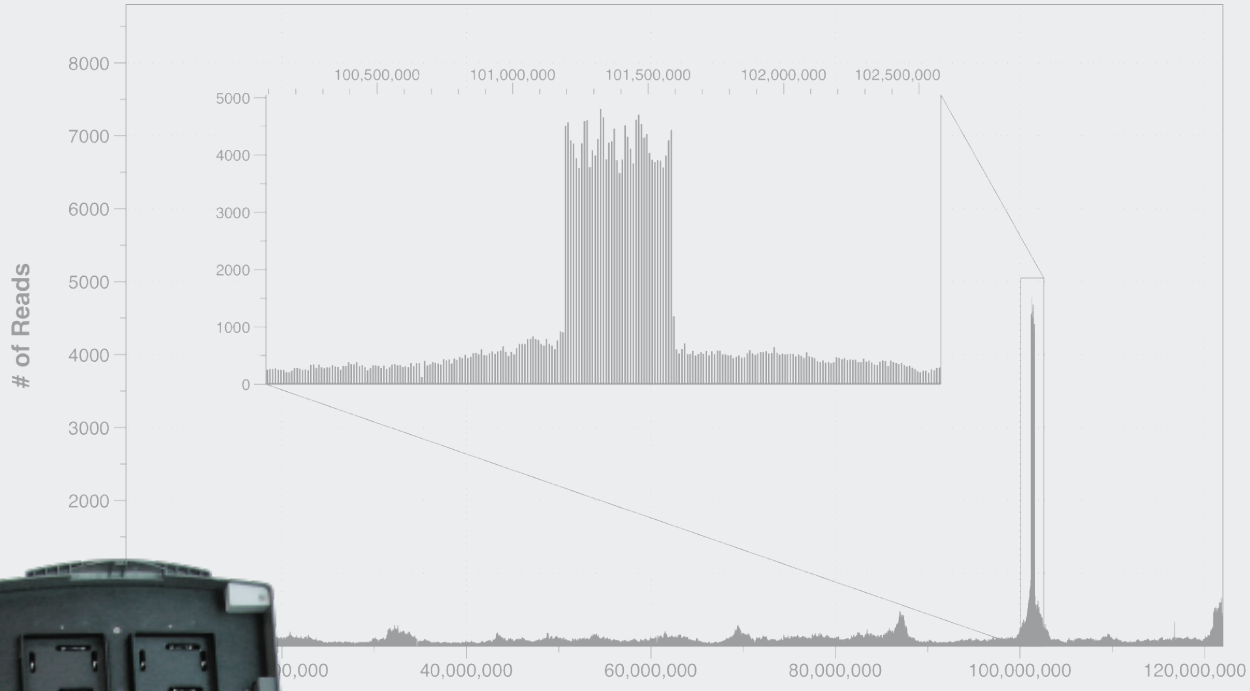
HLS-CATCH

HMW DNA Target Enrichment for Sequencing

- Pseudogenes
- Segmental Duplications
- Copy Number Variants



sageHLS™

HMW DNA Library System

sage science

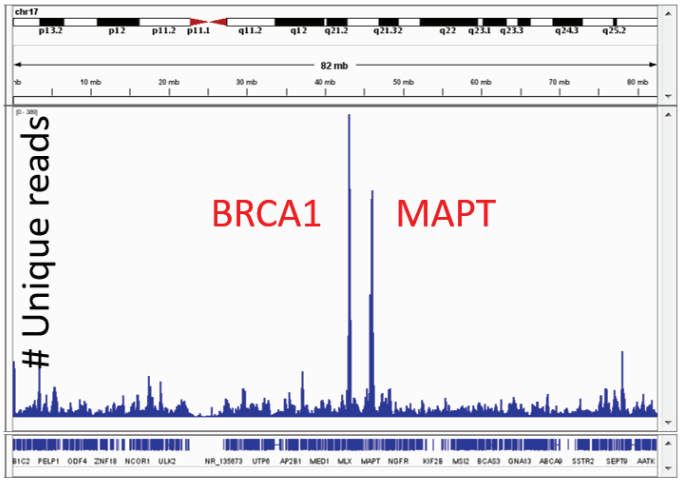
Comprehensive Coverage of Entire Gene Targets

