Beyond Basic Target Enrichment: New Tools to Fuel Your NGS Research

Sequencing, Finishing and Analysis in the Future 2012

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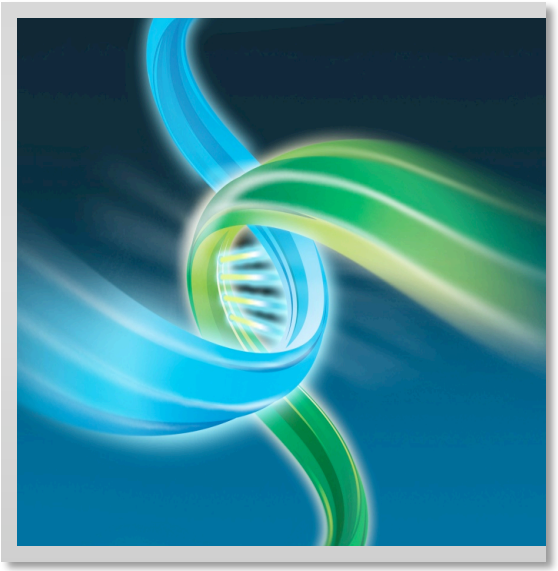
Agenda

1. New tool for DNA methylation
2. Deep RNA sequencing
3. Fast strategy with benefits



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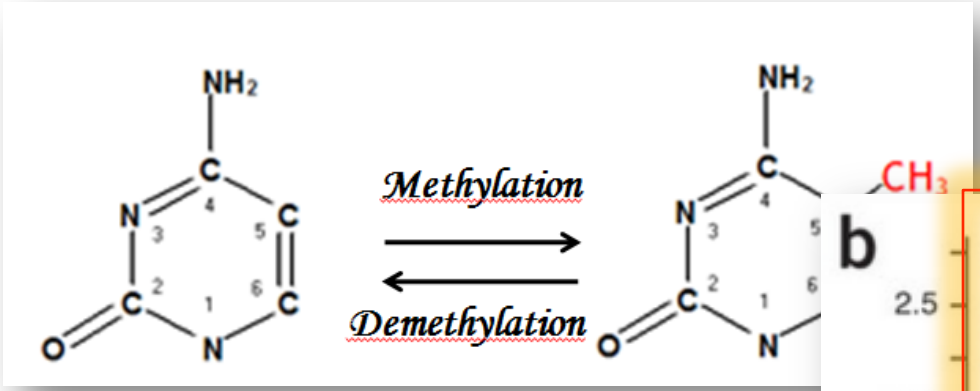
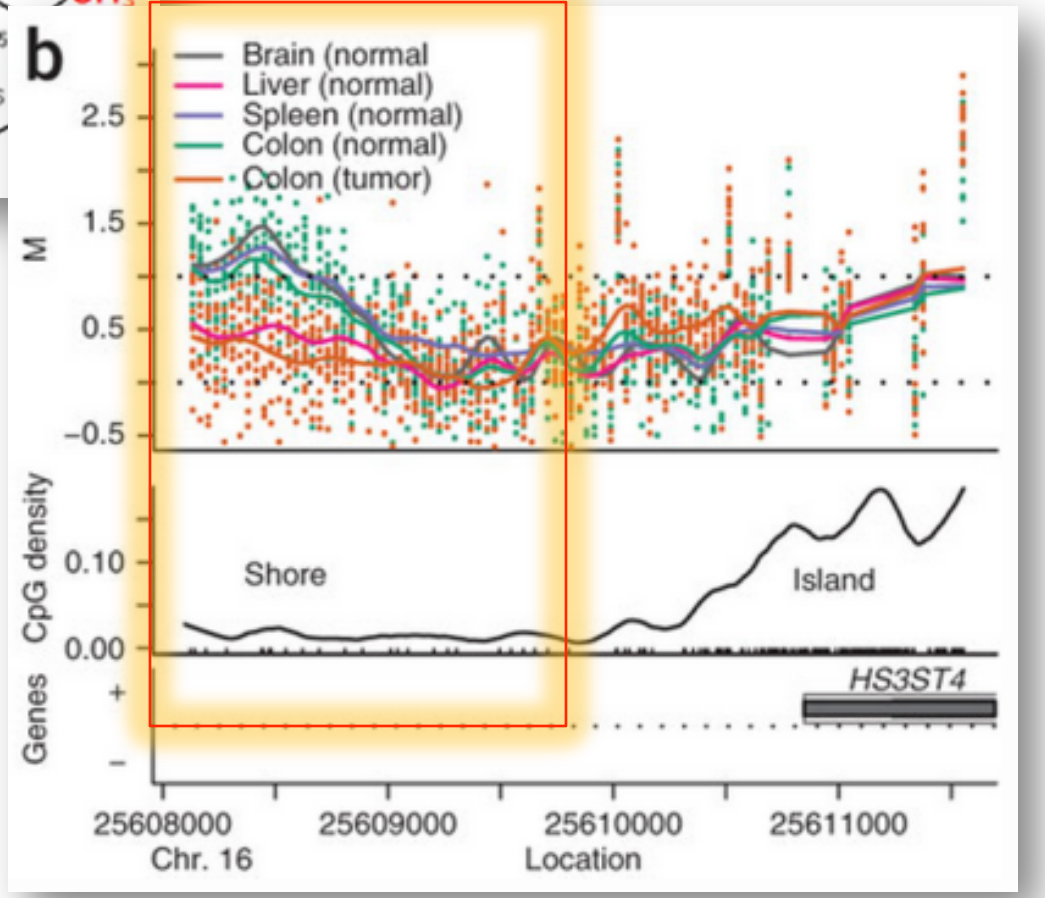
Differentially Methylated Regions (DMR)



