



# Patch PCR: A targeted sequencing approach to quantify breast cancer ctDNA



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University of Utah





# Disclosure

The technology and results I will describe are part of collaborative project with Kailos Genetics, Inc.

I am an inventor of the technology, and a founder and scientific advisor for Kailos Genetics, Inc. which entails personal financial interests.

In compliance with the University of Utah Individual Conflict of Interest Committee, non-conflicted members of the research team collect and analyze all data for this research.



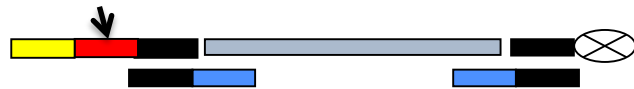
# Patch PCR

Restriction Digest  
Genomic DNA



Specifically Anneal Patch  
Oligos and Universal  
Adapters to Ends of  
Targeted Fragments

Unique Molecular Identifier



Left Patch Oligo

Right Patch Oligo

Exonuclease  
Resistant 3'  
Modification

Thermostable Ligase Links  
Universal Adapters to  
Targeted Fragments



Degrade Unselected DNA  
and Excess Adapters with  
Exonuclease



Universal PCR with Patient  
Specific DNA Barcodes and  
illumina primers

Illumina  
primer



Patient Specific  
Barcode

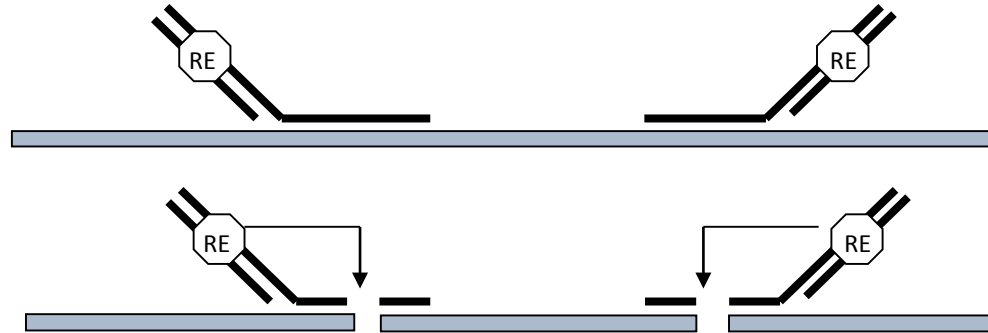
Illumina  
primer





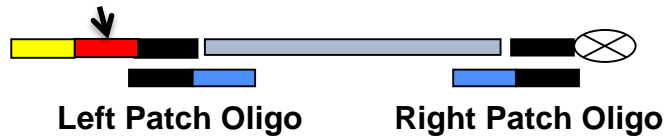
# Patch PCR

Oligo-directed digest of target loci with Type II restriction enzyme



Specifically Anneal Patch Oligos and Universal Adapters to Ends of Targeted Fragments

Unique Molecular Identifier



Exonuclease Resistant 3' Modification

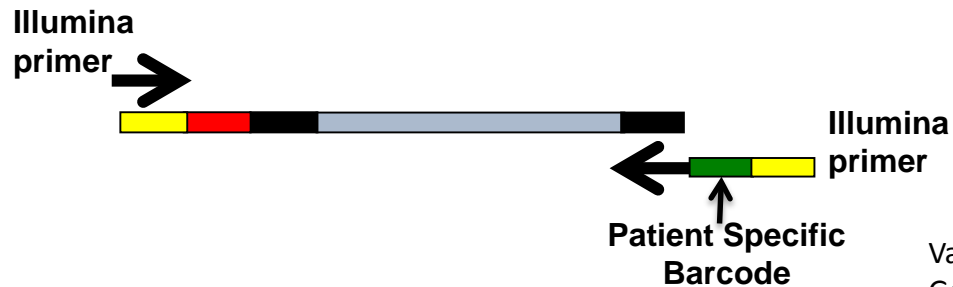
Thermostable Ligase Links Universal Adapters to Targeted Fragments



Degrade Unselected DNA and Excess Adapters with Exonuclease



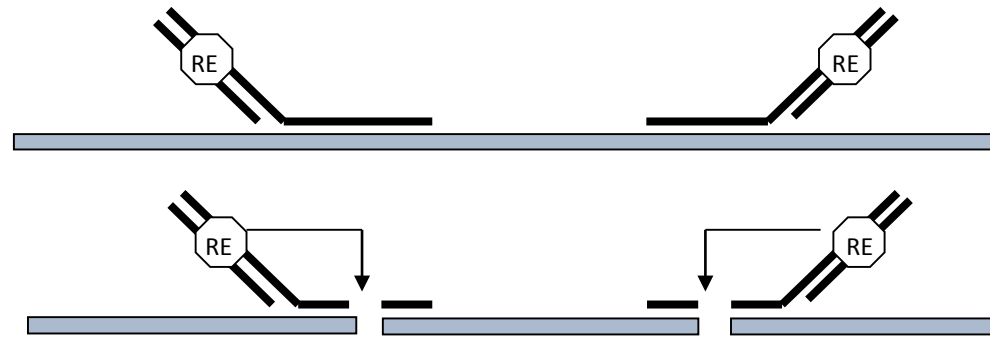
Universal PCR with Patient Specific DNA Barcodes and illumina primers





# Patch PCR

Oligo-directed digest of target loci with Type II restriction enzyme



High Specificity

Specifically Anneal Patch Oligos and Universal Adapters to Ends of Targeted Fragments

Unique Molecular Identifier



Exonuclease Resistant 3' Modification

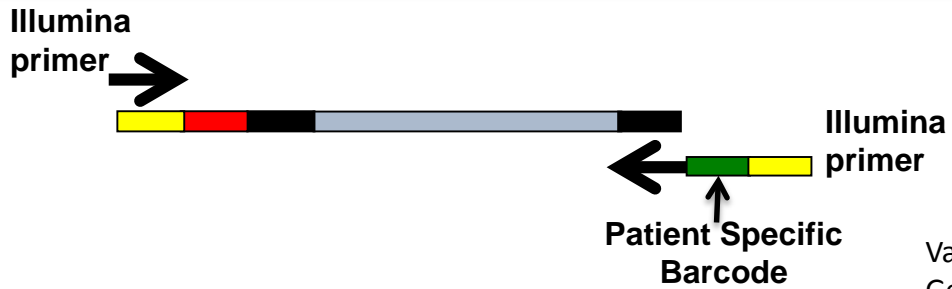
Thermostable Ligase Links Universal Adapters to Targeted Fragments



Degrade Unselected DNA and Excess Adapters with Exonuclease



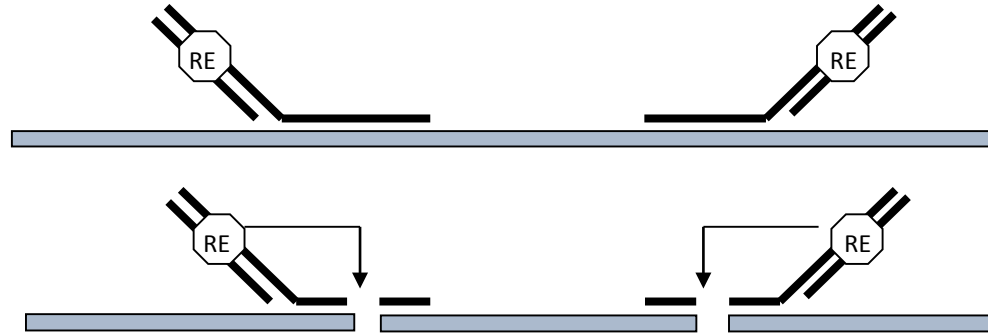
Universal PCR with Patient Specific DNA Barcodes and illumina primers





# Patch PCR

Oligo-directed digest of target loci with Type II restriction enzyme



Specifically Anneal Patch Oligos and Universal Adapters to Ends of Targeted Fragments

