Targeted isolation of long genomic DNA molecules

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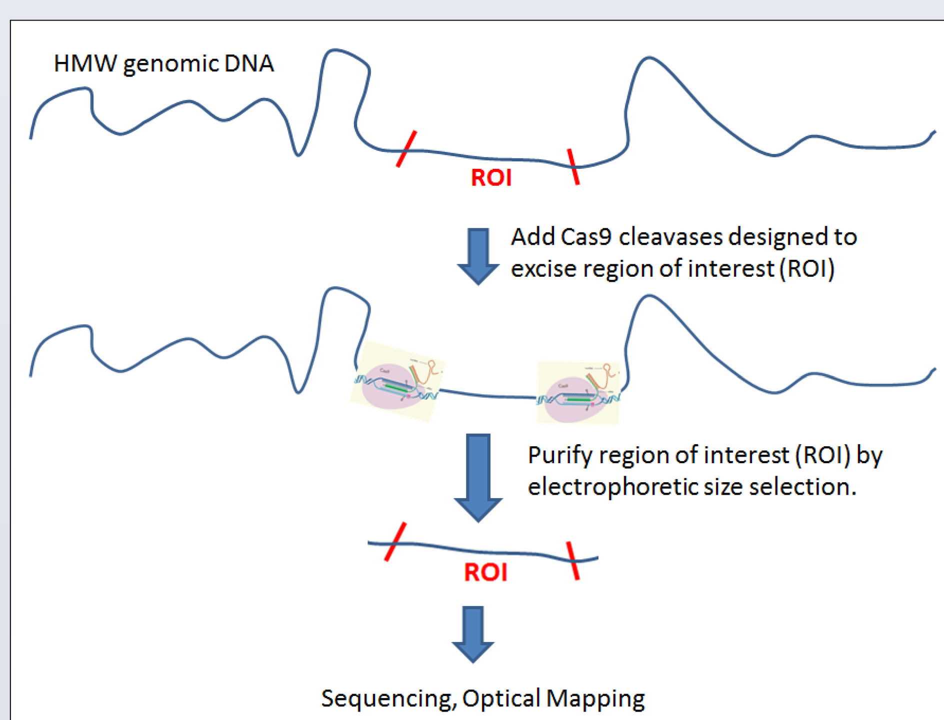
Introduction

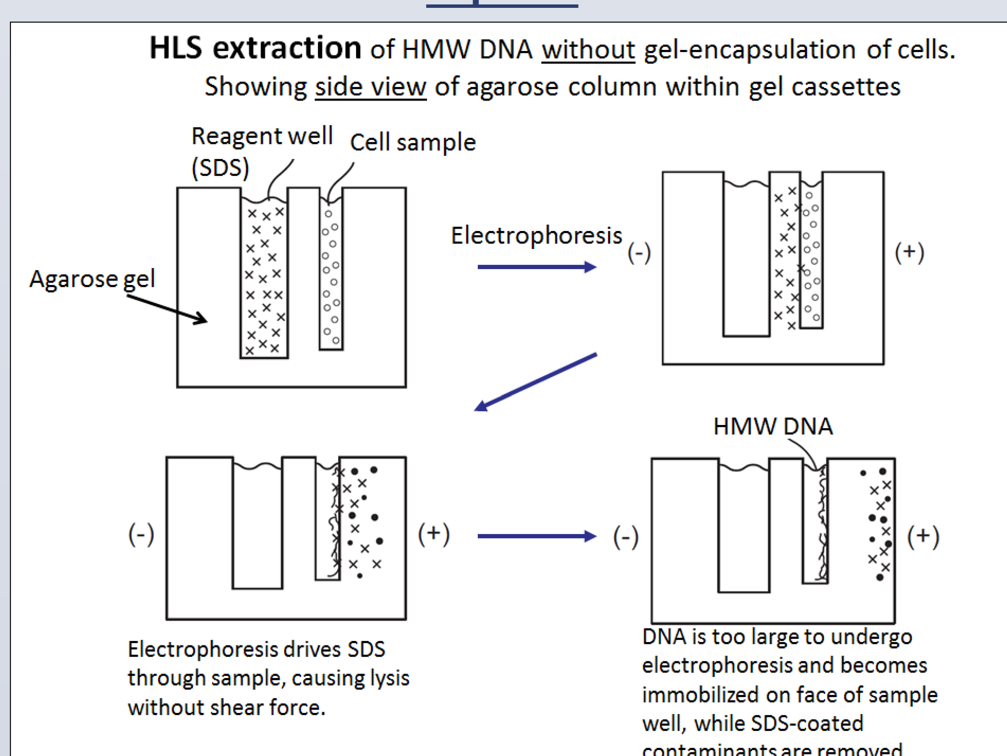
Targeted next-generation sequencing approaches have enabled a wide variety of genomic tests in the fields of oncology and inherited genetic disease. However, hybridization-based sequence capture methods have not yet been successfully applied to enrichment of long genomic DNA fragments, despite their potential benefits for long-read sequencing, optical mapping of single DNA molecules, and synthetic biology. The principal barriers to long molecule enrichment are (1) established methods of DNA purification can only produce molecules up to about 150kb in length, and (2) established enrichment methods require solution-phase capture of targeted DNA on microparticles, which can shear long molecules.