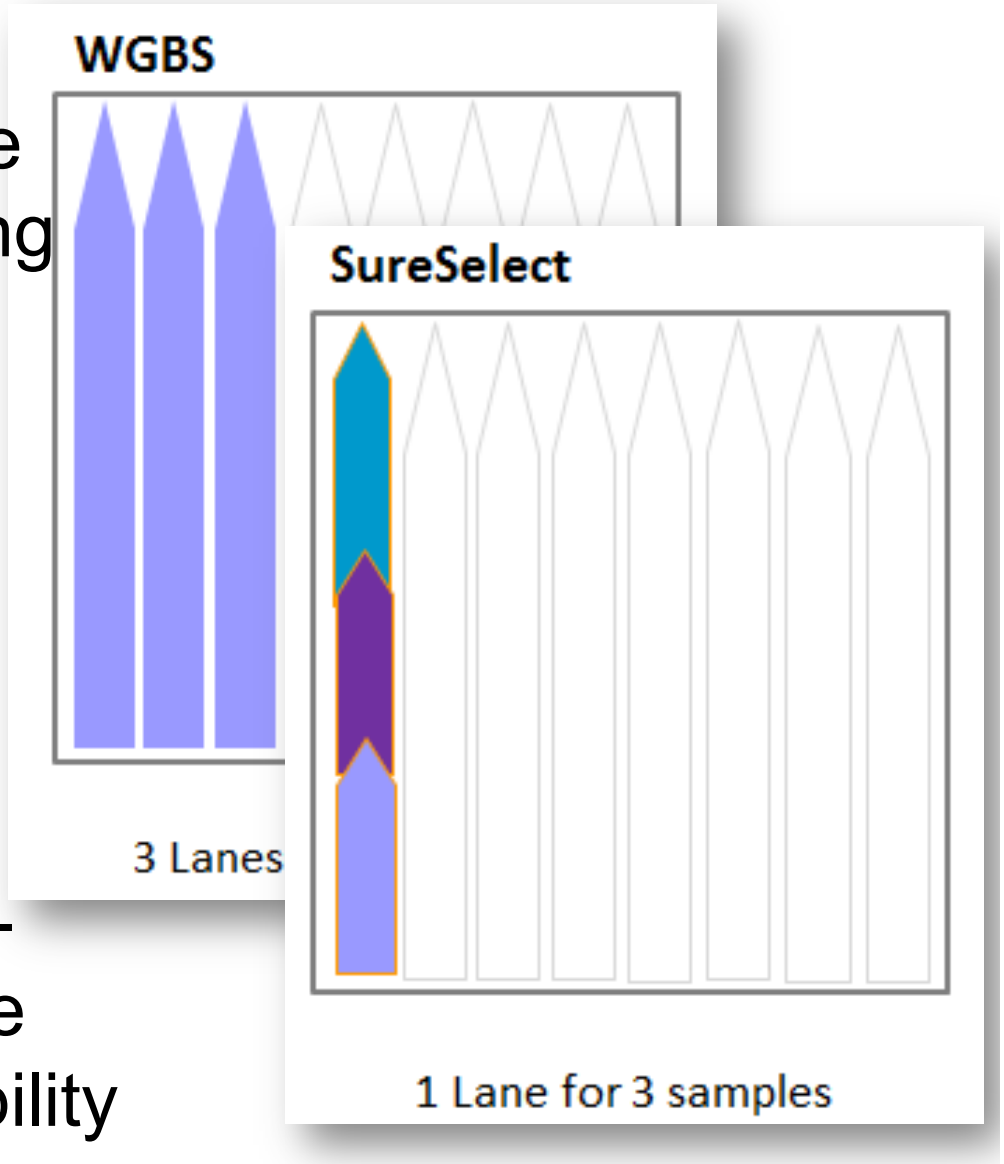
- CpG islands
- CpG island **shores** (~2kb away from islands)
- CpG island **shelves** (~4kb away from islands)
- CpG <u>shore</u> methylation is strongly related to gene expression



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

Whole Genome Bisulfite Sequencing (WGBS)

- Whole Genome Coverage
- Costly and time consuming
- Requires extensive bioinformatics
- Limited scalability per run



Target Enrichment:

- reduce costs/sample
- maintain similar whole-genome CpG coverage
- Have increased scalability

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SureSelect^{XT} Human Methyl-Seq

Discovery Tool

- Not methylation-state dependent
- No prior knowledge needed

Comprehensive design

- Not limited to CpG Islands.
- Comprehensive Content
- CpG Islands, Promoters and DMRs

