

# SageHLS CATCH Workflow for Targeted Nanopore Sequencing

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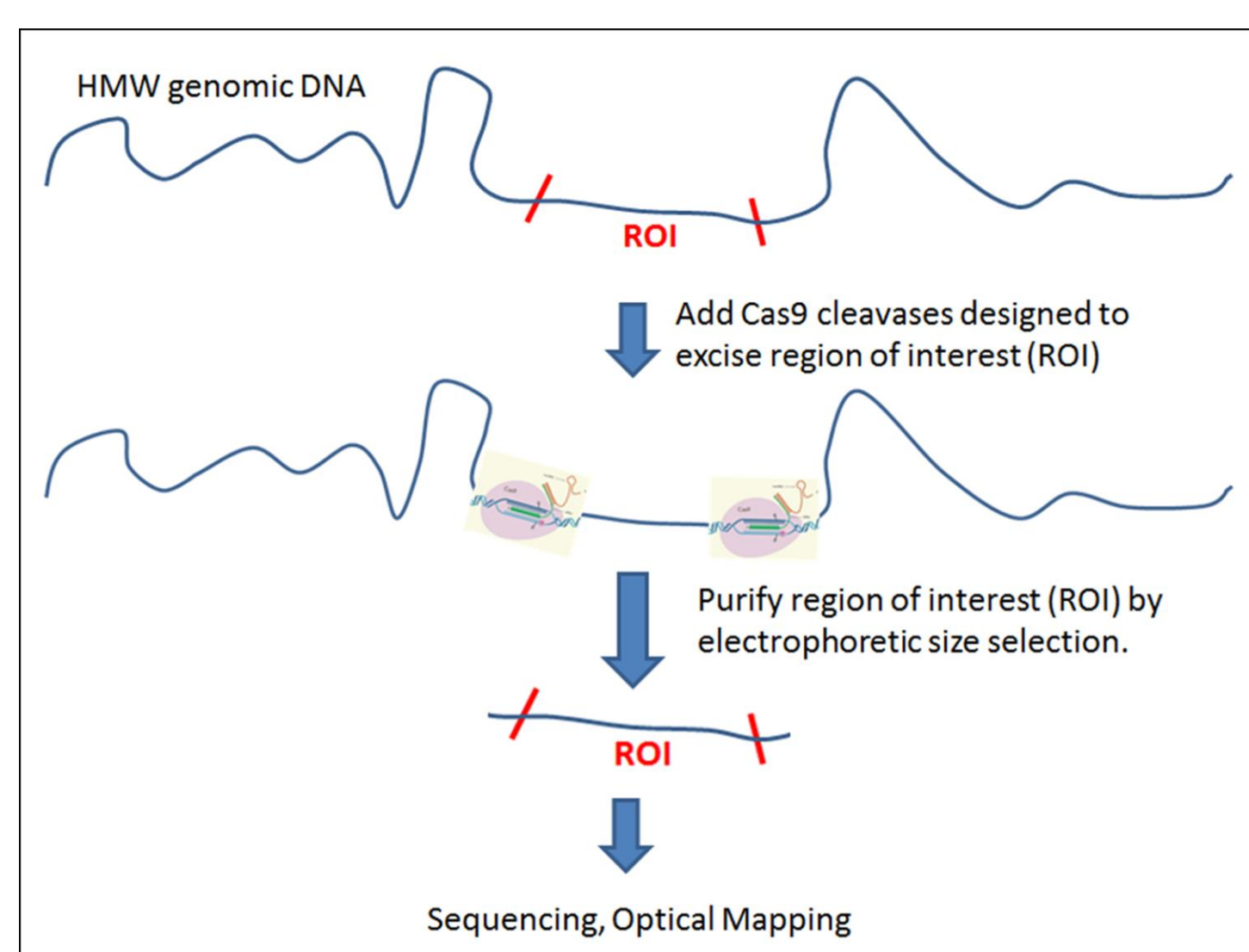
Sage Science, Beverly, MA

## Abstract

Sage has developed a semi-automated method for isolation of specific large genomic DNA fragments, called HLS-CATCH. The method utilizes a tightly coupled three-step procedure which is carried out in the SageHLS cassette: 1) HMW DNA is isolated and immobilized in the sample well wall of the HLS cassette; 2) the DNA is digested in the cassette with customized Cas9 nucleases designed to excise the fragment of interest from the genome; 3) the digested DNA is subjected to size-selection electrophoresis and electroelution of the HLS-CATCH products.

