



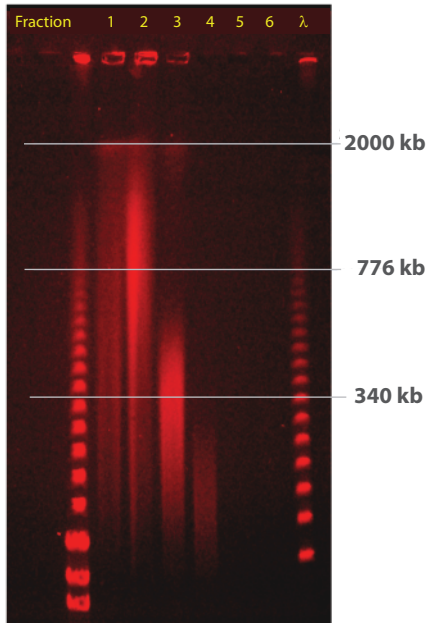
# sageHLS™ HMW DNA Library System

**Extract.  
React.  
Select.**

**One-Stop Sample Prep  
for Long-Range Genomics**

# Extract ultra-HMW DNA from Cells

Study DNA fragments as long as 2MB  
Treat genomic DNA with a random cleavase



Pulsed-field gel analysis

Input DNA: 8µg DNA equivalents

Fraction	DNA (ng)	% yield
1	428	5.3%
2	1581	19.8%
3	1691	21.1%
4	314	3.9%
5	24	0.3%
6	26	0.3%
<b>Total</b>	<b>4063</b>	<b>51%</b>

DNA Recovery per Collection Well

White blood cells were prepared from whole blood using standard centrifugation techniques. The cells (~6.6 x 10<sup>4</sup>) were resuspended in 70ml and loaded onto a SageHLS gel cassette. The purification and cleaving processes include several pipetting, electrophoresis, and incubation steps requiring about 90 minutes. Size selection required 3.5 hours. Total hands-on time was about 15 minutes.

## Genome Structure

- Rearrangements
- Copy Number Variation
- Haplotype Phasing

## Stay Tuned for More...

The SageHLS platform lends itself to new development as needs arise. New suspension kits and protocols are planned for numerous cell types, organisms, and tissues. The system is not limited to endonuclease treatment either: ligases, nickases, or polymerases could be used to modify genomic DNA into labeled or tagged libraries.

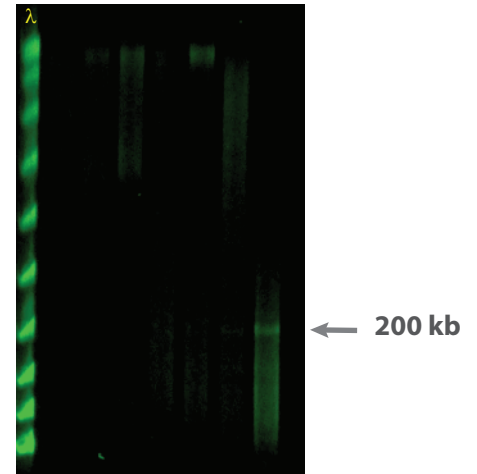
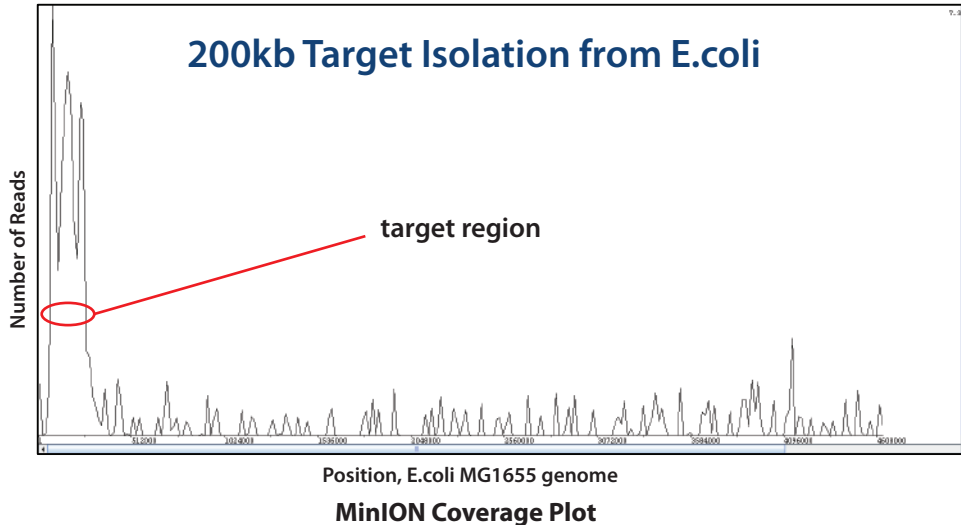
Specifications at a glance:

Run Times		Sample		Instrument	
Extraction	1 hr	DNA Load*	10µg	Power Req.	100-240 VAC
Treatment	30 min	Volume	70µl	Incubation Temp.	15-50°C
Collection	1-6 hr	Capacity	1-4/run	Certifications	CE