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

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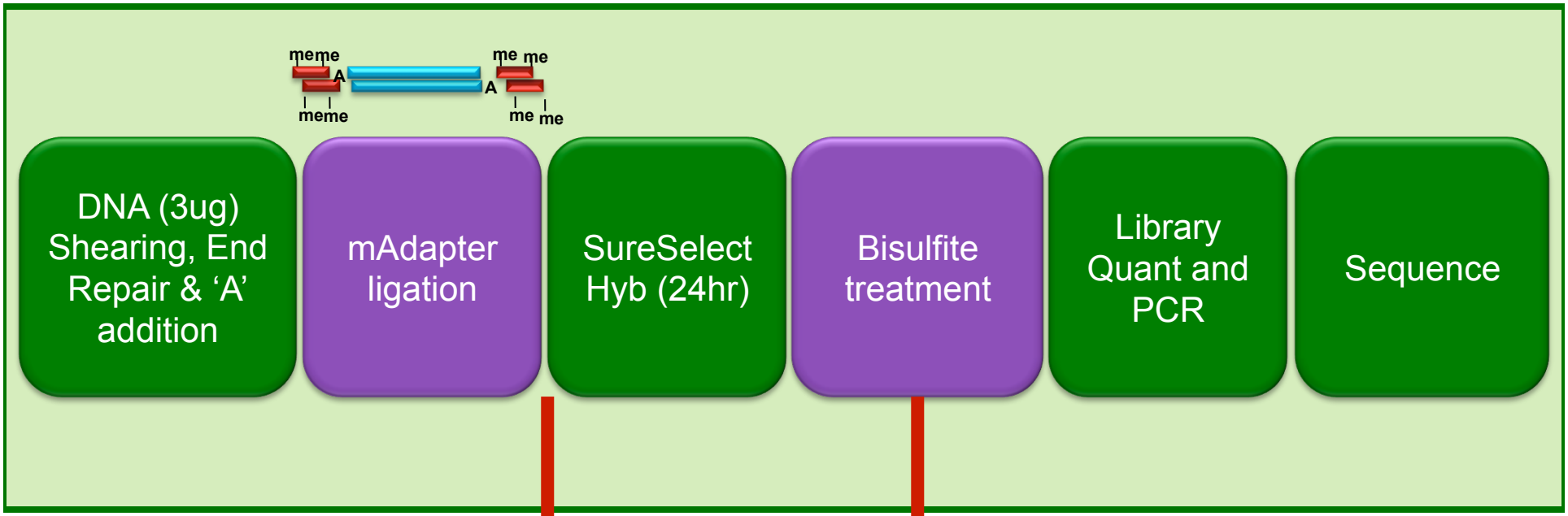
SureSelect Methyl-Seq Protocol



No PCR before Bisulfite treatment to preserve the Methylation state

Bisulfite treatment is performed after hybridization to maximize sample complexity

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Methyl-Seq Comparison with published WGBS data

Vol 462|19 November 2009|doi:10.1038/nature08514 nature

Human DNA methylomes at base resolution show widespread epigenomic differences

Ryan Lister[1]*, Mattia Pelizzola[1]*, Robert H. Dowen[1], R. David Hawkins[2], Gary Hon[2], Julian Tonti-Filippini[4], Joseph R. Nery[1], Leonard Lee[2], Zhen Ye[2], Que-Minh Ngo[2], Lee Edsall[2], Jessica Antosiewicz-Bourget[5,6], Ron Stewart[5,6], Victor Ruotti[5,6], A. Harvey Millar[4], James A. Thomson[5,6,7,8], Bing Ren[2,3] & Joseph R. Ecker[1]

Cell line: IMR90 (female lung fibroblast)

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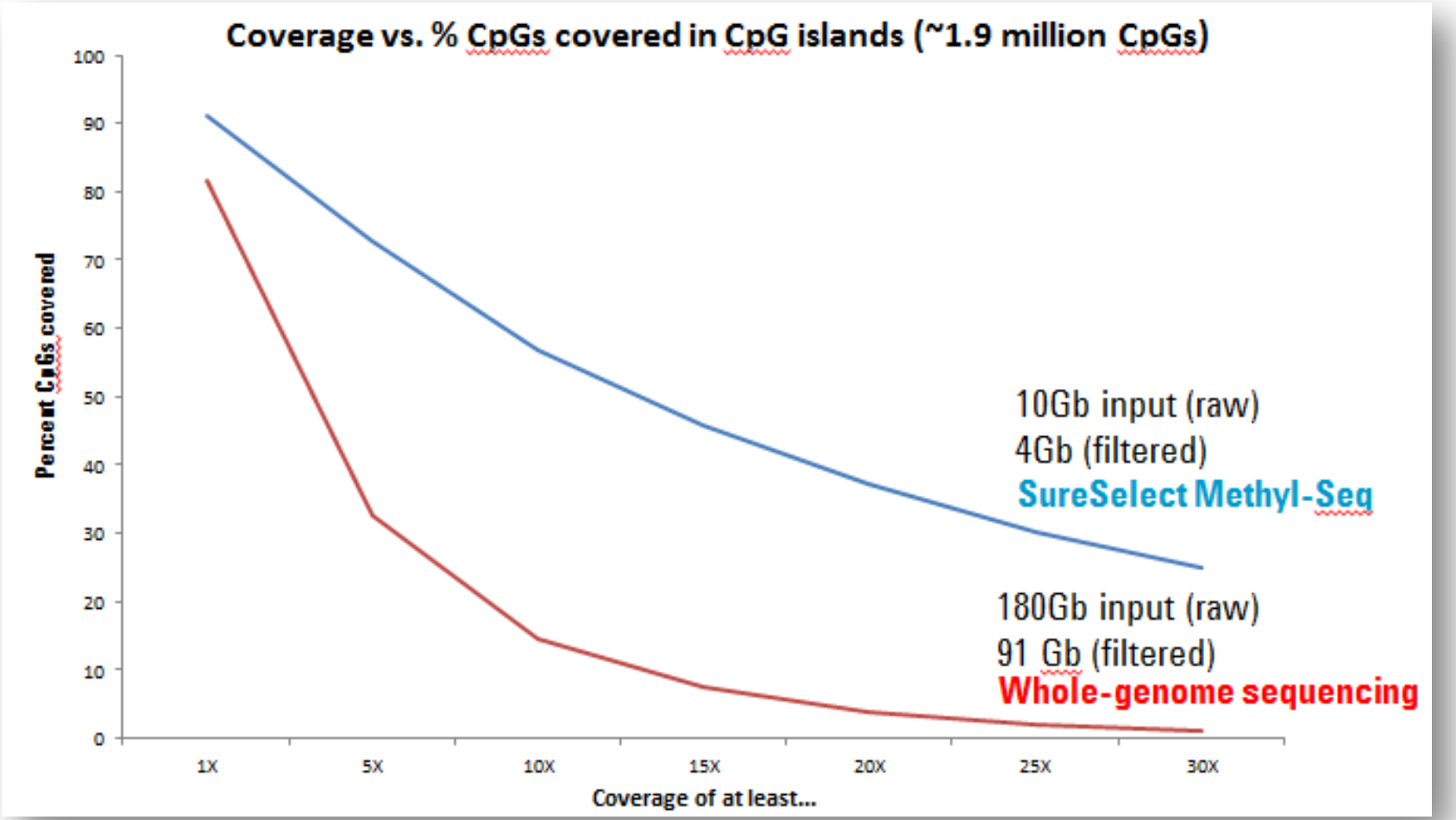
SureSelect vs. Whole-genome bisulfite sequencing