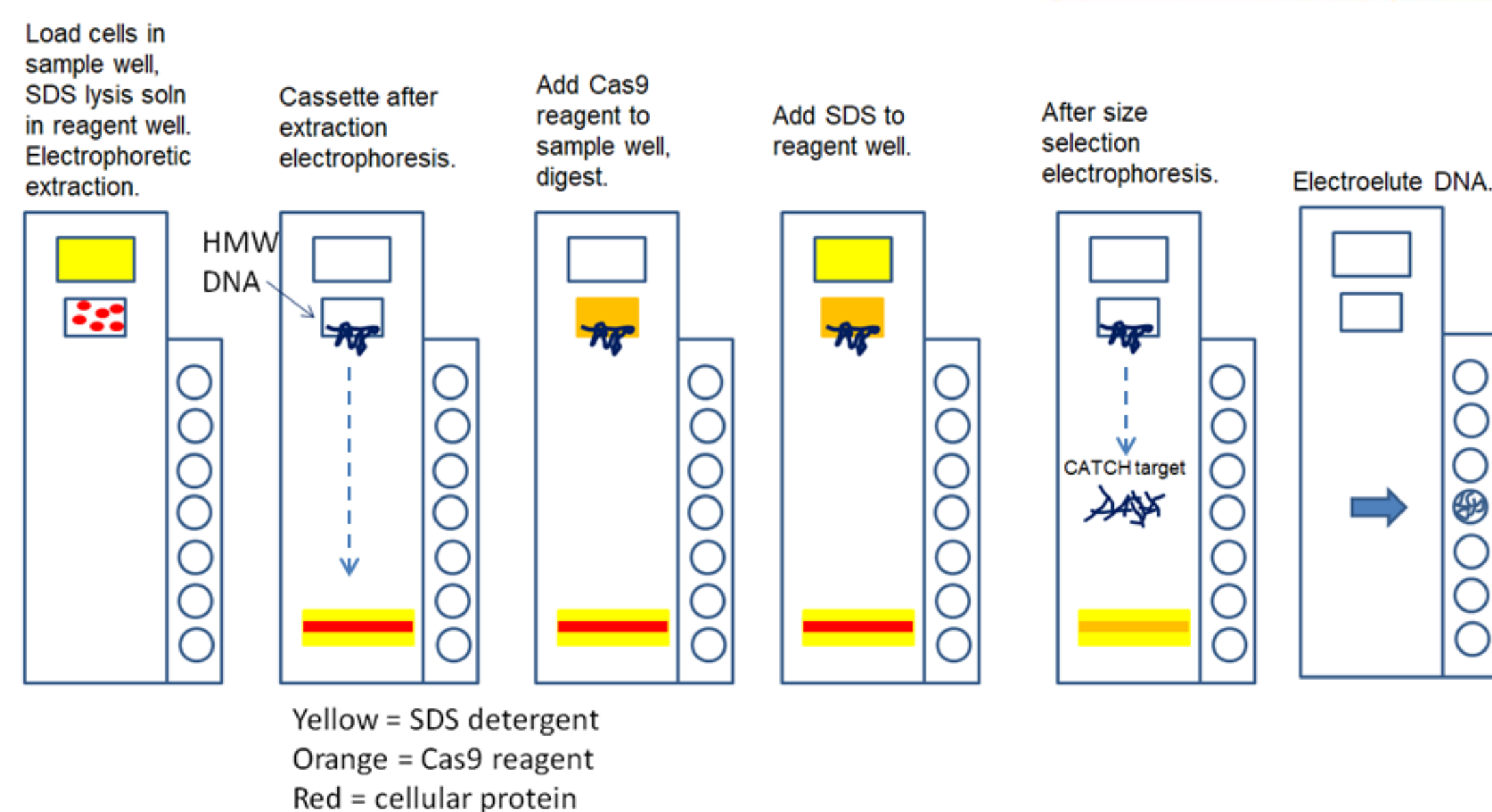
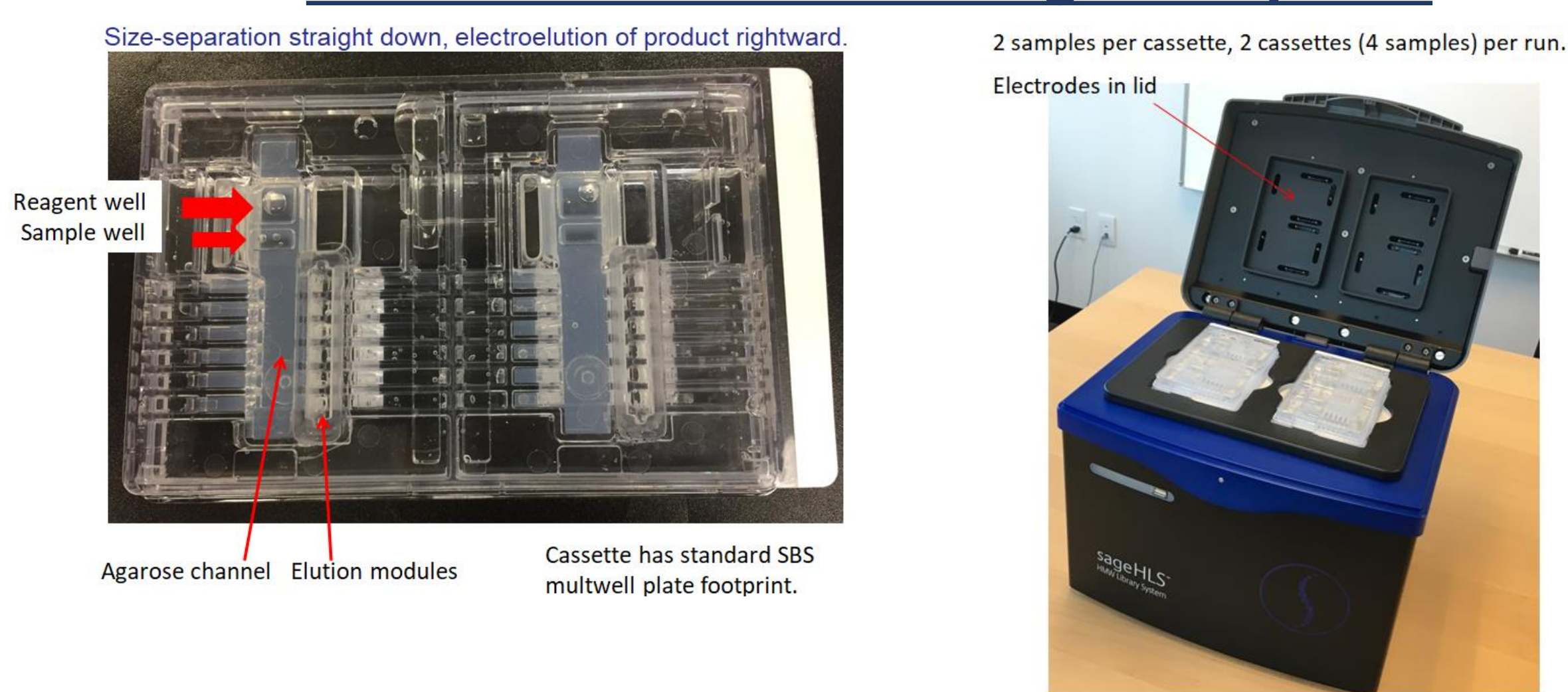
An important feature of the HLS-CATCH method is that the vast majority of the purified genomic DNA is not cleaved by the customized Cas9 cleavages, and remains immobilized in the sample well during size-selection. This allows significant enrichment (up to 800-fold) of the electrophoretically mobile HLS-CATCH targets. We demonstrate that the HLS-CATCH targets can be sequenced with good coverage using standard Minion ligation library methods.

