SureSelect
The Leader in Target Enrichment

Genomic Solutions Division

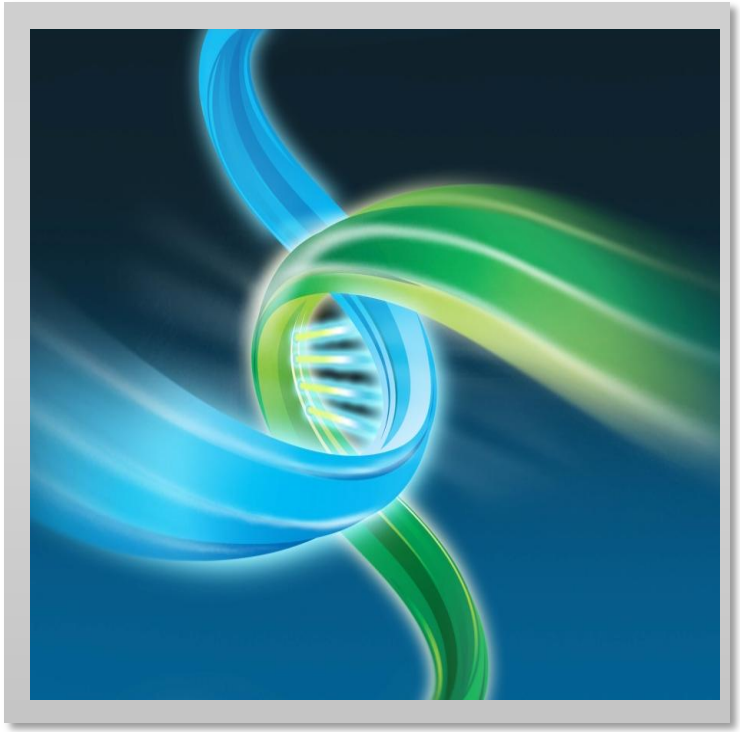
Yong Yi

Marketing Director

Doug Roberts

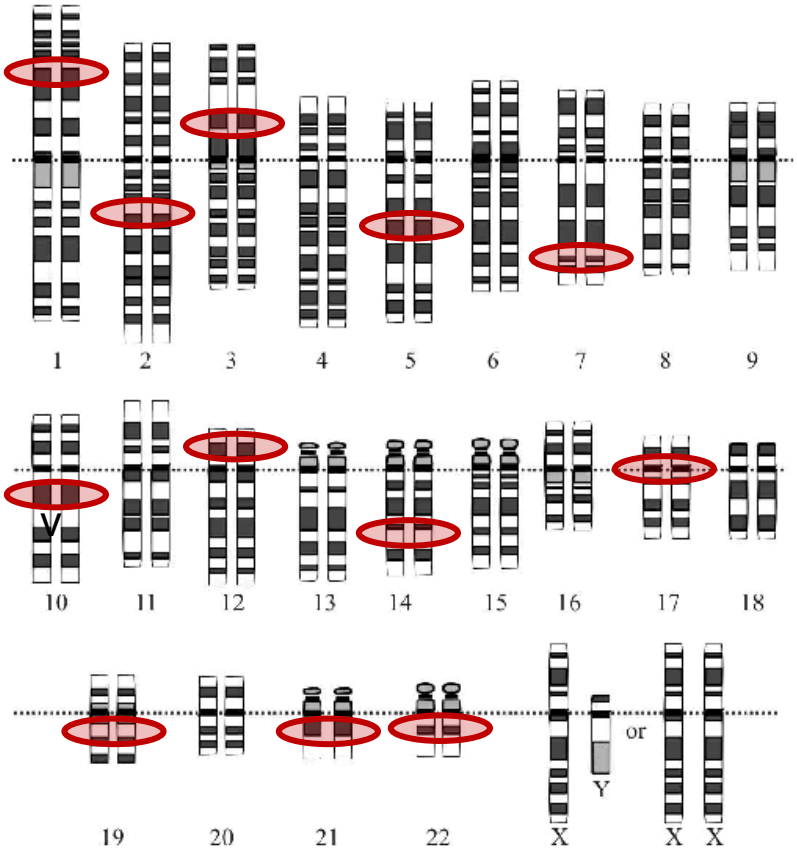
Manager, DNA Applications

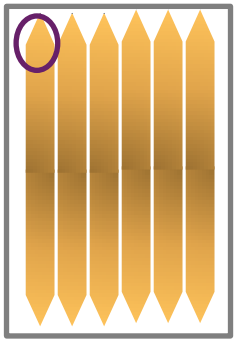
1. SureSelect Technology
2. Whole Exome Sequencing
3. Summary



Maximize Sequencing Efficiency

Required Throughput = Genome Size x Average Coverage





Target = 50Mb x 100 = 5Gb

Target = 5Mb x 100 = 500Mb

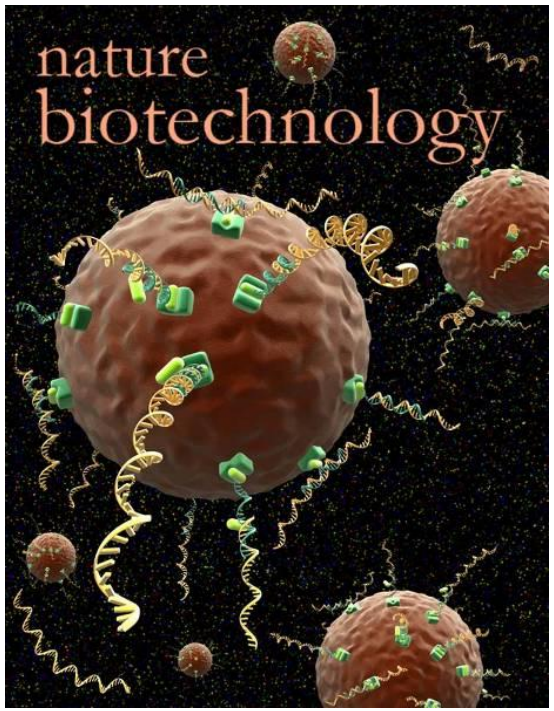
Target = 0.5Mb x 100 = 50Mb

Target =50Kb x 100 = 5Mb

Develop designs for any sequencing capacity:

- High Throughput or Desktop

SureSelect – 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 – The Leader in Target Enrichment

COMPLETE Solution

Best **PERFORMANCE**

FLEXIBLE Designs

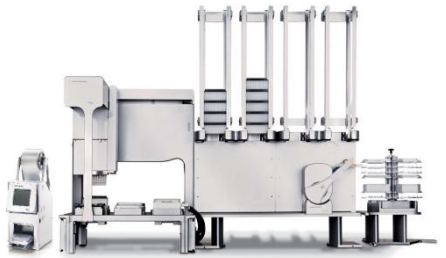
SureSelect - Most Complete Enrichment Solution

All Exon Designs

- All Exon V5
- All Exon V5+UTRs
- Non Human Exomes

Custom Solutions

- Custom DNA
- Custom RNA



Automation

Targeted Panels

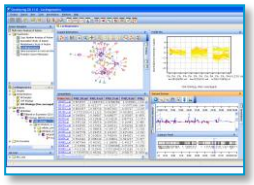
- Human Methyl-Seq
- Chrm. X
- Human Kinome