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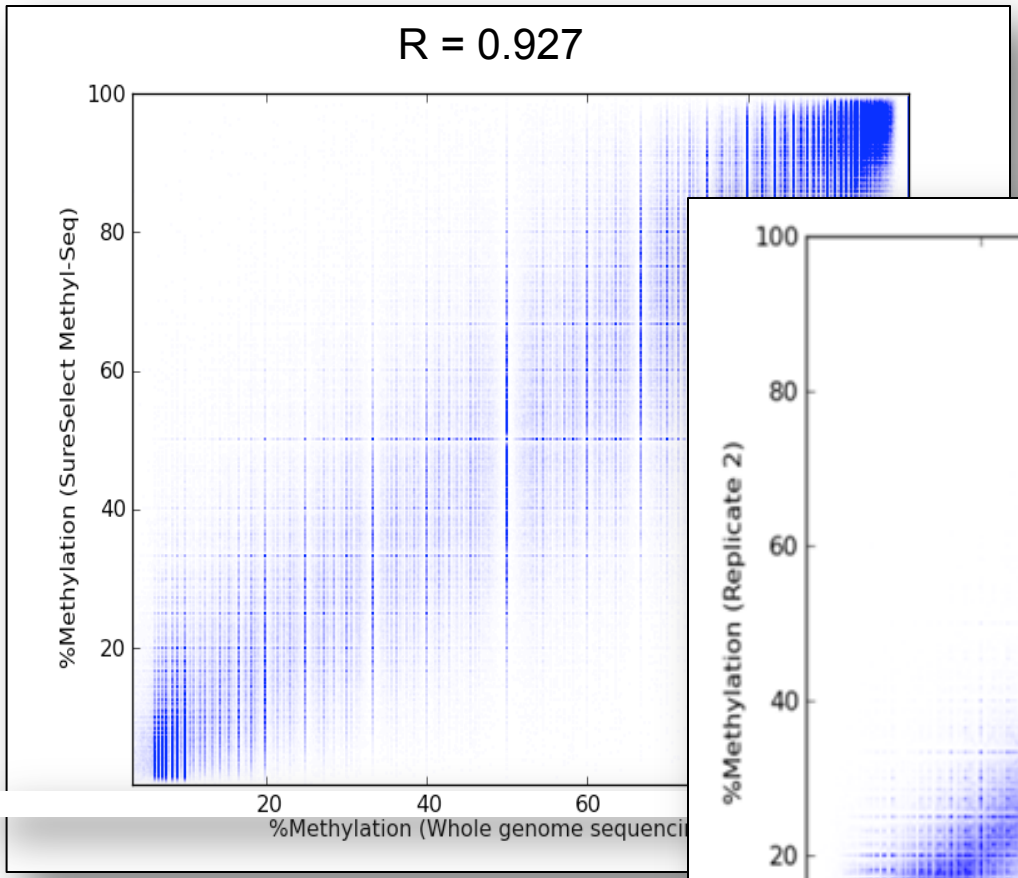
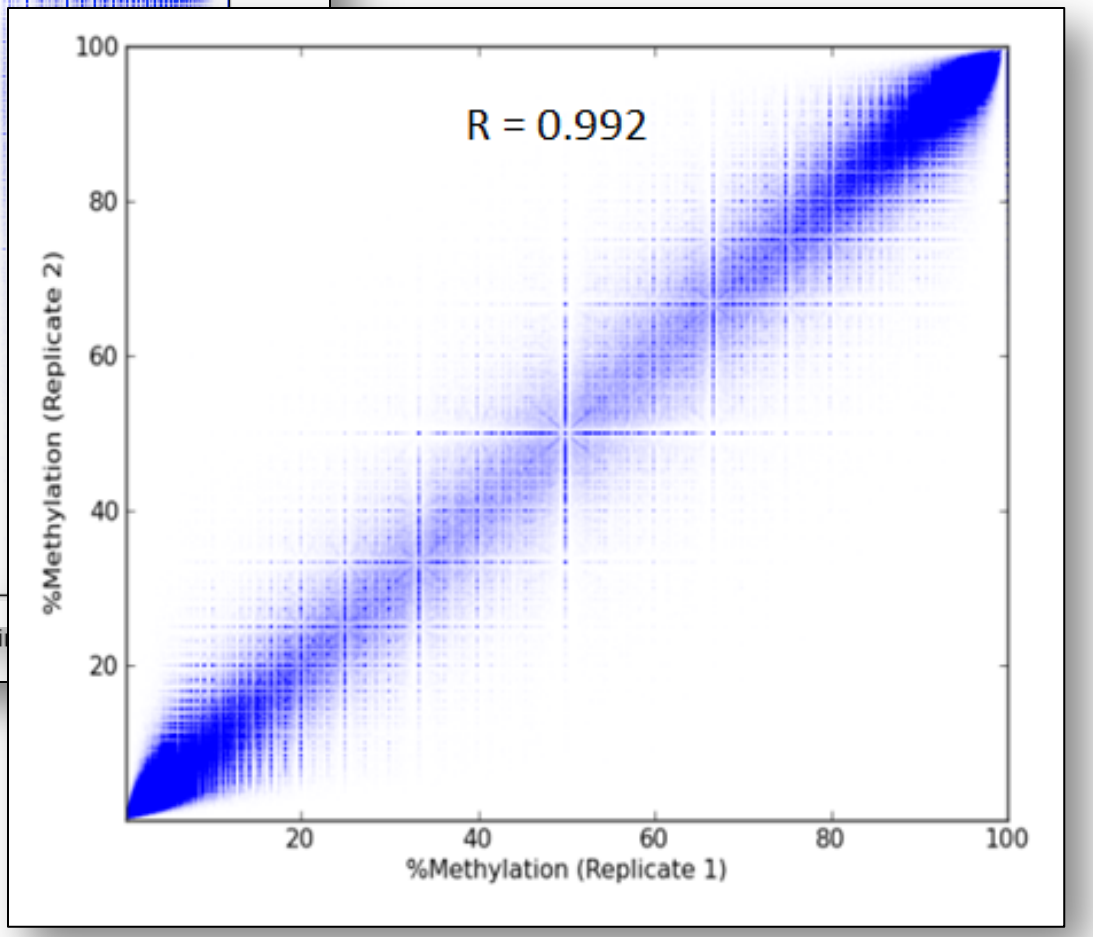
Concordance with WGBS data