## CATCH concept for isolation of genomic DNA targets



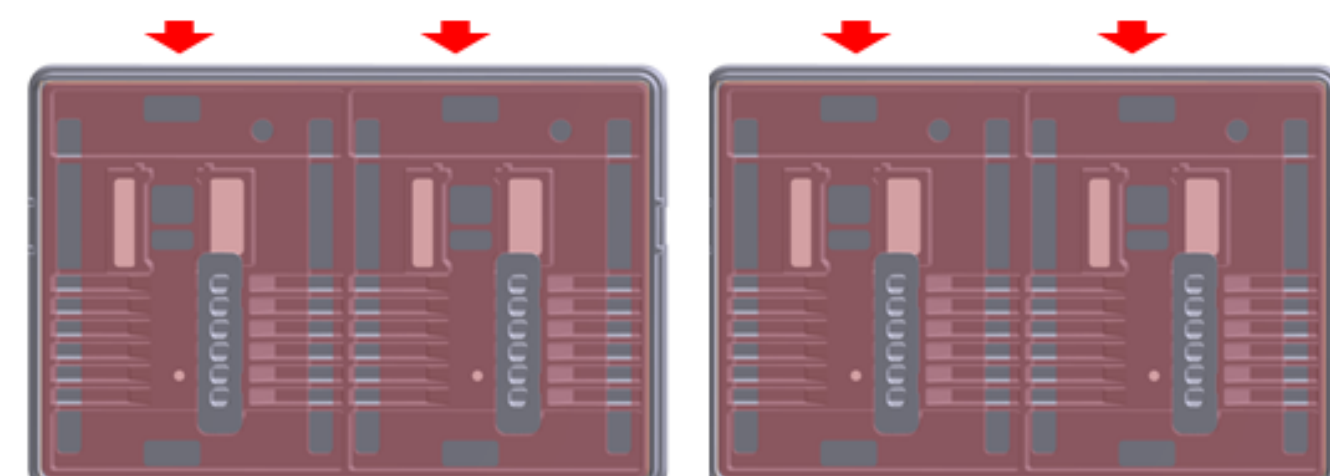
Jiang et al., Nature Communications, 2015, 6:8101, DOI: 10.1038/ncomms9101.  
Gabrieli, et al. 2017, bioRxiv DOI: <https://doi.org/10.1101/110163>  
Yuval Ebeinstein lab, Tel Aviv University