\+ **Library Prep**

Post-capture	Pre-capture
SureSelect *XT* Illumina	SureSelect *XT2* Illumina
SureSelect *XT* SOLiD	

SureSelect - Most Complete Workflow Solution

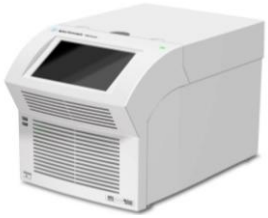
From Sample to Analysis



GeneSpring NGS

2100 Bioanalyzer

2200TapeStation



SureCycler 8800

MX3000

Agilent NGS Automation

SureSelect – The Leader in Target Enrichment

COMPLETE Solution

Best **PERFORMANCE**

FLEXIBLE Designs

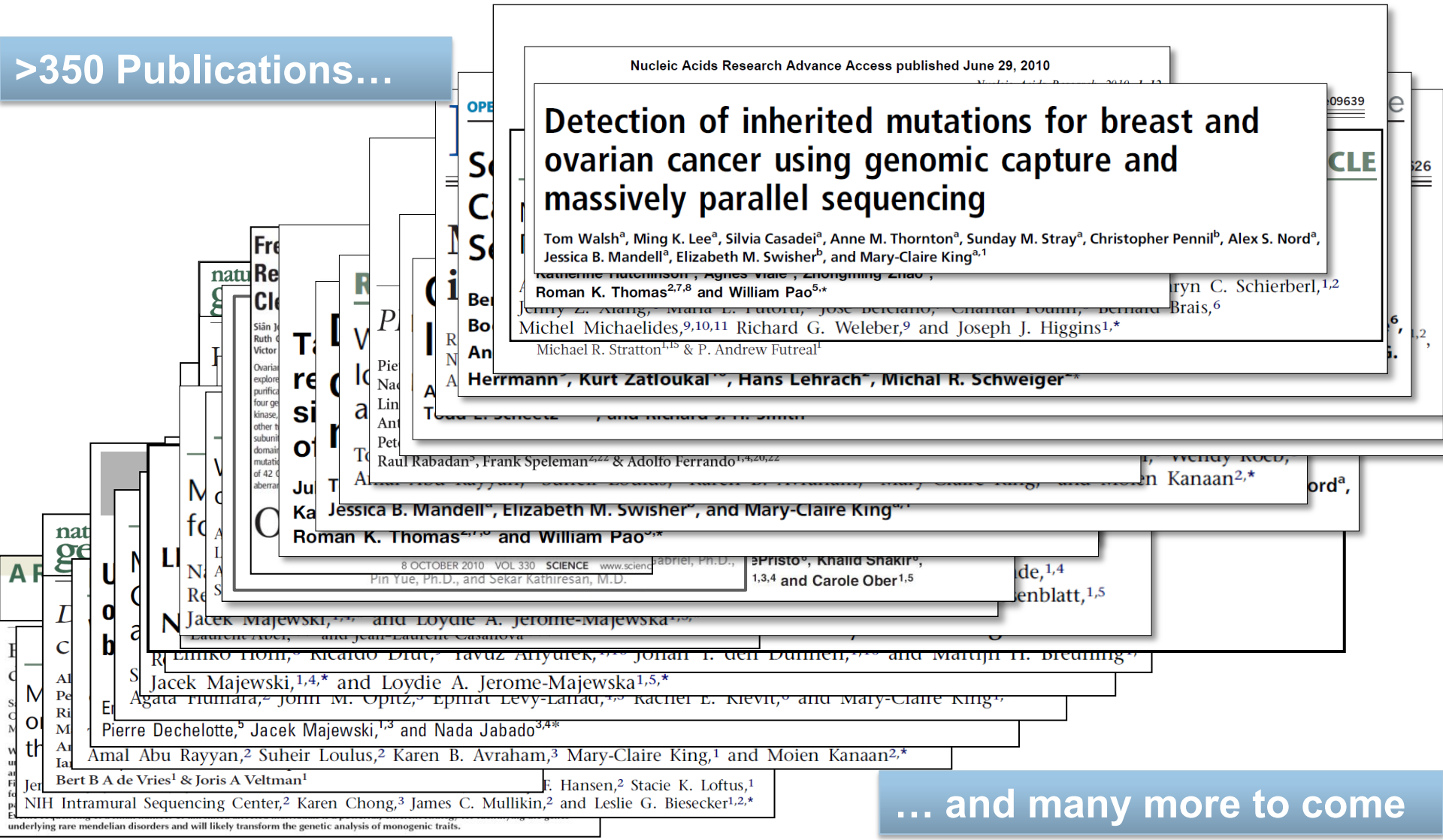
SureSelect Technology

The Best Performance

Core Technology	Benefits
Ultra-Long RNA Baits (120-mer) **Binding strength** **RNA:DNA > DNA:DNA**	**Better Sensitivity** Detect more SNP, InDels, CNV, fusions
	Better Workflow 16hr hybridization, other require up to 72hrs
	Better Allelic Balance Equal representation of both alleles

SureSelect: Enabling Scientific Discovery

>350 Publications…



… and many more to come

SureSelect – The Leader in Target Enrichment

COMPLETE Solution

Best **PERFORMANCE**

FLEXIBLE Designs

SureSelect - Most Flexible Enrichment Solution

Custom or Catalog Solutions for DNA and RNA targets

- Proven, Refined Design Algorithms
- ISO 13485 Reagents
- Validated, Scalable Automation Solution

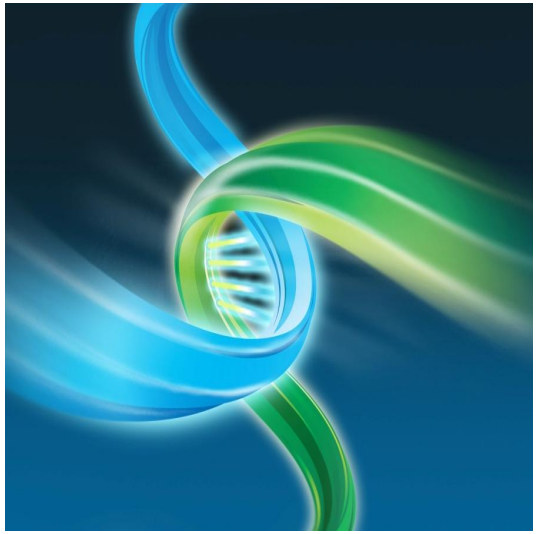
SureDesign –
New, intuitive software for custom capture designs

SureSelect – Flexible Solution

From High Throughput to Desktop Sequencers

SureSelect All Exon V5

- Design
- Performance
- Cancer Applications



Human All Exon V5 Design and Synthesis

- Updated Content: Refseq, Gencode, UCSC, TCGA, CCDS, miRBase
- Proprietary probe selection/placement algorithms based on GC, Tm, Entropy, Mapability
- Long 120mer probes printed in Agilent's microarray fabrication facility
  - V5 ~ 544K individual probes spanning 50.4 Mb
  - V5 +UTR ~ 882K individual probes spanning 74.6 Mb
- High GC content printed in higher replication to boost performance
- Matched for best performance with Agilent's library preparation
- DNA probes are converted into biotinylated RNA bait