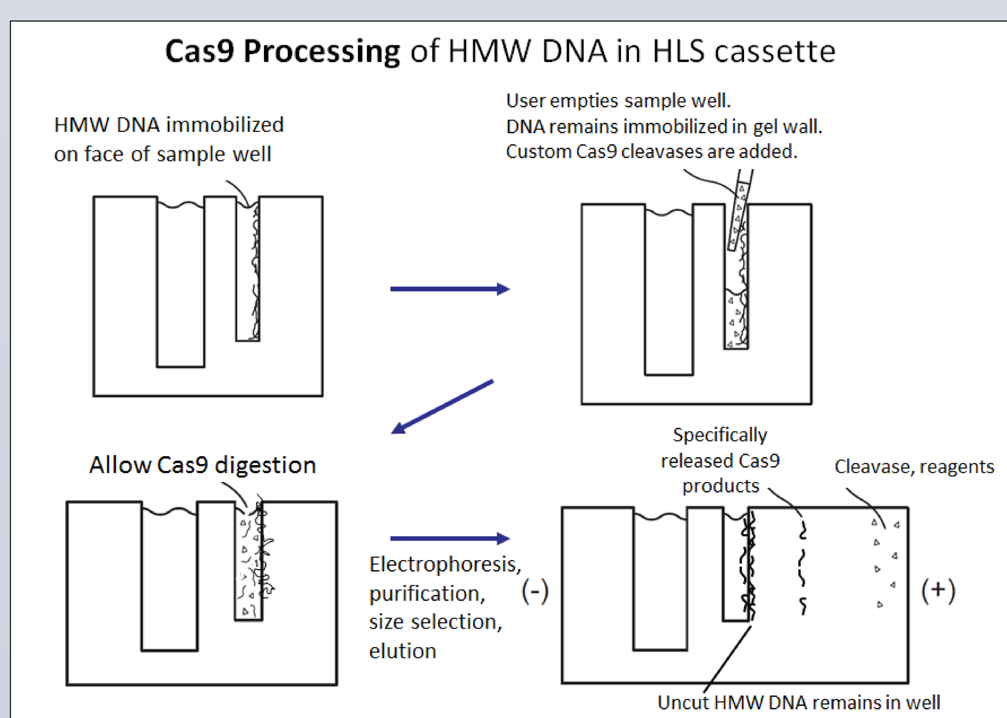
We present a new approach to long DNA enrichment, which utilizes CATCH (cas9-assisted targeting of chromosome segments, Jiang et al., Nature Communications, 2015, 6:8101, DOI: 10.1038/ncomms9101, www.nature.com/naturecommunications) in a novel sample preparation system (SageHLS System, Sage Science, Inc.), which enables integrated DNA purification and enzymatic processing of HMW DNA. Briefly, a suspension of intact cells is loaded into a disposable, gel-filled cassette. The cells are gently lysed using an electrophoretic process that leaves purified, large HMW genomic DNA molecules (>>10mb) immobilized in the sample well. Customized cas9-nucleases are used to cleave out the targeted genomic DNA fragments from the immobilized HMW DNA, and the cleaved fragments are electrophoretically separated and electroeluted into buffer-filled elution chambers for downstream analyses. We discuss progress on using the integrated CATCH-HLS process for targeted isolation of bacterial and human genomic fragments up to several hundred kb in length.