Unique Molecular Identifier



Left Patch Oligo

Right Patch Oligo

Thermocycling of ligation increases the number of target molecules captured (High Sensitivity)

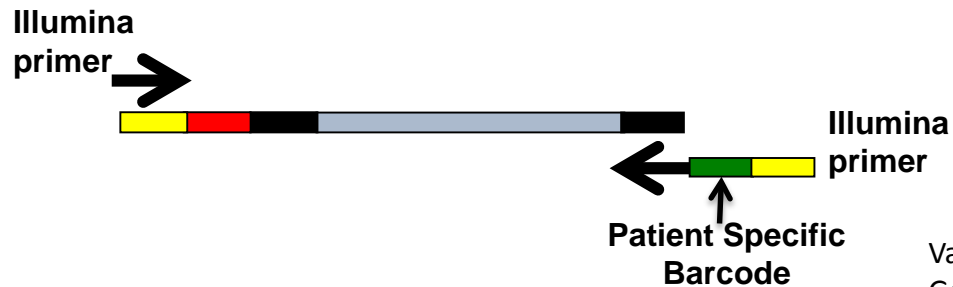
Thermostable Ligase Links Universal Adapters to Targeted Fragments



Degrade Unselected DNA and Excess Adapters with Exonuclease

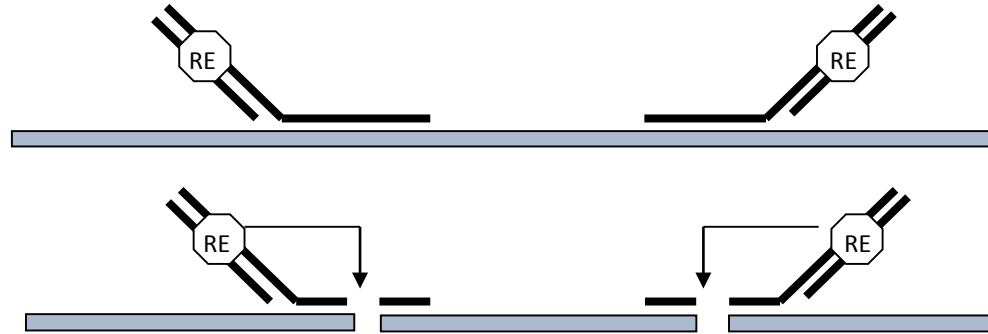
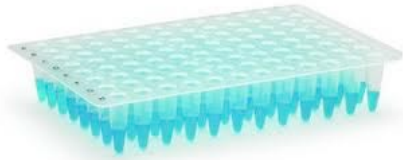


Universal PCR with Patient Specific DNA Barcodes and illumina primers





# Patch PCR



Unique Molecular Identifier



Left Patch Oligo

Right Patch Oligo



Illumina primer



Illumina primer



Patient Specific Barcode

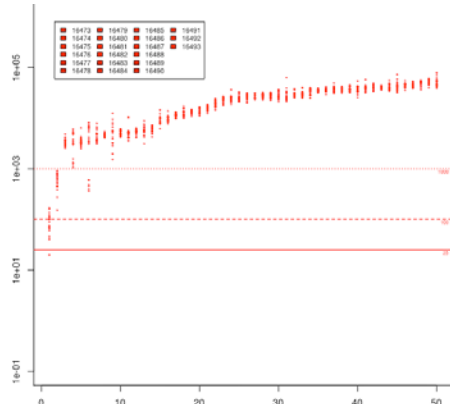


Clinical  
Laboratory  
Improvement  
Amendments

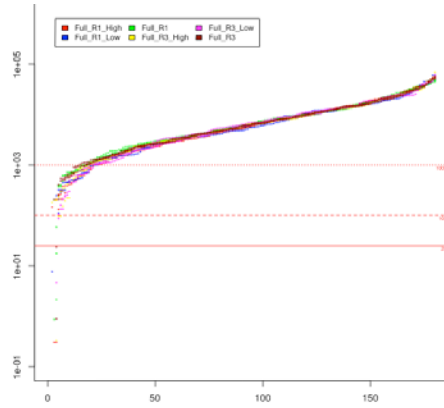


# Patch PCR

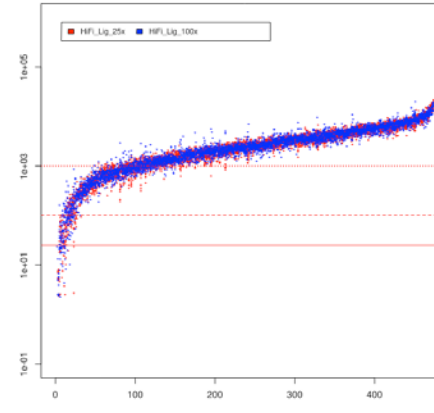
Ideal for targeted clinical-scale panels (10-1000 amplicons)



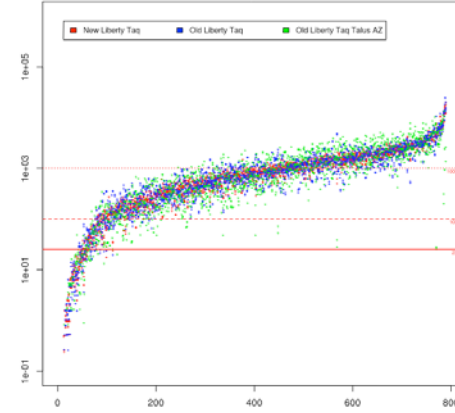
BRCA1 & BRCA2  
50 Amplicons



Pharmacogenetics  
180 Amplicons



Germline Cancer Risk  
474 Amplicons



GWAS Follow-up  
789 Amplicons

## High Specificity (On-Target):

99.2%

98.3%

99.6%

99.1%

## Affordable:

Standard synthesis oligos: \$15 per amplicon, enough for ~1,000 samples

Reagent cost: < \$50 per sample

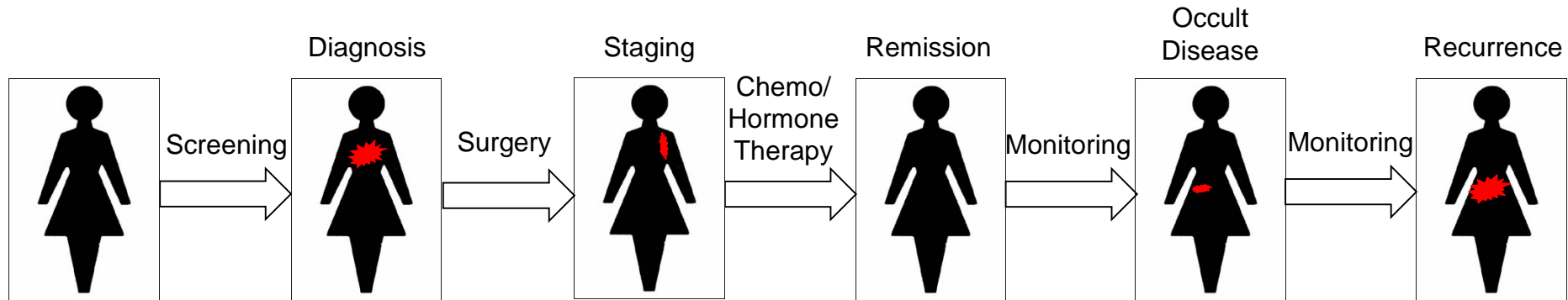
Sequencing cost: 20-48 samples per Miseq run (\$50 per sample), 200-300 samples per NextSeq Run







# Breast cancer ctDNA



39 million  
mammograms in  
the US per year  
(FDA MQSA National  
Statistics)

246,660  
New cases of  
invasive breast  
cancer in the US  
per year  
(NCI SEER)

