SureSelect The Leader in Target Enrichment

Genomic Solutions Division

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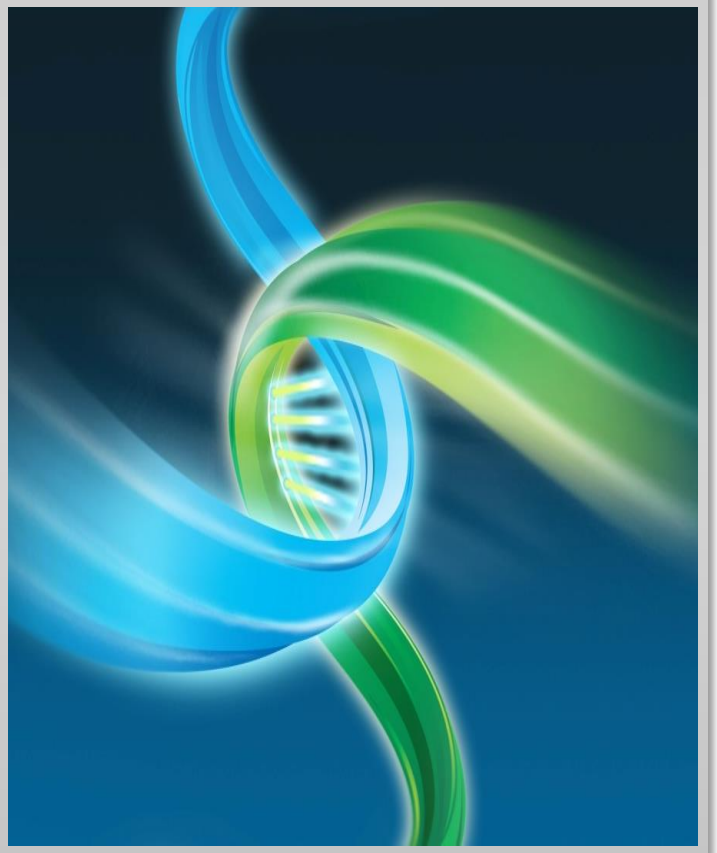
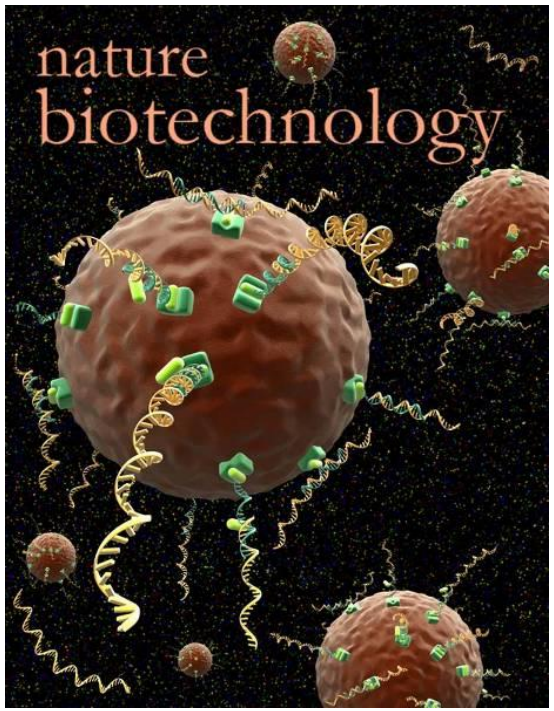
07-14-2016

1. SureSelect Technology
2. Whole Exome Sequencing
3. FFPE Samples
4. Custom DNA Panels
5. Methyl-Seq
6. Halo-HS
7. Custom Oligo Library Access



SureSelect Technology

Pioneer in NGS Target Enrichment



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

- Capture fragments with longest, most efficient 120-mer cRNA baits
- Probes can be designed to any regions of interest, samples can be multiplexed
- Easy to implement and compatible with validated automation solution

SureSelect Technology

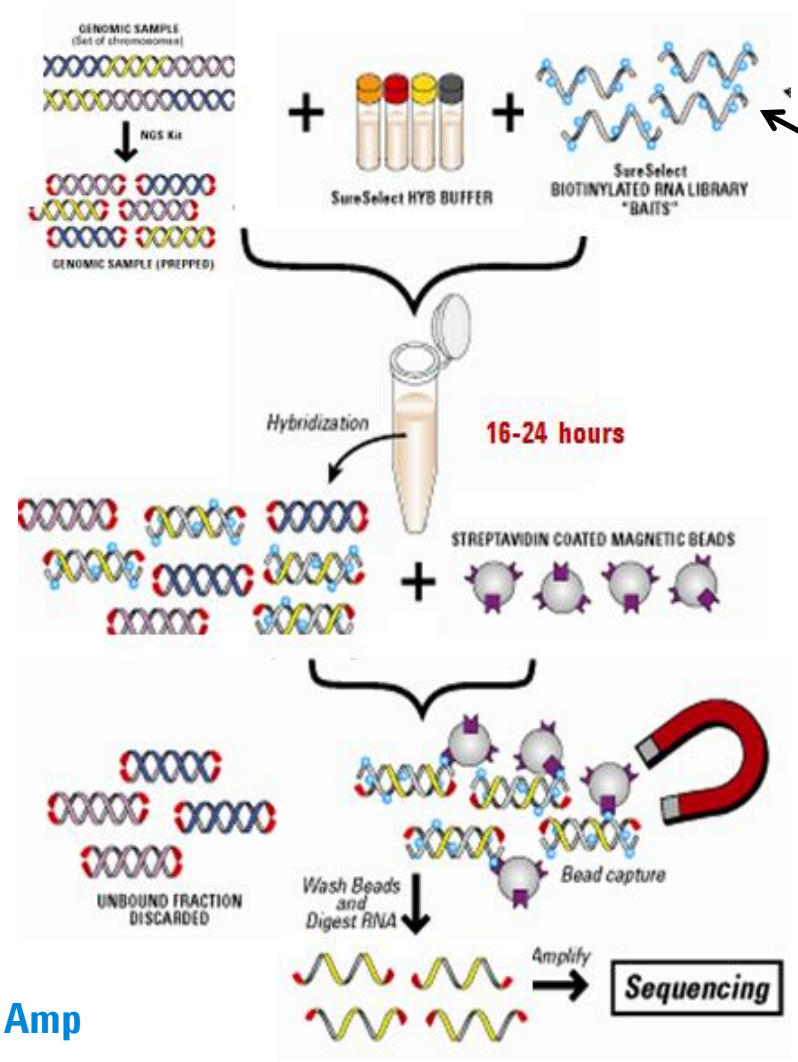
Simple Target Enrichment Workflow

Library prep
Hyb set-up

Hybridization

Capture

Wash / Elution / Amp



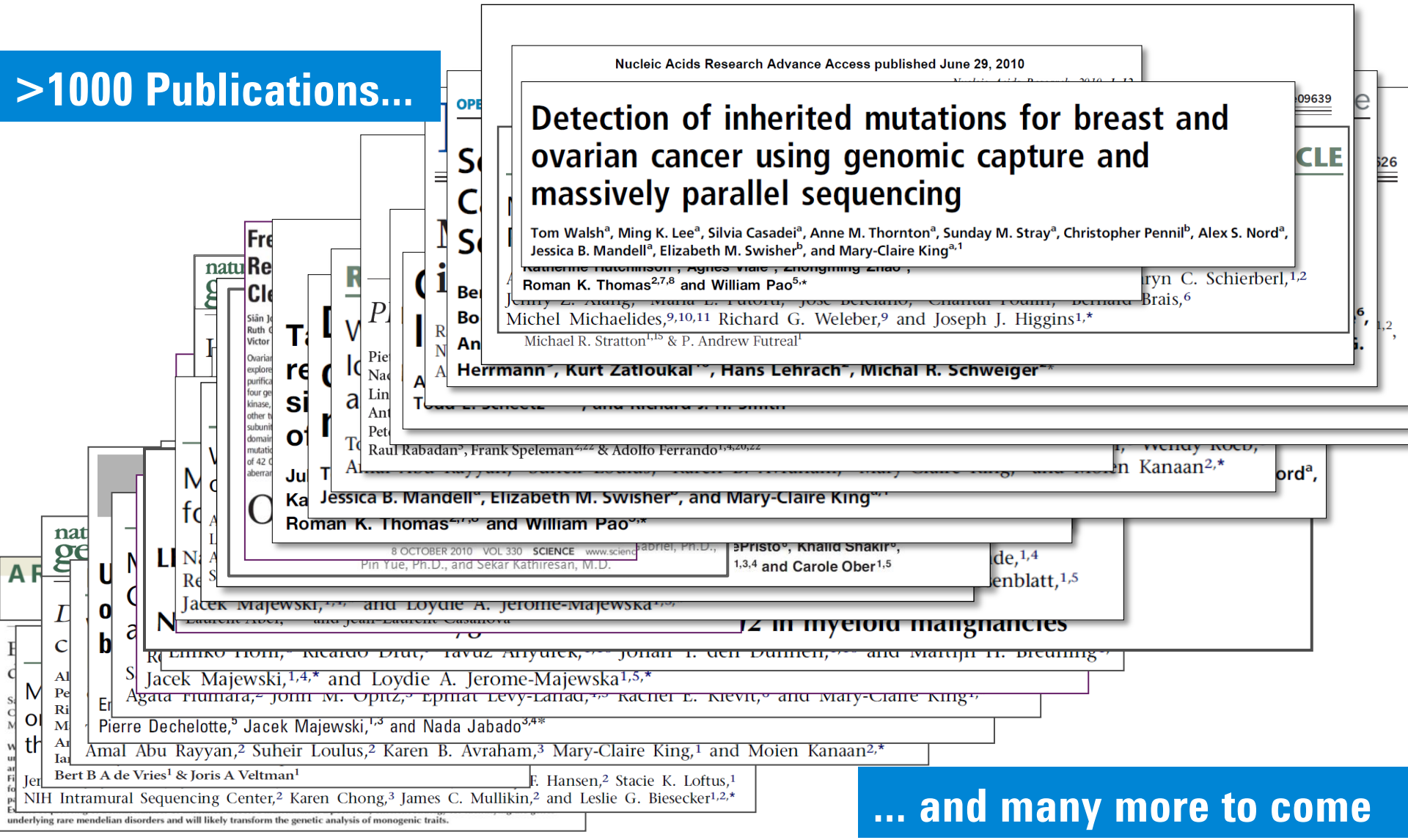
Baits:
- **cRNA probes**
- **Long (120bp)**
- **Biotin labeled**
- **User-defined**

>50 Mendelian Diseases uncovered by SureSelect

- Miller syndrome – USA - 2009
- TARP syndrome – USA - 2010
- Schinzel-Giedion syndrome - Netherlands - 2010
- Fowler Syndrome – Canada - 2010
- Terminal Osseous Dysplasia – Netherlands – 2010
- Hearing Loss – USA – 2010
- Perrault Syndrome – USA – 2010
- Kaposi sarcoma – USA – 2010
- Sensenbrenner Syndrome – Netherlands – 2010
- Hyperphosphatasia syndrome – Germany – 2010
- Kabuki syndrome - USA – 2010
- Van Den Ende-Gupta syndrome – Canada – 2010
- Neonatal Diabetes Mellitus – France – 2010
- Autoimmune lymphoproliferative syndrome – USA – 2010
- Familial Amyotrophic lateral sclerosis – USA – 2010
- Non-syndromic mental retardation – USA – 2010
- Osteogenesis Imperfecta – Germany/Netherlands – 2011
- Hajdu-Cheney syndrome – London /France– 2011
- Acne Inversa – China – 2011
- Leucoencephalopathy – Japan – 2011
- Taybi-Linder syndrome – France – 2011
- Ochoa syndrome – Saudi Arabia – 2011
- Spastic paraparesis - USA – 2011
- Distal Arthrogryposis – USA – 2011
- Amelogenesis Imperfecta – UK - 2011

The Most Published Target Enrichment Platform

>1000 Publications...



... and many more to come

Most Databases Covered for Comprehensive Variant Detection-Exons (Exome, WES)

	Clinical Research		Translational Research		Cancer Research
Parameter	**Clinical Research Exome**	**Focused Exome**	**All Exon V6+UTR**	**All Exon V6**	**All Exon V6+COSMIC**
Capture Size	54Mb	12Mb	79Mb	58Mb	64Mb
CCDS	X		X	X	X
RefSeq	X		X	X	X
GENCODE	X		X	X	X
miRBase	X		X	X	X
TCGA	X		X	X	X
UCSC	X		X	X	X
HGMD, OMIM, ClinVar	X	X	X*	X*	X*
COSMIC					X
Amount of Seq. (for 80% at 20x)	4Gb	<1Gb	6Gb	4Gb	4Gb
Overall Workflow	7h-1.5d	4.5 days	7h-1.5d	7h-1.5d	7h-1.5d
Add *Custom* Content	Yes	Yes	No	Yes	No

SureSelect – The Leader in Target Enrichment

COMPLETE Solution

Best **PERFORMANCE**

FLEXIBLE Designs

SureSelect Technology

Most Complete Enrichment Solution

All Exon Designs

- Human All Exon
- Non Human Exomes
- Clinical Research Exome

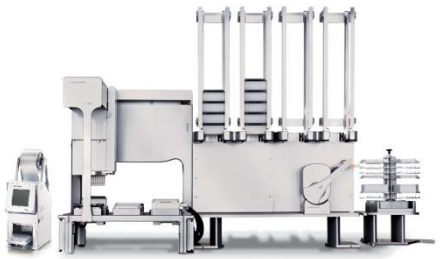
Custom Solutions

- Custom DNA
- Custom RNA

Targeted Panels

- Inherited Disease
- Human Kinome (DNA/RNA)
- Human Methyl-Seq
- ClearSeq Comprehensive Cancer

Post-capture	Pre-capture
SureSelect XT Illumina	SureSelect XT2 Illumina
SureSelect QXT Illumina	
SureSelect Strand Specific RNA Illumina	
SureSelect Ion Torrent PGM	



Automation

Sureselect Library Preparation kits

	CONSTITUTIONAL				
	CANCER				
	TARGET ENRICHMENT				WGS
	SureSelect^{XT}	**SureSelect^{XT} Fast**	**SureSelect^{XT2}**	**SureSelect^{QXT}**	**SureSelect^{QXT}**
Library Prep Features	• 3ug or 200ng • Best complexity	• 200ng input • High complexity • Tested with FFPE • 11hr sample to sequencing workflow	• 1ug or 100ng input • Streamlined: Precap Pooling + MM Reagents	• 50ng input • Single day sample to sequencing • Streamlined: transposase-based	• 50ng input • Single day sample to sequencing • Streamlined: transposase-based
Key Benefit	• Complexity for rare allele detection • Compatibility with low input	• Complexity for rare allele detection • Low input	• Cost effective	• Low input • One day workflow	• Low input • One day workflow

SureSelect

FFPE Samples

FFPE Samples Using SureSelect

FFPE Challenges

- DNA quality issues (age, storage, fixation process)
- Sample degradation can impact sequencing quality (sensitivity, quality, allelic balance, etc)

FFPE Samples Using SureSelect

Questions

- Can FFPE-derived material be used for targeted sequencing?
- Is there any bias introduced by using FFPE vs fresh-frozen samples?
- How does target-enriched sequencing data derived from FFPE samples compare to microarray data?