## CATCH workflow in the SageHLS system



## HLS-CATCH workflow, Minion sequencing, 180kb mouse Brca1 region

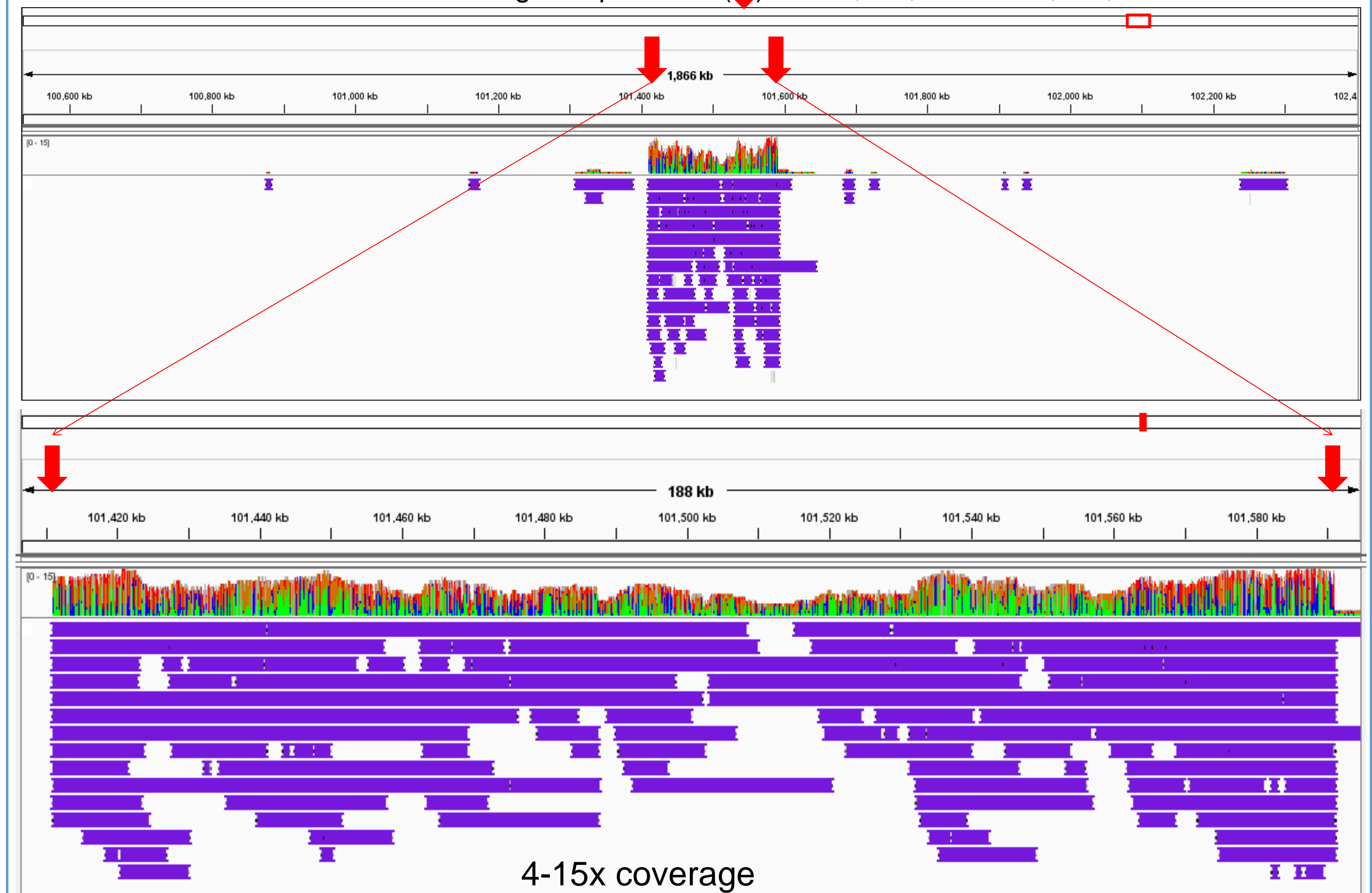
HLS-CATCH input = 375k cells per lane, total 4 lanes



- All in HLS Cassette { Extract and digest with Cas9 (2 gRNAs).  
Size select and electroelute in HLS cassette
- qPCR to quantify targets, pool peak target fractions.
- Pool has **12 ng total DNA, ~1m Brca1 target copies (200 pg).**
- 1D ligation library kit (SQK-LSK-108).
- Basecall w/ Albacore, align minimap2.

