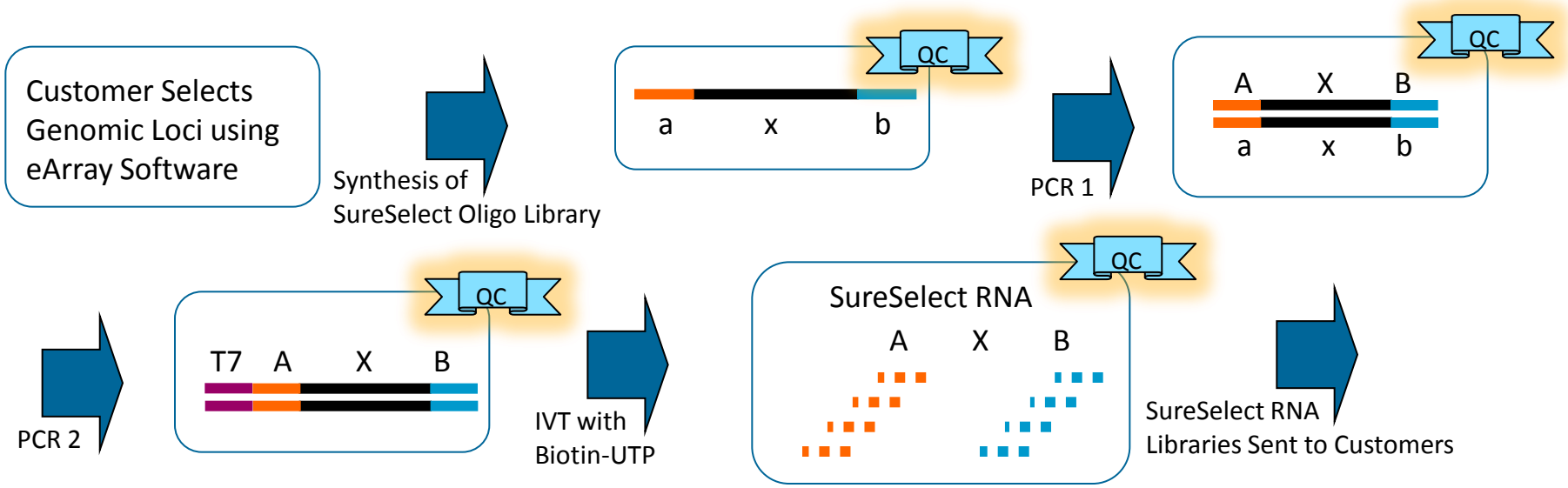



Agilent Technologies
eArray
Workspace
Help | Logout
Welcome Maithreyan Srinivasan (Agilent)
Home
Libraries
Bait Groups
Baits
Application Type: TargetEnrichment
Switch Application Type
Search
Libraries
Bait Groups
Library Name:
Species:
ELID:
Search
Reset
Refresh
View All
Next >>
Pending Jobs
Refresh
View All
Agilent Technologies. - Windows Internet Explorer
https://earray.chem.agilent.com/earray/
File Edit View Favorites Tools Help
Favorites
Suggested Sites
Agilent URL
Get More Add-ons
Welcome to Agilent Technolo...
Agilent Technologies.
Agilent Technologies
eArray
User Login
Login Name:
Remember my login name
Password:
LOGIN
Forgot Password
Request for Registration
Additional Information
- Frequently asked questions
- Information on how to do common tasks
- Download Agilent Genomic Workbench Standard Edition
- Download GeneSpring GX
- Target Enrichment Tutorial with Wizard
- Target Enrichment Tutorial without Wizard
NEW!
NEW!
Agilent Technologies - Windows Internet Explore
Create Library by Bait Tiling
Info
1. Library Options and Target Details
2. Upload Message
3. Define Library
4. Layout Baits
5. Create Library
Help
sapiens
sapiens, UCSC hg18, NCBI Build 36, Marc
Upload
Masked Regions
Info
Upload

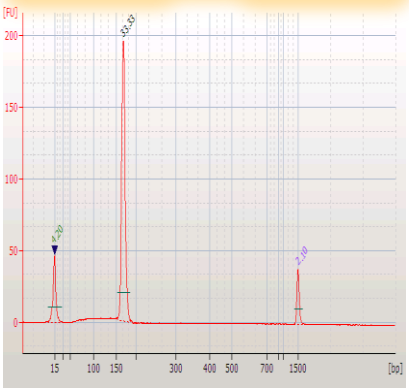
Santa Clara Manufacturing Facility

- **Industrial manufacturing – Class 10,000 clean-room**
- **Wired directly into eArray, allowing direct customer access to fully customizable products**
- **High-performance inkjet printing enables long oligo manufacturing**

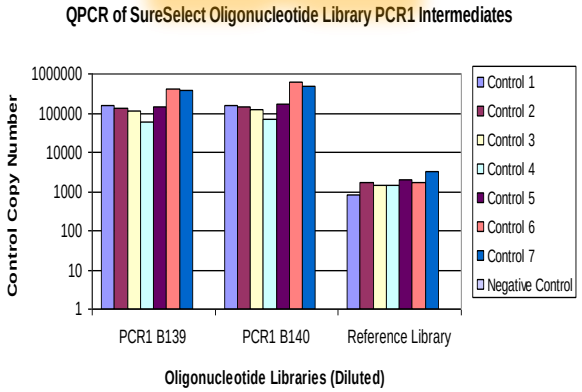
SureSelect Biotinlyated RNA Library Production & Quality Control



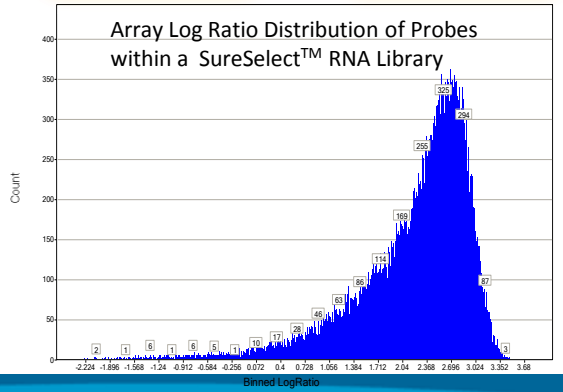
Bioanalyzer



qPCR



Arrays: Optional



Presentation Agenda

- Introduction to SureSelect™ target enrichment
- eArray and kit production
- Current SureSelect™ products
- NGS QPCR kits and automation

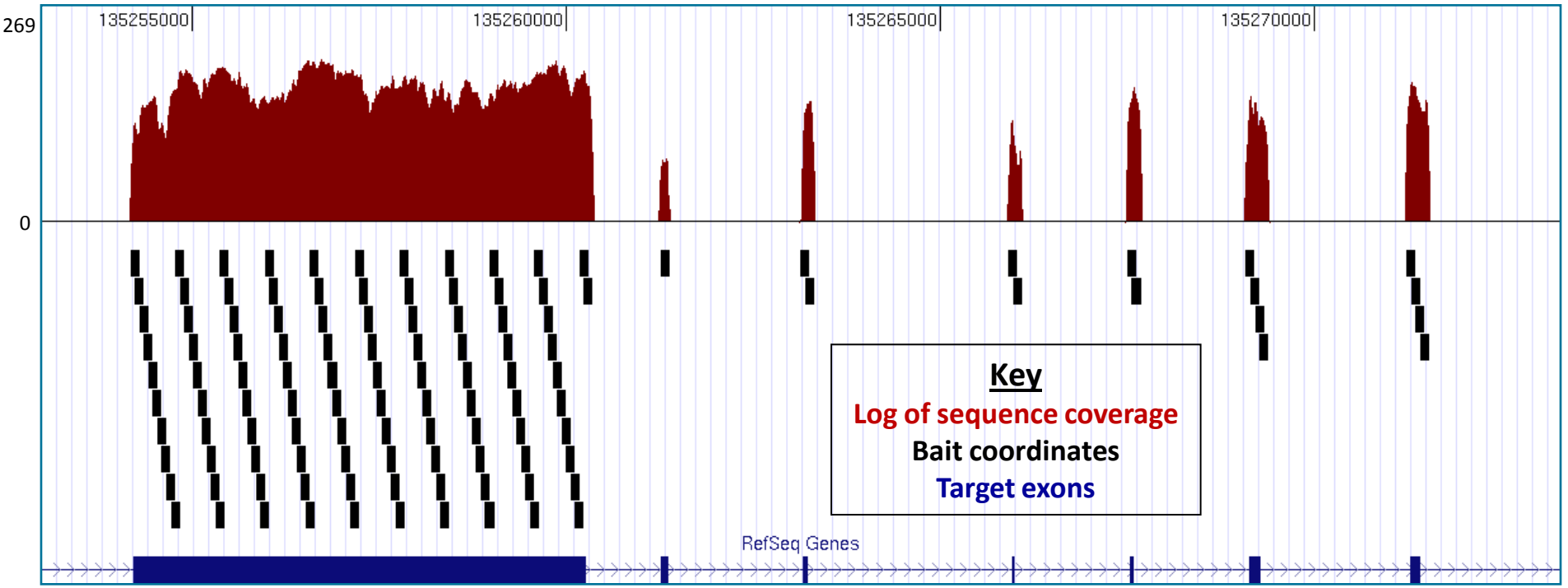
SureSelect™ Target Enrichment Products

- **Human X-Exon Demo kit**
  - 3 Mb
  - 5 reactions/kit (G4459A)
- **Human All Exon kits (v1&v2)**
  - V1 38 Mb (CCDS + >1,000 ncRNA)
  - V2 38 Mb (v1 + additional RefSeq)
  - 5 - 10,000 reactions/kit
- **Human All Exon Plus kit**
  - 38 Mb (CCDS + >1,000 ncRNA)
  - Plus add your custom content (up to 6.9 Mb)
  - Illumina, SOLiD
  - 5 - 10,000 reactions/kit
- **50 Mb Human All Exon kit**
  - 50 Mb GENCODE content
  - Illumina, SOLiD
  - 5 - 10,000 reactions/kit
  - Multiplexable