Human All Exon V5 and V5+UTRs

The Best Performing Exome
Ready for Sequencing the Next Day

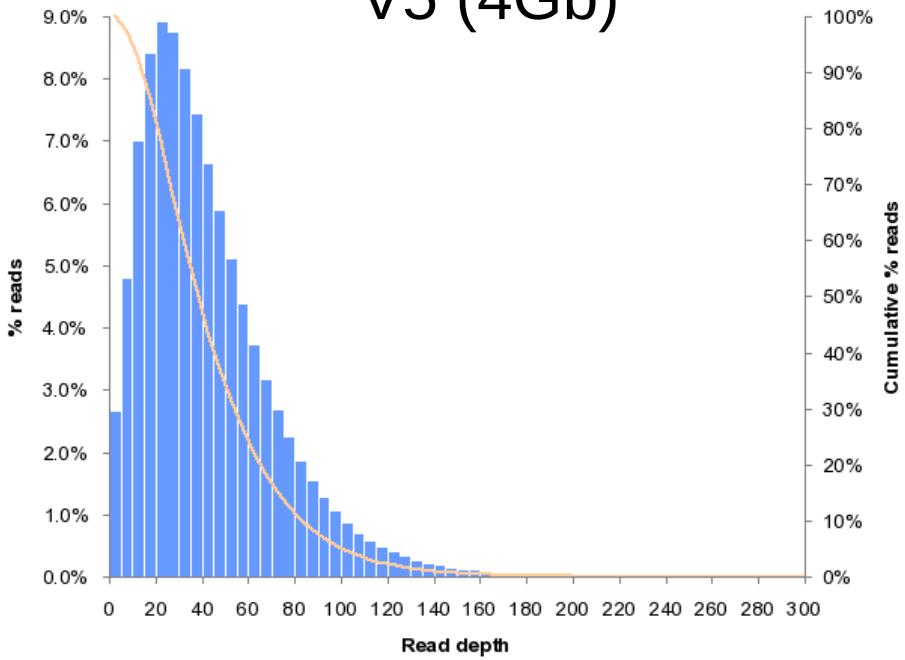
The Fastest Workflow

From sample to sequencing the next day

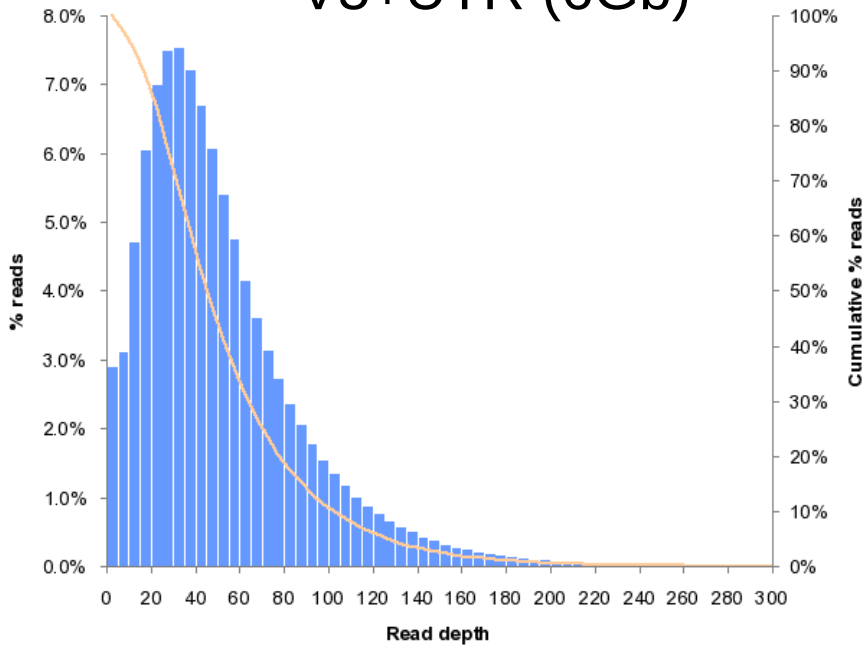
Overnight Hybridization Enables Sequencer Ready Samples the Next Day

Human All Exon V5: Distribution of Read Depths

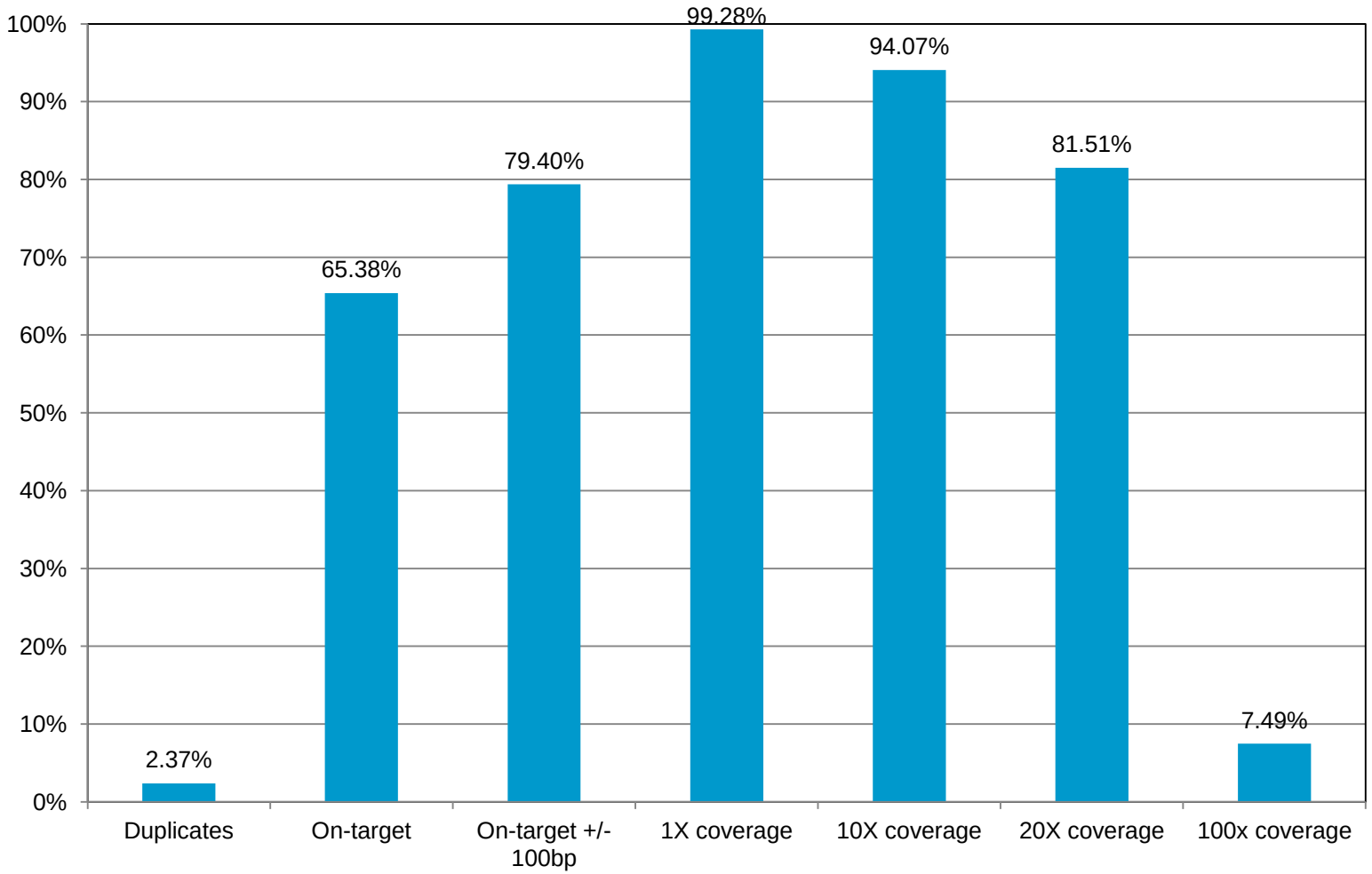
V5 (4Gb)