RESEARCH ARTICLE Open Access

Targeted high throughput sequencing in clinical cancer Settings: formaldehyde fixed-paraffin embedded (FFPE) tumor tissues, input amount and tumor heterogeneity

Martin Kerick[1†], Melanie Isau[1,2†], Bernd Timmermann[1], Holger Sültmann[3], Ralf Herwig[1], Sylvia Krobitsch[1], Georg Schaefer[4,5], Irmgard Verdorfer[5,6], Georg Bartsch[4], Helmut Klocker[4], Hans Lehrach[1] and Michal R Schweiger[1*]

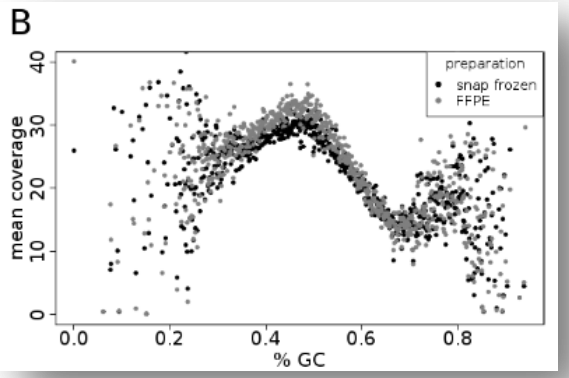
Method

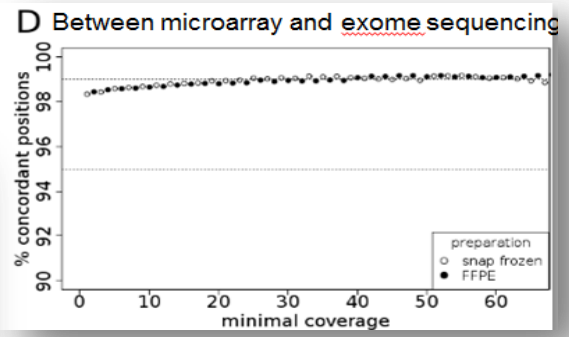
- 50Mb All Exon capture library and 3.9Mb custom capture
- 2 x 36bp single-end sequencing on Illumina GAIIx

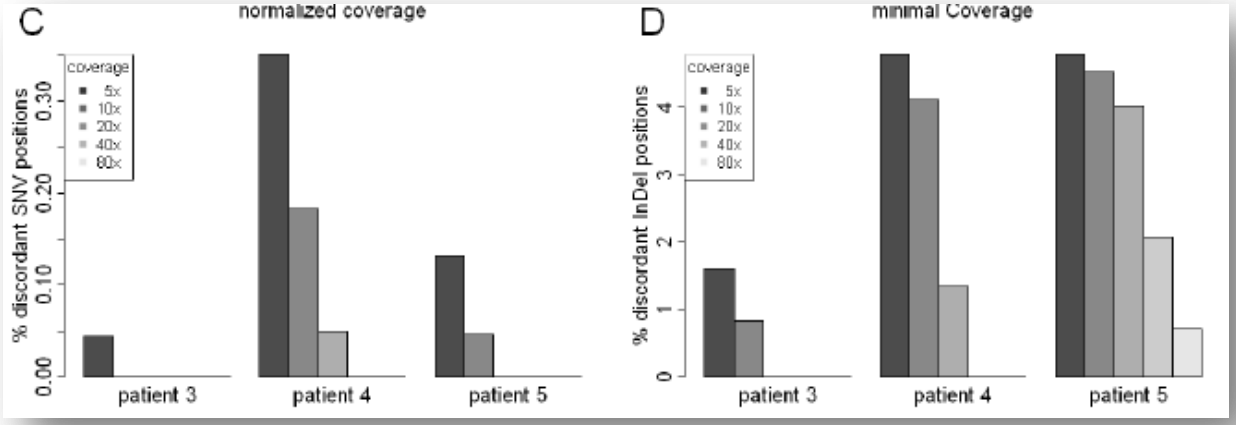
FFPE Samples Using SureSelect

Results

- Similar % reads on target (75.5% frozen; 74.7% FFPE)
- Comparable GC-dependent coverage profile
- High NGS SNP concordance to microarray
- 98% concordance in SNP detection across varying input
- At 20X depth, <0.05% discordant SNPs between 2 foci for all patients







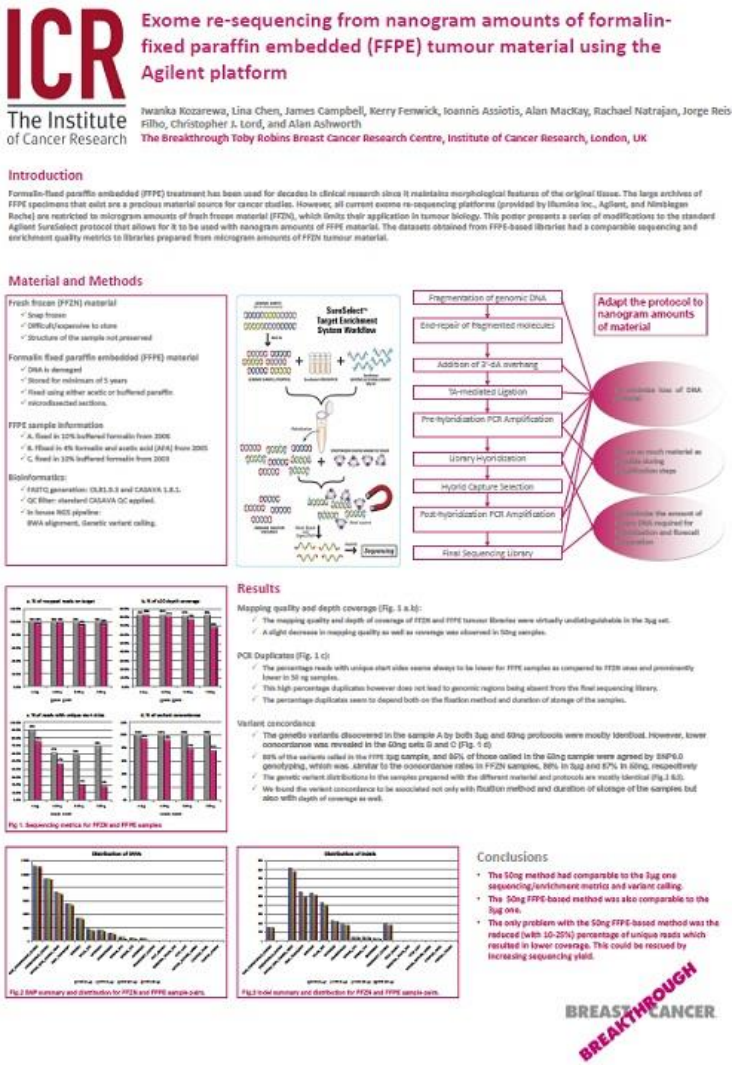
FFPE Samples Using SureSelect

Breakthrough in Brest Cancer Research

- Demonstration of FFPE samples from ICR with 50ng of input using SureSelect Human All Exon and modified protocol

Major conclusions

- The 50ng method had comparable to 3µg for sequencing/enrichment metrics and variant calling
- The 50ng FFPE-based method was also comparable to 3µg protocol


ICR
The Institute of Cancer Research

Exome re-sequencing from nanogram amounts of formalin-fixed paraffin embedded (FFPE) tumour material using the Agilent platform

Iwanka Kozarewa, Lina Chen, James Campbell, Kerry Fenwick, Ioannis Assiotis, Alan MacKay, Rachael Natrajan, Jorge Reis Filho, Christopher J. Lord, and Alan Ashworth

The Breakthrough Toby Robins Breast Cancer Research Centre, Institute of Cancer Research, London, UK

Introduction

Material and Methods

Fresh frozen (FFZN) material

Formalin fixed paraffin embedded (FFPE) material

FFPE sample information

Bioinformatics:

SureSelect Target Enrichment System Workflow

Fragmentation of genomic DNA
End-repair of fragmented molecules
Addition of 3'-dA overhang
TA-mediated Ligation
Pre-hybridization PCR Amplification
Library Hybridization
Hybrid Capture Selection
Post-hybridization PCR Amplification
Final Sequencing Library

Adapt the protocol to nanogram amounts of material

Results

Mapping quality and depth coverage (Fig. 1 a, b):

PCR Duplicates (Fig. 1 c):

Variant concordance

Conclusions

- The 50ng method had comparable to the 3µg one sequencing/enrichment metrics and variant calling.
- The 50ng FFPE-based method was also comparable to the 3µg one.
- The only problem with the 50ng FFPE-based method was the reduced (with 10-20%) percentage of unique reads which resulted in lower coverage. This could be rescued by increasing sequencing yield.

BREAST CANCER BREAKTHROUGH

SureSelect Custom DNA

The Best Performance and Flexibility

Capture only what you want — Custom content from <200Kb up to 24Mb+

Higher sample throughput per run — >1,000 samples/run with 96 indexes!

Cost effective — Minimize enrichment and sequencing costs

Faster time from sample to results — Greater sample throughput, easier data analysis

SureSelect – Proven High Sensitivity

Capture SNPs and Indels, while maintaining allelic balance

Science AAAS

Frequent Mutations of Chromatin Remodeling Gene *ARID1A* in Ovarian Clear Cell Carcinoma

Siân Jones,[1] Tian-Li Wang,[2] Ie-Ming Shih,[3] Tsui-Lien Mao,[4] Kentaro Nakayama,[5] Richard Roden,[3] Ruth Glas,[6] Dennis Slamon,[6] Luis A. Diaz Jr.,[1] Bert Vogelstein,[1] Kenneth W. Kinzler,[1]* Victor E. Velculescu,[1]* Nickolas Papadopoulos[1]*

Table 1. Mutations in *ARID1A*, *KRAS*, *PIK3CA*, and *PPP2R1A* in human ovarian clear cell carcinomas.*

Sample†	Gene	Transcript accession	Nucleotide (genomic)‡	Nuc (cl
OCC01PT	*ARID1A*	CCDS285.1	g.chr1:26972561_26972562insA	c.3854_3855insA
OCC02PT	*ARID1A*	CCDS285.1	g.chr1:26896034C>T	c.[illegible]3C>T
OCC02PT	*ARID1A*	CCDS285.1	g.chr1:26978879-26978880dupGT	c.5903_5904dupGT
			g.chr1:26972009_26972034delTGATGGGGCG	c.3659_3684delTGATGGGGCG
OCC03PT	*ARID1A*	CCDS285.1	CATGTCCTATGAGCCA (hom)	CATGTCCTATGAGCCA
OCC07PT	*ARID1A*	CCDS285.1	g.chr1:26896066C>A	c.585C>A
OCC08PT	*ARID1A*	CCDS285.1	g.chr1:26970389delC	c.3391delC
OCC10PT	*ARID1A*	CCDS285.1	g.chr1:26972790_26972792dupGCA (hom)	c.4001_4002dupGCA (hom)
OCC10PT	*ARID1A*	CCDS285.1	g.chr1:26979804_26979805delTG (hom)	c.682[illegible]
OCC11PT	*ARID1A*	CCDS285.1	g.chr1:26930334_26930335insCCTAC	c.145[illegible]
OCC13PT	*ARID1A*	CCDS285.1	g.chr1:26974233_26974234insTGGC	c.492[illegible]
OCC14PT	*ARID1A*	CCDS285.1	g.chr1:26972886_26972887_delTT (hom)	c.401[illegible]
OCC15PT	*ARID1A*	CCDS285.1	g.chr1:26973940G>A	c.4635G>A
OCC15PT	*ARID1A*	CCDS285.1	g.chr1:26978178T>A	c.5202T>A
OCC16PT	*ARID1A*	CCDS285.1	g.chr1:26895967_26895973delCGCCGCC (hom)	c.486_492delCGCCGCC (hom)
OCC18PT	*ARID1A*	CCDS285.1	g.chr1:26971925delA	c.3575delA
OCC20PT	*ARID1A*	CCDS285.1	g.chr1:26970221delG	c.3223delG
OCC22PT	*ARID1A*	CCDS285.1	g.chr1:26979694dupG	c.6718dupG
OCC23PT	*ARID1A*	CCDS285.1	g.chr1:26896379_2689637980_insCGTC	c.898_899insC
OCC23PT	*ARID1A*	CCDS285.1	g.chr1:26979686_26979687insT	c.6710_6711insT
OCC24PT	*ARID1A*	CCDS285.1	g.chr1:26930542C>T	c.1663C>T
OCC27PT	*ARID1A*	CCDS285.1	g.chr1:26896263_26896272delCGTCGTCTTC	c.782_791delCGTCGTCTTC
OCC27PT	*ARID1A*	CCDS285.1	g.chr1:26971984_26971994delCAGCCCAGTAT	c.3634_3644delCAGCCCAGTAT

25bp deletion

7bp deletion

10bp deletion

11bp deletion

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

Table 3. Genomic deletions and duplication identified by the assay

		Mutant sites identified by assay				
Gene	Genomic event	Chromosome	Start*	End*	Size (bp)	Ratio†
BRCA1	Deletion exons 1–15	17	41,226,145	41,327,157	101,013	0.509
BRCA1	Duplication exon 13	17	41,230,562	41,235,836	[illegible],275	1.578
BRCA1	Deletion exons 14–20	17	41,203,975	41,229,297	[illegible],323	0.519
BRCA1	Deletion exon 17	17	41,219,596	41,219,755	160	0.495
BRCA2	Deletion exons 1–2	13	32,889,020	32,890,900	1,881	0.489
BRCA2	Deletion exon 21	13	32,950,734	32,952,070	1,337	0.544

*Breakpoints are flanked by *Alu* and other repeats, which are not captured.
†Reads per base pair for deletion or duplication/reads per base pair for wild-type genotype.