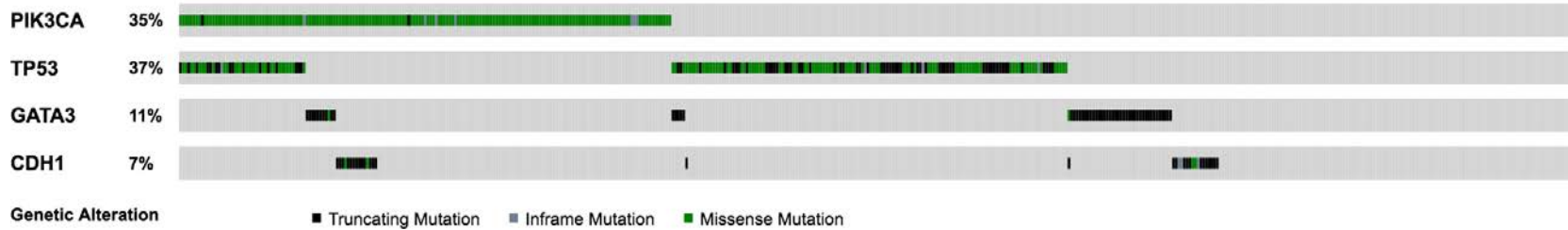
~50,000  
women per  
year develop  
hormone  
therapy  
resistance

~1.5 million  
women undergo  
monitoring for  
breast cancer  
recurrence in the  
US every year

~86,000  
women per  
year develop  
disease  
recurrence  
per year

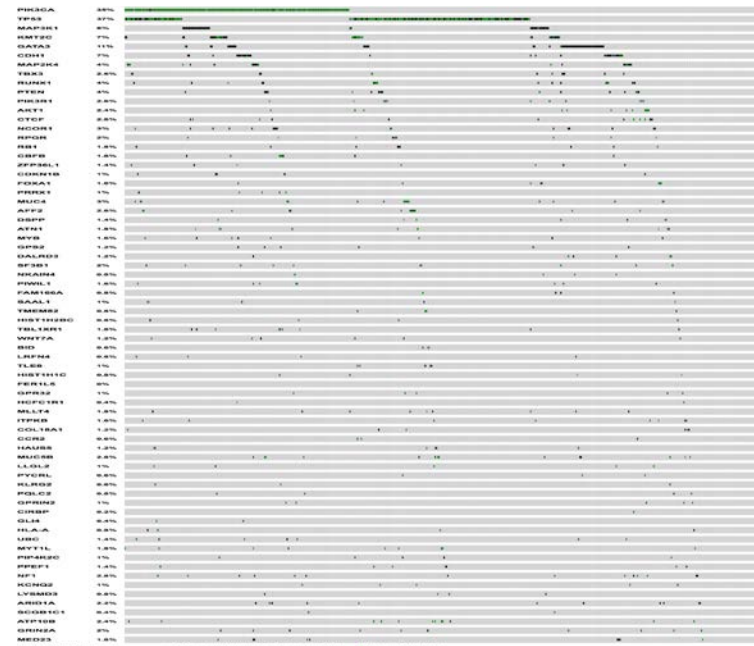
# Common breast cancer mutations

Four genes are mutated in 75% (378/507) of patients TCGA Nature 2012, cbioportal.org

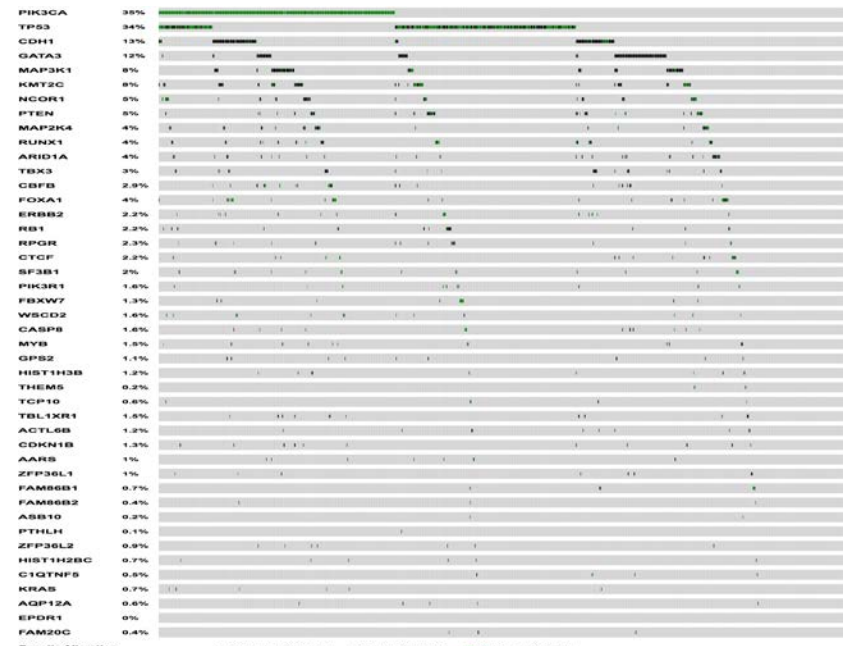


Increasing the panel size to include all significantly mutated genes yields diminishing returns.

TCGA Invasive Ductal Breast Cancer, Nature 2012  
All Significantly Mutated Genes  
Altered in 461 (91%) of 507 cases/patients

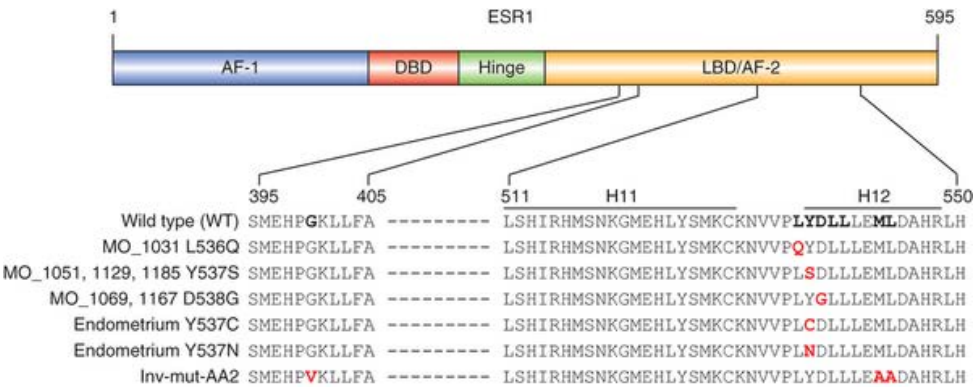


TCGA Invasive Lobular Breast Cancer, Cell 2015  
All Significantly Mutated Genes  
Altered in 716 (88%) of 817 cases/patients

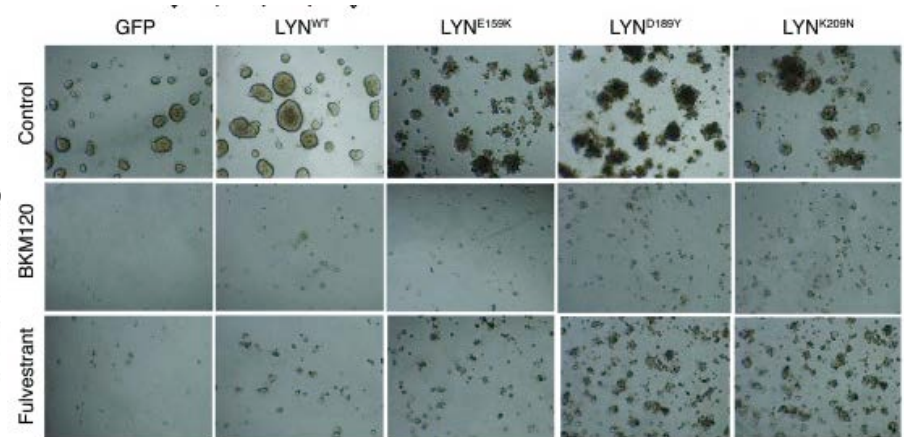


# Mutations that confer hormone therapy resistance

## Hotspot Mutations in the Estrogen Receptor (ESR1) Ligand Binding Domain



## Hotspot Mutations in the LYN inhibitory SH2 domain (SRC family kinase)



J Clin Invest. 2014 Dec;124(12):5490-502. doi: 10.1172/JCI72573. Epub 2014 Nov 17.