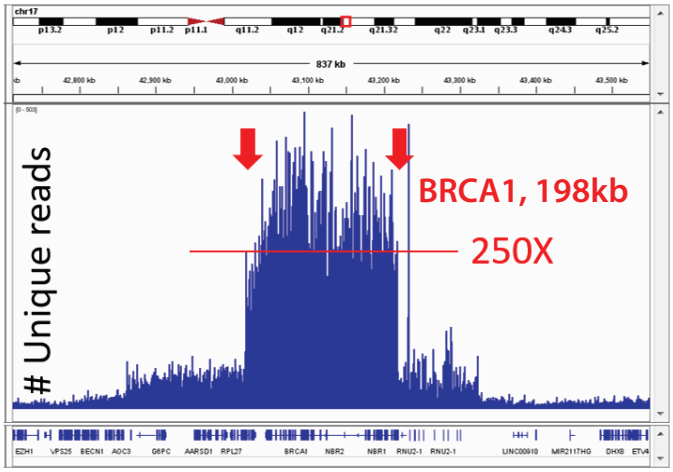
The CATCH* process provides researchers with the most direct access to genes for study. Target regions are defined with the simple design of guide-RNAs to flank regions of interest — and enriched directly from cells. With intact full-length targets, genes can be deep sequenced with direct short read methods and/or analyzed with long range approaches.

*Cas9-Assisted Targeting of CHromosomal segments

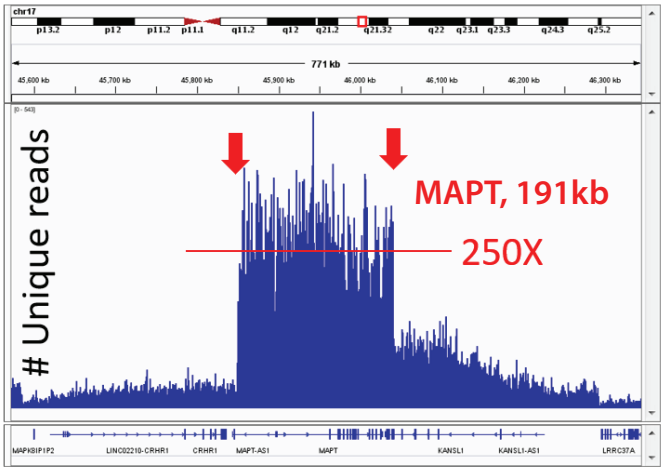
Jiang et. al., 2015, Nature Communications, doi:10.1038/ncomm9101

Alignment to Chromosome 17





NA12878 cell line samples (~275,000 cells 2.5µg equivalent) were loaded into SageHLS sample lanes. SageHLS Elution fractions with target fragments were identified by qPCR. Target fractions (10 ng total DNA) were used for library generation using the Agilent SureSelectXT Low Input Target Enrichment System for Illumina Paired-End Multiplexed Sequencing Library kit (using manufacturer protocol vs B0, using 11 cycles of pre-enrichment PCR) without SureSelectXT enrichment and secondary amplification steps. [1]



Arrows indicate positions of guide RNAs.

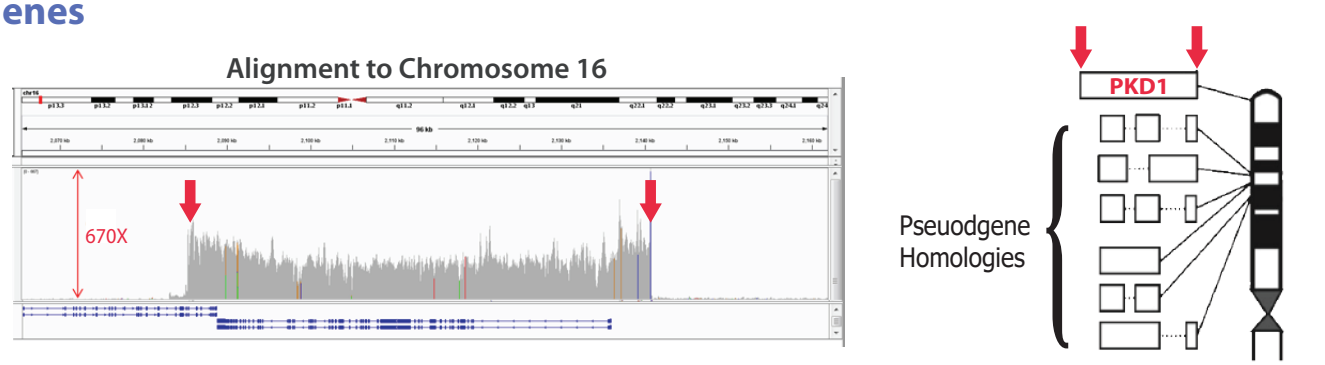
[1]**Collaborators gratefully acknowledged:** Melissa Smith, Ethan Ellis, James Powell, Ayesha Rasool, Maya Stahl, and Robert Sebra
Icahn Institute and Dept. of Genetics & Genomic Sciences, Icahn School of Medicine at Mount Sinai