V5+UTR (6Gb)



Human All Exon V5 Performance Statistics

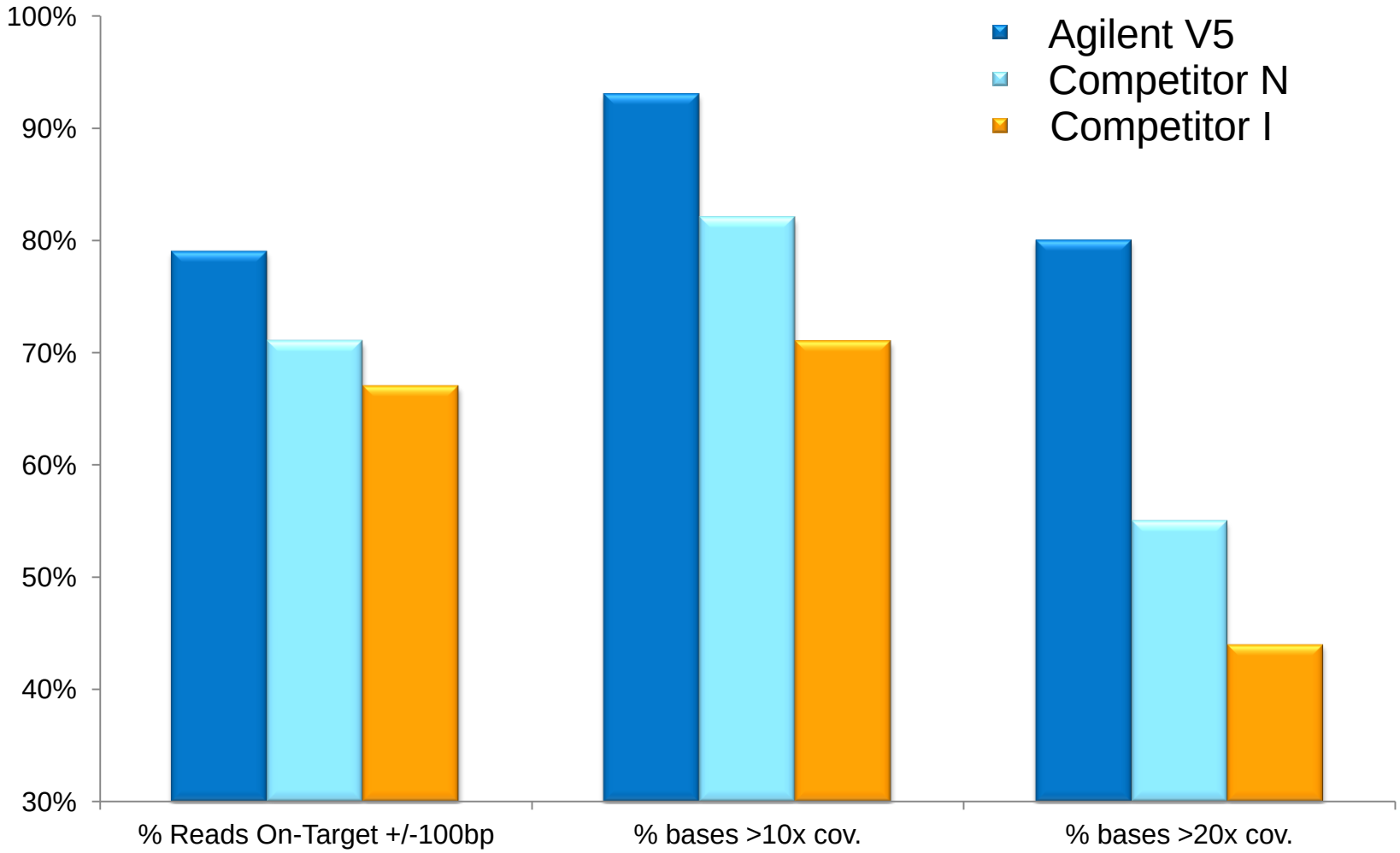


The Best Performance and Fast Workflow

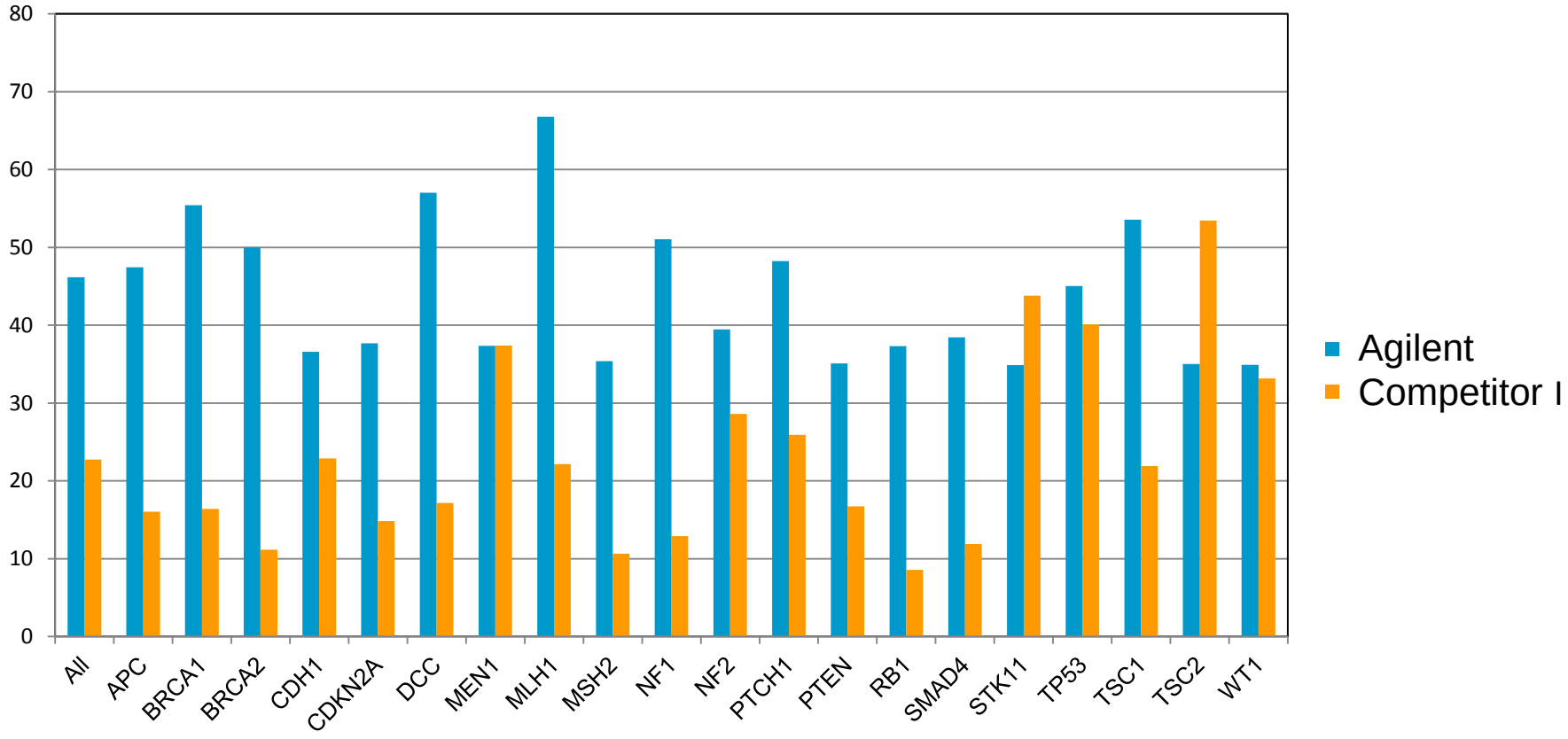
Parameter	All Exon V5	All Exon V5+UTRs	Competitor N	Competitor I
Target Size	50Mb	75Mb	64Mb	62Mb
# Genes	21,522	21,522	>20,000	20,794
# Targeted Exons	357,999	359,555	-	201,121
CCDS	X	X	X	X
RefSeq	X	X	X	X
GENCODE	X	X	X	X
miRBase	X	X	X	X
TCGA	X	X		
UCSC	X	X		
Amount of Seq. (total)	4Gb	6Gb	6Gb	10Gb