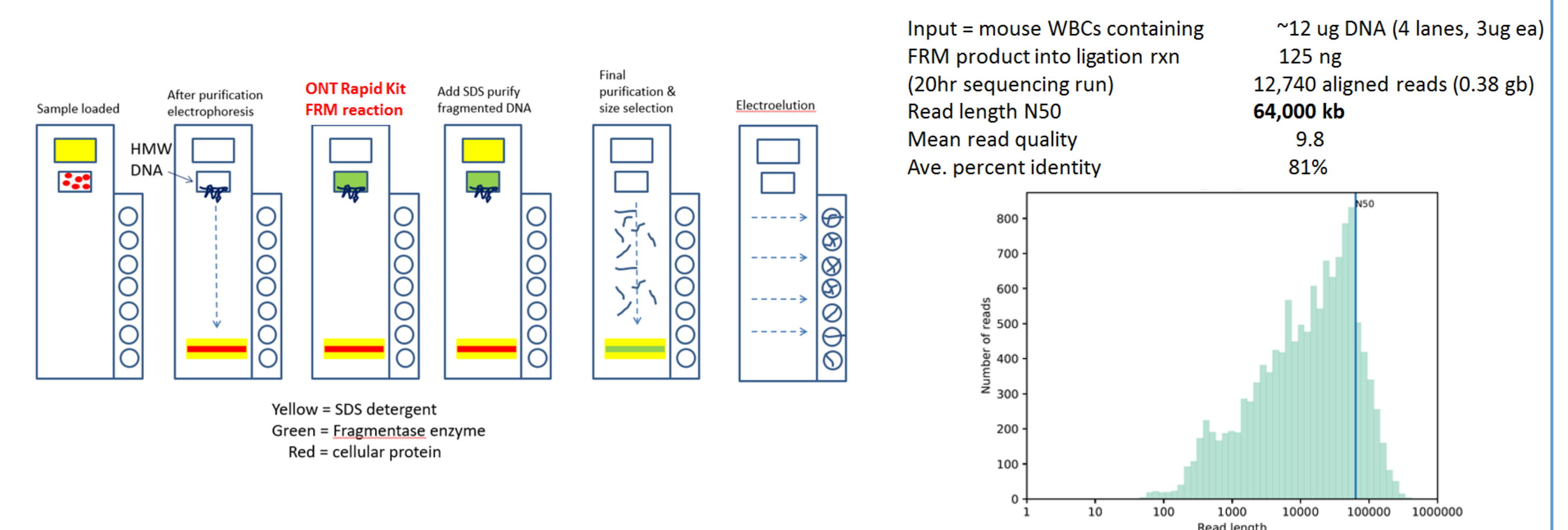
## Targeted Minion sequencing – 180kb mouse Brca1 region

Mouse chr11 around Brca1 locus: gRNA positions (↓) at 101,411,140 & 101,591,000.