Unravel Difficult Genomic Targets

Some genes or genomic regions remain poorly characterized and are not easily analyzed by hybridization-based targeted sequencing or whole genome sequencing. The CATCH method allows researchers to use to zero in on these areas, many of which are implicated in cancers and inherited diseases.

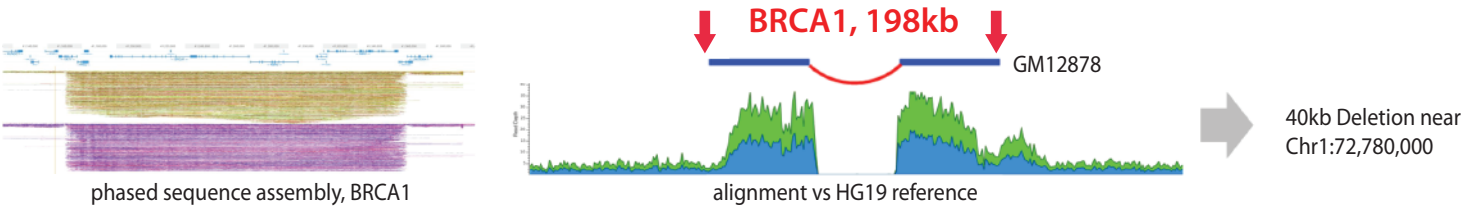
Zombie Genes, Dead Zones, and Dark Regions

Pseudogenes



Analysis of the PKD1 gene, implicated in polycystic kidney disease, is complicated by six nearby pseudogenes. Using the HLS-CATCH process PKD1 was targeted and enriched, eliminating ambiguity in the subsequent sequence analysis. The test used NA12878 cells, Kapa HyperPrep library construction kit, and Illumina MiSeq sequence analysis (Sage Science internal study).

Phasing, SVs, and Repeats

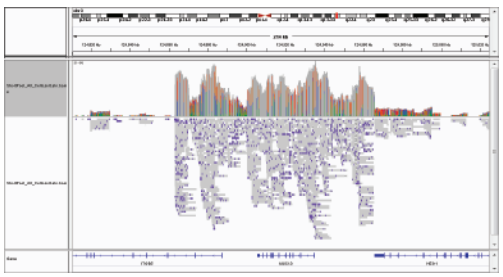


phased sequence assembly, BRCA1

alignment vs HG19 reference

Linked-read sequencing requires low DNA input amounts and benefits from long fragments. In this study, HLS-CATCH BRCA1 targets were pipetted directly onto the 10X Genomics Chromium platform and sequenced. This method captured large structural variants and provided haplotype phasing information.[2]

MUC13, 103kb



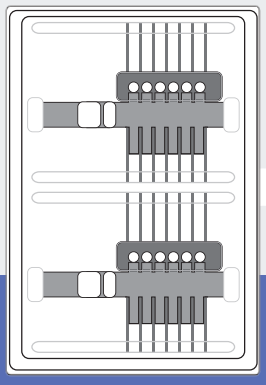
Long-read single molecule sequencing can provide accurate analysis of repeating genomic elements. PacBio sequencing of the MUC13 target provided average read lengths (N50) of ~10kb in this test.[1]