Principle of CATCH: Cas9-Targeted DNA Isolation



Principle of HLS-CATCH – Integrated HMW extraction & targeted capture

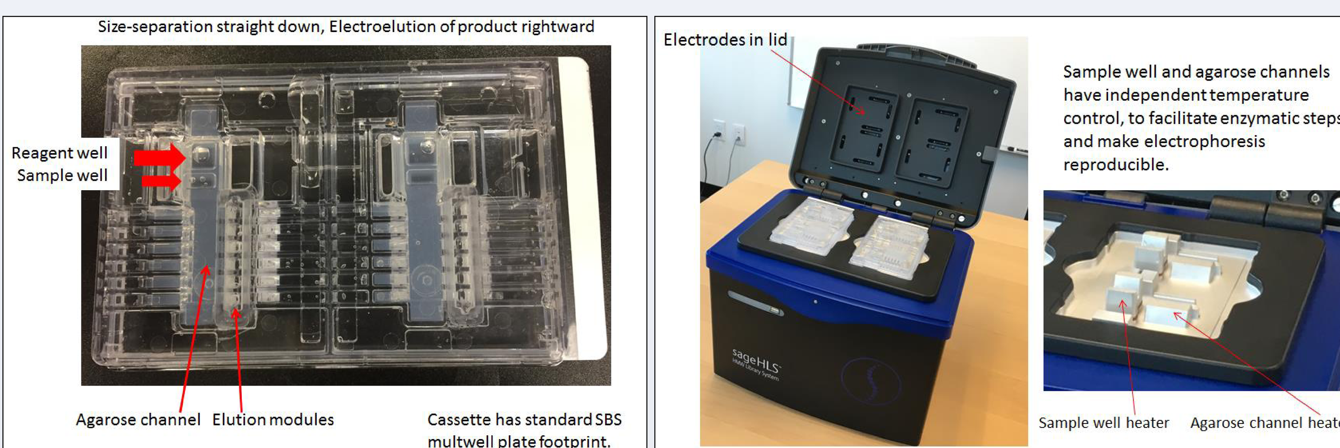




SageHLS System

Cassettes are based on a two-dimensional electrode geometry. Purification and size selection electrophoresis are carried out top to bottom (in this image), and final electroelution of product is carried out from left to right, into an array of buffer-filled elution modules on the right side of the gel column.

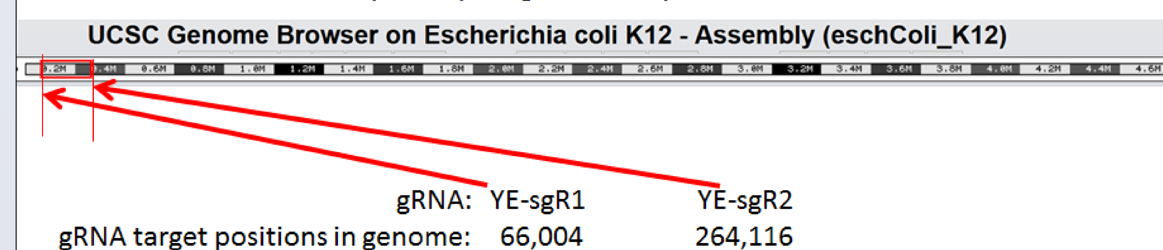
System capacity is 2 samples per cassette, 2 cassettes (4 samples) per instrument run, ~3-7hr run (depending on target size),



Example 1: Isolation of 200kb E.coli genomic fragment

Experimental overview:

1) E.coli spheroplasts were loaded into HLS cassette.
2) Genomic DNA was electrophoretically purified in HLS cassette.
3) Genomic DNA was digested in cassette with wt SpCas9 (NEB) assembled with gRNAs (IDT, Alt-R™) bordering a 198kb ROI (4.2% of genome).
4) DNA released by Cas9 digestion was size selected & electroeluted in HLS cassette, and analyzed by PF gel electrophoresis.