New

- **Custom Indexing kits**
  - Capture 0.2, 0.5, 1.5, 3 Mb, 6.9 Mb
  - 10 - 5,000 reactions per kit
  - eArray web portal interface
  - Illumina, SOLID
  - Significant cost savings $

Agilent SureSelect™ Target Enrichment Efficacy: X Chromosome Kit Sample Coverage Plot



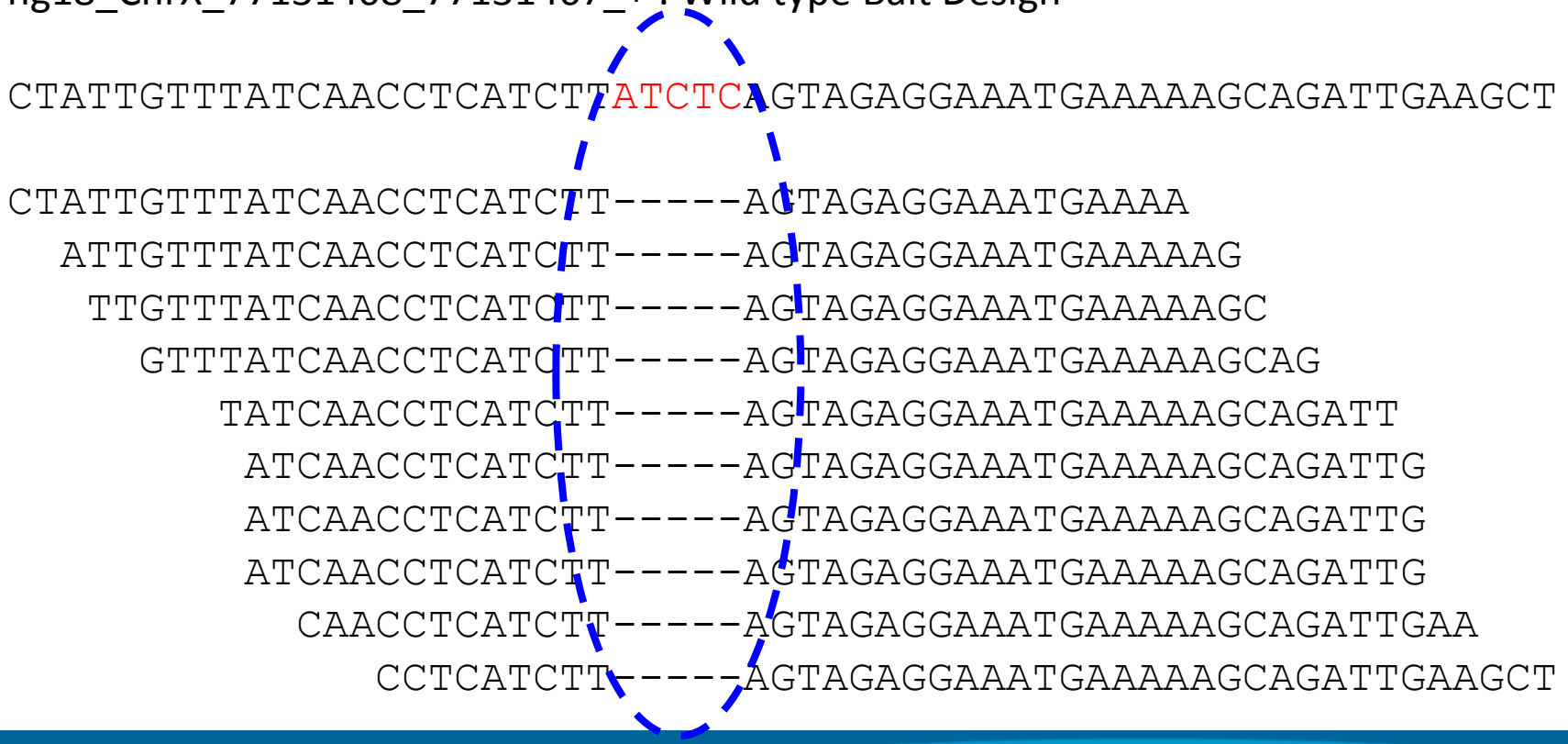
- UCSC Genome Browser sequence coverage for a portion of the SureSelect Target Enrichment demo kit
- Coverage of the RefSeq exons on the non-pseudoautosomal portion of Chromosome X using 2x tiling
- Sequence coverage is higher for those exons that are covered by more than one overlapping bait

Efficient Capture of 5 bp Deletion on Chr X: Menke's Syndrome

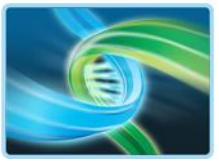
SureSelect™ Target Enrichment Kit Efficiently Captures 5 bp Mutant

Readout on Illumina GA

hg18_ChrX_77131408_77131467_+ : Wild type Bait Design



Exon Capture is a Powerful Tool to Study Mendelian Diseases



- **Mendelian diseases are caused by coding mutations (with some exceptions)**
- **Exons are only ~1-1.4 % of human genome (30-50Mb)**
- **Primarily protein coding regions**

Advantages:

- Much less sequencing
- ~5% of WGS, so up to 20x more samples

Disadvantage:

- Miss non-coding variants

Why coding+?

- More interpretable
- Easier to follow up
- Especially adapted to study of Mendelian diseases

SureSelect X-Demo kit™

- **All Exons on X chromosomes**
- **7674 exons**
- **3 Mb**

SureSelect Human All Exon ™

- **CCDS exons – v1**
- **CCDS + RefSeq – 38 Mb v2 (Broad)**
- **GENCODE – 50 Mb (Sanger)**
- **Includes ncRNA**

Applications to Mendelian Disorders

ARTICLE

Massively Parallel Sequencing of Exons on the X Chromosome Identifies *RBM10* as the Gene that Causes a Syndromic Form of Cleft Palate

Jennife
NIH Ir

REPORT

Whole Exome Sequencing and Homozygosity Mapping Identify Mutation in the Cell Polarity Protein GPSM2 as the Cause of Nonsyndromic Hearing Loss DFNB82

Tom Walsh,[1]
Amal Abu Ra

nature genetics

De novo mutations of *SETBP1* cause Schinzel-Giedion syndrome

Alexander Hoischen[1,14], Bregje W M van Bon[1,14], Christian Gilissen[1,14], Peer Arts[1], Bart van Lier[1], Marloes Steehouwer[1], Petra de Vries[1], Rick de Reuver[1], Nienke Wieskamp[1], Geert Mortier[2], Koen Devriendt[3], Marta Z Amorim[4], Nicole Revencu[5], Alexa Kidd[6], Mafalda Barbosa[7], Anne Turner[8], Janine Smith[9], Christina Oley[10], Alex Henderson[11], Ian M Hayes[12], Elizabeth M Thompson[13], Han G Brunner[1], Bert B A de Vries[1] & Joris A Veltman[1]

Mutations in the DBP-Deficiency Protein HSD17B4 Cause Ovarian Dysgenesis, Hearing Loss, and Ataxia of Perrault Syndrome

Sarah B. Pierce,[1,7] Tom Walsh,[1,7] Karen M. Chisholm,[1,8] Ming K. Lee,[1] Anne M. Thornton,[1] Agata Fiumara,[2] John M. Opitz,[3] Ephrat Levy-Lahad,[4,5] Rachel E. Klevit,[6] and Mary-Claire King[1,*]

RAPID C

Dent[4],

Unexpected Allelic Heterogeneity and Spectrum of Mutations in Fowler Syndrome Revealed by Next-Generation Exome Seq