**LYN-activating mutations mediate antiestrogen resistance in estrogen receptor-positive breast cancer.**

Schwarz LJ, Fox EM, Balko JM, Garrett JT, Kuba MG, Estrada MV, González-Angulo AM, Mills GB, Red-Brewer M, Mayer IA, Abramson V, Rizzo M, Kelley MC, Meszoely IM, Arteaga CL.

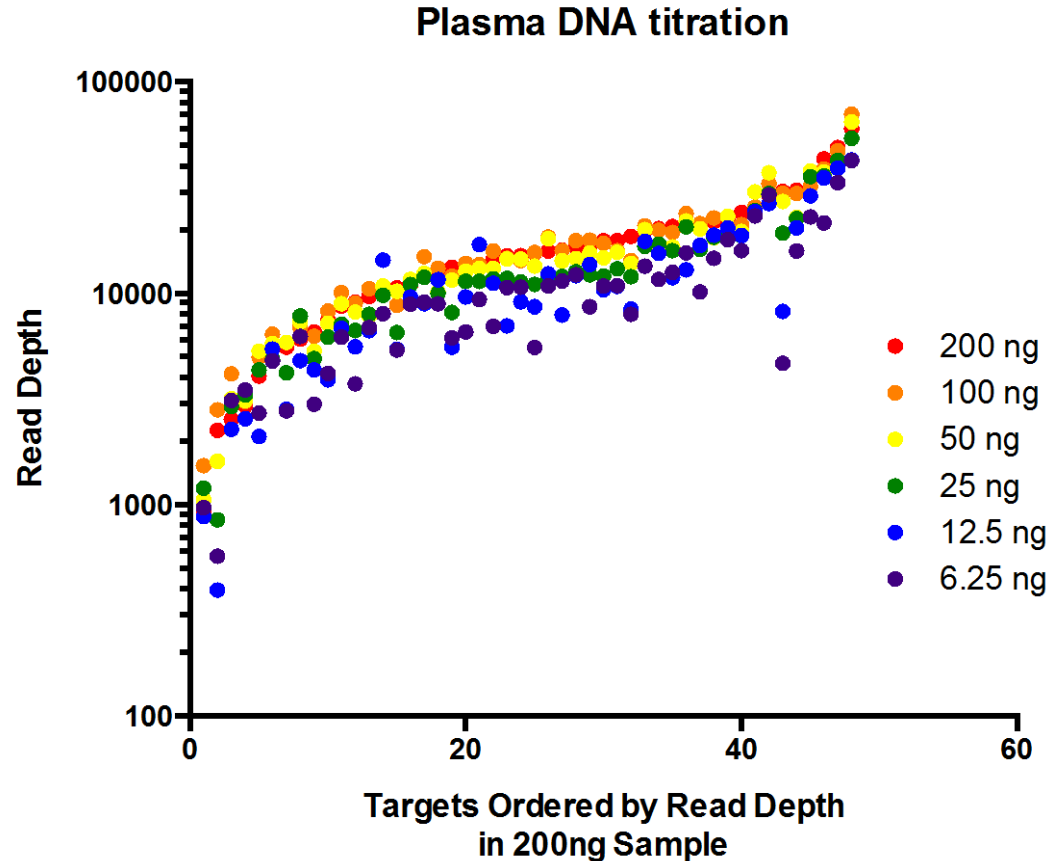
ER ligand-binding domain mutations in ~25% of breast mets

- Li, S. *et al. Cell Reports* **4**, 1116–1130 (2013)
- Toy, W. *et al. Nat. Genet.* **45**, 1439–1445 (2013)
- Robinson, D.R. *et al. Nat. Genet.* **45**, 1446–1451 (2013)
- Jeselsohn, R. *et al. Clin. Cancer Res.* (2014)
- Merenbakh-Lamin, K. *et al. Cancer Res.* **73**, 6856–6864 (2013)



# Breast Cancer Mutation Panel

48 amplicons: TP53 CDS, PIK3CA CDS, ESR1 LBD, LYN SH2 Domain  
100% of targets captured with 1<sup>st</sup> design

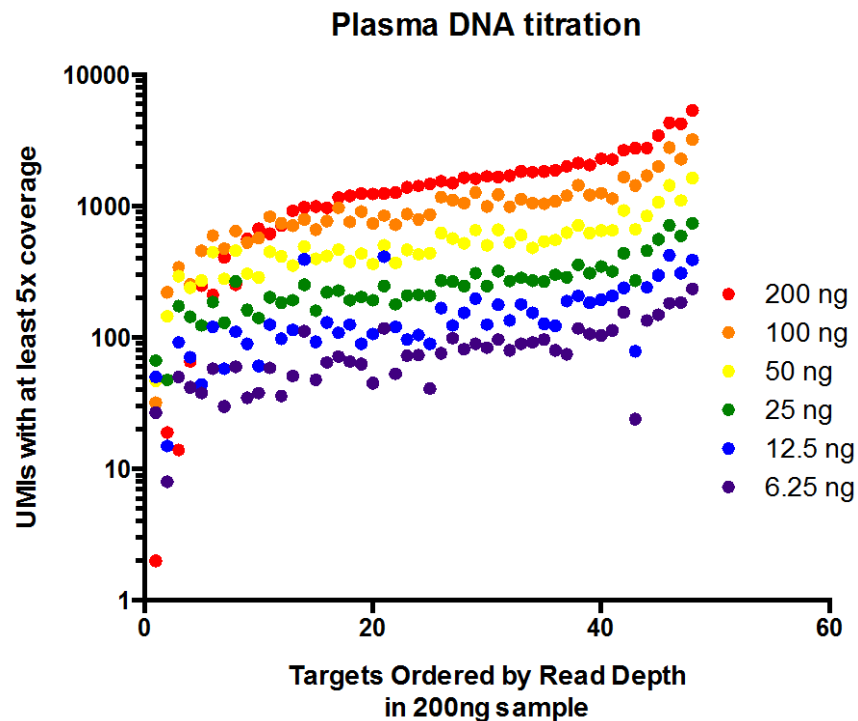
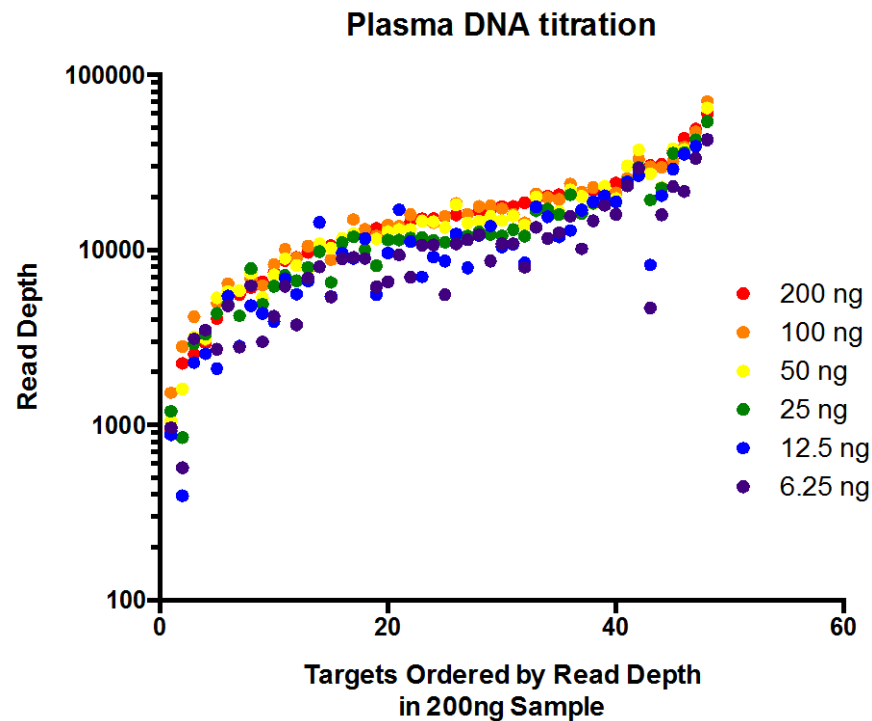


| Uniformity   | 200 ng | 100 ng | 50 ng | 25 ng | 12.5ng | 6.25ng |
|--|--------|--------|-------|-------|--------|--------|
| Coverage Uniformity<br>(Fraction of Amplicons with<br>>20% of the mean coverage) | 0.92   | 0.94   | 0.92  | 0.96  | 0.94   | 0.96   |
| Coefficient of Variation (CV)  | 0.70   | 0.71   | 0.73  | 0.75  | 0.77   | 0.77   |