[2]**Collaborators gratefully acknowledged:** GiWon Shin, Stephanie U Greer, Li C Xia, HoJoon Lee, and Hanlee P Ji. *Stanford University School of Medicine.* Assembly of Mb-size genome segments from linked read sequencing of CRISPR DNA targets, 2018, BioRxiv preprint doi.org/10.1101/373142

Specifications at a Glance

Run Times	Sample
Extraction 1-3 hr	DNA Load* 2.5µg
Treatment 30 min	Volume 70µl
Collection 1-6 hr	Capacity 1-4/run

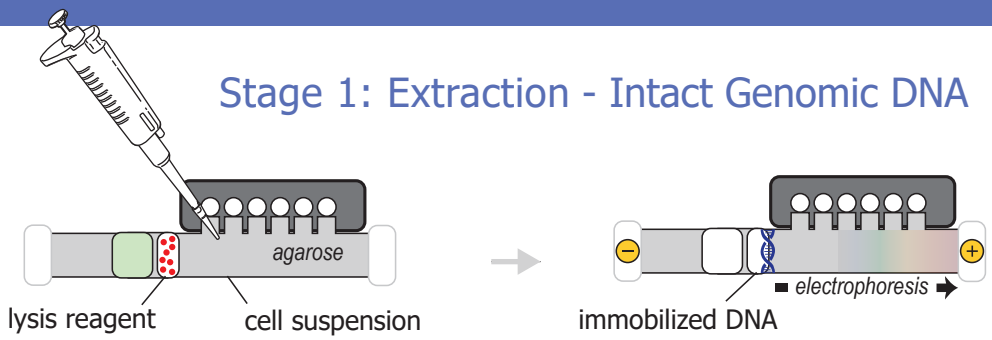
**recommended DNA equivalency per cell suspension*



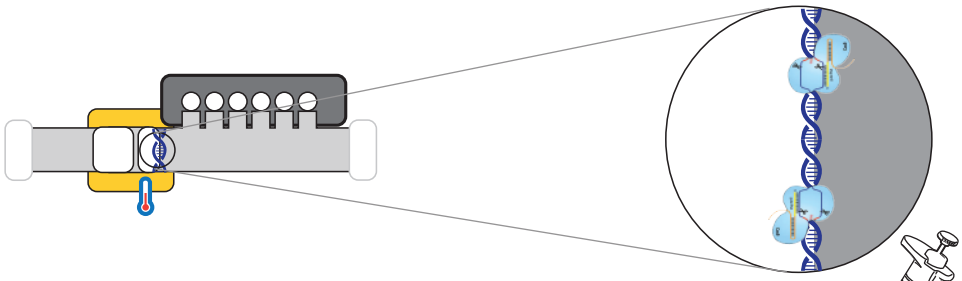
agarose gel cassette

SageHLS Workflow

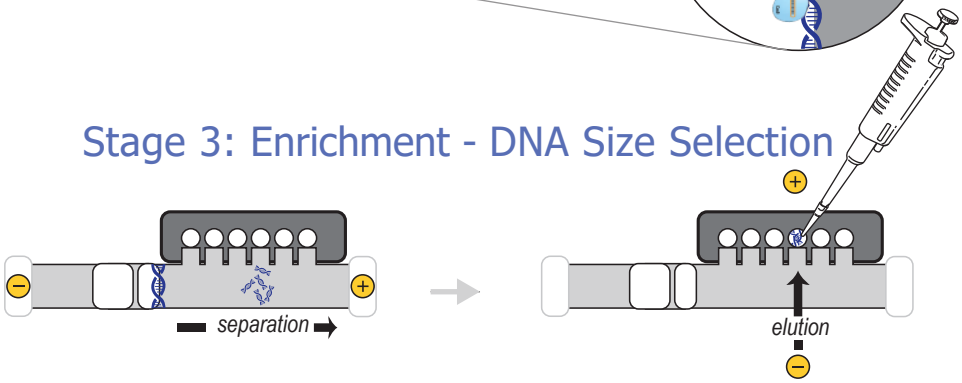
Stage 1: Extraction - Intact Genomic DNA



Stage 2: Cas9 Targeting - Regions up to 1MB



Stage 3: Enrichment - DNA Size Selection



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