Overall Workflow	1.5 days	1.5 days	4.5 days	3.5 days
Add Custom Content	Yes	No	Yes	No

The Best Performance

Outstanding coverage with less sequencing

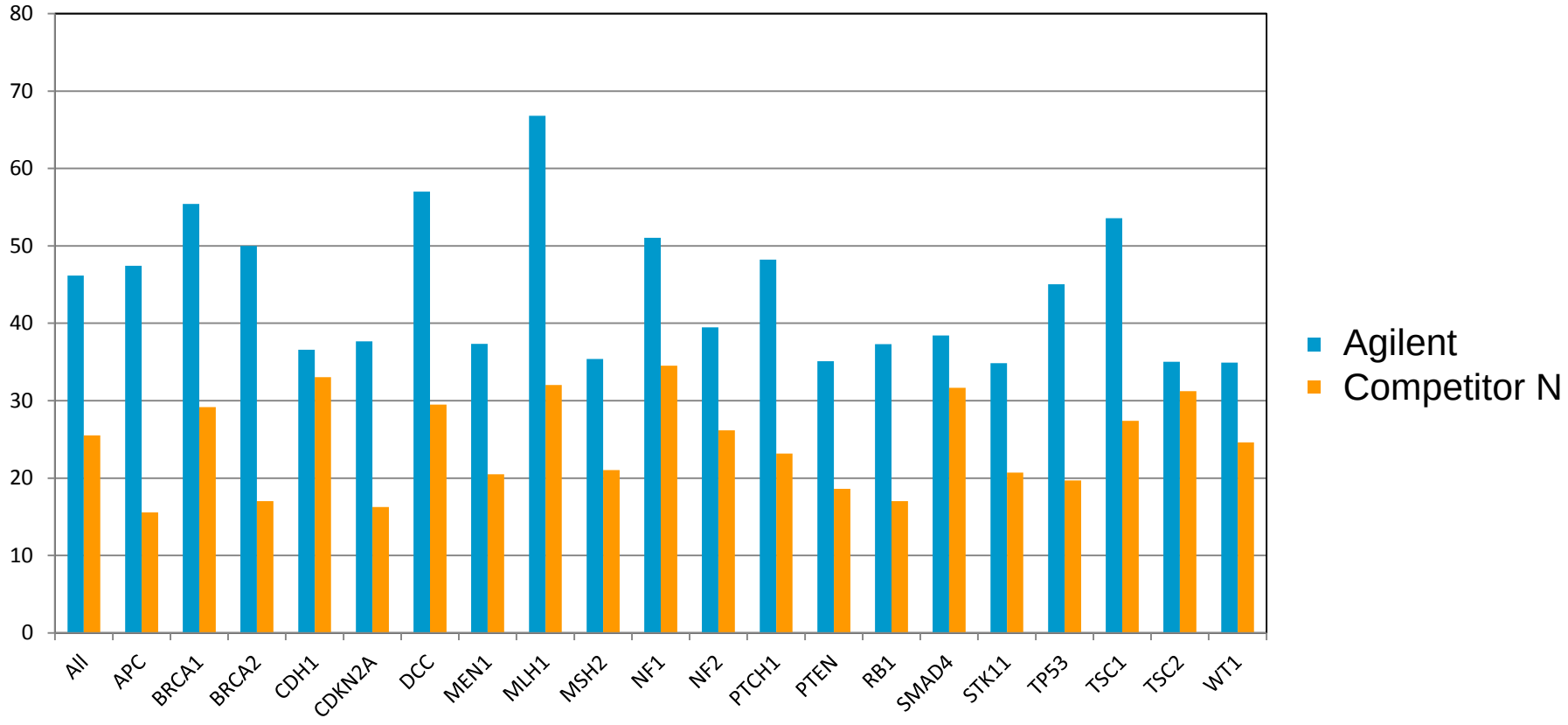


Human All Exon V5: Coverage in Tumor Suppressor Genes



- Agilent displays higher depth of coverage with the same amount of sequencing (4Gb)

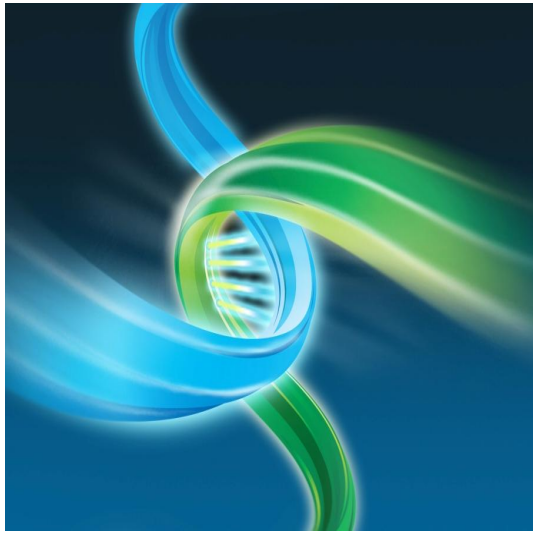
Human All Exon V5: Coverage in Tumor Suppressor Genes



- Agilent displays a higher depth of coverage with the same amount of sequencing (4Gb)