Large deletions and duplications

PNAS

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- Inherited mutations in the tumor suppressor genes BRCA1, BRCA2, and multiple other genes predispose to high risks of breast and/or ovarian cancer.
- Developed an **Custom Panel** to detect all mutations in **21 genes** that predispose to breast of **ovarian cancer**, including BRCA1 and BRCA2
- There were **zero false-positive calls.**

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

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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

PNAS

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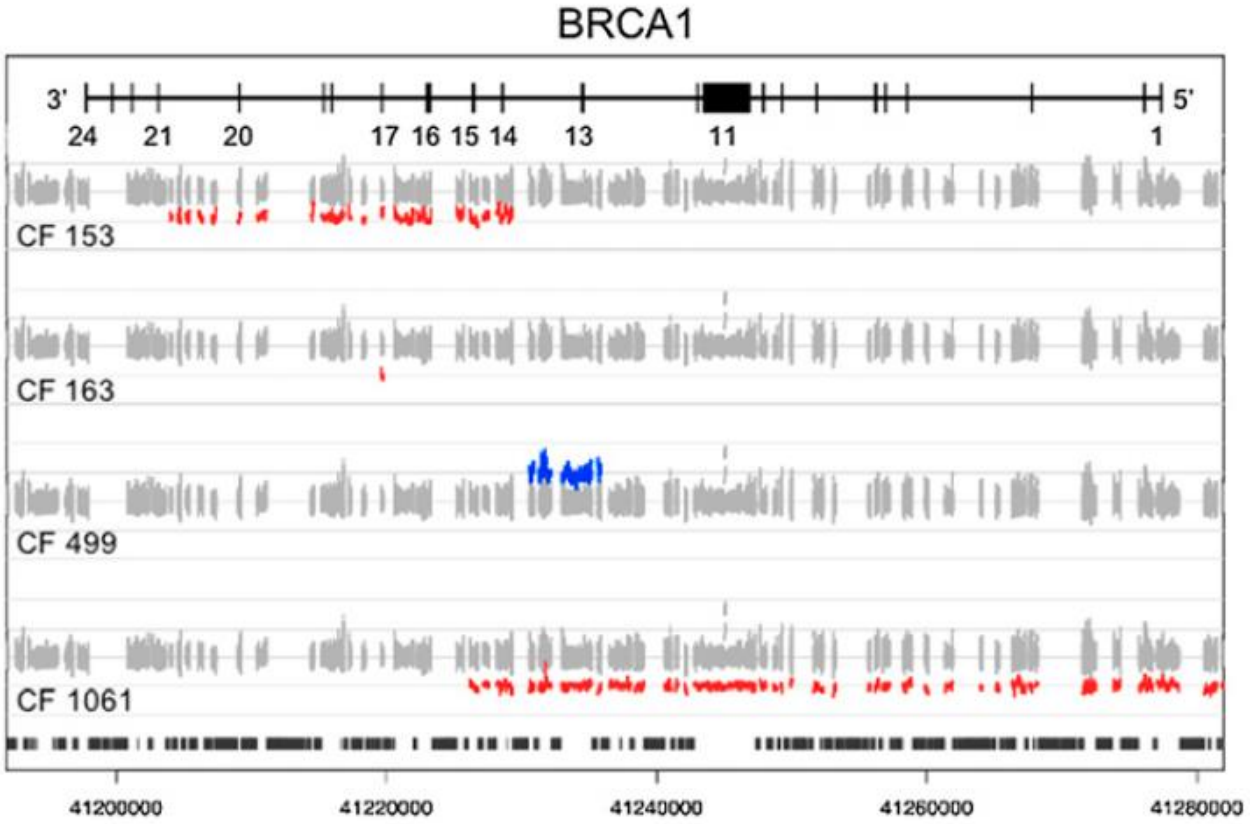
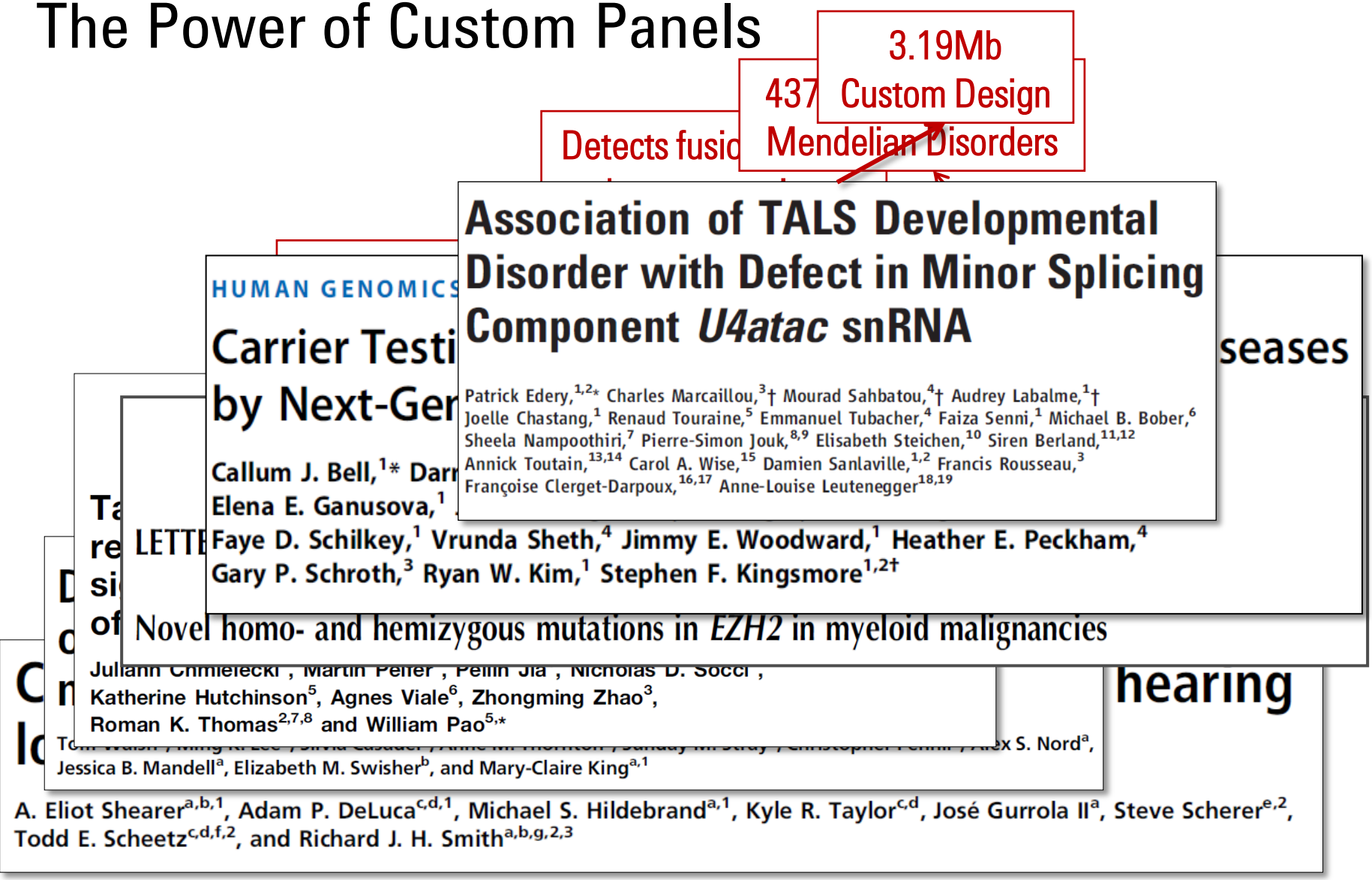


- CNVs can be measured by comparing read depth to a reference
- Ability to measure both deletions and duplications
- Variants range from 100s bp – +100kb

The Power of Custom Panels



Easily Create Your Custom Design

NEW!
Streamlined
Design Software

SureDesign
Free Web Portal

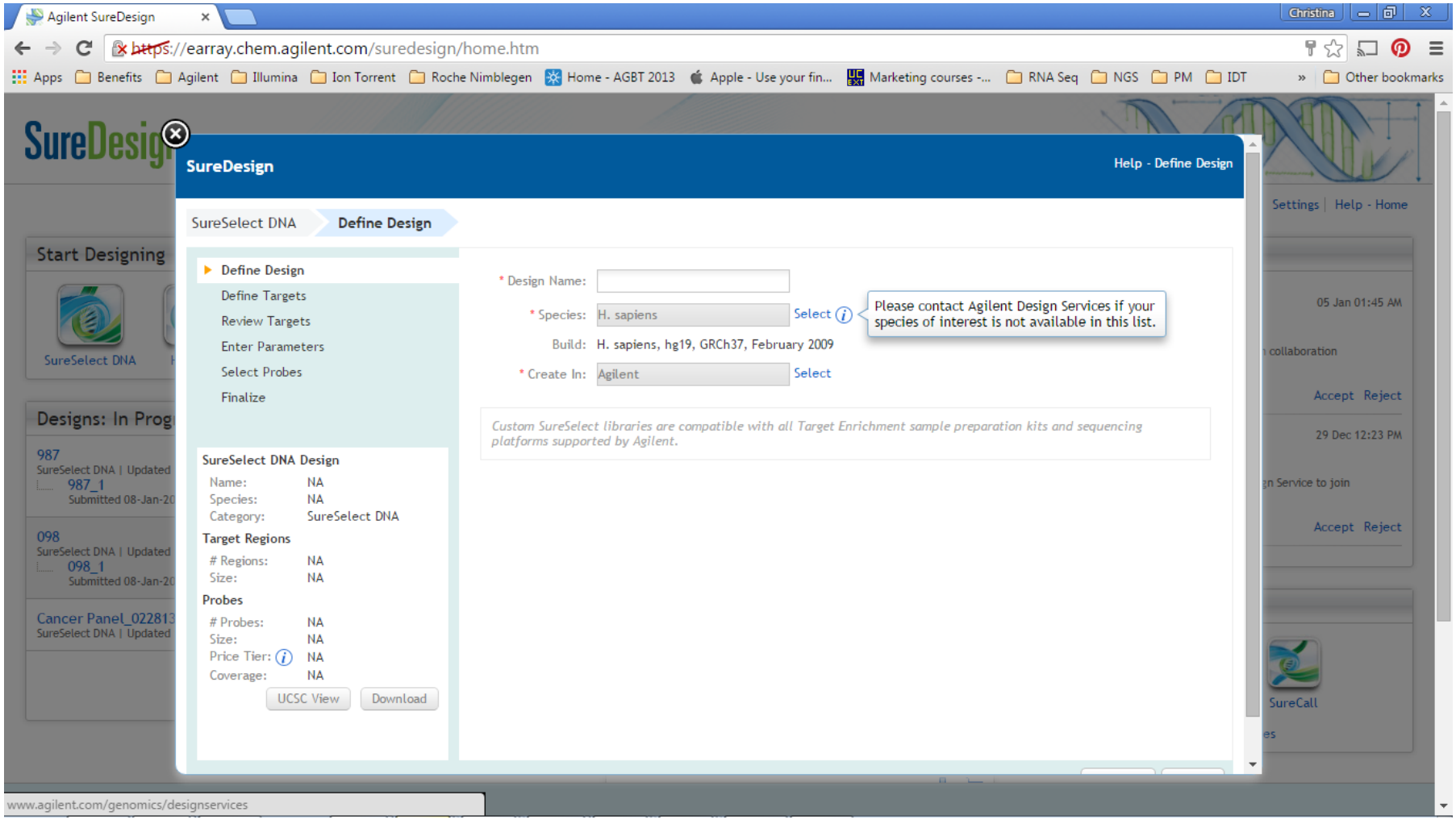
Genomic Coordinates
Regions you want to capture

Design Services
For Complex Designs

Your Custom SureSelect Kit
A bait library to capture your regions of interest

Custom Design Service

Link



The Power of Custom Panels

For any species!

Neves *et al. BMC Proceedings* 2011, **5**(Suppl 7):O48
http://www.biomedcentral.com/1753-6561/5/S7/O48

BMC Proceedings

ORAL PRESENTATION Open Access

Targeted sequencing in the loblolly pine (*Pinus taeda*) megagenome by exome capture

Leandro Neves[1*], John Davis[2], Brad Barbazuk[3], Matias Kirst[2]

From IUFRO Tree Biotechnology Conference 2011: From Genomes to Integration and Delivery
Arraial d'Ajuda, Bahia, Brazil. 26 June - 2 July 2011

Saintenac *et al. Genome Biology* 2011, **12**:R88
http://genomebiology.com/2011/12/9/R88

Genome Biology

RESEARCH Open Access

Targeted analysis of nucleotide and copy number variation by exon capture in allotetraploid wheat genome

Cyrille Saintenac, Dayou Jiang and Eduard D Akhunov*

SureSelect Human Methyl-Seq

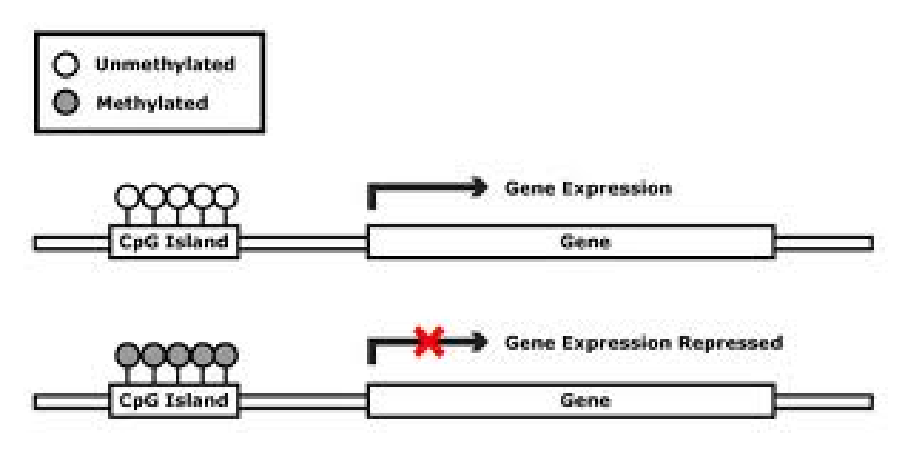
for Illumina Sequencing

Agilent Gene Regulation Solutions

CpG Dinucleotides & Their Genomic Locations

CpG islands

- High frequency of CpG dinucleotides
  - ✓ > 500bp & GC content > 55% & observed/expected CpG ratio > 0.65
- In or near about 40% of promoters of mammalian genes

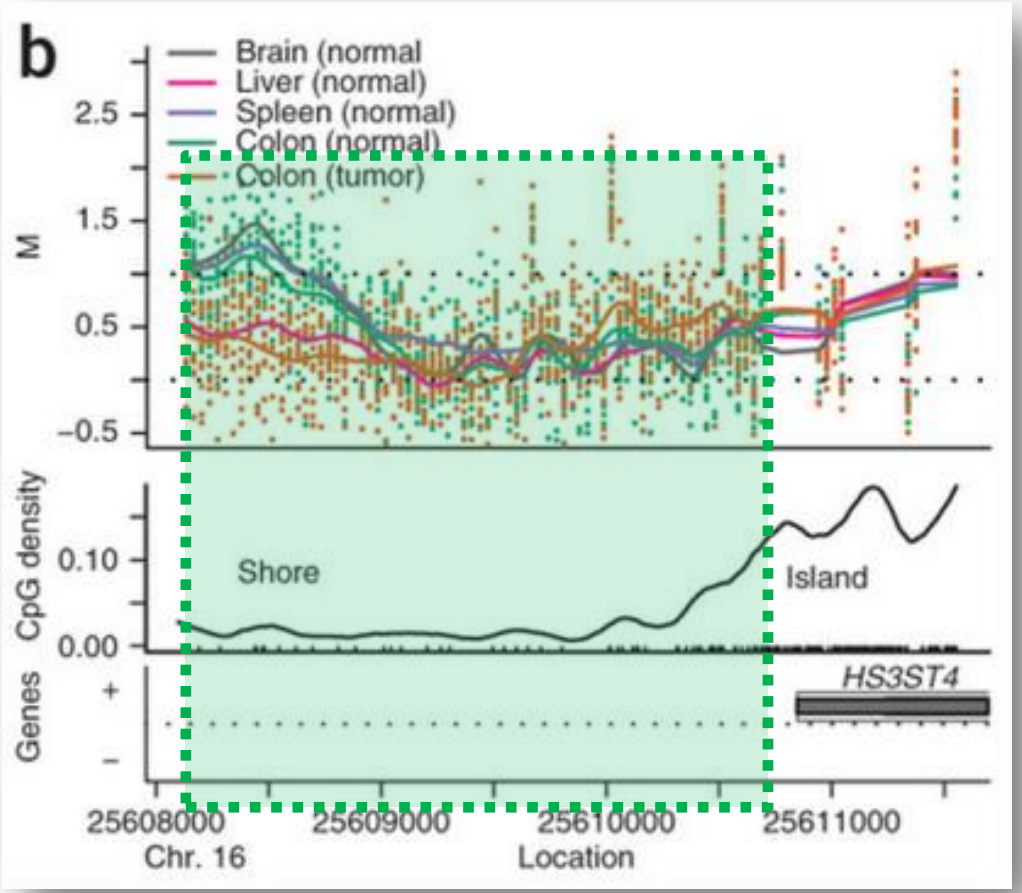


Promoters

- 75% of transcriptional start sites have CpG-rich regions
- 88% of active promoters are associated with CpG-rich sequences