HGVS HUMAN GENOME VARIATION SOCIETY

e for a rare mendelian disorder of independent kindreds, we captured

Emilie Lalonde,[1,3†] Steffen Albrecht,[2†] Kevin C.H.
Pierre Dechelotte,[5] Jacek Majewski,[1,3] and Nada J

[1]*McGill University and Genome Quebec Innovation Centre,*
McGill University Health Center, Montreal, Canada; [3]*Depart*
[4]*Departments of Pediatrics, Montreal Children's Hospital, M*
Anatomy, CHU Clermont–Ferrand, Université d'Auvergne, Fr

REPORT

Terminal Osseous Dysplasia Is Caused by a Single Recurrent Mutation in the *FLNA* Gene

Yu Sun,[1,11] Rowida Almomani,[1,11] Emmelien Aten,[1] Jacopo Celli,[1] Jaap van der Heijden,[1] Hanka Venselaar,[2] Stephen P. Robertson,[3] Anna Baroncini,[4] Brunella Franco,[5,6] Lina Basel-Vanagaite,[7] Emiko Horii,[8] Ricardo Drut,[9] Yavuz Ariyurek,[1,10] Johan T. den Dunnen,[1,10] and Martijn H. Breuning[1,*]

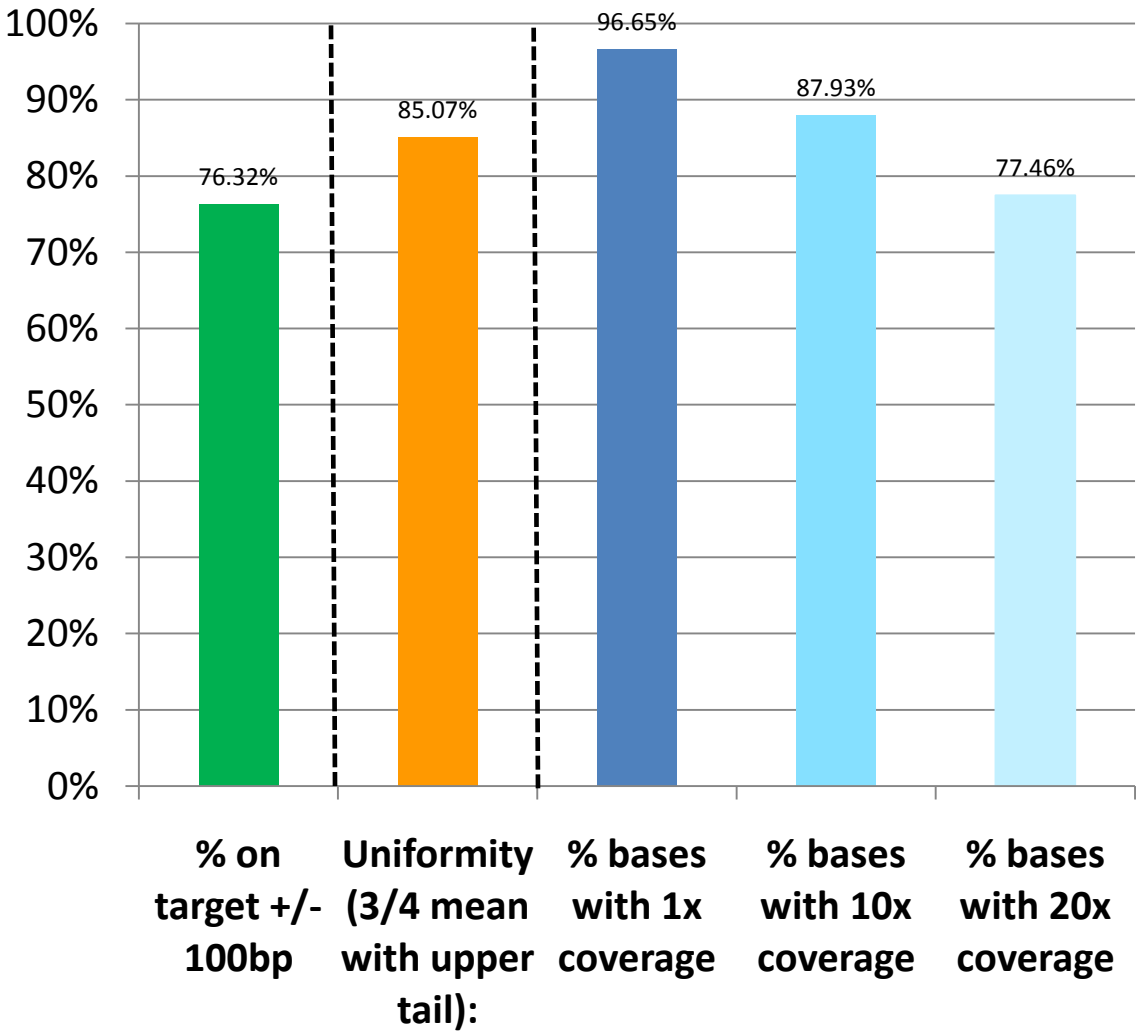
Agilent Technologies

Human All Exon Kits – Comprehensive Coverage

	Original design	Exome V2	50 Mb design (New)
	CCDS Sept. 2008	CCDS Sept. 2008 + additional RefSeq content including CCDS Sept. 2009 exons	GENCODE and Sanger (includes CCDS and Broad defined v2 content as well)
CCDS (Sept. 2009)	**93.76%**	**99.01%**	**99.86%**
CNV (Mar. 2010)	**23.98%**	**27.49%**	**30.62%**
Ensembl (6/16/2010)	**65.58%**	**71.37%**	**75.24%**
miRNA (miRBase 14)	**90.00%**	**90.00%**	**92.78%**
GenBank (6/16/2010)	**75.96%**	**89.07%**	**90.74%**
RefSeq Genes (6/16/2010)	**86.69%**	**93.29%**	**96.47%**
RefSeq Transcripts (6/16/2010)	**88.85%**	**95.07%**	**97.50%**
Total	**37Mb**	**38Mb**	**50Mb**
Developped with	**Broad**	**Broad**	**Sanger**

- Human All Exon kits can be customized (PLUS) with up to 6.9 Mb additional custom content
- Human All Exon kits can be multiplexed on SOLiD4 and HiSeq2000