# Breast Cancer Mutation Panel

48 amplicons: TP53 CDS, PIK3CA CDS, ESR1 LBD, LYN SH2 Domain



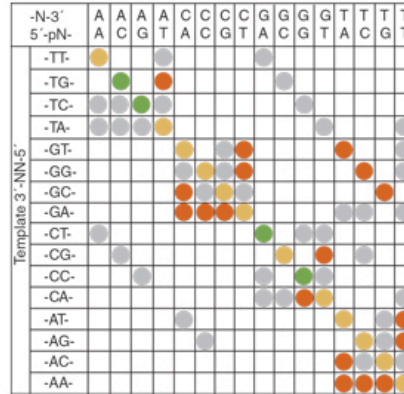
| Template Molecule  | 200 ng | 100 ng | 50 ng | 25 ng | 12.5 ng | 6.25ng |
|--------------------|--------|--------|-------|-------|---------|--------|
| Capture Percentage |        |        |       |       |         |        |
| Minimum            | 0.00   | 0.11   | 0.31  | 0.63  | 0.40    | 0.42   |
| Mean               | 2.56   | 3.42   | 3.63  | 3.58  | 4.16    | 4.31   |
| Maximum            | 8.85   | 10.66  | 10.82 | 9.82  | 11.22   | 12.46  |

# Breast Cancer Mutation Panel Optimization

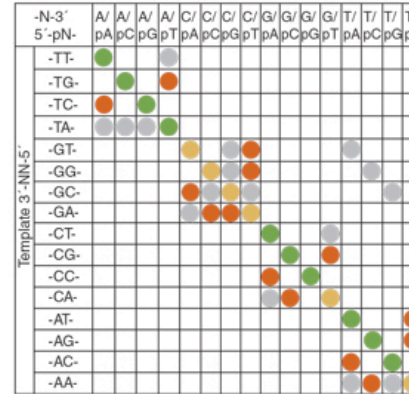
New Thermostable  
Ligase



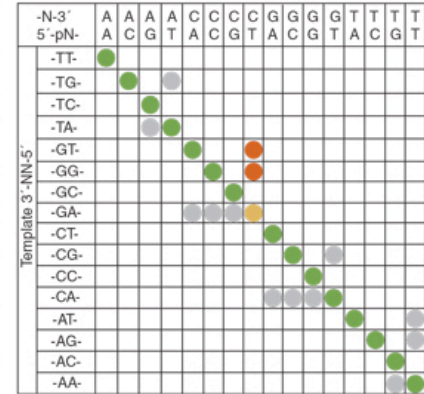
epicentre  
an illumina company  
Ampligase®



Taq DNA Ligase



HiFi Taq DNA Ligase



Optimized Adapter and  
Patch Concentrations



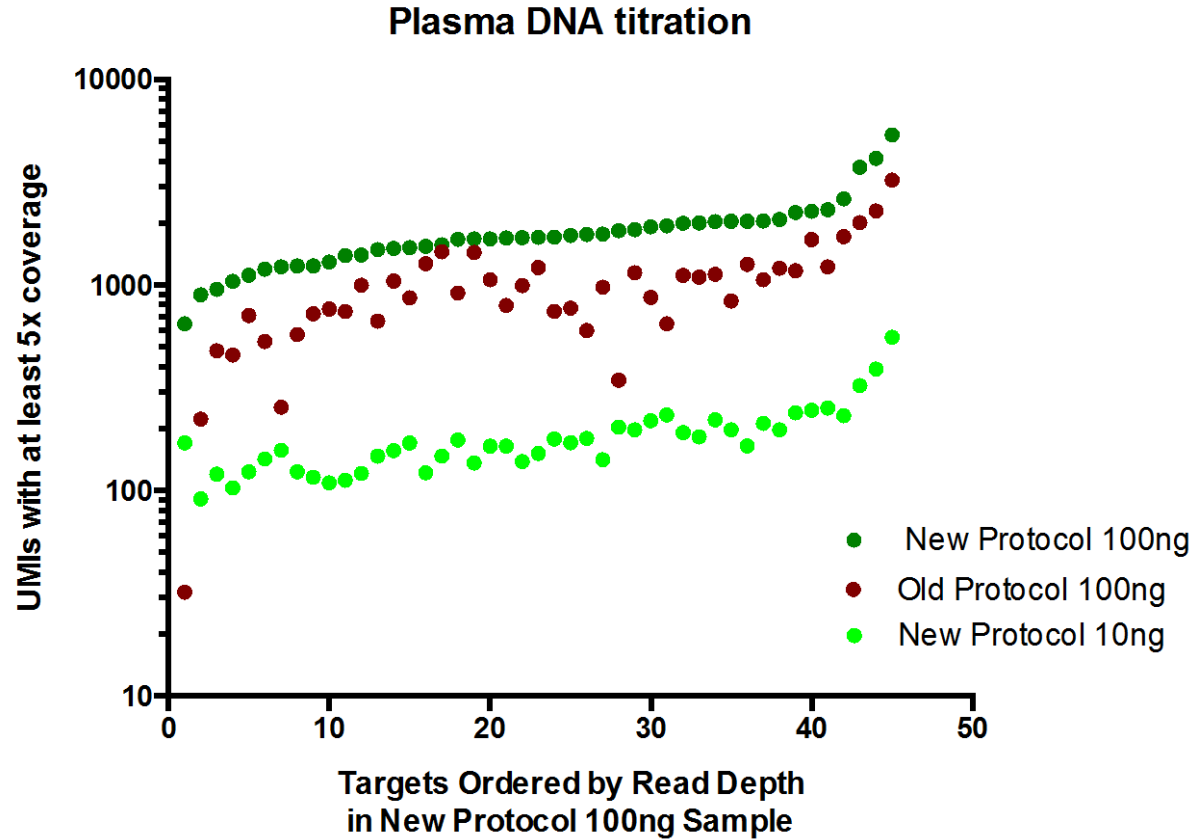
New Polymerase

- Platinum Taq (Life Technologies)
- PfuUltra II (Agilent)
- Vent DNA Polymerase (NEB)
- KAPA HiFi (Kapa Biosystems)