Differentially Methylated Regions (DMR)

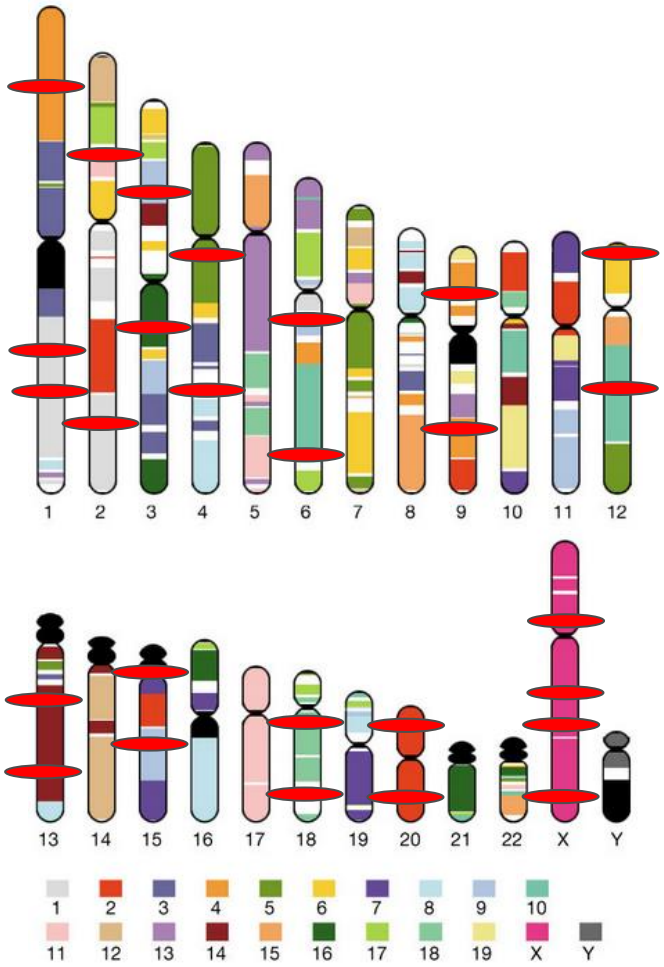
- CpG islands (e.g. 4~8 % tissue-specific differentially methylated regions or T-DMR)
- CpG island **shores** (~2kb away from islands, e.g. 76% of T-DMRs in shores)
- CpG island **shelves** (~4kb away from islands)



HS3ST4 : heparan sulfate D-glucosaminyl 3-O- sulfotransferase 4

Irizarry RA et al. Nature Genetics 2009

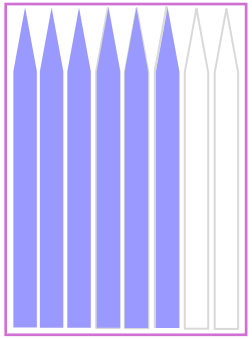
Target Known Methyl Regions



Less than 3% of the genome is reported to vary it's methylation state

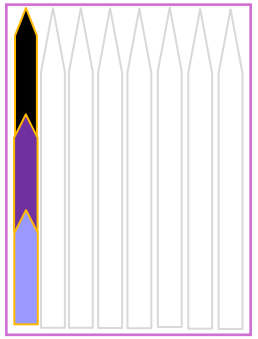
- Focus your research with SureSelect Methyl-Seq

Whole Genome Bisulfite Sequencing



3-6 lanes for one sample
90-180Gb per sample

SureSelect Human Methyl-Seq



3 samples per lane
10Gb per sample

9-18x more efficient with greater depth per region

SureSelect^{XT} Human Methyl-Seq

The First Comprehensive Methylation Discovery System

Discovery Tool

- Probes independent of Methylation state
- Determine methylation state of all methyl sites in region

Comprehensive Design

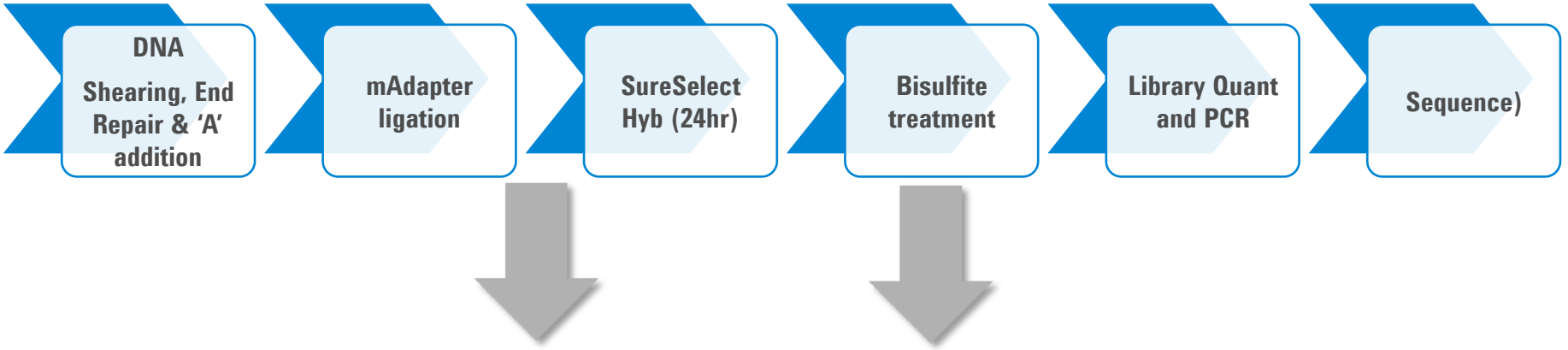
- CpG Islands
- Promoter regions
- DMRs (Differentially Methylated Regions)

DESIGN CONTENT - 84 Mb Design, 3.7M

CpGs

- CpG islands
- Cancer, Tissue-specific DMRs
- GENCODe promoters
- DMRs or regulatory features in:
  - ✓ CpG Islands, shores and shelves ±4kb
  - ✓ DNAseI hypersensitive sites
  - ✓ Refseq Genes
  - ✓ Ensembl Regulatory Features

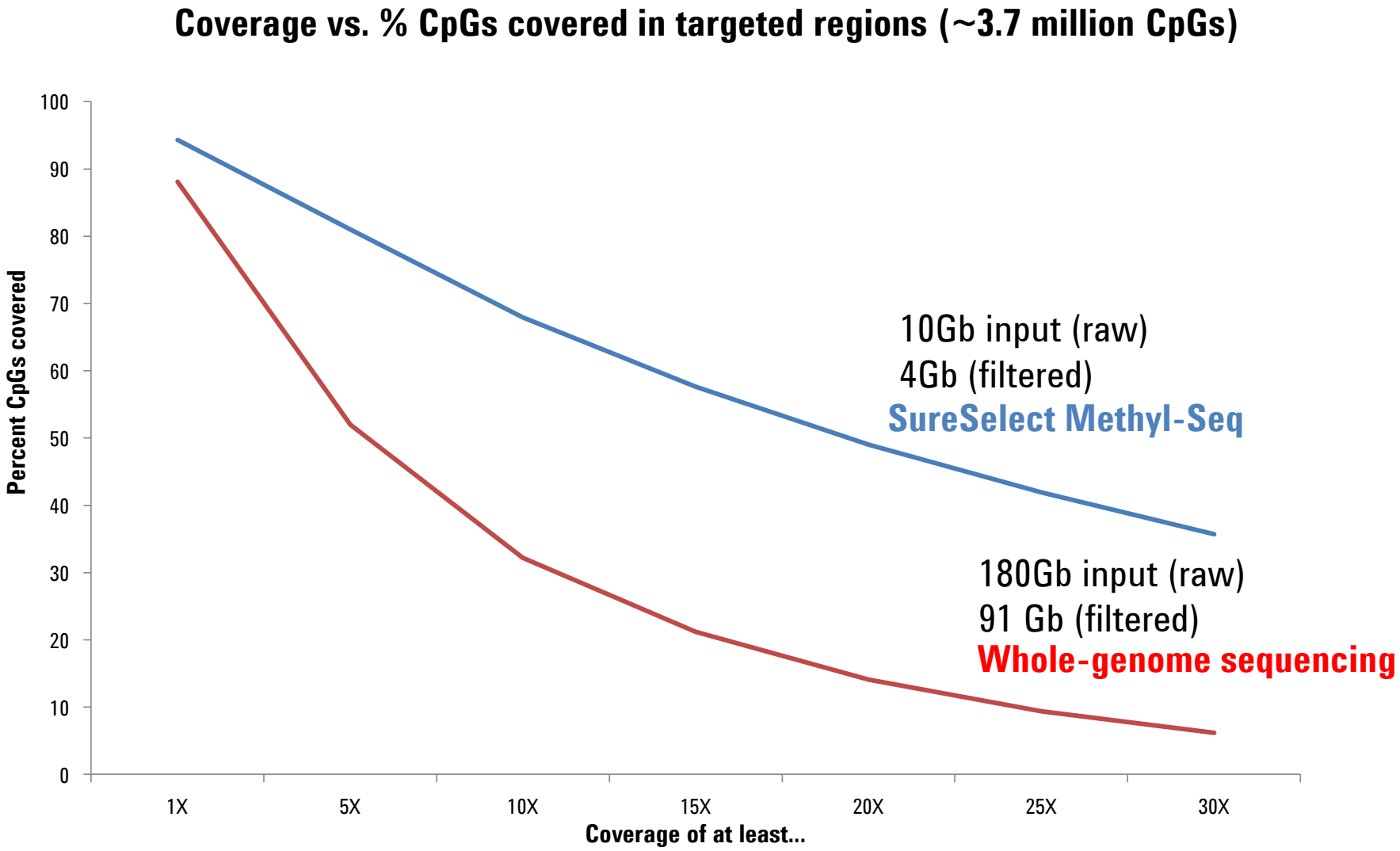
SureSelect Methyl-Seq Protocol



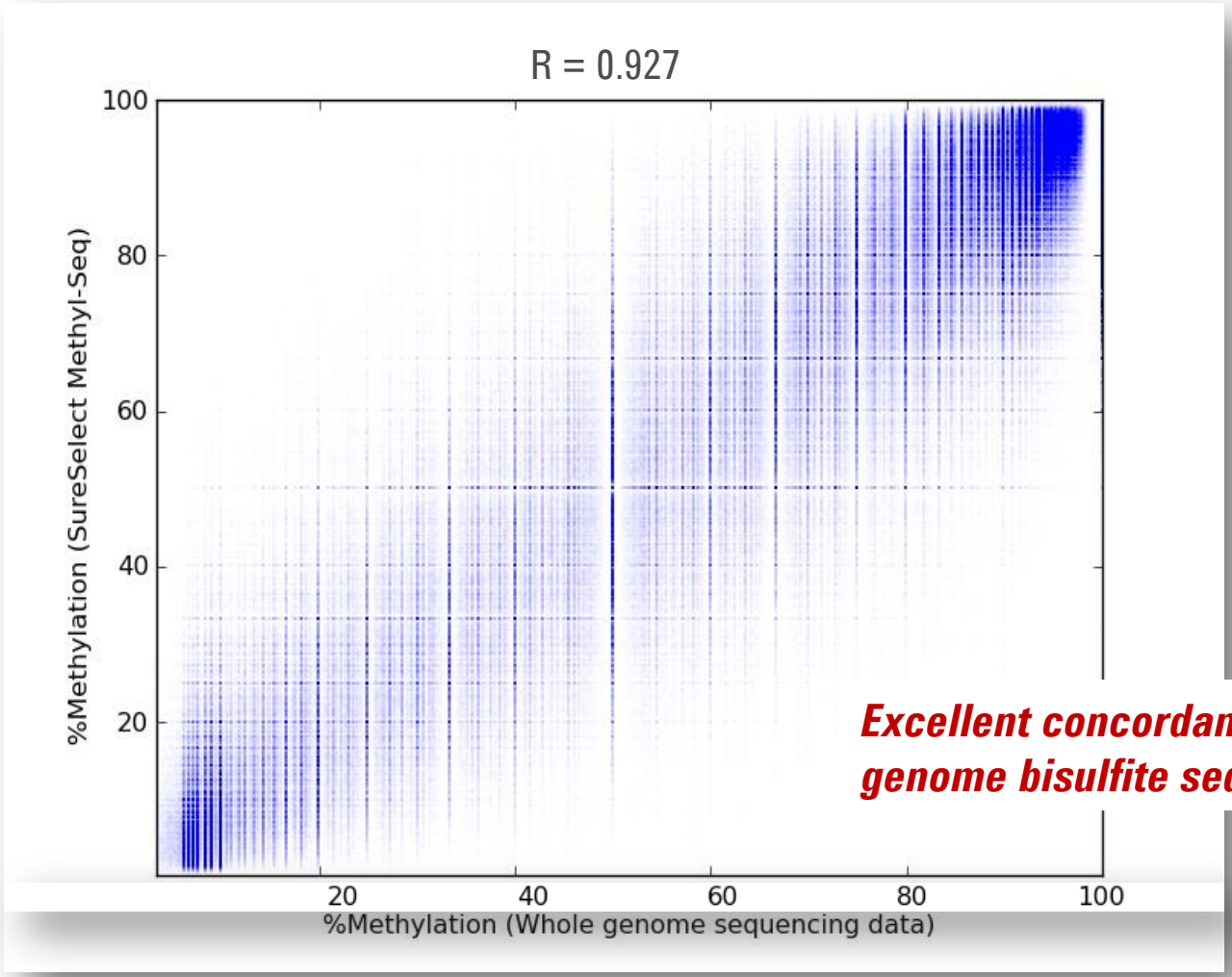
No PCR before Bisulfite treatment to preserve the Methylation state

Bisulfite treatment is performed after hybridization to maximize sample complexity