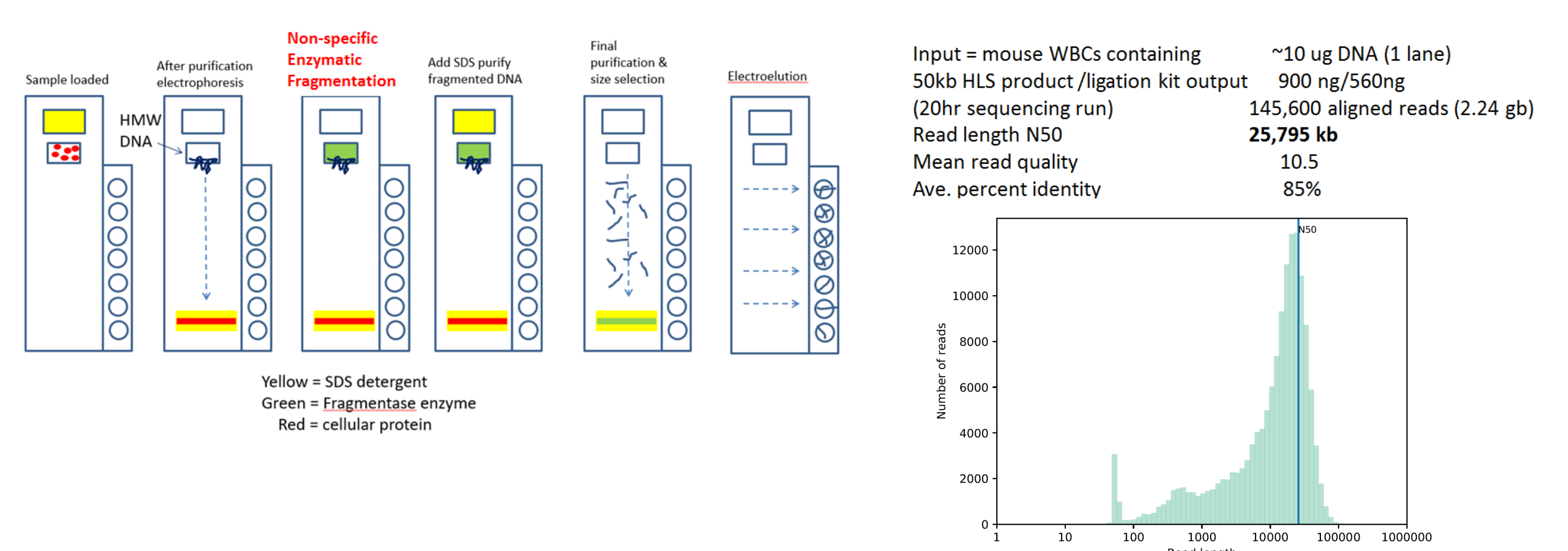


## New SageHLS workflows for whole genome sequencing

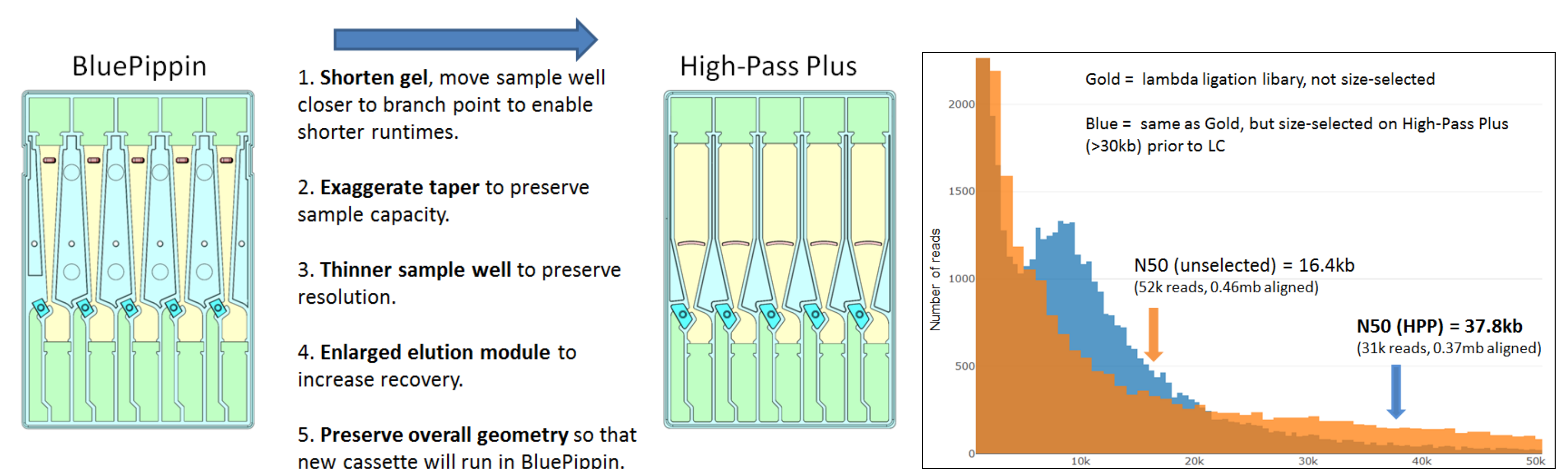
Using Oxford Rapid kit FRM enzyme for fragmentation:



Using a novel enzyme mix for fragmentation, in development from New England Biolabs:



## New BluePippin cassette: High-Pass Plus



## Acknowledgements and Contact

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