Reproducibility



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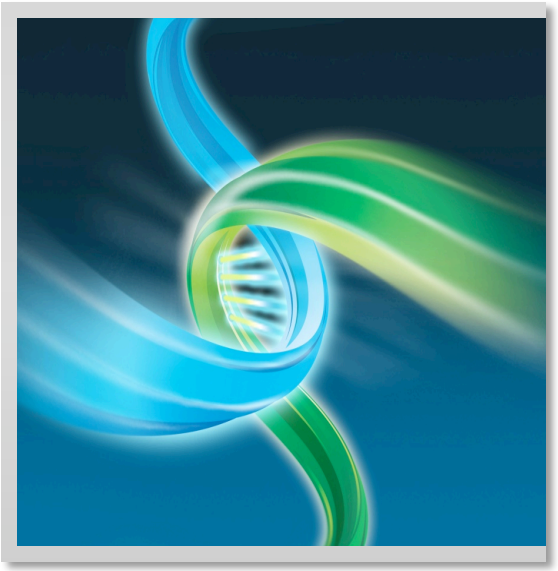

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RNA-Seq Transcriptome Target Enrichment in Cancer

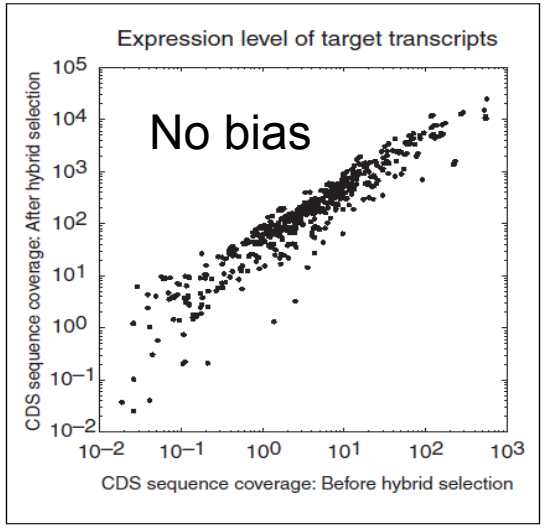
SureSelect RNA

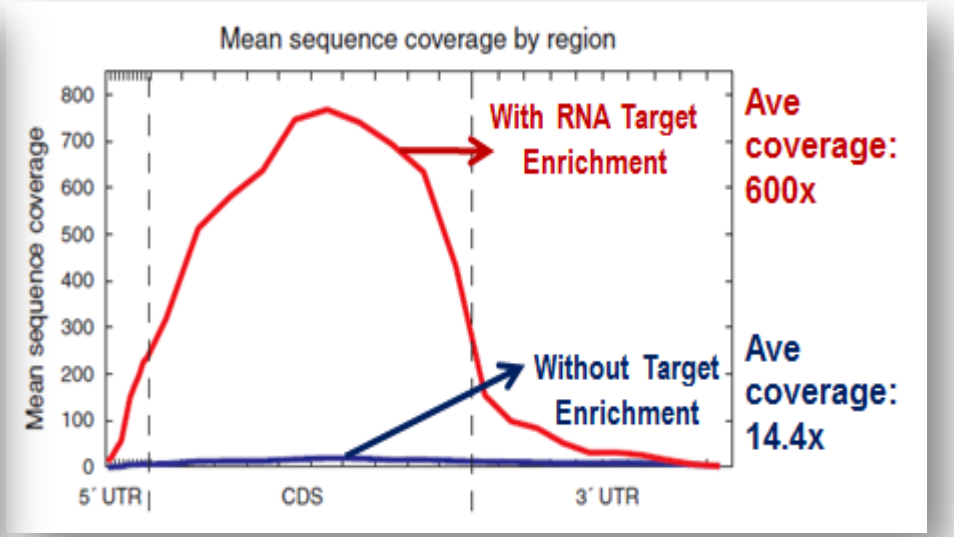
Targeted next-generation sequencing of a cancer transcriptome enhances detection of sequence variants and novel fusion transcripts

Joshua Z Levin*, Michael F Berger†, Xian Adiconis*, Peter Rogov*, Alexandre Melnikov*, Timothy Fennell‡, Chad Nusbaum*, Levi A Garraway†§ and Andreas Gnirke*

Genome Biology 2009, 10:R115

- **Aim:** RNA Target Enrichment of 467 Cancer Genes (~all Tyr Kinases + Genes from Cancer Gene Census); Overall >800 target transcripts
- **Method:** Enrich cDNA from K-562 CML cell line cDNA libraries and compare results from before and after target enrichment





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Benefits of RNA-Seq Target Enrichment in Cancer

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Feature	Without RNA Target Enrichment	With RNA Target Enrichment
Avg. Coverage	14.4x	606x