Example Studies: Target Enrichment in Cancer

- **Target Enrichment for Novel Discovery**
  Diffuse Large B cell Lymphoma
- **Intratumor Heterogeneity**
  Renal Cell Carcinoma



Diffuse Large B cell Lymphoma (DLBCL)

- Aggressive Non-Hodgkin lymphoma
- Affects 30,000 new patients in the US every year
- Current Standard of Care –R-CHOP
  - Rituximab (anti CD20), cyclophosphamide, doxorubicin, vincristine, and prednisone
- 3 year event-free survival ~60% , remaining 40% die of disease

Exome sequencing of Non-Hodgkin Lymphoma

PNAS PNAS PNAS

Discovery and prioritization of somatic mutations in diffuse large B-cell lymphoma (DLBCL) by whole-exome sequencing

Jens G. Lohr[a,b], Petar Stojanov[a,b], Michael S. Lawrence[a], Daniel Auclair[a], Bjoern Chapuy[b], Carrie Sougnez[a], Peter Cruz-Gordillo[a], Birgit Knoechel[a,b,c], Yan W. Asmann[d], Susan L. Slager[d], Anne J. Novak[d], Ahmet Dogan[d], Stephen M. Ansell[d], Brian K. Link[e], Lihua Zou[a], Joshua Gould[a], Gordon Saksena[a], Nicolas Stransky[a], Claudia Rangel-Escareño[f], Juan Carlos Fernandez-Lopez[f], Alfredo Hidalgo-Miranda[f], Jorge Melendez-Zajgla[f], Enrique Hernández-Lemus[f], Angela Schwarz-Cruz y Celis[f], Ivan Imaz-Rosshandler[f], Akinyemi I. Ojesina[a], Joonil Jung[a], Chandra S. Pedamallu[a], Eric S. Lander[a,g,h,1], Thomas M. Habermann[d], James R. Cerhan[d], Margaret A. Shipp[b], Gad Getz[a], and Todd R. Golub[a,b,g,i]

[a]Eli and Edythe Broad Institute, Cambridge, MA 02412; [b]Dana–Farber Cancer Institute, Boston, MA 02115; [d]Mayo Clinic College of Medicine, Rochester, MN 55902; [c]Children's Hospital Boston, Boston, MA 02115; [e]University of Iowa College of Medicine, Iowa City, IA 52245; [f]Instituto Nacional de Medicina Genómica, 14610 Mexico DF, Mexico; [g]Harvard Medical School, Boston, MA 02115; [h]Massachusetts Institute of Technology, Cambridge, MA 02142; and [i]Howard Hughes Medical Institute, Chevy Chase, MD 20815

Contributed by Eric S. Lander, December 29, 2011 (sent for review November 22, 2011)

To gain insight into the genomic basis of diffuse large B-cell lymphoma (DLBCL), we performed massively parallel whole-exome sequencing of 55 primary tumor samples from patients with DLBCL and matched normal tissue. We identified recurrent mutations in genes that are well known to be functionally relevant in DLBCL, including *MYD88*, *CARD11*, *EZH2*, and *CREBBP*. We also identified somatic mutations in genes for which a functional role in DLBCL has not been previously suspected. These genes include *MEF2B*, *MLL2*, *BTG1*, *GNA13*, *ACTB*, *P2RY8*, *PCLO*, and *TNFRSF14*. Further, we show that *BCL2* mutations commonly occur in patients with *BCL2/IgH* rearrangements as a result of somatic hypermutation normally somatic mutations in oncogenes and tumor-suppressor genes in B lymphocytes.

Traditionally, DLBCL has been classified by the morphology and immunophenotype of the malignant B-cells but more recently, molecular classifications have been reported. Specifically, gene expression-based classification of DLBCL has been proposed (5, 6), and the prognostic relevance for this has been demonstrated (7). It has been suggested that distinct signal transduction pathways are affected in the subtypes that are defined in this way, and that certain genetic defects preferentially occur in specific subtypes defined by the presumed cell of origin of the tumors (8–12).

PNAS 109 3879-3884 (2012)

Exome sequencing of DLBCL and Follicular Lymphoma (FL)

Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma

Ryan D. Morin[1]*, Maria Mendez-Lago[1]*, Andrew J. Mungall[1], Rodrigo Goya[1], Karen L. Mungall[1], Richard D. Corbett[1], Nathalie A. Johnson[2], Tesa M. Severson[1], Readman Chiu[1], Matthew Field[1], Shaun Jackman[1], Martin Krzywinski[1], David W. Scott[2], Diane L. Trinh[1], Jessica Tamura-Wells[1], Sa Li[1], Marlo R. Firme[1], Sanja Rogic[2], Malachi Griffith[1], Susanna Chan[1], Oleksandr Yakovenko[1], Irmtraud M. Meyer[3], Eric Y. Zhao[1], Duane Smailus[1], Michelle Moksa[1], Suganthi Chittaranjan[1], Lisa Rimsza[4], Angela Brooks-Wilson[1,5], John J. Spinelli[6,7], Susana Ben-Neriah[2], Barbara Meissner[2], Bruce Woolcock[2], Merrill Boyle[2], Helen McDonald[1], Angela Tam[1], Yongjun Zhao[1], Allen Delaney[1], Thomas Zeng[1], Kane Tse[1], Yaron Butterfield[1], Inanç Birol[1], Rob Holt[1], Jacqueline Schein[1], Douglas E. Horsman[2], Richard Moore[1], Steven J. M. Jones[1], Joseph M. Connors[2], Martin Hirst[1], Randy D. Gascoyne[2,8] & Marco A. Marra[1,9]