SureSelect vs. Whole-Genome Bisulfite Sequencing

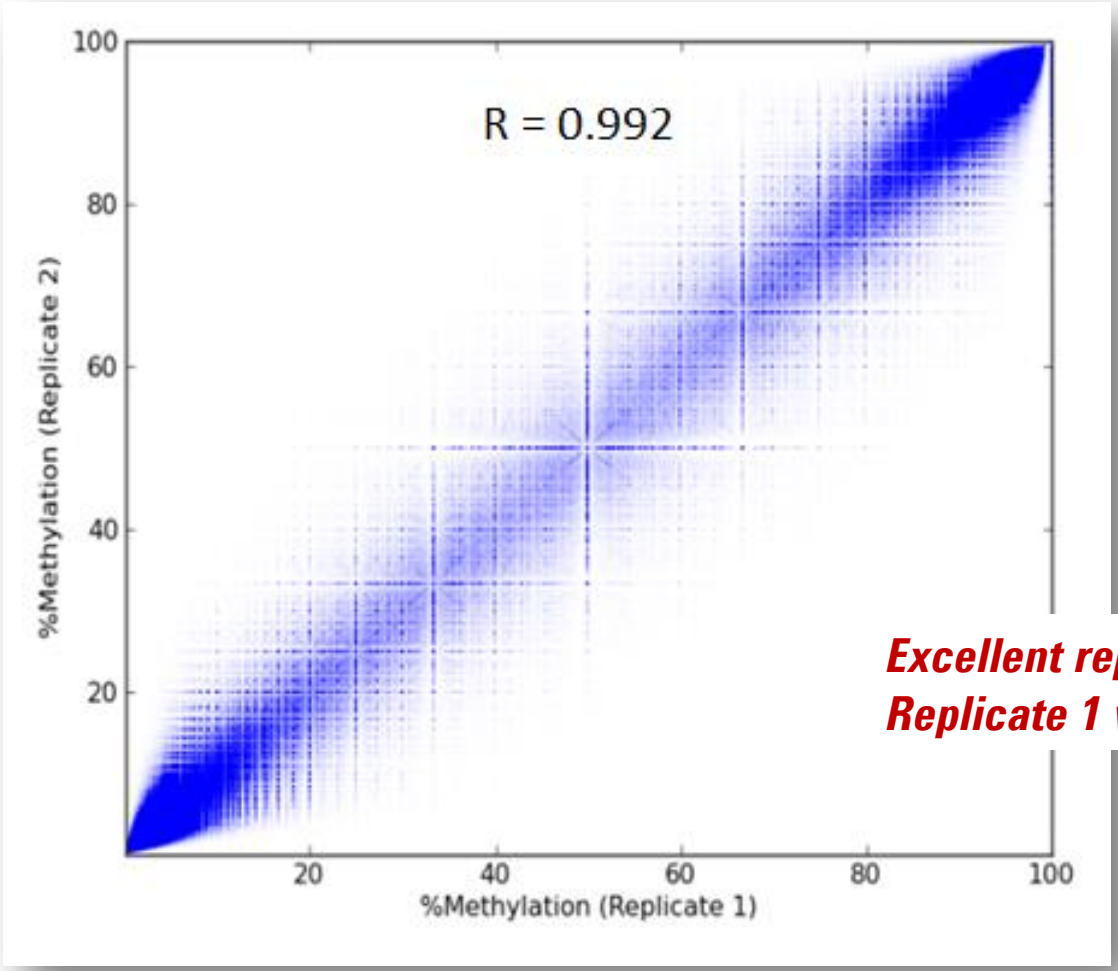


Good Concordance between WGBS & SureSelect Methyl-Seq



Excellent concordance with whole genome bisulfite sequencing data.

Excellent Reproducibility

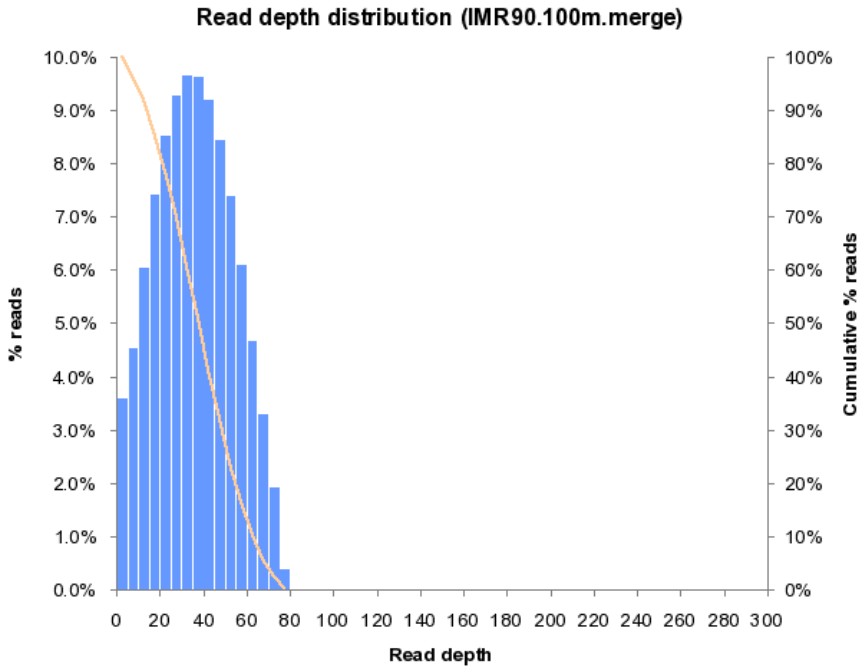


Excellent reproducibility for IMR90
Replicate 1 vs. 2

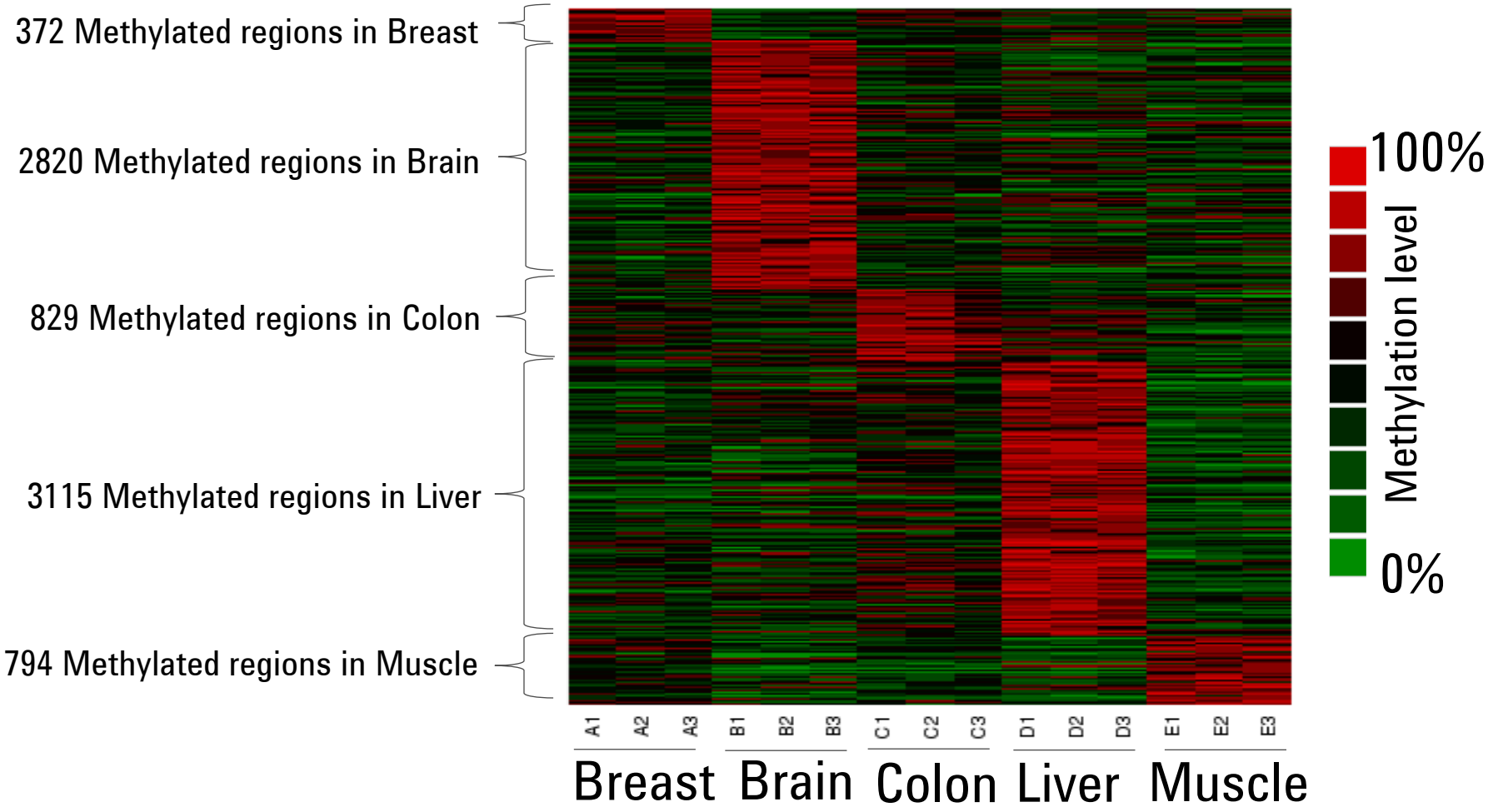
Capture performance

Input 10Gb Raw sequence

Percentage reads in targeted regions:	82.0%
Percentage reads in regions +/- 100bp:	93.6%
Percent of genome targeted:	2.7%
Enrichment in targeted regions:	30.07
Uniformity (3/4 mean with upper tail):	91.4%
Number of bases in targeted regions:	84,367,621
Percentage of targeted bases covered by...	
...at least 1 read:	98.7%
...at least 10 reads:	91.4%
...at least 20 reads:	78.9%



Tissue Specific DMRs

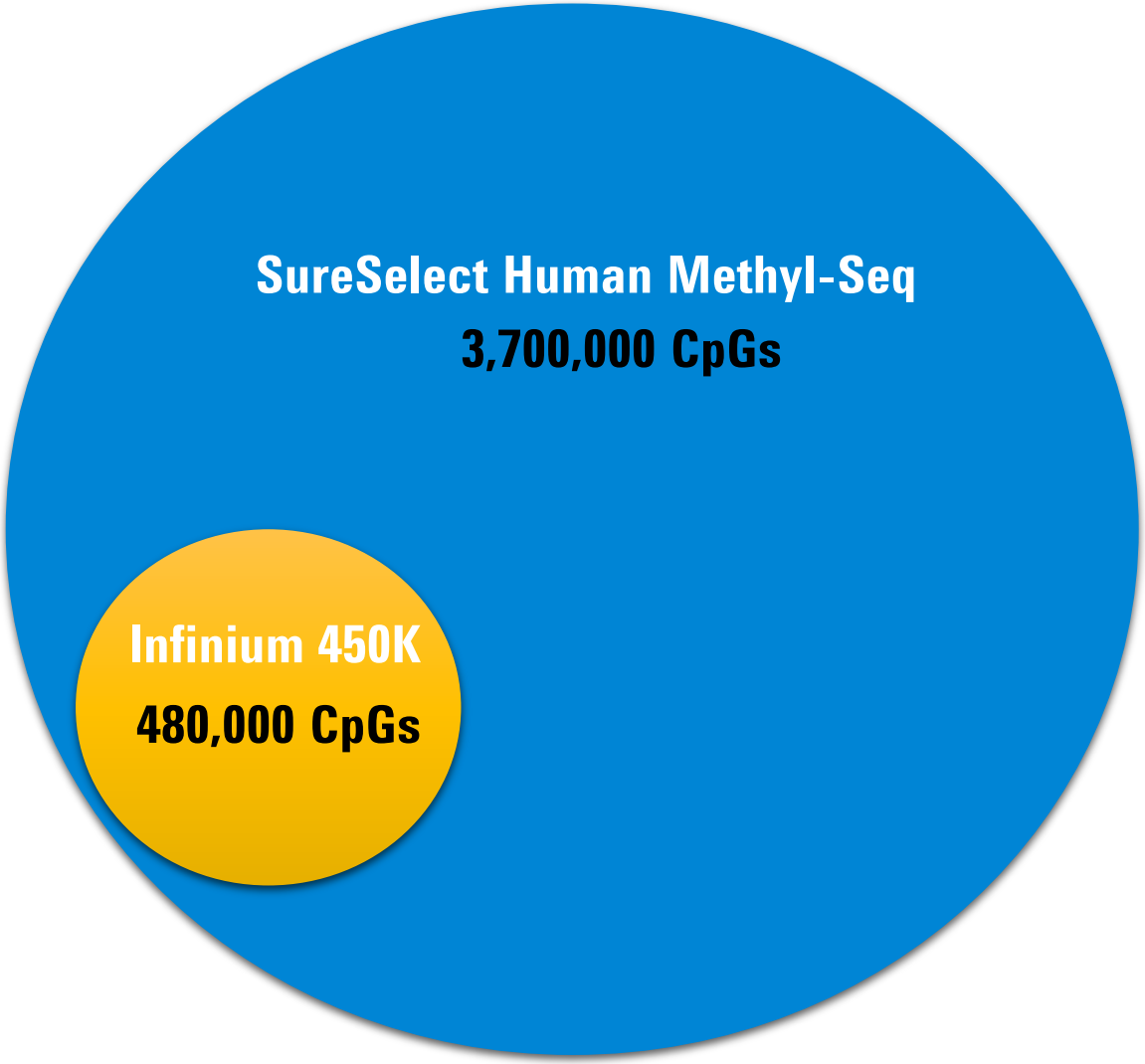


MeDip-Seq & Reduced Representation BiSulfite Sequencing (RRBS)

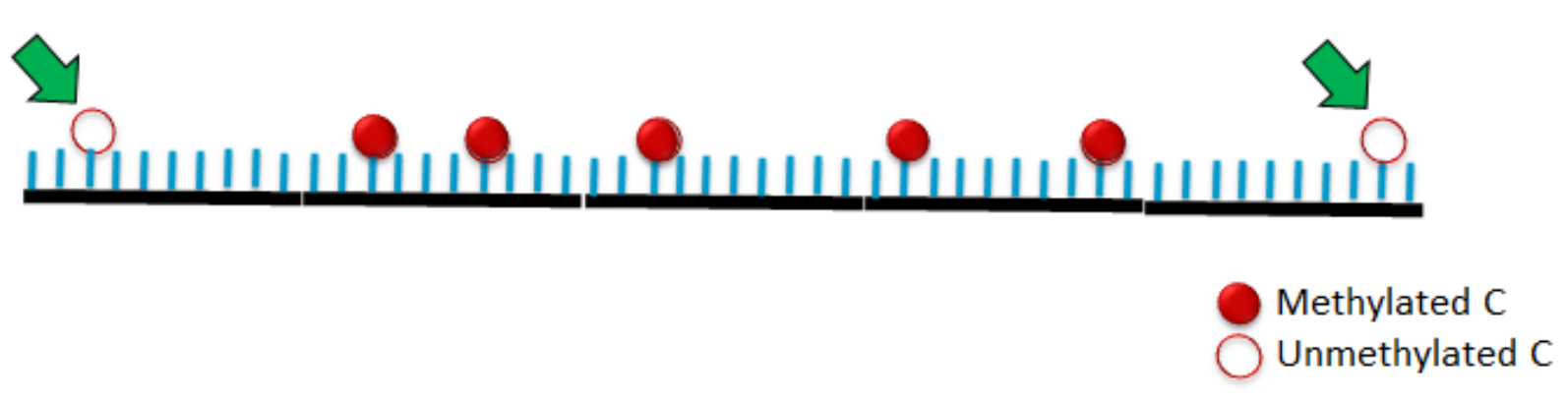
Limitations

- Cannot target specific regions (i.e. DMRs in Shelf and Shore regions)
- Biased towards methylated regions, Repeat sequences & CpG-rich sequences
- Can miss under-methylated regions
- Difficult to design since knowledge of methylation state for the target region is needed