Human All Exon 50Mb – 2x76 bp, 50-60M HQ Reads



The most comprehensive Human All Exon content available

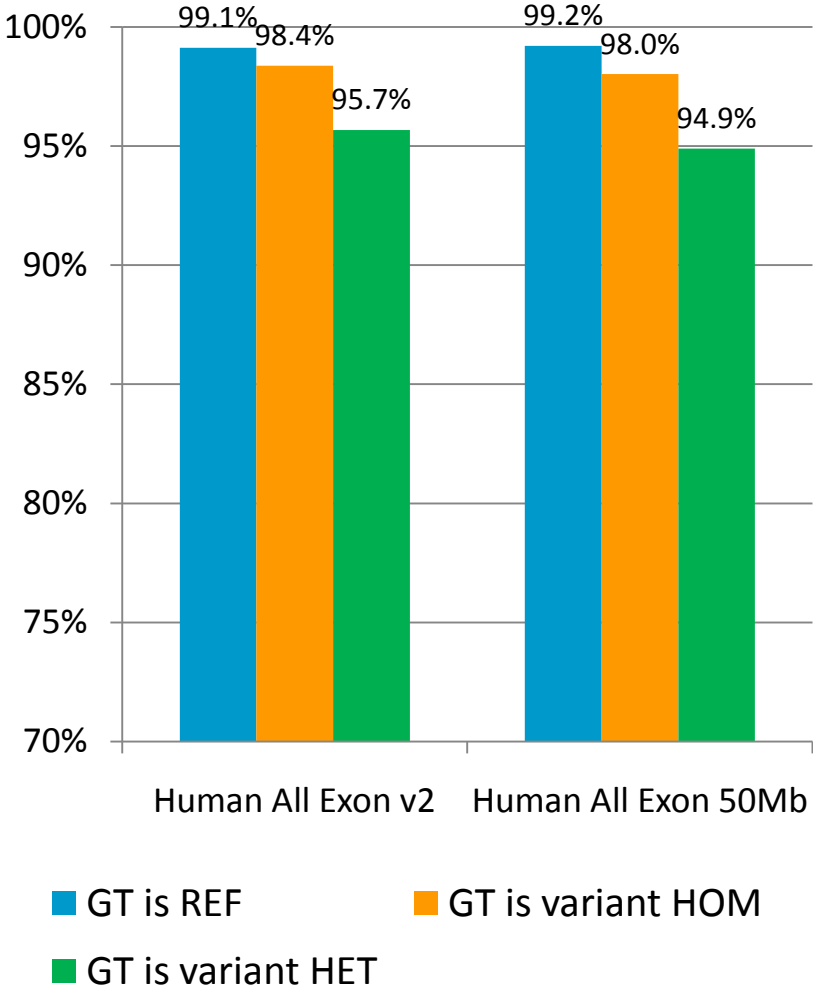
38 Mb design = a subset of 50 Mb

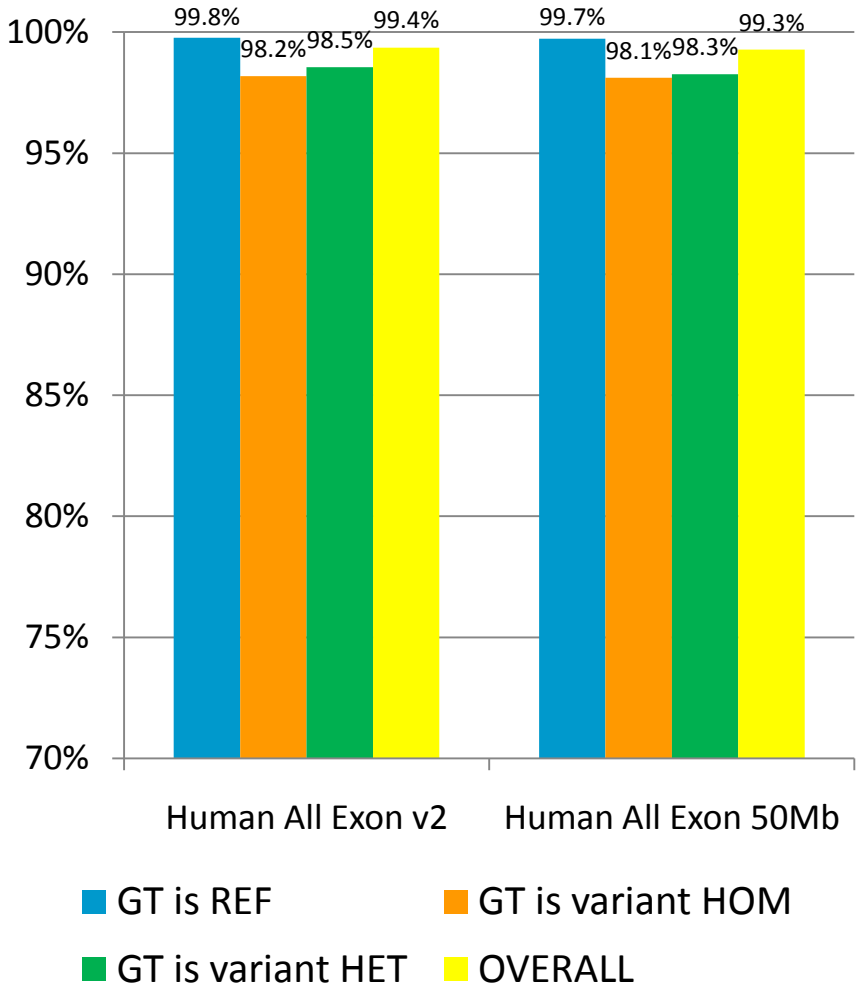
Sequencing capacity:
- 0.5-1 sample / lane GAIIx
- 1-3 samples / lane HiSeq
- 5-10 samples /full slide SOLiD4

Chemistry recommended:
- PE 2x76 bp Illumina v4
- PE 50+25 SOLiD

Multiplexing:
- Illumina
- SOLiD

Comparison of SNP Calls with HapMap





Agilent Technologies

Current 38 Mb Human Exon vs. New 50 Mb Design

	Original design	50 Mb design
	CCDS Sept. 2008 (%)	GENCODE and Sanger (includes CCDS and Broad defined v2 content as well)
CCDS (Sept. 2009)	93.76	99.86
CNV (Mar. 2010)	23.98	30.62
Ensembl (6/16/2010)	65.58	75.24
miRNA (miRBase 14)	90.00	92.78
GenBank (6/16/2010)	75.96	90.74
RefSeq Genes (6/16/2010)	86.69	96.47
RefSeq Transcripts (6/16/2010)	88.85	97.50
Total	38 Mb	50 Mb
Developed with	Broad	Sanger

With new content we now more accurately represent CCDS, GenBank, RefSeq Genes and RefSeq Transcripts databases

All Exon Plus

Is the Human All Exon Kit not hitting all of your regions of interest?

Enter Your Custom Regions in eArray

All Exon Library

CCDS exons
>1000 ncRNAs
38 Mb

+

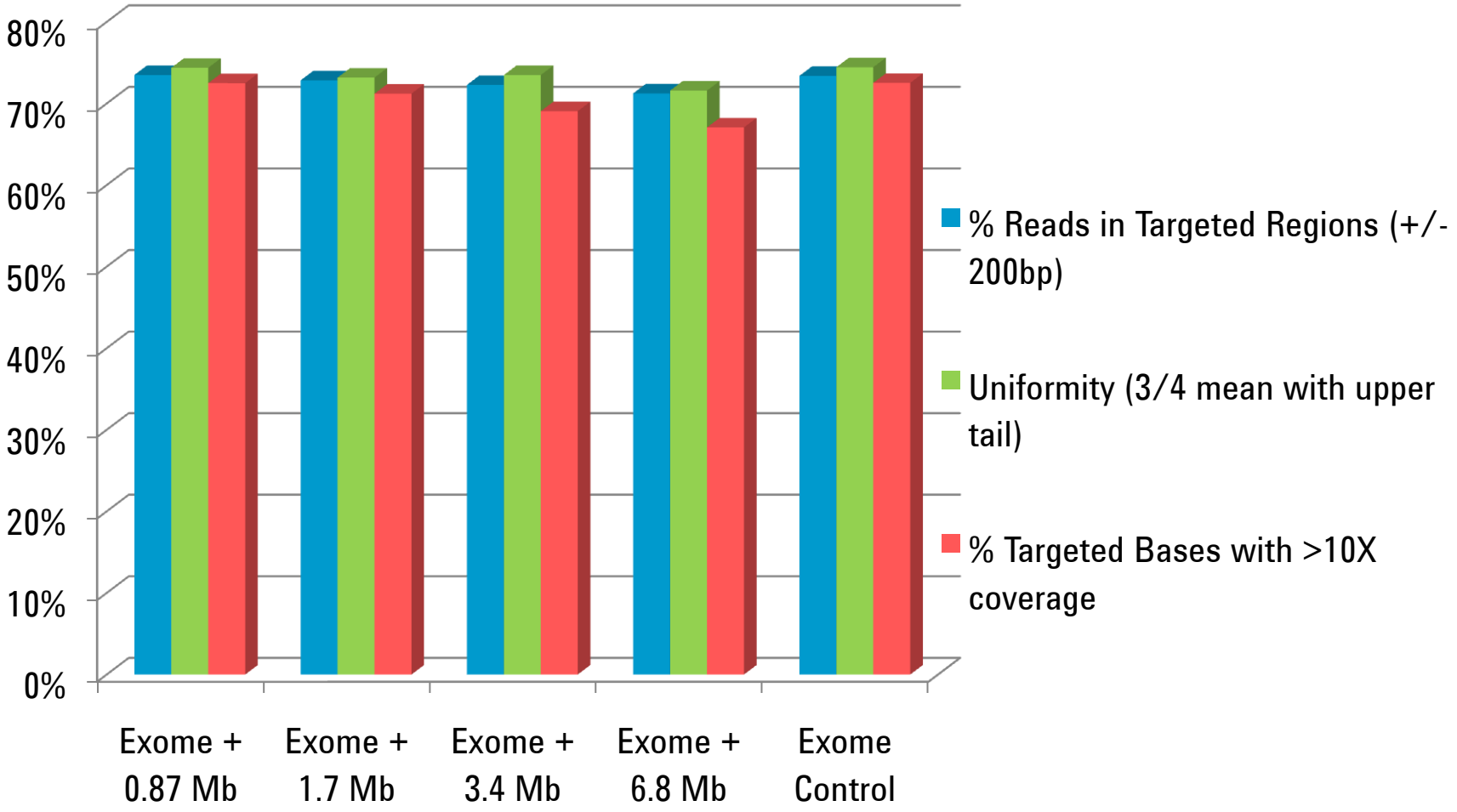
Your Custom Library

Your regions of interest (6.9 Mb)