Fold-Enrichment	N/A	42x
Regions with 20x coverage	13%	63%
Common SNPs	76	257
Novel Variants	4	16
Splice-Junctions	2958	4720
Alternatively spliced-genes	52	177
Gene Fusions	2	6

42X greater coverage

More sensitive variant detection

- Increased coverage improves ability to find rare transcripts and novel fusions
- Whole transcriptome analysis requires >40x more sequencing to achieve same depth for targeted regions

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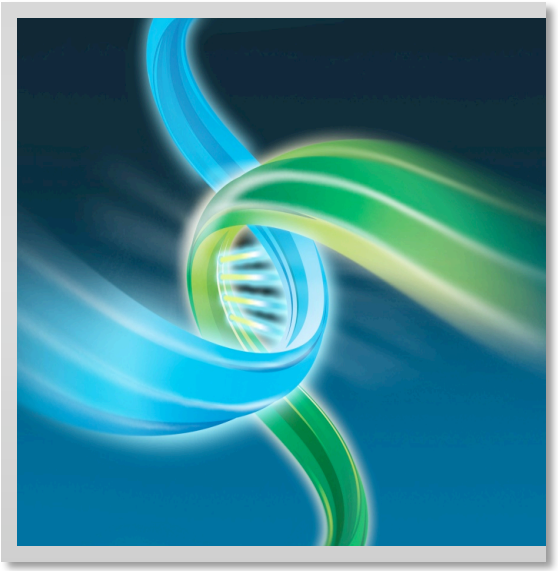

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HaloPlex – Simplicity of Next Generation PCR

HaloPlex

SIMPLE WORKFLOW
PREMIUM PERFORMANCE

- ✓ ***Low input (200ng) DNA***
- ✓ ***Library-prep free***
- ✓ ***Less than 6 hours***
- ✓ **96 indexed samples for throughput**
- ✓ Simple Design Wizard
- ✓ Single tube prep
- ✓ Any Desktop Sequencer
- ✓ **Any Targets up to 5Mb**

New!

New!

Compared to SS:

- **Faster to results due to no library prep and short**
- **Small panels**
- **Interrogates repeats**
- **Human only**

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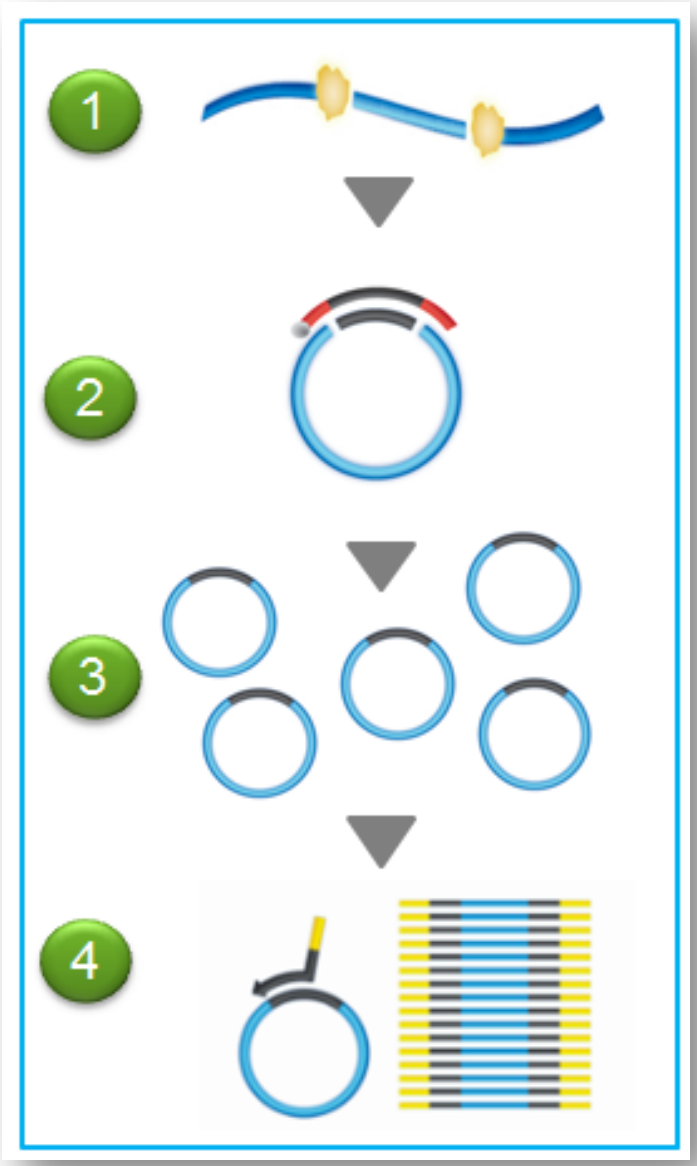
HaloPlex: 4-step protocol