More Coverage of CpGs Compared to Infinium 450K Array



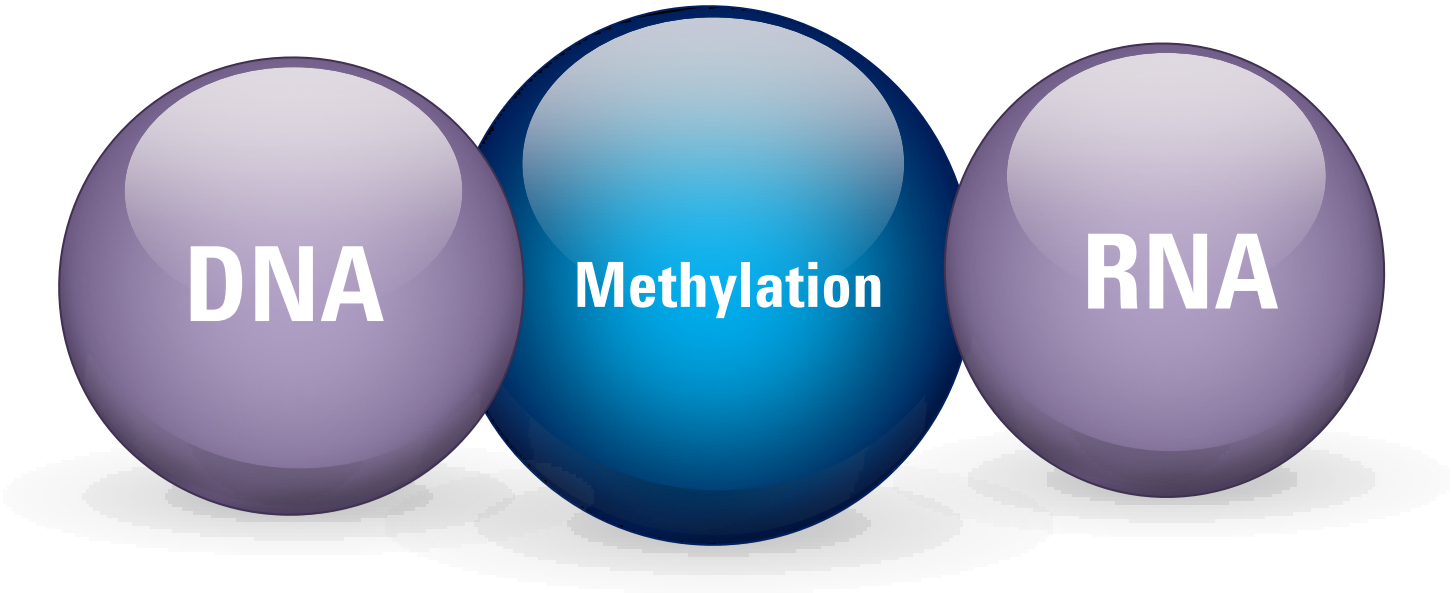
Microarray-Based Method: Infinium 450K Array



- Improves costs and throughput over whole-genome bisulfite
- Does not report individual methylation status
- Not whole-genome
- Does not cover ‘shores and shelves’ known to be important in DMR
- What are you missing?

SureSelect – "Omics" Solution

- **DNA:** Genetic variation
- **RNA:** Gene Expression
- **Methylation:** Effects on Gene Expression



HaloPlex^{HS}

Get to Know Your DNA. Every Single Fragment.

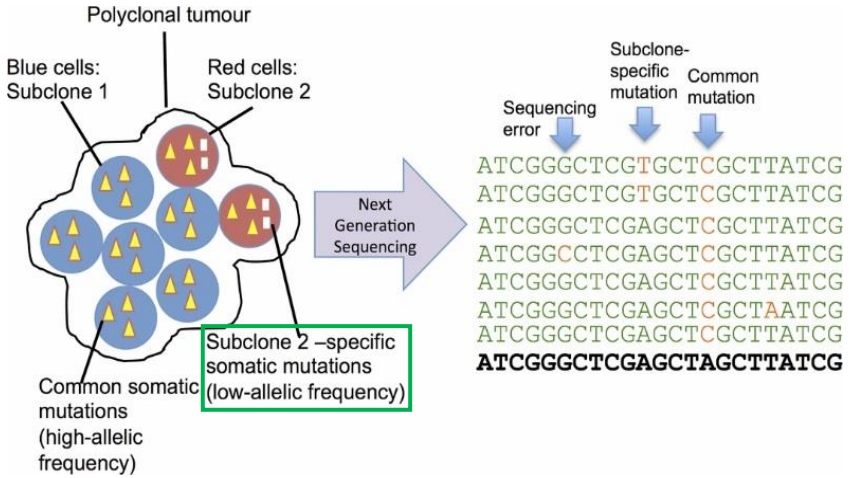
Low allele frequency variants

What are low allele frequency variants?

- Variants present at a frequency below 3%

What are low allele frequency variants implicated in?

- Clonal evolution and pathogenesis
- Tumor subclonal heterogeneity
- Immunological diversity



Adapted from Stead *et al* (2013) Human Mutation 34: 1432-1438

Low allele frequency variants

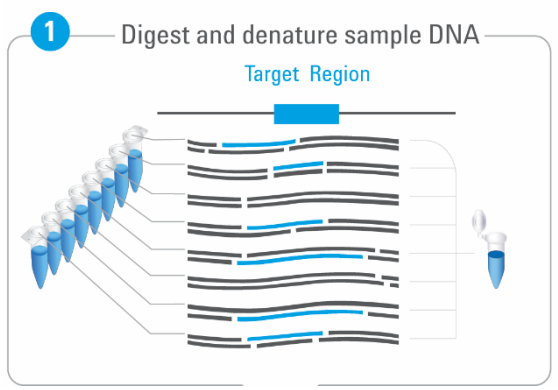
- Low allele frequency variants are difficult to detect by conventional NGS methods
- Relatively high error rate of sequencers (1 wrong base call in 100-1000 sequenced bases)

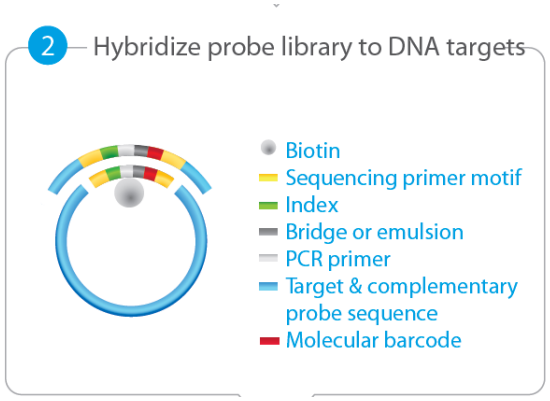
Platform	Primary error type	Background (%)
Pacific Biosciences	G/C deletions	16
Life Ion Torrent	Short deletions, homopolymers	1
ABI SOLiD	A-T bias	0.2
Illumina MiSeq	Single nucleotide	0.1
Illumina HiSeq	Single nucleotide	0.1

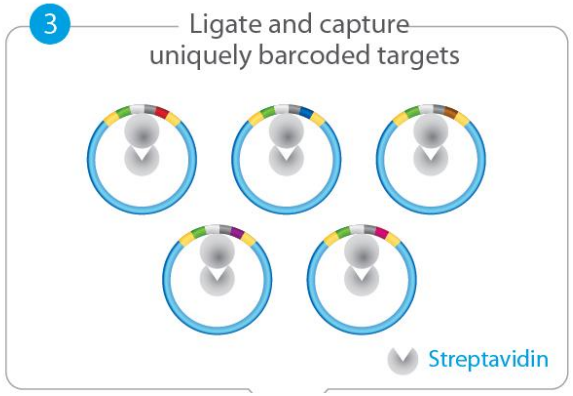
Kennedy *et al* (2014) Nature Protocols 9: 2586 - 2606

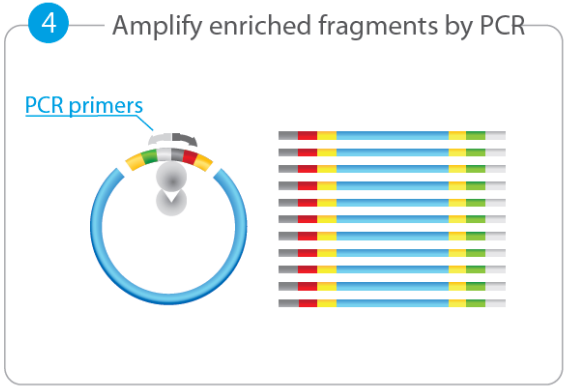
Requires molecular barcodes for increased sensitivity and accuracy

How HaloPlex^{HS} works





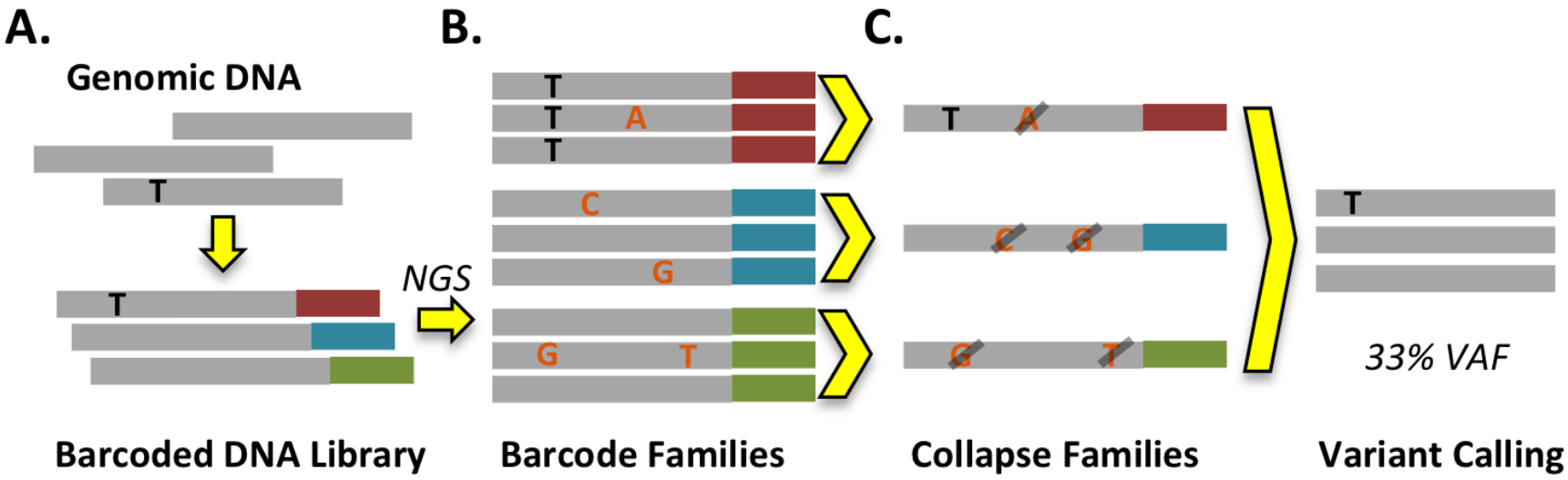




Molecular Barcodes

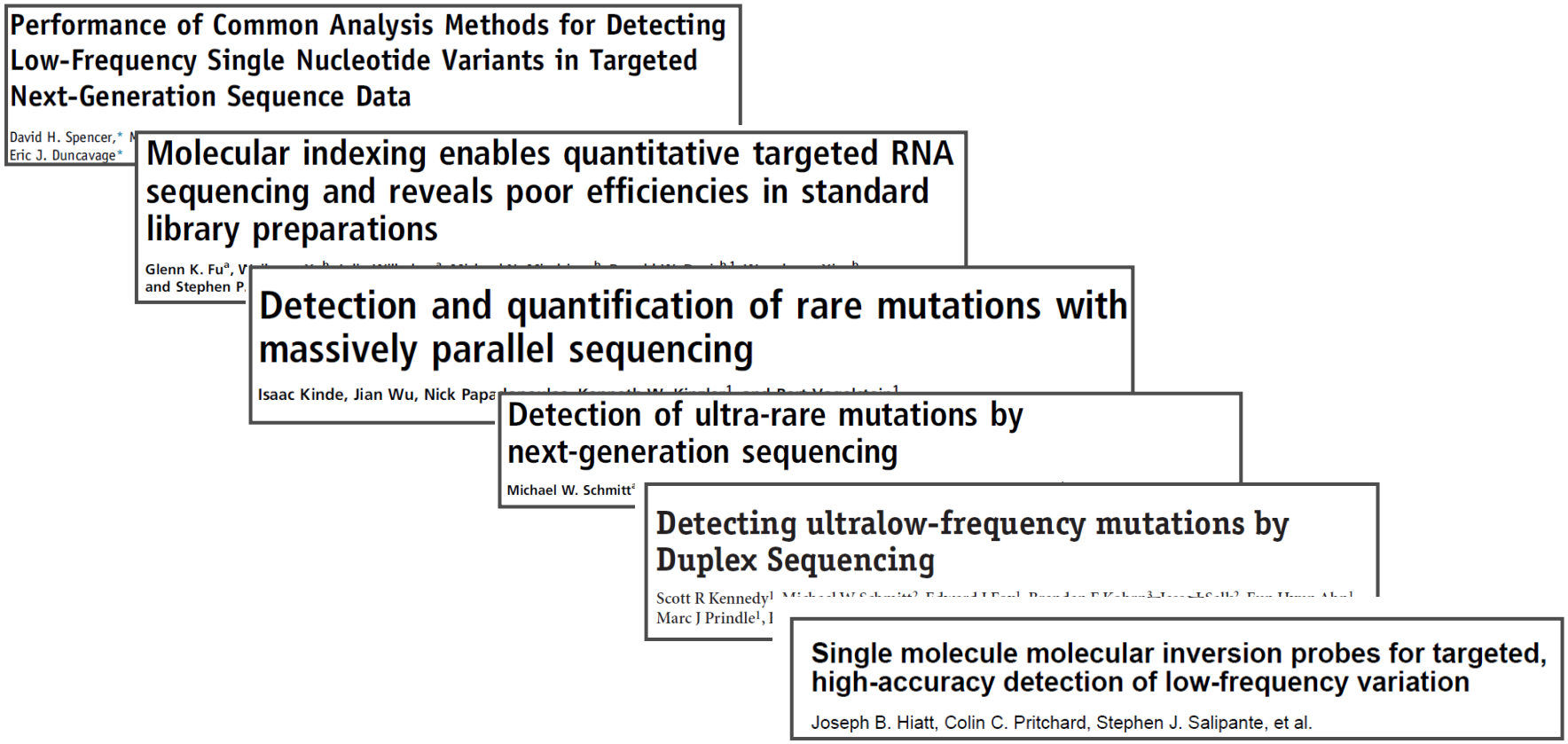
Molecular barcodes are degenerate oligonucleotide sequences (10-16bp) attached to individual DNA molecules