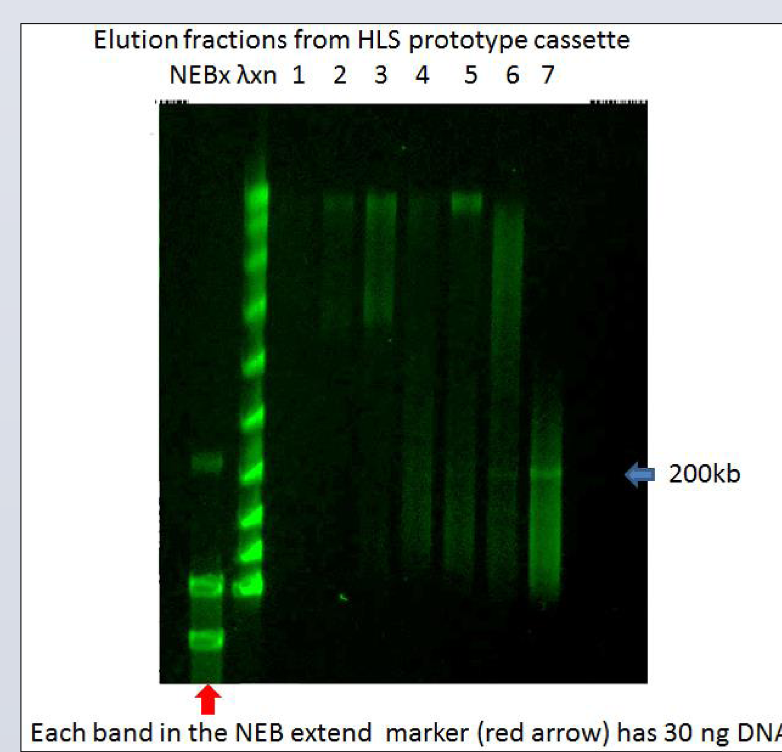


	gRNA: YE-sgR1	YE-sgR2
gRNA target positions in genome:	66,004	264,116

Two HLS-CATCH isolations were carried out. The output of the first isolation was analyzed by PF gel electrophoresis. The second one was analyzed by qPCR and Oxford Nanopore sequencing.

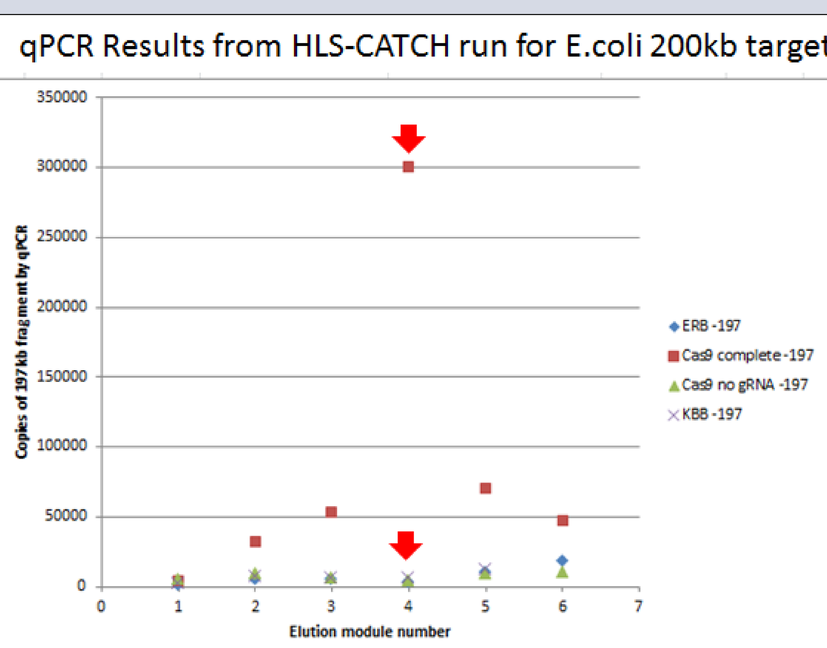
PF analytical gel of first isolation is shown below:

Elution fractions from HLS prototype cassette



Each band in the NEB extend marker (red arrow) has 30 ng DNA.