HaloPlex

1. Digest DNA
2. Hybridize Probes
3. Purify / Ligate
4. Amplify



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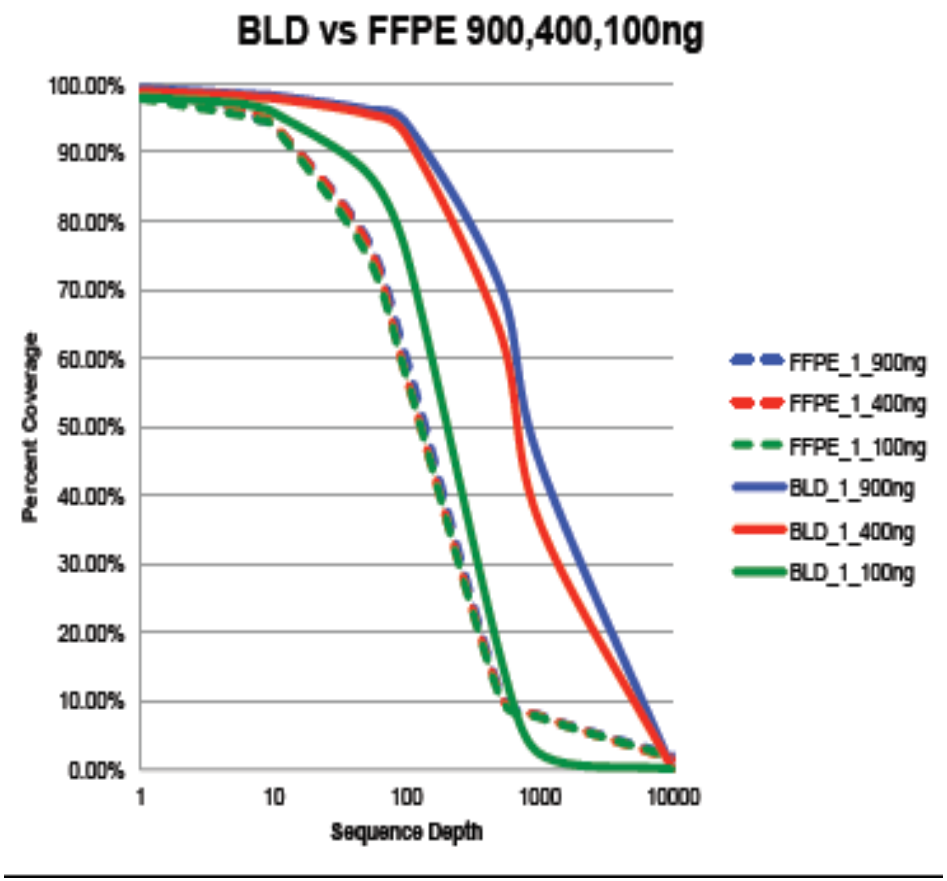
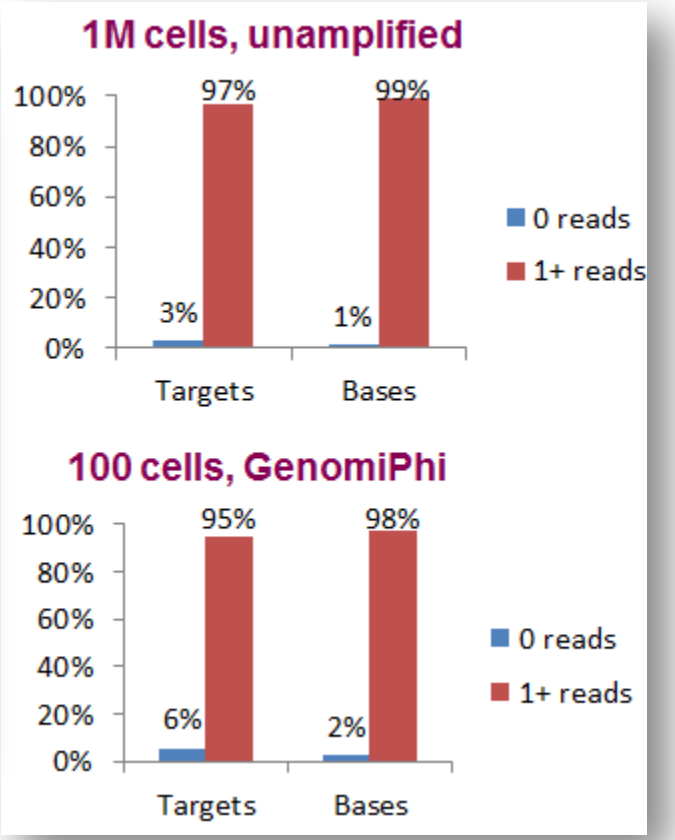
HaloPlex with FFPE Samples and WGA Samples