Allow for accounting of sequencer and PCR errors in high coverage NGS data



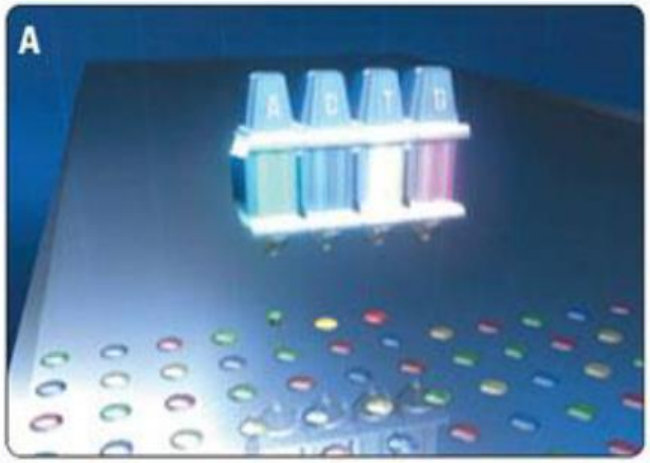
Courtesy of Dr. Eric Duncavage (AGBT 2015)

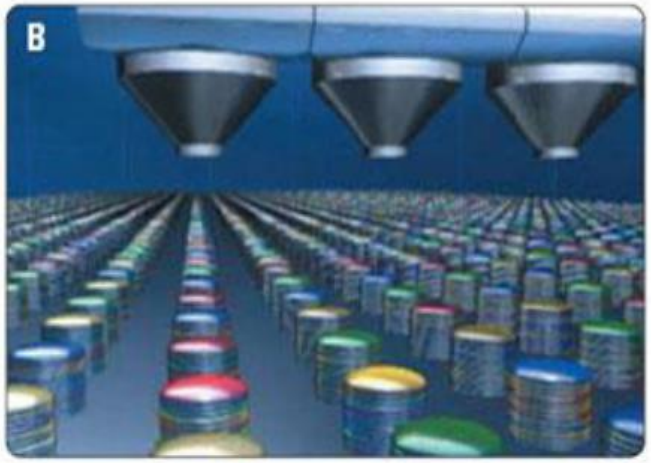
The need for sensitivity and accuracy

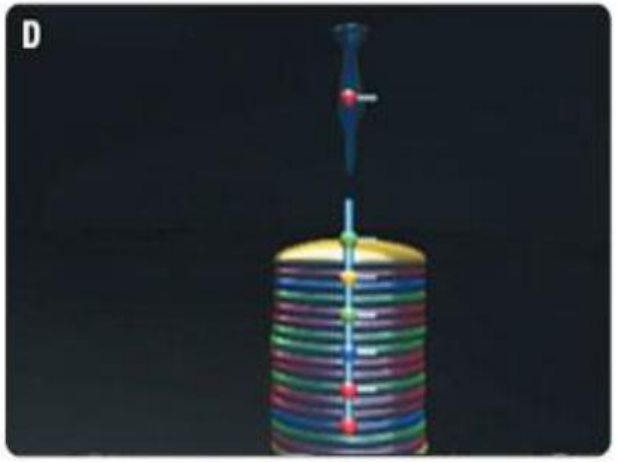


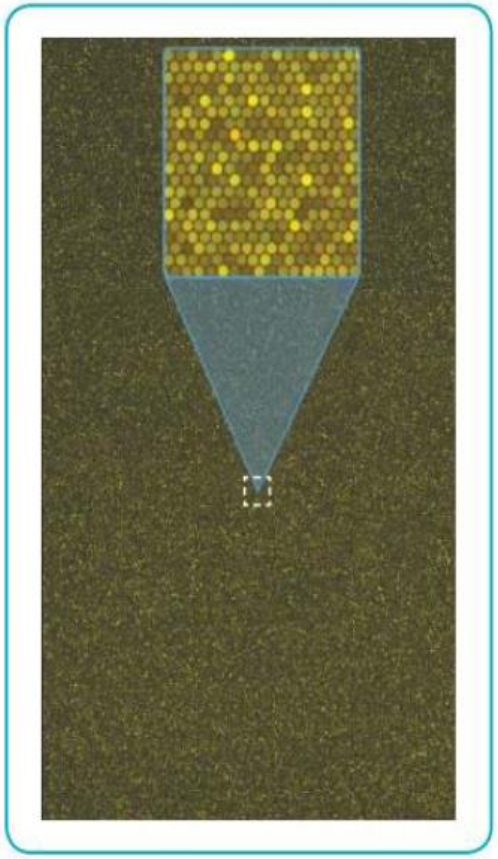
Oligo Library Access Program

SurePrint Technology



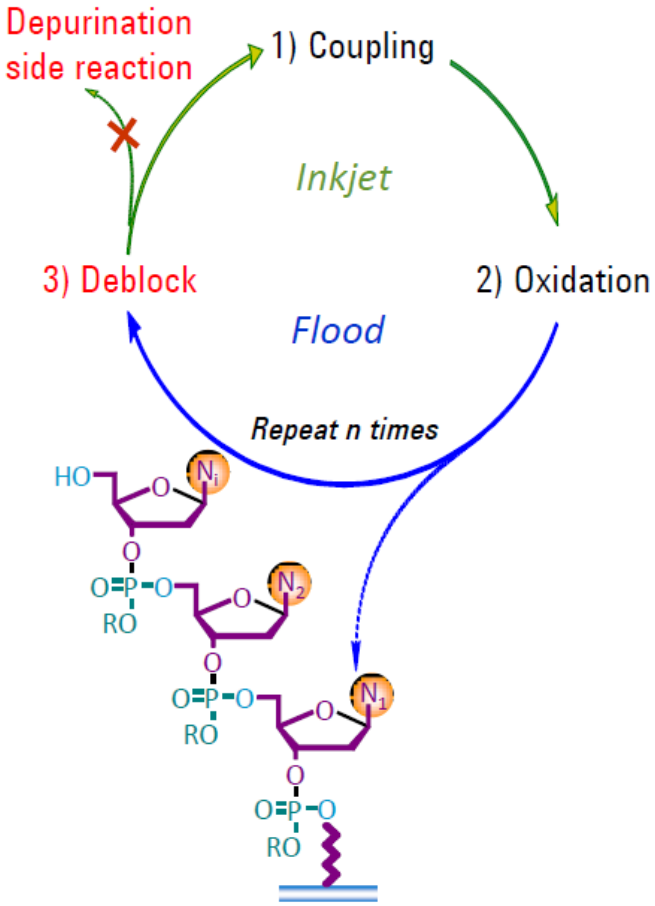






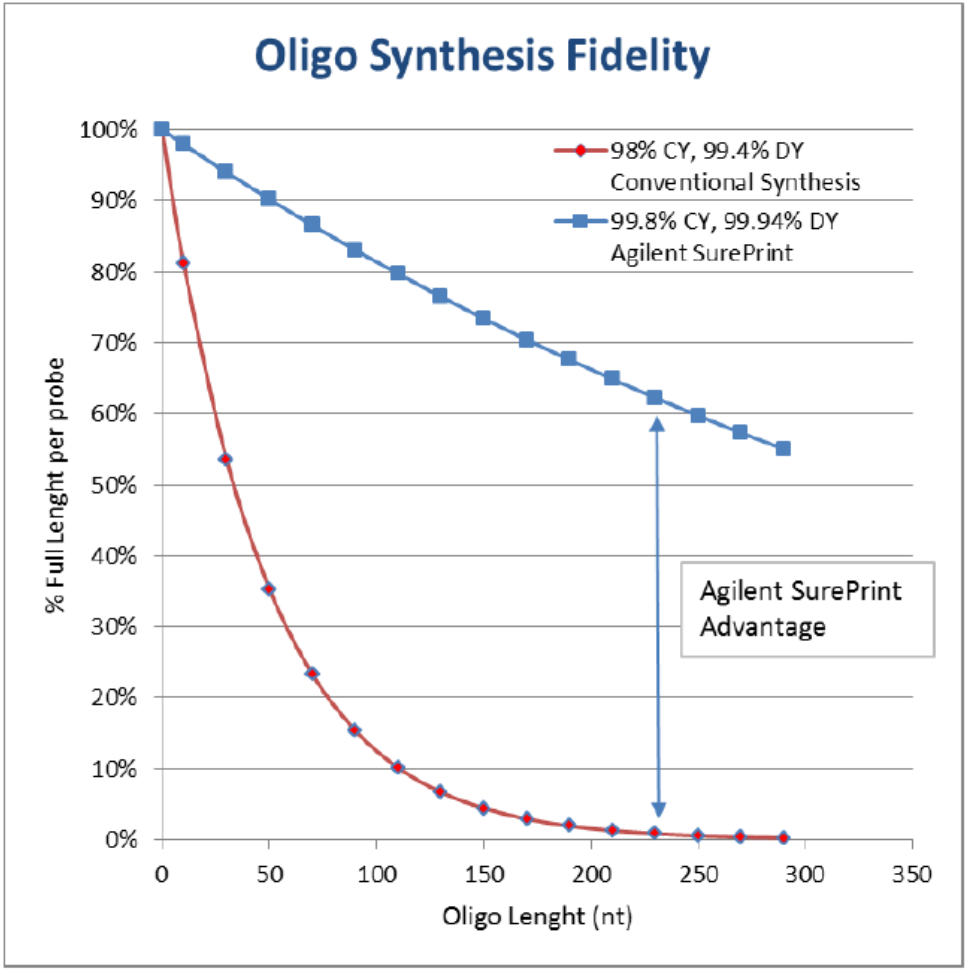
Agilent's million-featured microarray. Inset shows a magnified view of a small region.

Chemical Synthesis: Achieving High Sequence Fidelity



Long length synthesis is achieved by improved cycle yield

- ↑ Coupling efficiency
- ↓ Depurination
- ↑ Consistency

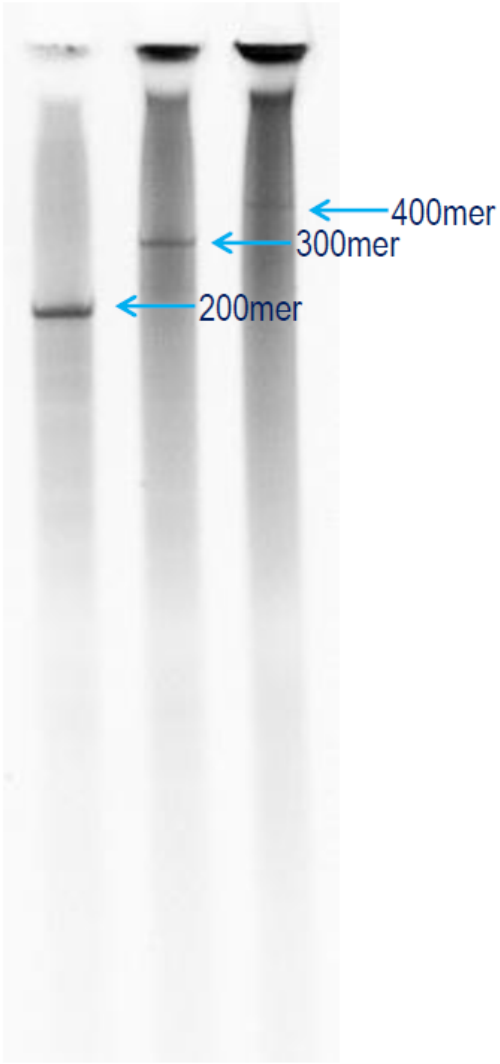


$$\%FL = (CY * DY)^{nt}$$

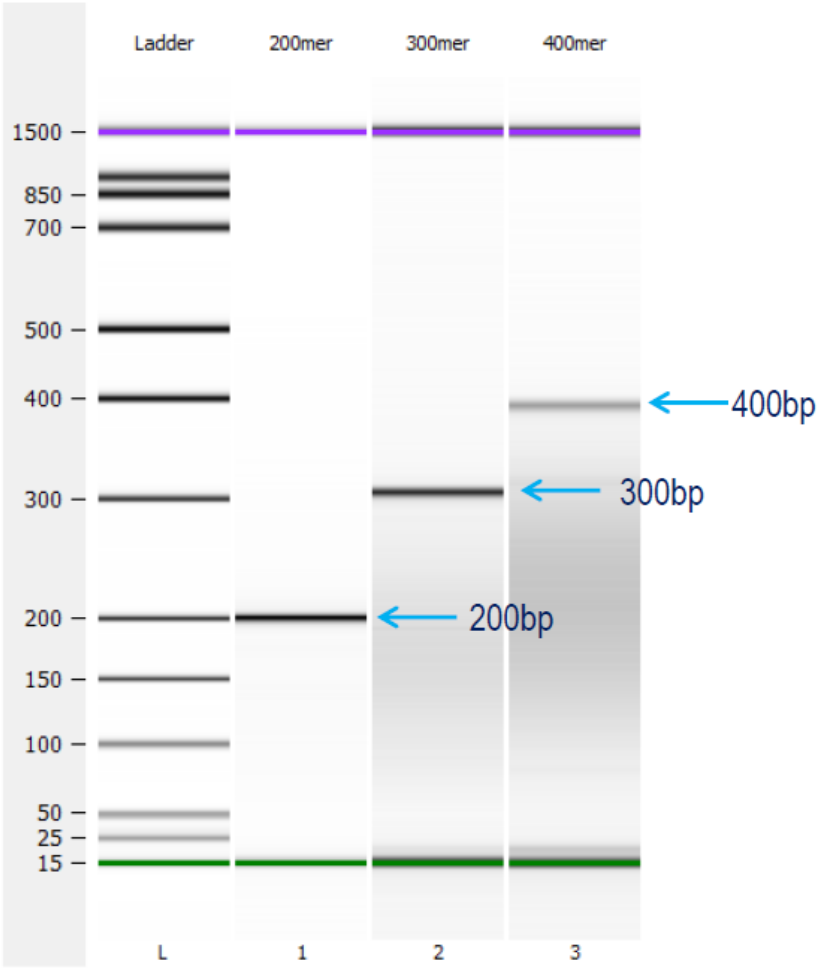
%FL= %Full Lenght
CY=Synthesis Cycle Yield
DY=Depurination Cycle Yield