ALL EXON PLUS

Human All Exon Plus Performance

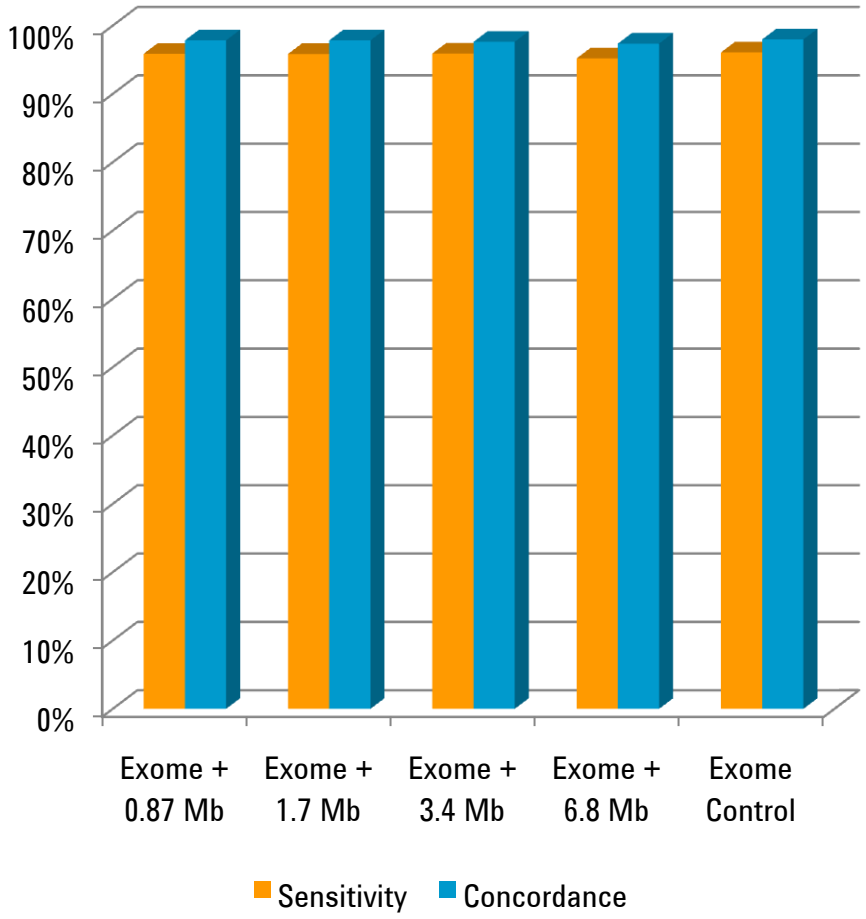
1 tube capture, 1 lane seq. at 2x76 bp on GAIIx = ~2 Gb



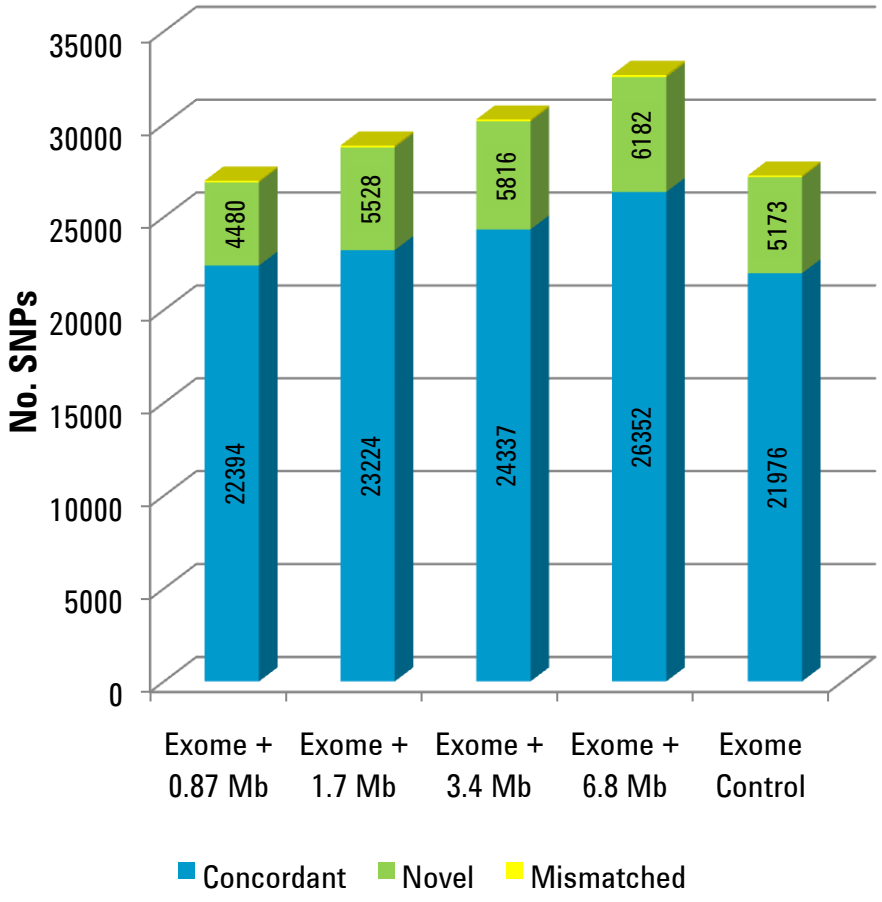
Human All Exon Plus Performance

1 tube capture, 1 lane seq. at 2x76 bp on GAIIx = ~2 Gb

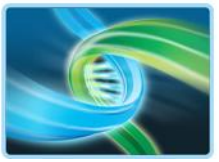
SNP Analysis vs. HapMap



SNP Analysis vs. dbSNP



Other Applications of Targeted Re-Sequencing



- Capture any custom genomic regions (introns, exons, UTRs, regulatory, etc.)
- Ideal for biomarkers discovery and profiling (e.g. cancer)
- Ideal for custom SNP follow-up
- Ideal for characterization of large sample cohorts

Key enabling features:

- High throughput
  - 12 Illumina indexes / up to 96 samples per run
  - 16 SOLiD barcodes / up to 128 samples per run
- Only pay what you capture, scalable from 0.2 to 6.9 Mb (sweet spot for 3rd Gen Seq)
  - <0.2 Mb
  - 0.2 – 0.5 Mb
  - 0.5 – 1.5 Mb
  - 1.5 – 3 Mb
  - 3 – 6.9 Mb
- Very reproducible, excellent allelic balance for accurate heterozygote calls
- Custom and catalog content (kinome)
- Automation (library prep and capture)

PNAS

Detection of inherited mutations for breast and ovarian cancer using genomic capture and massively parallel sequencing

Tom Walsh[a], Ming K. Lee[a], Silvia Casadei[a], Anne M. Thornton[a], Sunday M. Stray[a], Christopher Pennil[b], Alex S. Nord[a], Jessica B. Mandell[a], Elizabeth M. Swisher[b], and Mary-Claire King[a,1]

[a]Departments of Medicine and Genome Sciences and [b]Obstetrics and Gynecology, University of Washington, Seattle, WA 98195

- Inherited loss-of-function mutations in the tumor suppressor genes BRCA1, BRCA2, and multiple other genes predispose to high risks of breast and/or ovarian cancer. Cancer-associated inherited mutations in these genes are collectively quite common, but individually rare or even private.
- To determine whether massively parallel, "next-generation" sequencing would enable accurate, thorough, and cost-effective identification of inherited mutations for breast and ovarian cancer, we developed a genomic assay to capture [with Agilent's custom SureSelect], sequence, and detect all mutations in 21 genes, including BRCA1 and BRCA2, with inherited mutations that predispose to breast of ovarian cancer.
- There were zero false-positive calls of nonsense mutations, frameshift mutations, or genomic rearrangements for any gene in any test sample.
- This approach enables widespread genetic testing and personalized risk assessment for breast and ovarian cancer.

Detection of inherited mutations for breast and ovarian cancer using genomic capture and massively parallel sequencing

Tom Walsh[a], Ming K. Lee[a], Silvia Casadei[a], Anne M. Thornton[a], Sunday M. Stray[a], Christopher Pennil[b], Alex S. Nord[a], Jessica B. Mandell[a], Elizabeth M. Swisher[b], and Mary-Claire King[a,1]

[a]Departments of Medicine and Genome Sciences and [b]Obstetrics and Gynecology, University of Washington, Seattle, WA 98195