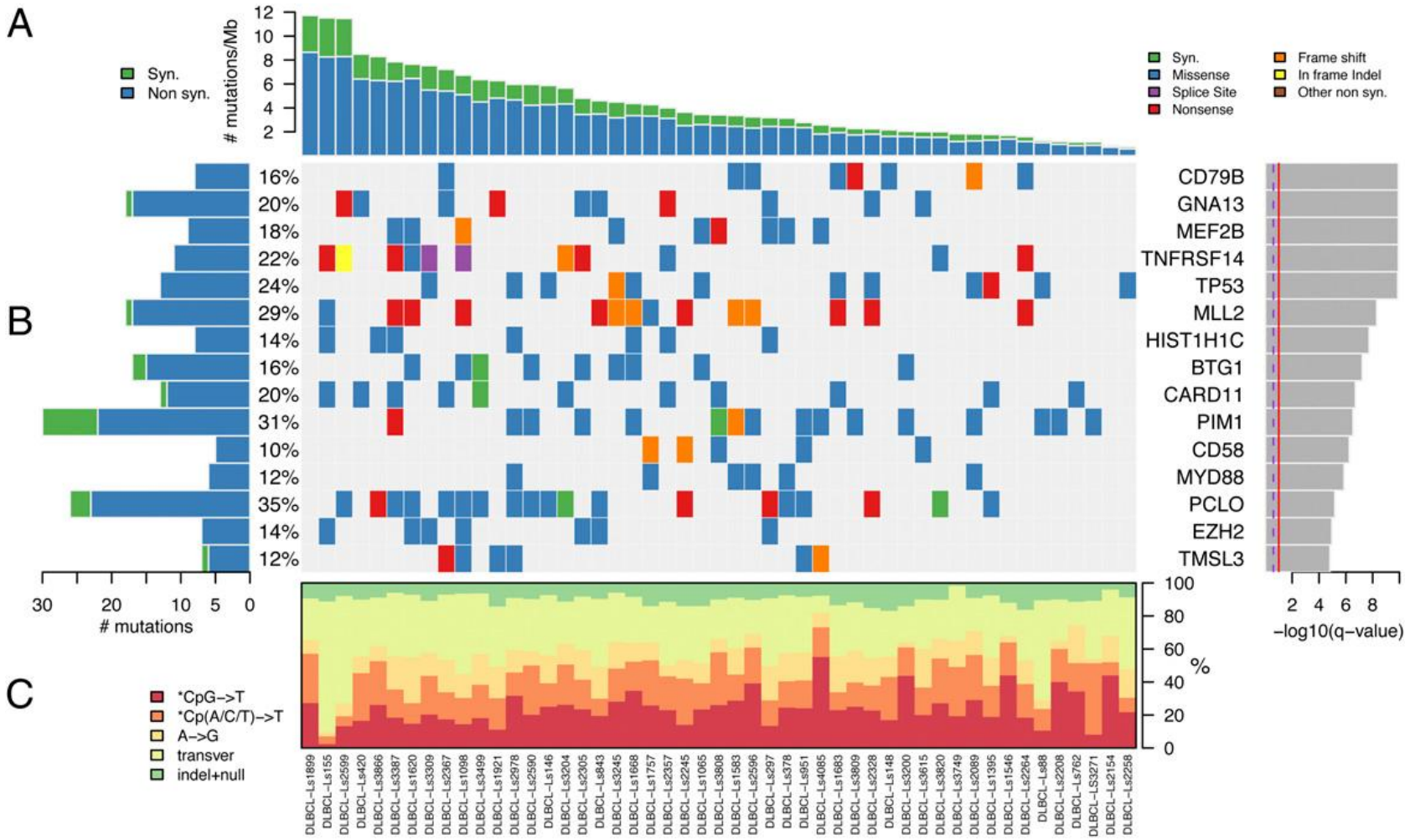
Follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL) are the two most common non-Hodgkin lymphomas (NHLs). Here we sequenced tumour and matched normal DNA from 13 DLBCL cases and one FL case to identify genes with mutations in B-cell NHL. We analysed RNA-seq data from these and another 113 NHLs to identify genes with candidate mutations, and then re-sequenced tumour and matched normal DNA from these cases to confirm 109 genes with multiple somatic mutations. Genes with roles in histone modification were frequent targets of somatic mutation. For example, 32% of DLBCL and 89% of FL cases had somatic mutations in *MLL2*, which encodes a histone methyltransferase, and 11.4% and 13.4% of DLBCL and FL cases, respectively, had mutations in *MEF2B*, a calcium-regulated gene that cooperates with CREBBP and EP300 in acetylating histones. Our analysis suggests a previously unappreciated disruption of chromatin biology in lymphomagenesis.

Nature 476 298-303 Aug 2011

DLBCL Exome Sequencing Details

- *Lohr et al.*
  - 55 patients paired tumor and germline(normal) DNA
  - Captured with Agilent's exome capture
  - 150-fold mean sequence coverage of targeted exons
- *Morin et al.*
  - 117 patients FL and DLBCL tumor DNA
  - Captured with Agilent's exome capture

DLBCL mutation frequency



PNAS 109 3879-3884 (2012)

Agreement between two studies

- Common Pathways and Genes Identified in Both Studies
  - Known contributing genes: *CD79B, TP53, CARD11, MYD88, EZH2*
  - Suggested Pathogenic role: *MLL2, TNFRSF14, BTG1, MEF2B, GNA13*
  - Selected Common Novel findings: *HIST1H1C, PIM1*
  - Selected Novel Findings (*Lohr et al*): *PCLO, TMSL3,*
  - Selected Novel Findings (DLBCL, *Morin et al*): *SGK1, BCL2*

Frequent nonsynonymous mutations in chromatin remodeling genes, suggesting potential cooperation in lymphomagenesis

-HDAC inhibitors under investigation (phaseI/II)

Nature 476 298-303 Aug 2011

PNAS 109 3879-3884 (2012)

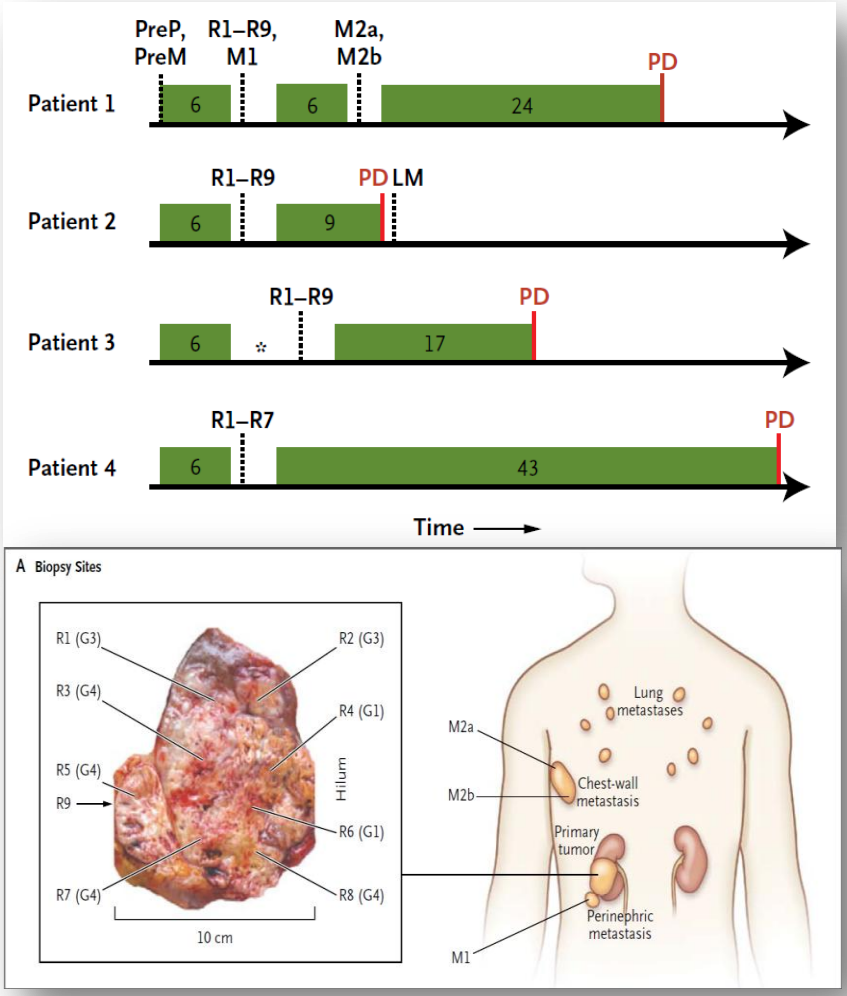
Conclusions

- High Coverage of exonic content in two large cohorts of DLBCL
- Confirmed mutations in known and novel sites
- Excellent agreement between studies
- Spurred new efforts to understand potential previously unknown drivers
- Progress toward new therapeutic avenues and potentially increased survival rates