# Breast Cancer Mutation Panel

Optimized to improve template molecule capture efficiency

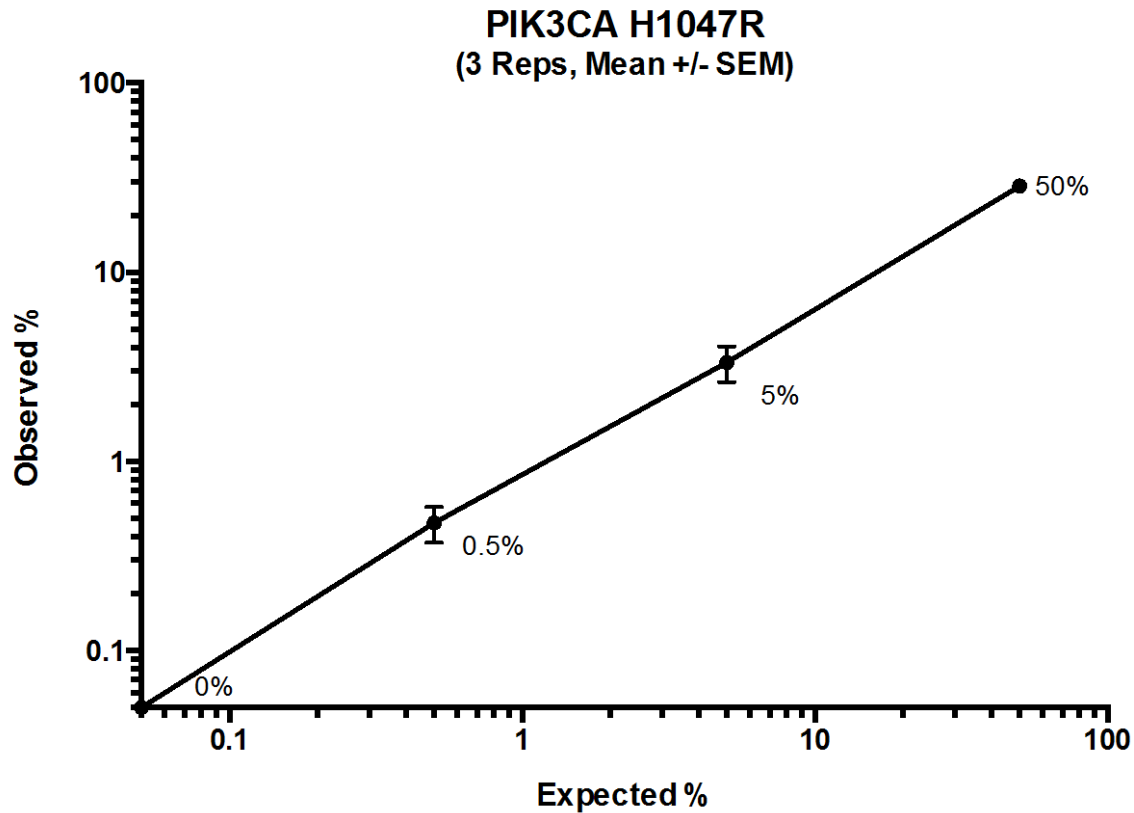


| Template Molecule Capture Percentage | Old Protocol 100ng | New Protocol 100ng | New Protocol 10ng | New Protocol Improvement |
|--------------------------------------|--------------------|--------------------|-------------------|--------------------------|
| Minimum                              | 0.11               | 4.27               | 6.01              | 48x                      |
| Mean                                 | 3.39               | 12.15              | 12.15             | 3.6x                     |
| Maximum                              | 10.66              | 35.44              | 36.77             | 3.4x                     |



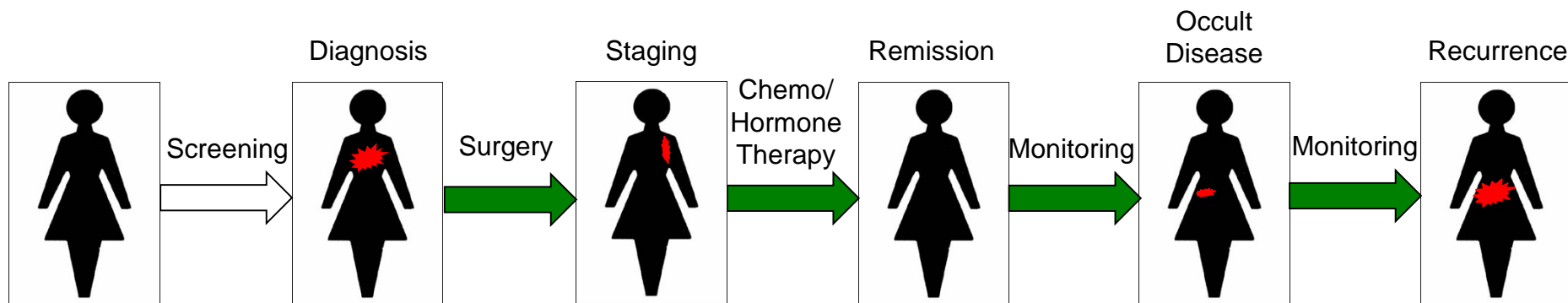
# Sensitive and Specific Quantification of Rare Mutations

1. Obtain Reference DNA containing 50% PIK3CA H1047R from Horizon Discovery
2. Sonicate to shear to average 170bp
3. Spike into 50ng Healthy Donor Plasma DNA at varying frequencies





# Blood plasma collection at Huntsman Cancer Institute (Year 1)



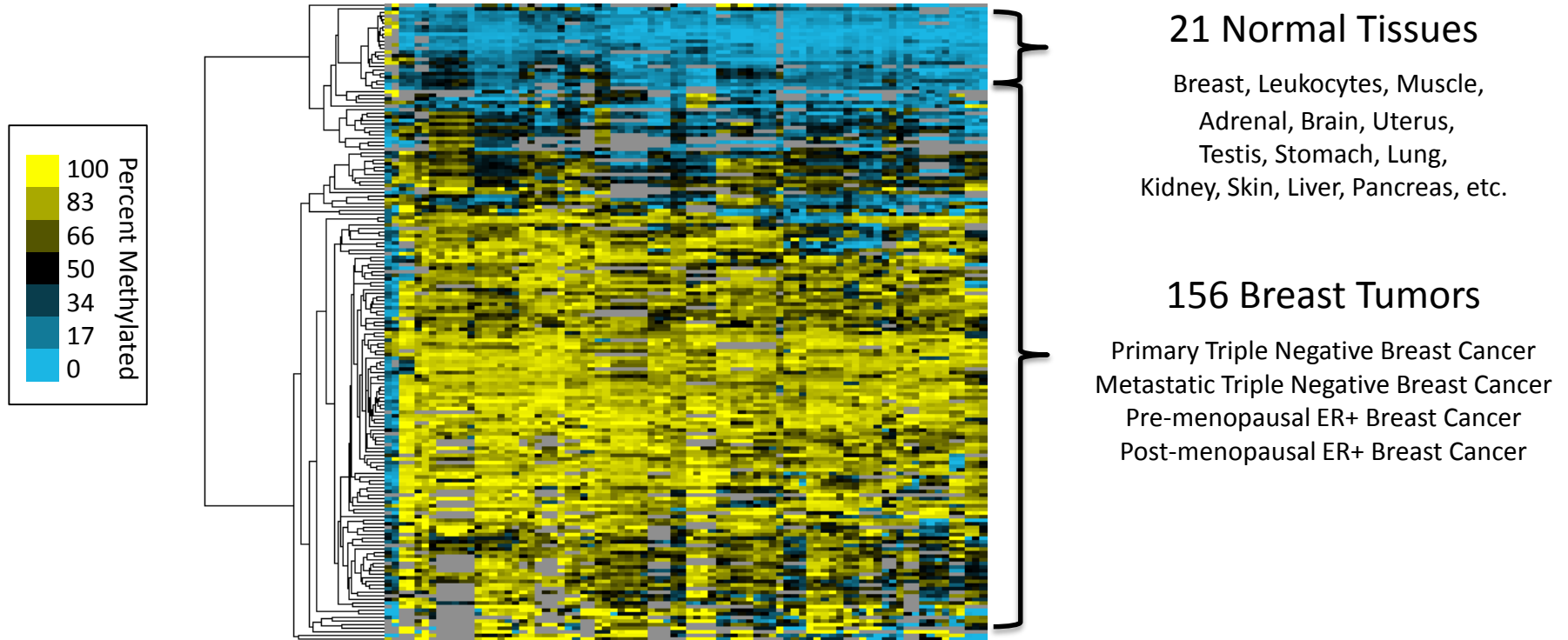
| Invasive Breast Cancer Cohorts     |  | Number of Patients in Year 1          |
|------------------------------------|--|---------------------------------------|
| Molecular Margins Residual Disease | Surgery (Pre-op and 14 days Post-op)                       | 84                                    |
| Monitoring Therapy Response        | Neoadjuvant chemotherapy (longitudinal)                    | 19                                    |
|                                    | Chemotherapy for residual localized disease (longitudinal) | 58                                    |
|                                    | Chemotherapy for metastatic disease (longitudinal)         | 72                                    |
| Monitoring for Recurrence          | Post-treatment follow-up appointments (longitudinal)       | Research-only draws recently approved |

| Ovarian Disease Cohorts  |  | Number of Patients in First 6 Months |
|--|--|--------------------------------------|
| Predict Diagnoses Before Surgery<br>Molecular Margins Residual Disease | Surgery with cancer diagnosis (Pre-op and Post-op draws) | 6                                    |
|  | Surgery with non-cancer diagnosis (Pre-op only)          | 8                                    |
| Monitoring Therapy Response  | Chemotherapy for ovarian cancer (longitudinal)           | 47                                   |