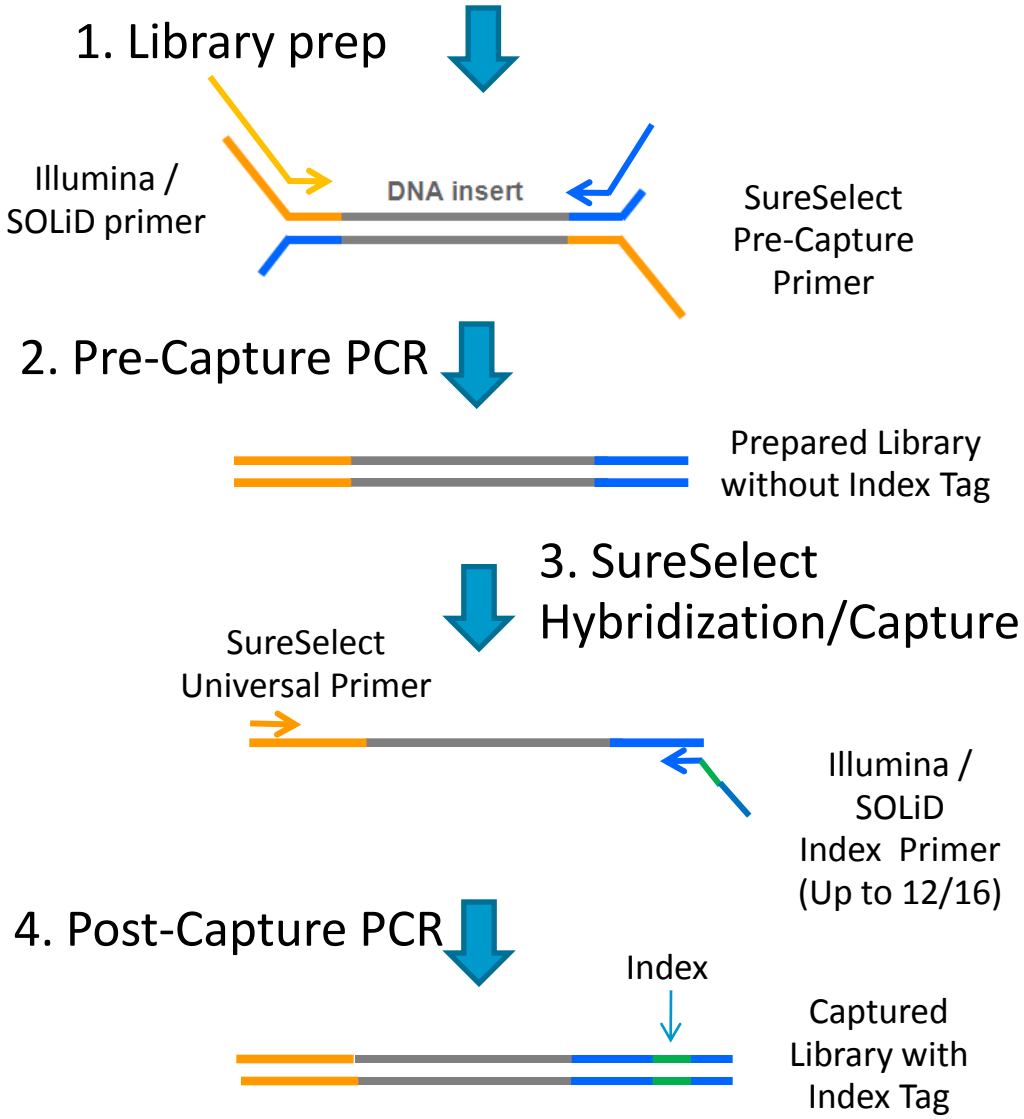
Excellent allelic balance

Table 2. Point mutations and small insertions and deletions identified by the assay

Deletion up to 19bp

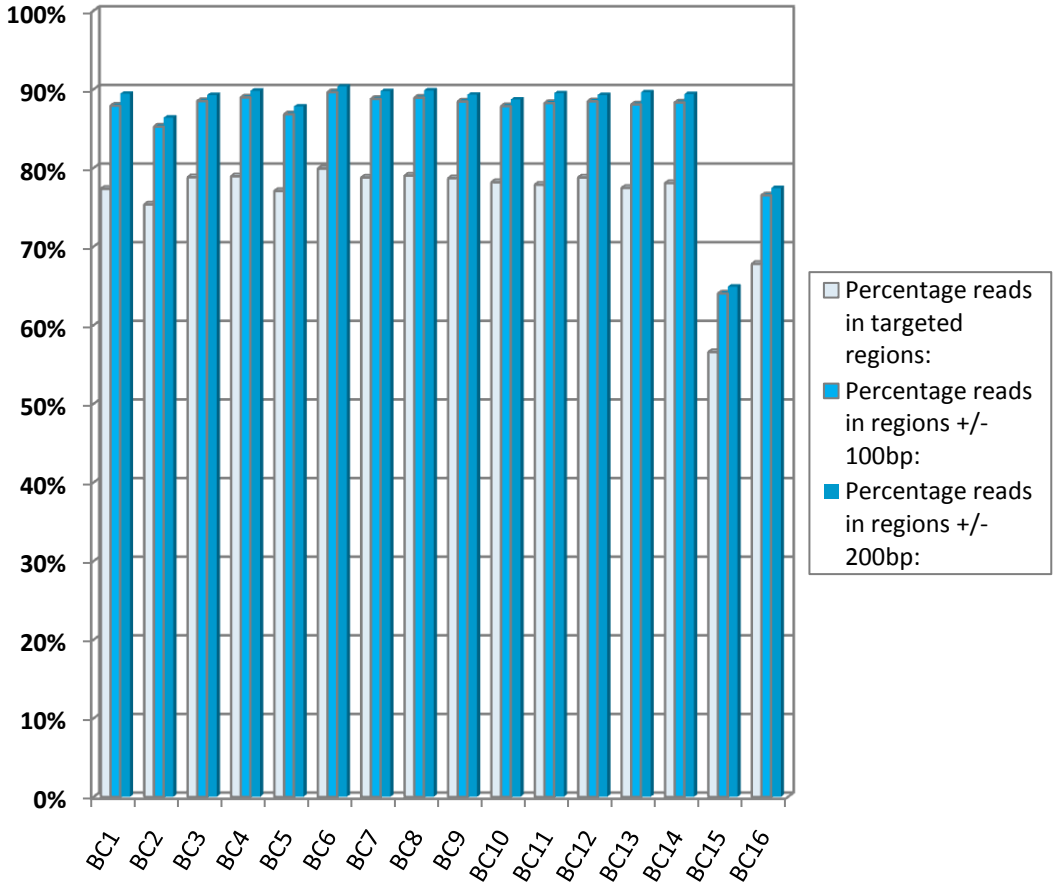
				Mutant sites identified				No. of reads		
Gene	Nucleotide	Effect	Type	Size (bp)	Chromosome	Start	End	Wild type	Variant	% Variant
BRCA1	4510 del3ins2	1465 stop	Deletion-insertion	1	17	41,228,596	41,228,597	525	596	0.53
BRCA1	5083 del19	1657 stop	Deletion	19	17	41,222,949	41,222,968	700	644	0.48
BRCA1	5382 insC	1829 stop	Insertion	1	17	41,209,080	41,209,081	606	596	0.50
BRCA2	999 del5	273 stop	Deletion	5	13	32,905,141	32,905,146	363	229	0.39
BRCA2	1983 del5	585 stop	Deletion	5	13	32,907,366	32,907,371	304	258	0.46
BRCA2	6174 delT	2003 stop	Deletion	1	13	32,914,438	32,914,439	565	661	0.54
BRCA2	9179 C > G	2984 stop	Nonsense	1	13	32,953,650		391	361	0.48
BRIP1	3401 delC	1149 stop	Deletion	1	17	59,761,006	59,761,007	651	486	0.43
CDH1	591 G > A	157 stop	Nonsense	1	16	68,842,406		421	359	0.46
CHEK2	1100 delC	381 stop	Deletion	1	22	29,091,857	29,091,858	3,293	586	0.15
MLH1	ivs14(-1) G > A	568 stop	Splice	1	3	37,083,758		1,024	683	0.40
MSH2	1677 T > A	537 stop	Nonsense	1	2	47,693,895		575	552	0.49
p53	721 G > A	R175H	Missense	1	17	7,578,406		449	306	0.41
PALB2	509 delGA	183 stop	Deletion	2	16	23,647,357	23,647,359	1,283	1,233	0.49
STK11	ivs6(-1) G > A	316 stop	Splice	1	19	1,221,947		722	572	0.44

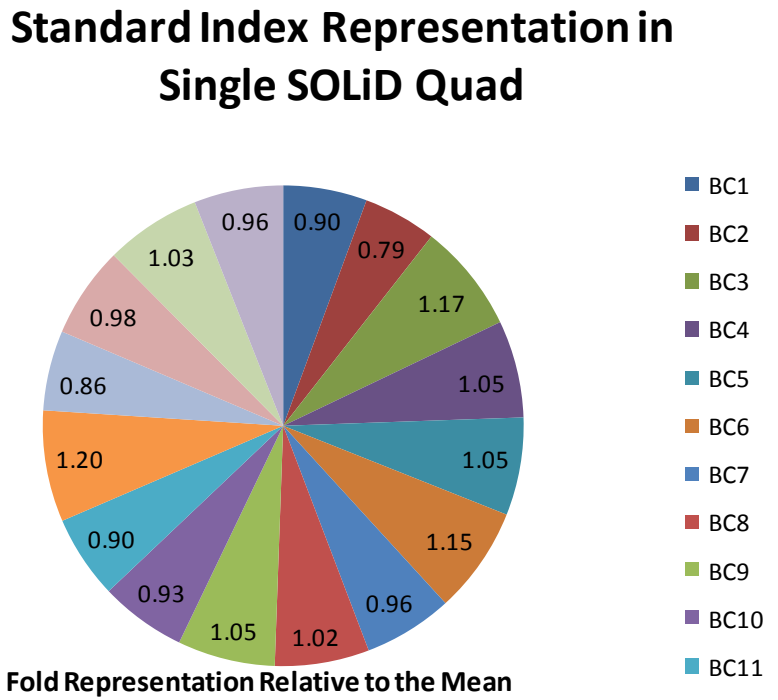
Indexing/Barcoding Procedure with SureSelect