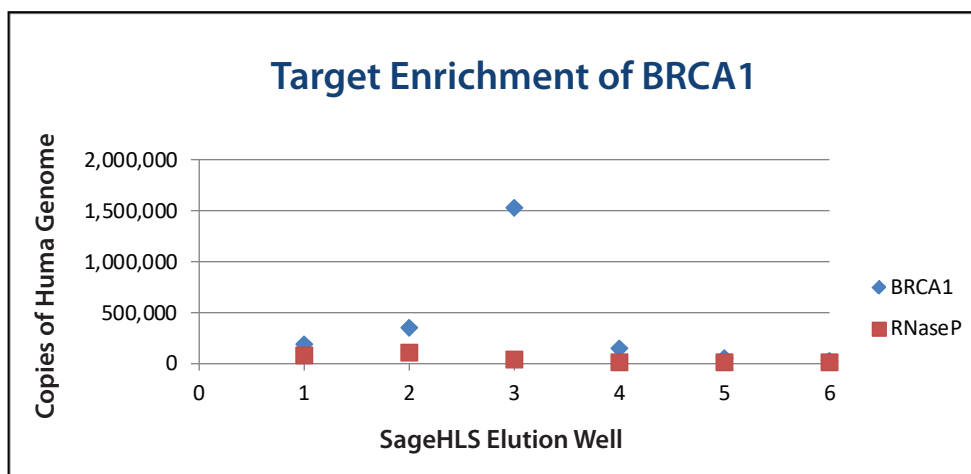
\* recommended DNA equivalency per cell suspension

# Target Large Genomic Regions with Cas9

**HLS-CATCH\*: CRISPR/Cas9, *In Vitro***  
*You supply the guide RNAs, we supply the rest*



A 200 Kb region was isolated from the E. coli genome using HLS-CATCH. A spheroplast suspension was prepared in SageHLS-compatible buffer using a Sage Science kit. The suspension was lysed under electrophoretic conditions, purifying the genomic DNA and immobilizing it within the agarose on the sample well wall. The DNA was then treated with wild type spCas9 (New England Biolabs) that had been assembled with guide RNAs (IDT, Alt-R™) bordering a 198 Kb region of interest (4.2% of the genome). DNA released by the Cas9 digestion was electro-eluted with the SageHLS cassette and sequenced with an Oxford Nanopore MinION™ sequencer.



A pool of five effective gRNAs were used in an HLS-CATCH experiment to excise the BRCA1 fragment from 1.5e06 Raji cells (input gDNA content about 10µg). The elution products from each elution well were evaluated by qPCR (ABI Taqman® kits, RNaseP gene as control).

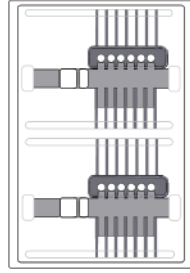
## Critical Research

- Cancer Genomics
- Inheritable Disease
- Plant Genomics

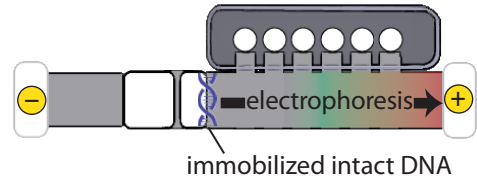
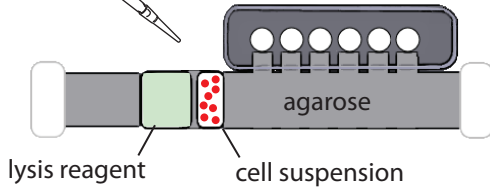
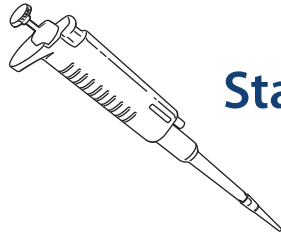
\*Cas9-assisted targeting of chromosome segments  
Jiang et. al., 2015, Nature Communications, doi:10.1038/ncomm9101

# SageHLS Workflow:

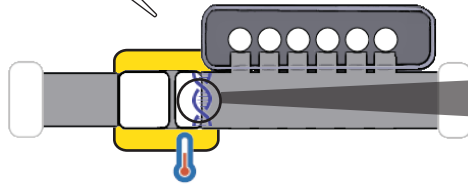
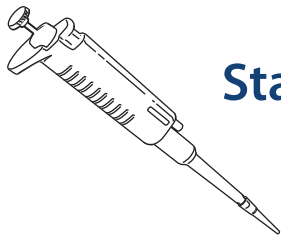
agarose gel cassette



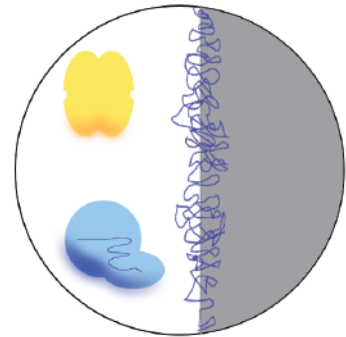
## Stage 1: DNA Extraction



## Stage 2: Enzymatic Reaction



cleavase  
or  
Cas9 + gRNAs



## Stage 3: DNA Size Selection