# Breast cancer specific DNA methylation

## 80 CpGs in 29 Genomic Regions

Differentially methylated between breast cancer and normal tissue (RLM  $p < 5 \times 10^{-6}$ )  
Difference in average methylation between tumors and normals  $> 50$

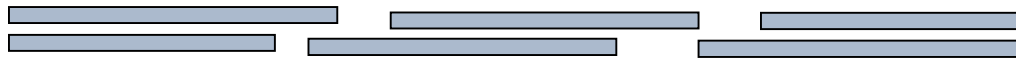


All tumors have methylation at many loci –  
providing a robust signal



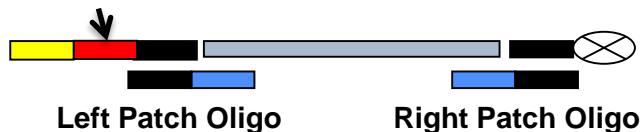
# Bisulfite Patch PCR

Restriction Digest  
Genomic DNA



Anneal Patch Oligos  
and Universal Primers  
to Targeted Fragment

Unique Molecular Identifier



Exonuclease Resistant  
3' Modification

Ligate Universal Primers  
to Targeted Fragment

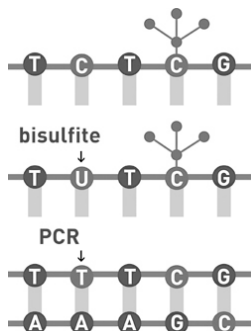
Illumina  
primers



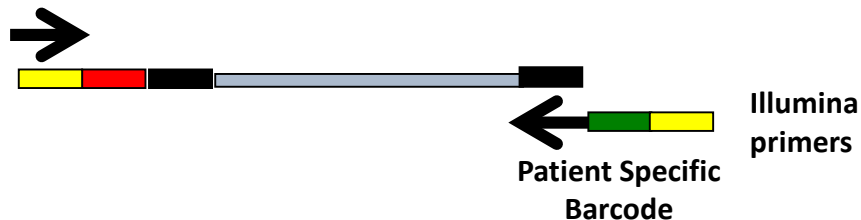
Degrade Unselected DNA  
with Exo I & III



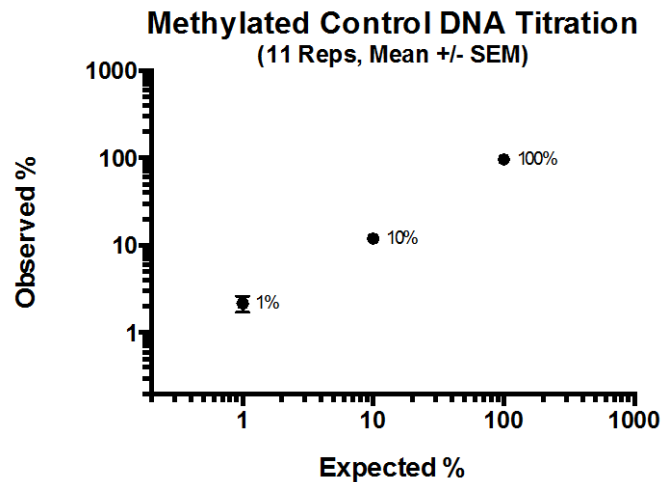
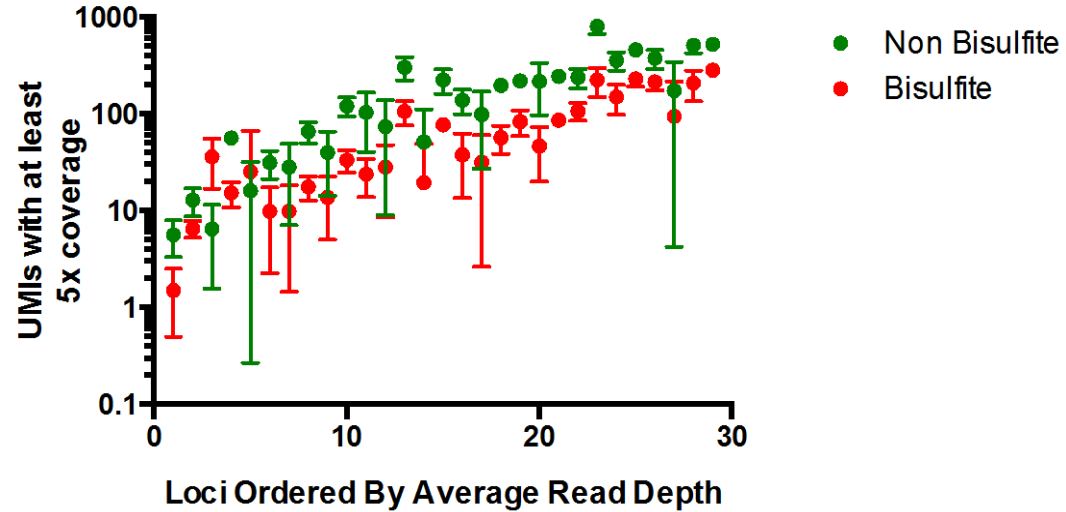
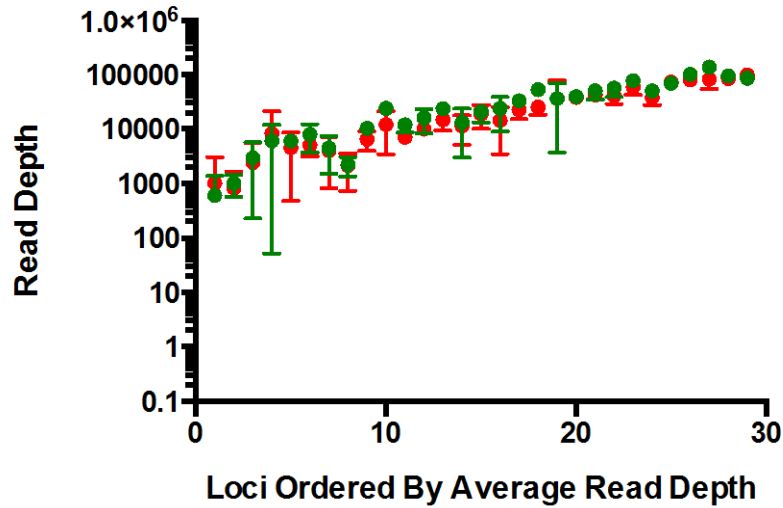
Sodium Bisulfite Treatment



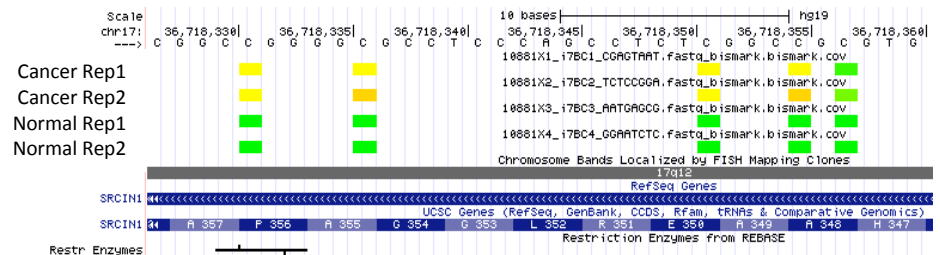
Universal PCR with Patient  
Specific DNA Barcodes and  
illumina primers