- **For optimum performance:**
  - Capture
  - Index
  - Pool
  - Sequence
- **Combine multiple samples per sequencing lane**
- **Save on capture costs with production scale**
- **Pay only for the Mb you capture:**
  - <0.2 Mb ➔ 12-16 samples
  - 0.2 – 0.5 Mb
  - 0.5 – 1.5 Mb
  - 1.5 – 3 Mb ➔ 3-4 samples
  - 3 – 6.9 Mb

SOLiD Barcoding – 16 barcodes of 0.2 Mb Capture in 1 SOLiD Quad, 1x50 bp, 30M reads

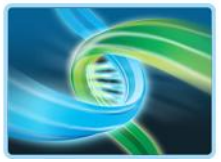


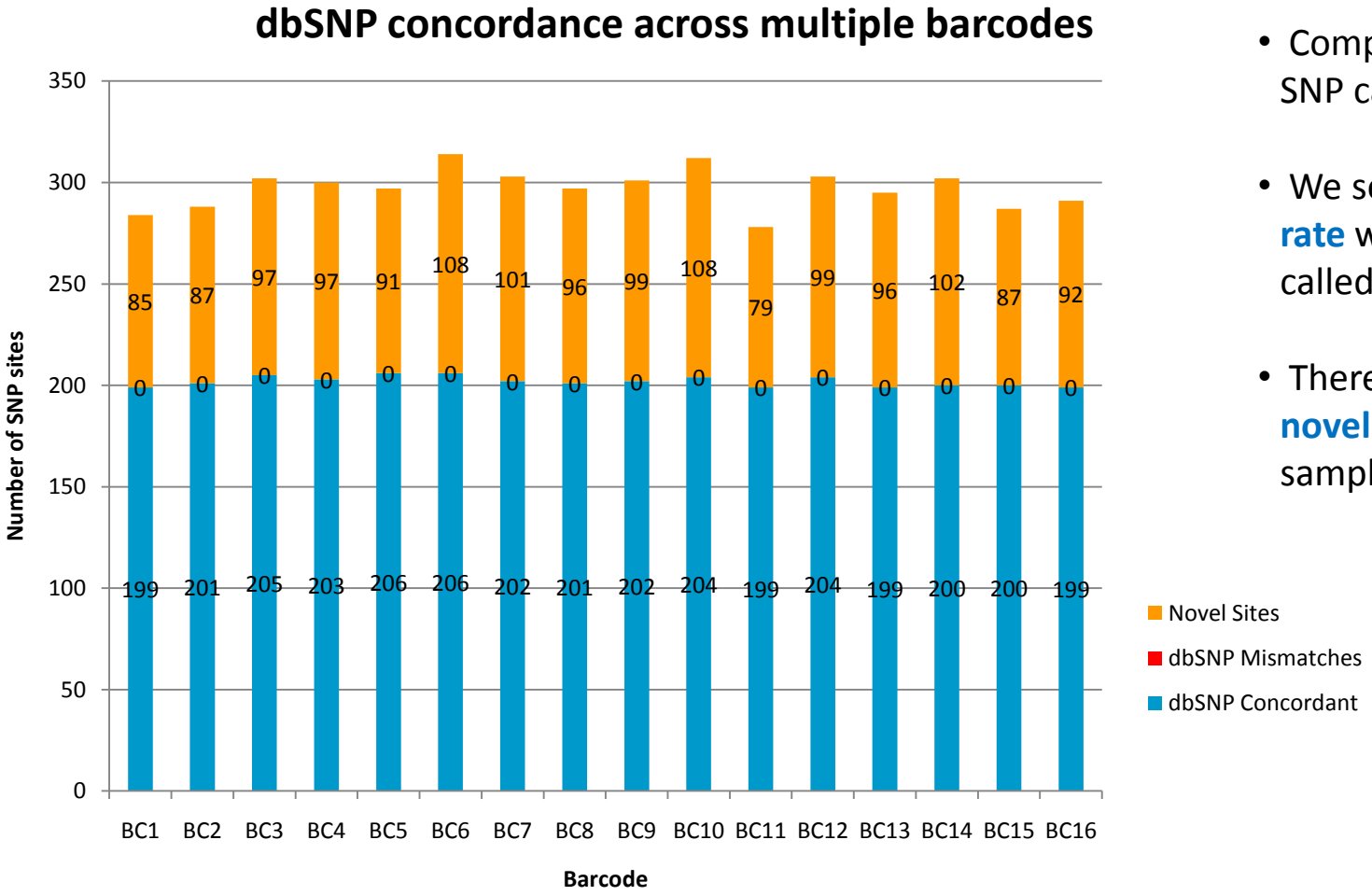


	HQ Reads
Mean/Barcode	1,844,819
Median/Barcode	1,843,950
Total/Quad	29,517,104

SOLiD Barcoding – Comparison with dbSNP

16 barcodes of 0.2 Mb Capture in 1 SOLiD Quad, 1x50 bp, 50M reads

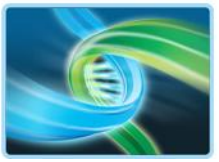




- Comparison of observed SNP calls vs. dbSNP 130
- We see **100% concordance rate** with dbSNP across all called SNPs.
- There are, on average, **~80 novel SNPs** called for each sample.

http://www.broadinstitute.org/gsa/wiki/index.php/The_Genome_Analysis_Toolkit

SureSelect™ Target Enrichment Kit Configurations



Product	Target amount (catalog number)	Reactions/kit	Product Definition
X-demo	3 Mb	5	Exons in the human X-chr
All Exon v1	38 Mb	5-10,000	Catalog content from CCDS + >1000 ncRNA
All Exon Plus	38 Mb + up to 6.9 Mb of custom content	5-10,000	Add custom content to All Exon catalog content
All Exon v2	38 Mb + RefSeq	5-10,000	CCDS Sept. 2009 + additional RefSeq
All Exon 50 Mb	50 Mb	5-10,000	GENCODE content – Most comprehensive coverage Multiplexable
Kinome	<3 Mb	5-10,000	All kinases
Indexed custom content	<0.2 Mb, 0.2-0.49 Mb, 0.5-1.49 Mb, 1.5- 2.9 Mb 3 – 6.9 Mb	10 – 5,000	Cost-saving custom offering – Illumina (12 indexes) and SOLiD (16 barcodes)

Presentation Agenda

- Introduction to SureSelect™ target enrichment
- eArray and kit production
- Current SureSelect™ kit offerings
- NGS QPCR kits and automation

NGS QPCR Kits

QPCR NGS Library Quantification Kit (illumina GA)

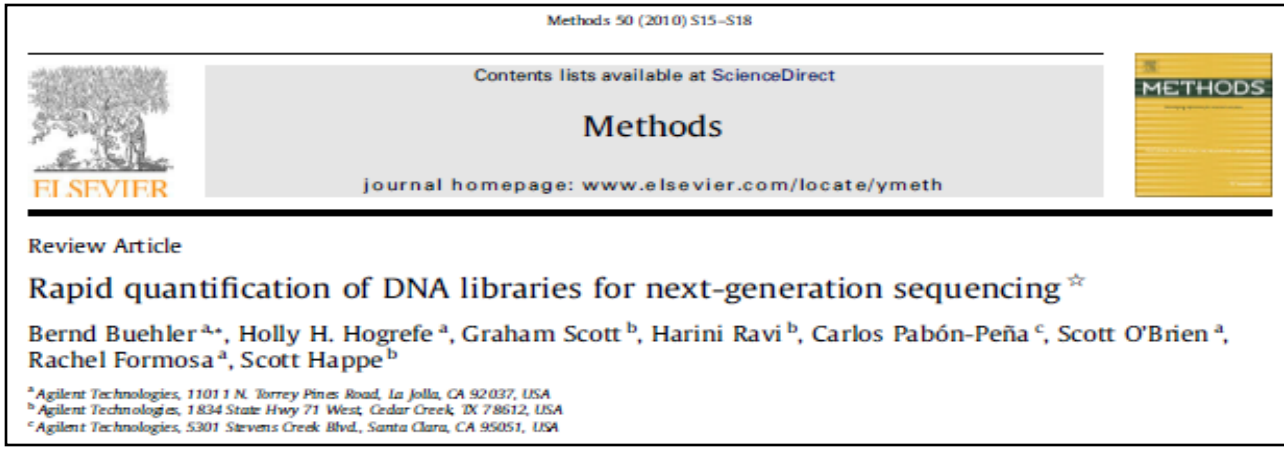
Part Number G4880A

QPCR NGS Library Quantification Kit (AB SOLiD)