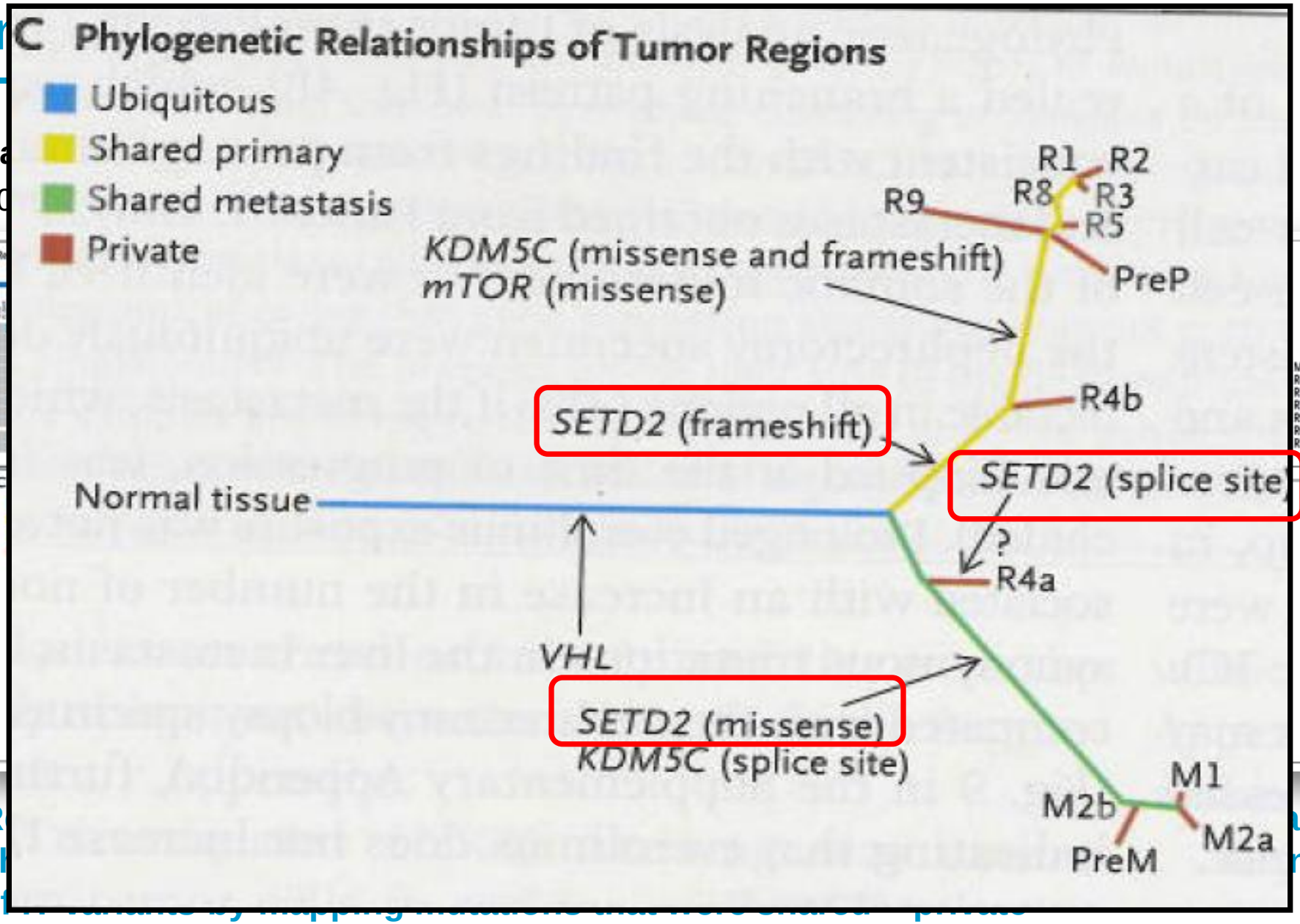
Discovery Using Target Enrichment:
Renal Cell Carcinoma

Aim:
To characterize *intra*-tumor heterogeneity that may foster tumor evolution/adaptation

Method:
SureSelect Exome sequencing of:
- 4 patient samples, 7-15 biopsies
- Pre-treatment (Primary vs. Metastasis)
- Intratumor sections (R1-R9)
- Multiple metastases



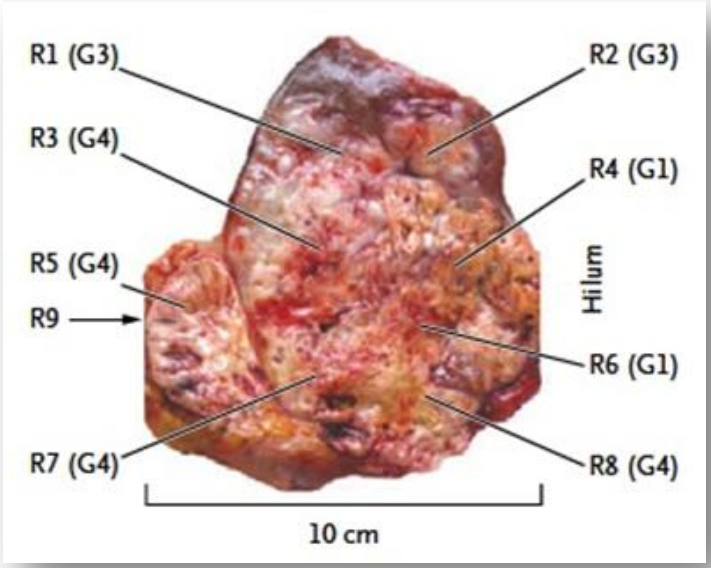
Gerlinger et.al. N Eng J Med 2012 366 (883-892)


C Phylogenetic Relationships of Tumor Regions
Ubiquitous
Shared primary
Shared metastasis
Private
R1
R2
R8
R3
R9
R5
PreP
KDM5C (missense and frameshift)
mTOR (missense)
R4b
SETD2 (frameshift)
SETD2 (splice site)
Normal tissue
?
R4a
VHL
SETD2 (missense)
KDM5C (splice site)
M1
M2b
M2a
PreM

Discovery Using Target Enrichment:
Renal Cell Carcinoma

Results/Conclusions:

- Identified driver mutations (*VHL, KDM5C, SETD2, MTOR, PTEN*)
- Only *VHL* was mutated ubiquitously in all analyzed regions
- Different *regions* of the tumor have different mutations in the very *same driver* gene, e.g. *MTOR, SETD2*
- Intra-tumor heterogeneity may contribute to failure of targeted chemotherapy
- Suggests potential need for multiple assessments of tumor



Summary

- SureSelect exome target enrichment
  - Optimized probe design/production for improved capture
  - High coverage of exons with only 4Gb of sequencing
  - Samples ready for sequencing the next day

- Enables large cancer sequencing studies
  - Allows in-depth determination of nucleotide variations
  - Driving discovery of new potential targets and therapies
  - Increasing understanding of tumor evolution

Thank You!