Pushing the limits of Chemical Synthesis in Research

OLS on denaturing gel



PCR of OLS up to 400mer

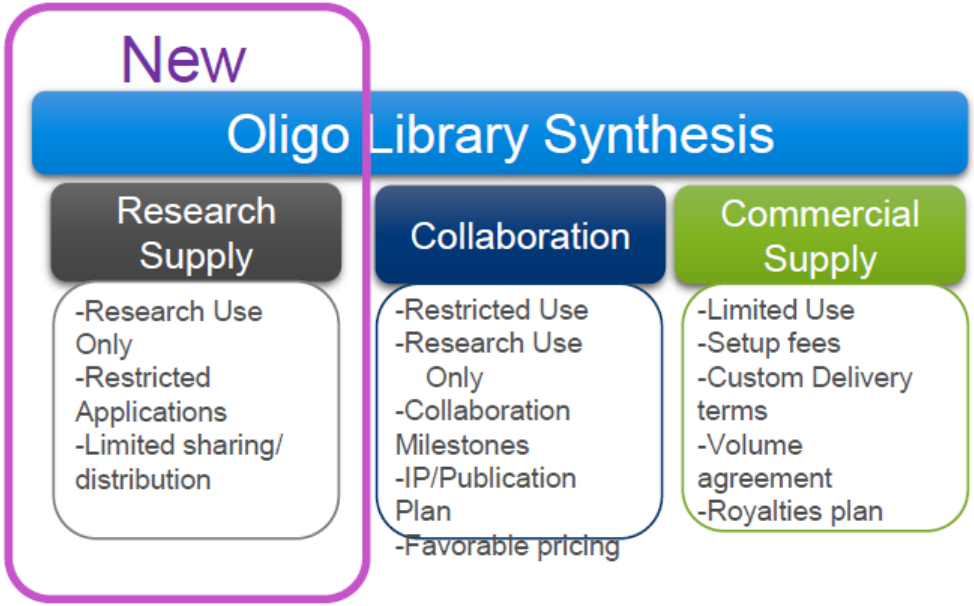


Oligo Library Synthesis Program (New Program)

Three paths to Agilent Custom Oligo libraries:

- **Research Supply:** Enable research applications using Agilent Oligo Library Synthesis products
- **Collaboration agreement**: Partner with Agilent in developing Novel Oligo libraries applications, typically leading to publication/Patent filling.
- **Commercial Supply:** Partner sees value on a continuous supply of Agilent Oligo libraries with negotiated use terms and agrees to a minimum annual volume. An upfront fee covers the setup of the products and agreement.

Common Terms: Partner should verify that it has obtained all third party required licenses for the given applications that might be covered under third party Intellectual Property.



Oligo Library Technology Access Program: Research Supply

Enable research applications using Agilent Oligo Library Synthesis products

In scope for this program:

- Research Use Only (contact us for other uses)
- Oligo libraries up to 230nt long of complexities of 7500, 15000, 100000 and 244000 unique oligos/library on amounts of approx. 10pmols/library
- 4-5 week delivery time
- Competitive pricing
- Outside of Restricted Applications (see below), no IP / publication restrictions by Agilent. Customer is responsible for any third party licenses that might be needed for the intended use/application. Agilent will be indemnified by customer in case of infringements claims.

Restrictions:

- Libraries cannot be resold/distributed outside of purchasing institution
- Purchase and Use Restricted to applications NOT involving the following fields*:
  - Target enrichment/capture
  - In Situ Hybridization (ISH) and variants
  - Gene Assembly (assemble of 2 or more oligos into a longer DNA construct)
  - Genome editing applications, including Functional Genomics screening libraries
  - Site Directed Mutagenesis

*For use in Restricted Applications contact Agilent for alternative Products or Programs

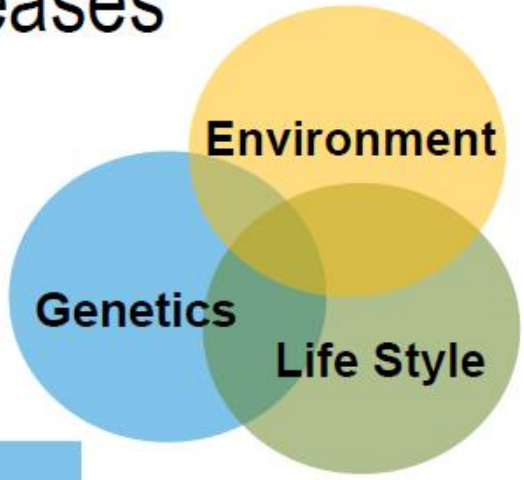
SurePrint Oligo Library Supply

- **Content**:
  - Library Complexities (Unique Sequences): **7.5k, 15k, 100K, 244K**
  - Length: **20-230nt**. Sequences can have different length.
  - Amount: **10pmol** (total library amount)
  - Delivery Format: Library is supplied dried out in a single tube
  - Pricing starting at 90bases
    - **Academic research as low as 0.04¢/base (**$0.4/kb**).** Additional sub-library charges may apply.
- **Library Design**:
  - Free design content, as long as not in conflict with restricted applications
  - It can include priming regions to amplify the library, additional charge for multiple sub libraries

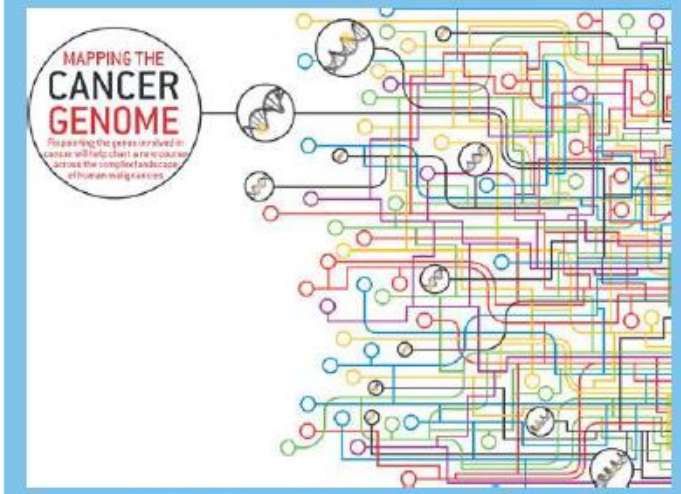
CRISPR Libraries: Early Access

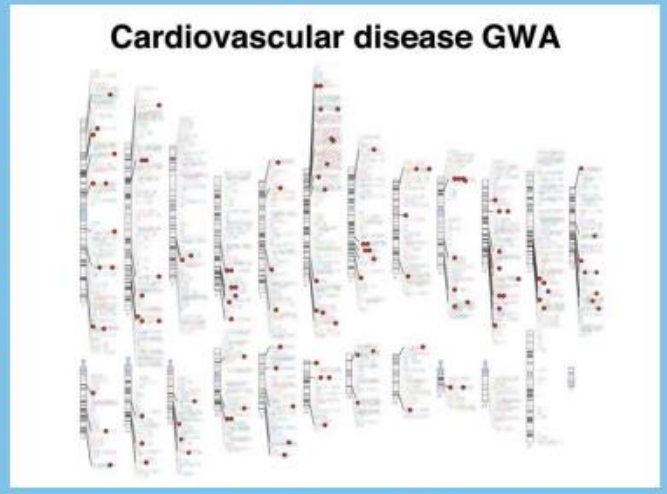
Life Sciences Research: Complex Diseases

- Cancer
- Cardiovascular disease
- Neurological disorders
- Metabolic disorders
- Autoimmune disorders



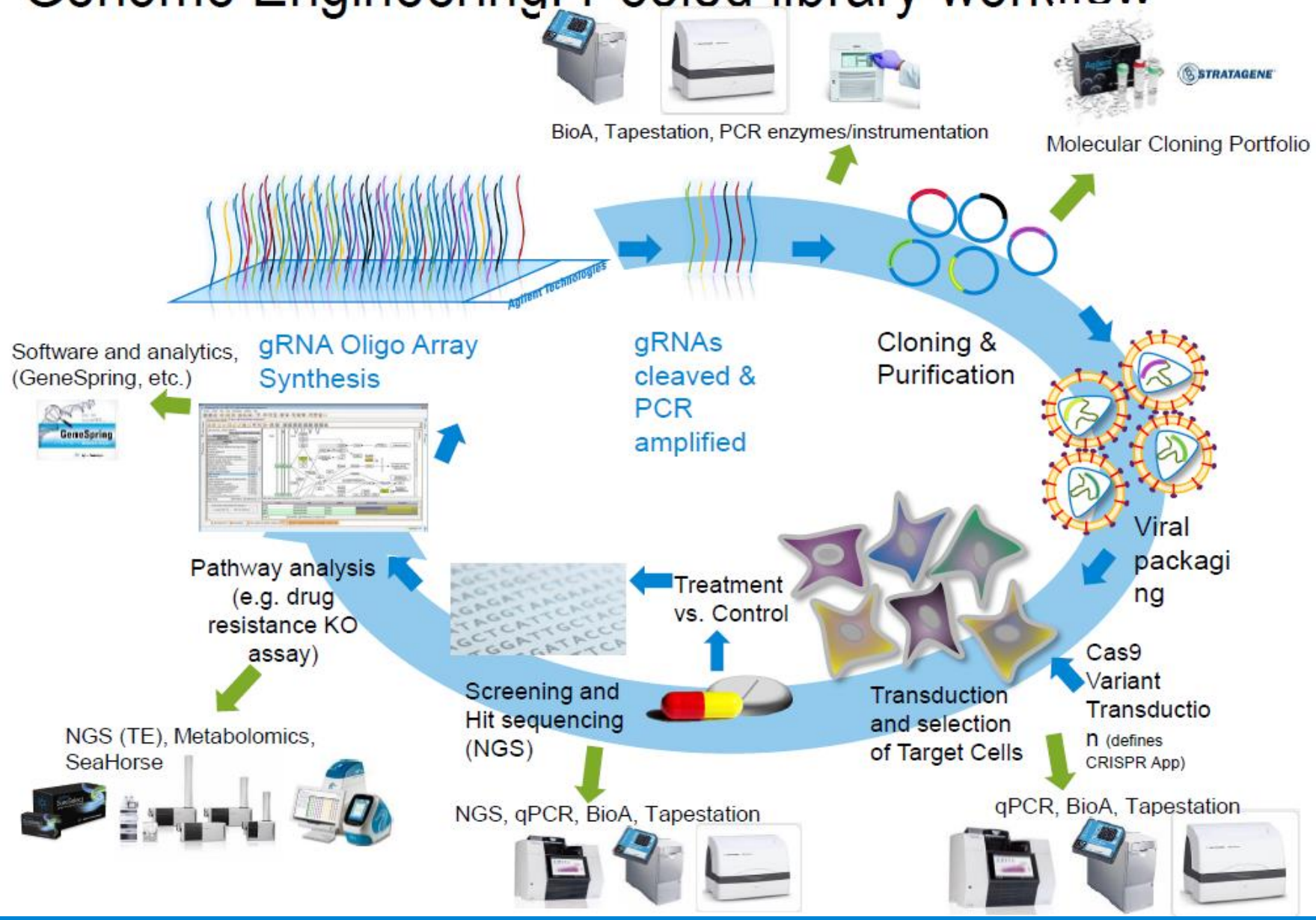
Genome sequencing has yielded 1000s of disease associated candidate genes





Understanding the functions and roles of these genes has been challenging

Genome Engineering: Pooled library workflow



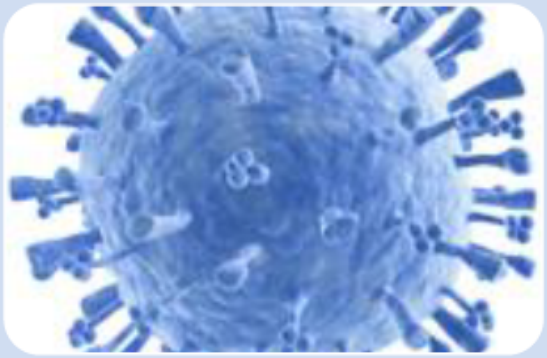
SureGuide CRISPR libraries

	When (Early Access)	Usage	Application	Strengths
Catalog hExome	Now	Functional genomics/target ID	Knock-out libraries	Highest Quality, Good Value
Custom hExome	Now	Functional genomics/target validation	Knock-out subsets	Highest Quality / Custom Designs
Custom hGenomeWide	Now	**Genome regulatory networks**	CRISPR i/a	Highest Quality / Custom Designs
Catalog mExome	Now	Functional genomics/target ID	Knock-out libraries	Highest Quality / Custom Designs
Custom mGenomeWide	Now	**Genome regulatory networks**	CRISPR i/a	Highest Quality / Custom Designs
Custom any Organism/Vector	Now	Various, fully custom	Customer design	Highest Quality / Custom Designs
HR Donor libraries	Now	**Donor DNA for knock-ins, fully custom**	**Genome wide tagging, reporters**	**Quality/ Design freedom**

Enabling scientist to generate and test genomics hypothesis

SureGuide EA Products

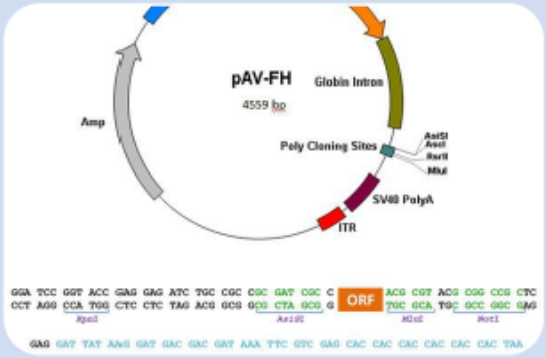
Ready-to-Package



Catalog Libraries

- Plasmid Library
- GeCKOv2
- Human and Mouse
- Cloned into lentivirus vector with hU6 promoter

Ready-to-Clone



Custom Libraries

- Pre-amplified OLS library
- User defined subset or designed

Human and mouse

- Compatible with SureVector cloning

Ready-to-Amplify

Custom Libraries

- Unamplified oligo pool
- Any species, any cloning method
- Entirely custom by user design

Agilent's Advantage

Quality

Guide Representation

- Are all the guides present in the pool?

99.99%

Guide Fidelity

- Are the guides all the correct sequence?

Highest fidelity oligo synthesis

Customization

Custom Content

- What if I have my own set of guides/targets?

Design service and fully custom

Workflow flexibility

- What if I want to delivery guides using my own technique?

Customization at catalog prices

Summary

- Best performing sureselect Exomes with option to add UTR and additional content
- Fastest workflow
- Performs well with low amounts of starting DNA for both Fresh frozen and FFPE samples
- Easy to customize
- Sureselect target enrichment for any species
- Halo-HS power of molecular barcodes enables to identify rare mutation with <1% frequency
- Sureselect Metyl seq is an ideal tool for genome wide methylation analysis and customizable.
- Pooled Sure guide libraries for Human and Mouse
- Best quality Custom Oligo Library to fit your need

Thank You!