Part Number G4881A

- All sequencing platforms require accurate quantification NGS libraries to ensure high-quality reads and efficient generation of data
- Too much DNA = mixed signals, un-resolvable data, lower number of reads
- Too little DNA = reduced sequencing coverage/read depth, empty runs, increased cost/run,& wastes time

The Agilent QPCR NGS Library Quantification kits provide an accurate and sensitive method for quantifying Illumina and AB SOLiD NGS libraries



Methods 50 (2010) S15–S18

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

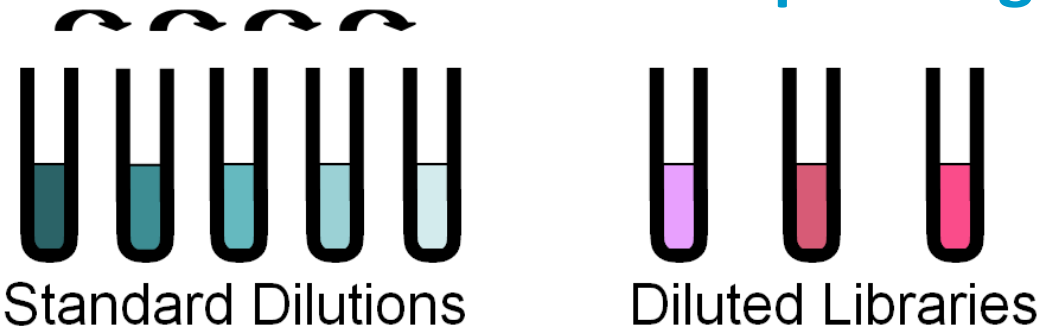
Review Article

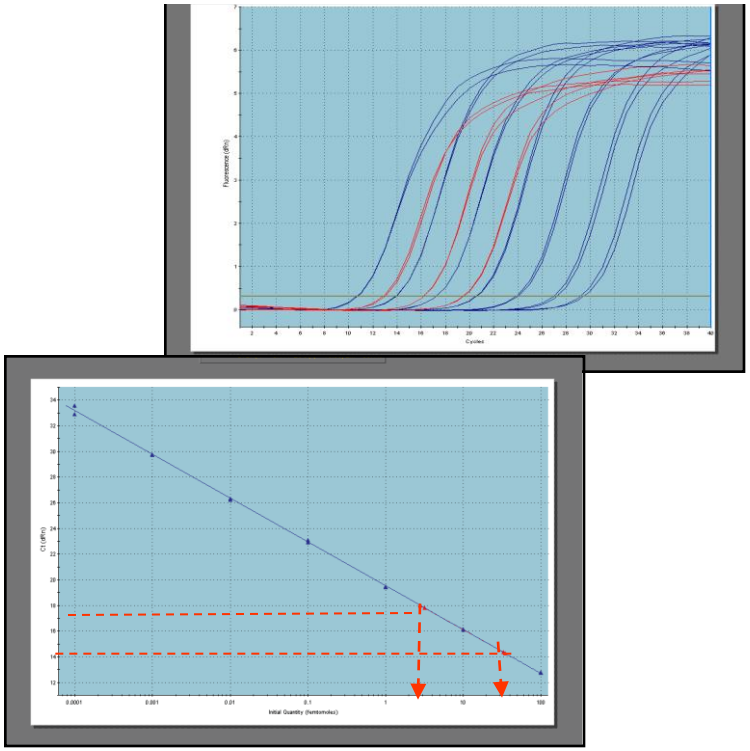
Rapid quantification of DNA libraries for next-generation sequencing ☆

Bernd Buehler [a,*], Holly H. Hogrefe [a], Graham Scott [b], Harini Ravi [b], Carlos Pabón-Peña [c], Scott O'Brien [a], Rachel Formosa [a], Scott Happe [b]

[a] Agilent Technologies, 11011 N. Torrey Pines Road, La Jolla, CA 92037, USA
[b] Agilent Technologies, 1834 State Hwy 71 West, Cedar Creek, TX 78612, USA
[c] Agilent Technologies, 5301 Stevens Creek Blvd., Santa Clara, CA 95051, USA

Quantification of DNA Libraries for Next-Generation Sequencing





Dilute the standard and the library to a pM range or lower

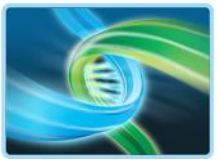
Run qPCR of an aliquot of the dilutions and determine the Ct values

Determine concentration of the library dilution based on the standard curve and correct for the dilution

Agilent Technologies

Illumina Library Prep and SureSelect Enrichment on the Bravo Automated Liquid Handling Platform

Next-Gen Sequencing and SureSelect Overview



Shear Genomic DNA

Repair Ends

3’-A Addition

Adapter Ligation

PCR Enrichment

Prepped Library

Automated Purification

Automated Purification

Automated Purification

Automated Purification

Automated Purification

SureSelect Oligo Capture Library

Library Hybridization

Automated Bead Capture

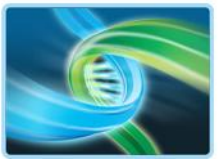
PCR

QA

Illumina Cluster & Sequence

Agilent Automation Bravo Liquid Handler

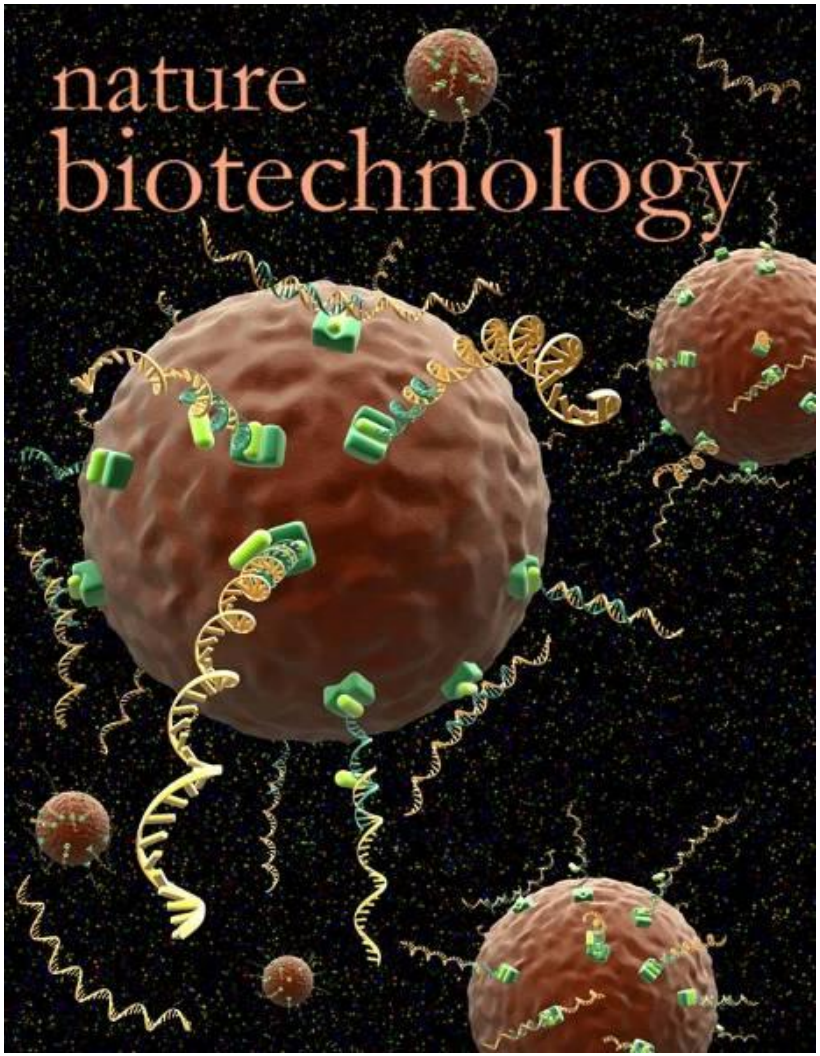
Summary: Efficient Enrichment for Re-Sequencing



- **Most comprehensive offering of Human All Exon catalog products**
  - New 50 Mb catalog content
  - All kits multiplexable (HiSeq and SOLiD4)
  - Exon Plus has the option to add up to 6.9 Mb of custom content

- **Enables Mendelian disease discovery**
  - Available for SE and PE on Illumina and SOLiD
  - Indexing/Barcoding for Illumina and SOLiD
  - Scalable and affordable from 0.2 – 6.9 Mb
  - Free web portal, eArray, enables fully custom design

- **Fastest way to your biological answer**
  - Low DNA input
  - Accurate SNP calls
  - Fast, reproducible and automatable



Thank You!

http://genomics.agilent.com