HaloPlex





Cancer Panel developed by OICR
19 Genes, ~60Kb size

Cancer Panel developed at Karolinska Institutet 78 Genes, ~197 Kb size

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Complete NGS Workflow

Library Prep | **Target Enrichment** | **Sequencing** | **Data Analysis**

SureSelect Kits

SureSelect Kits

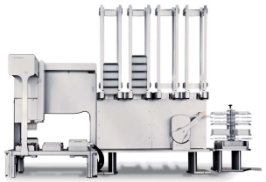
HaloPlex Kits

QC: Bioanalyzer/ TapeStation

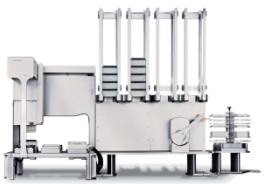
QC: Bioanalyzer/ TapeStation

PCR: Surecycler 8800
qPCR: Mx3005P

PCR: Surecycler 8800
qPCR: Mx3005P



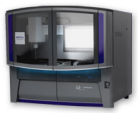
Agilent NGS Automation



Agilent NGS Automation

Compatible with:

Illumina GAIIx/HiSeq

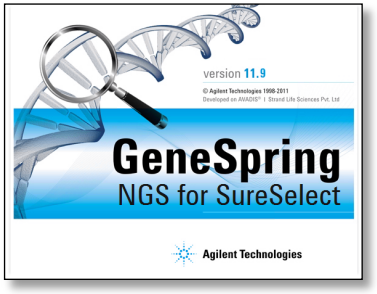


SOLiD/5500

Roche 454/GS Junior

Ion Torrent PGM/MiSeq

Pacific Biosciences



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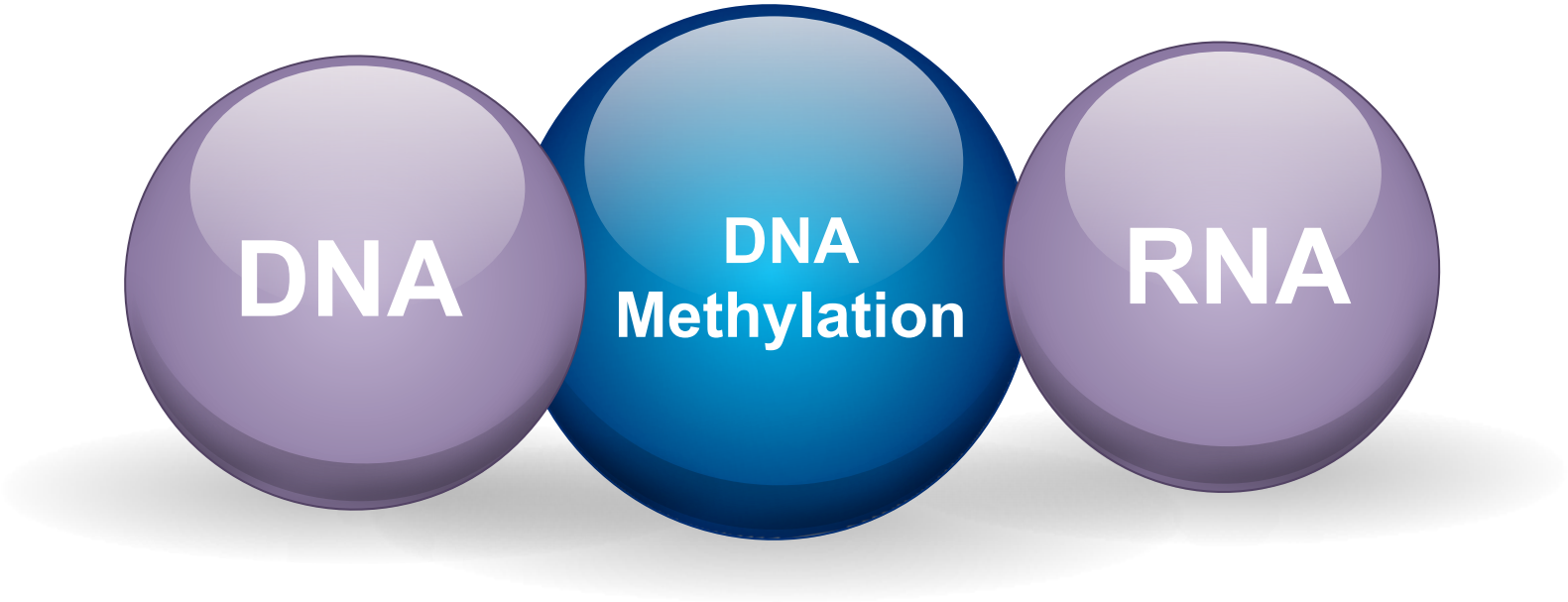
SureSelect: Complete "Omics" Solution

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



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Thank you.
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