# Breast Cancer Methylation Panel (Old Protocol)



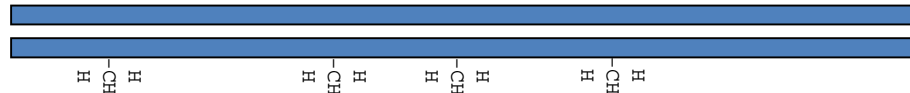
## Reproducible detection of aberrant DNA methylation in breast cancer patient



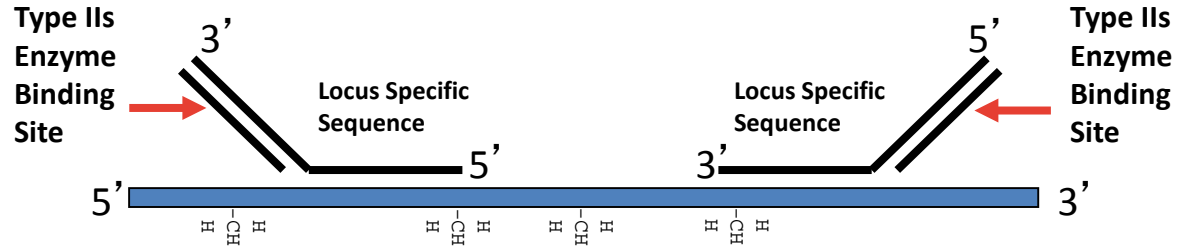


# PCR-Free Patch Libraries for Direct Detection of Methylation on the PacBio

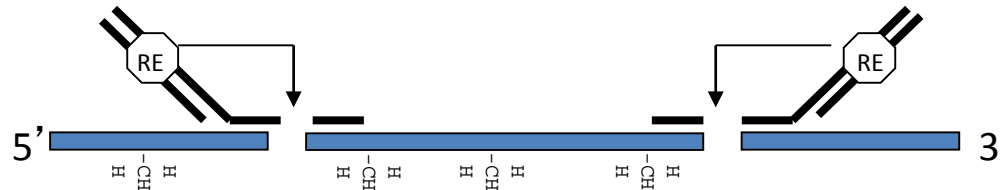
Genomic DNA



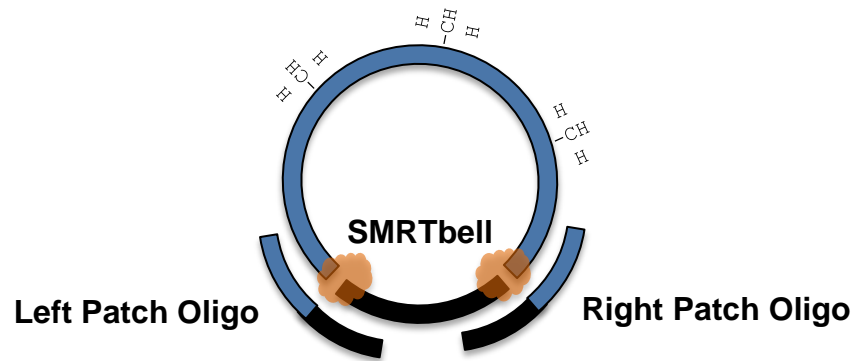
Denature & anneal oligos to target loci



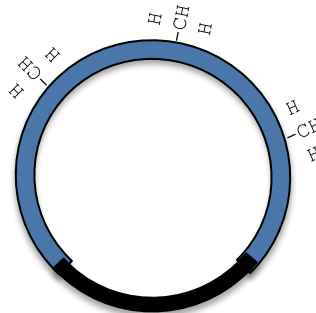
Oligo-directed digest of target loci with Type IIs restriction enzyme



Selectively ligate target loci to SMRTbell adapter using patch oligos as a bridge and thermostable ligase.



Digest unselected DNA with Exonuclease III and Exonuclease VII



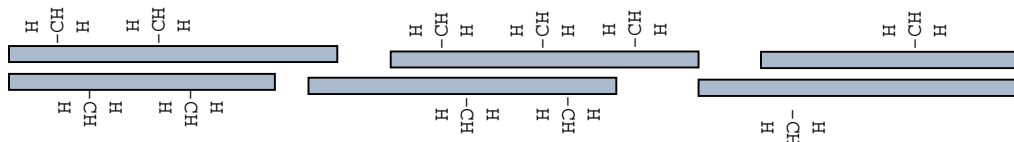
Sequel System





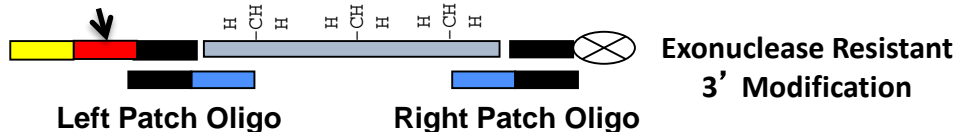
# PCR-free libraries for Direct Detection of Methylation on the Oxford Nanopore

**Restriction Digest  
Genomic DNA**

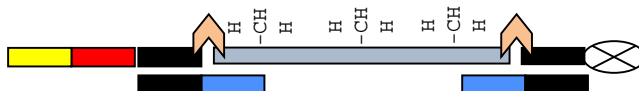


**Anneal Patch Oligos  
and Universal Primers  
to Targeted Fragment**

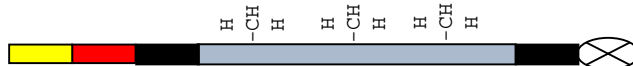
**Unique Molecular Identifier**



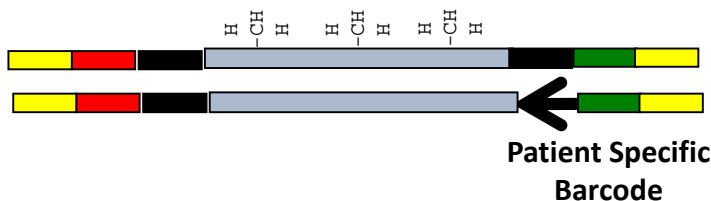
**Ligate Universal Primers  
to Targeted Fragment**



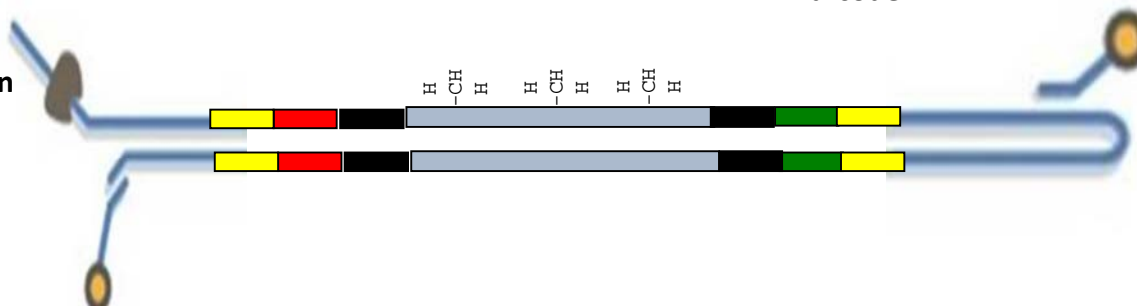
**Degrade Unselected DNA  
with Exo I & III**



**Isothermal Fill-in with primers  
containing patient barcode**



**Oxford Adapter Ligation**



**MinION**



# Acknowledgements

VARLEY LAB

Blake Atwood



Holden Wagstaff



Kris Berrett



Ashley Farre



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HEALTH SCIENCES

Mary Bronner



Keith Gligorich



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Molecular Pathology

Huntsman Cancer Institute



NATIONAL  
CANCER  
INSTITUTE

1R01CA204253-01



Cancer Center Support Grant