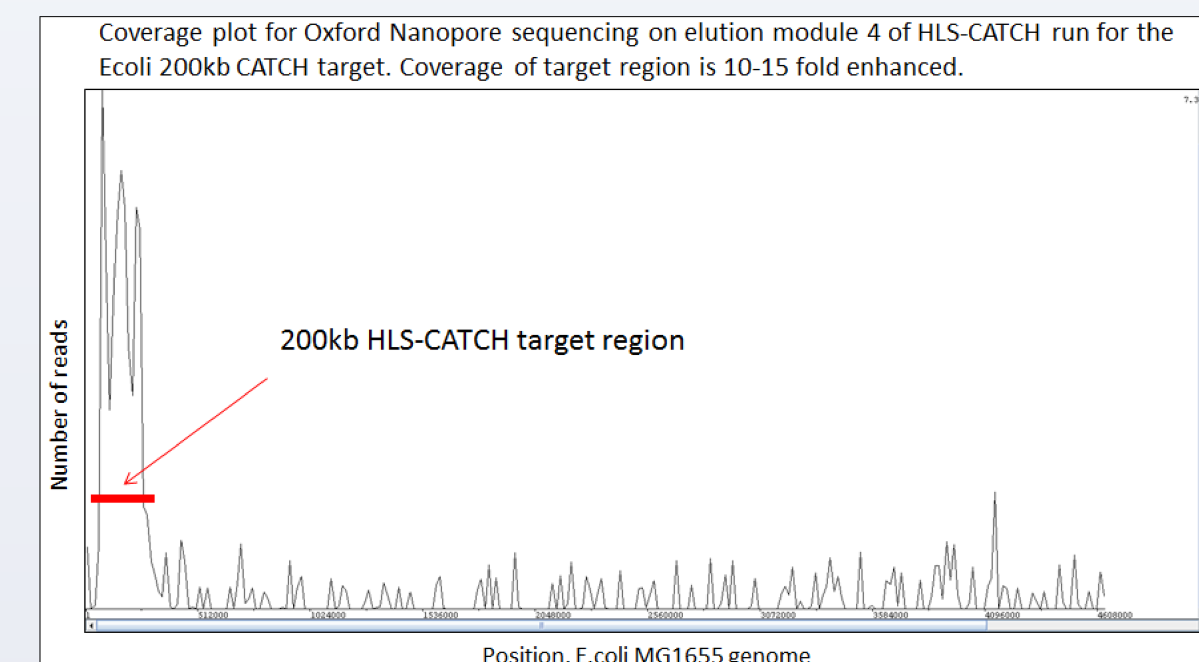
qPCR localization and quantification of target recovery on 2nd HLS-CATCH isolation.



"ERB" = mock digestion with ERB buffer, no cas9
"Cas9 complete" = digestion with cas9 + gRNAs 197kb-sgR1,R2
"Cas9 no gRNA" = digestion with Cas9 enzyme without any gRNA.
"KBB-197" = mock digestion with electrophoresis buffer only
(non-target qPCR results not shown similar to ERB controls)

Example 1: Isolation of 200kb E.coli fragment (cont.)

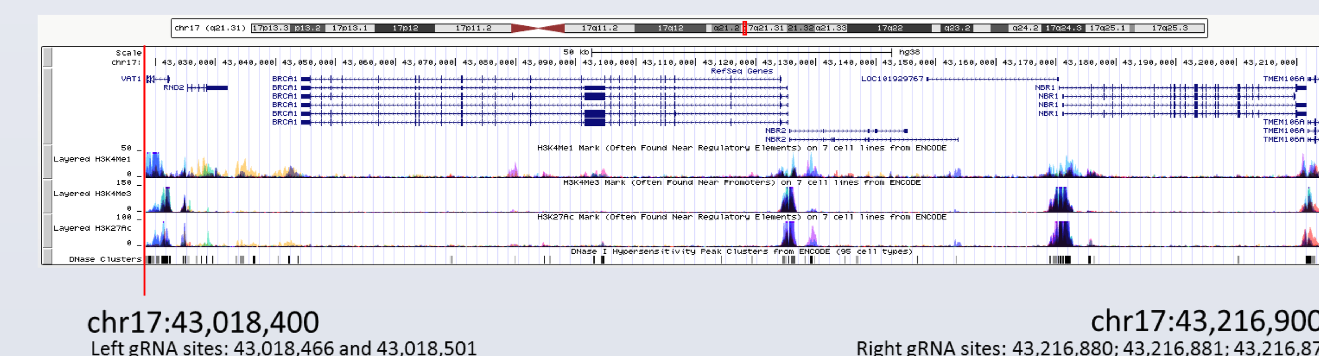
Oxford Nanopore Minion sequencing on product from elution module #4 of 2nd HLS-CATCH isolation.



Example 2. HLS-CATCH isolation of human 200kb BRCA1 region

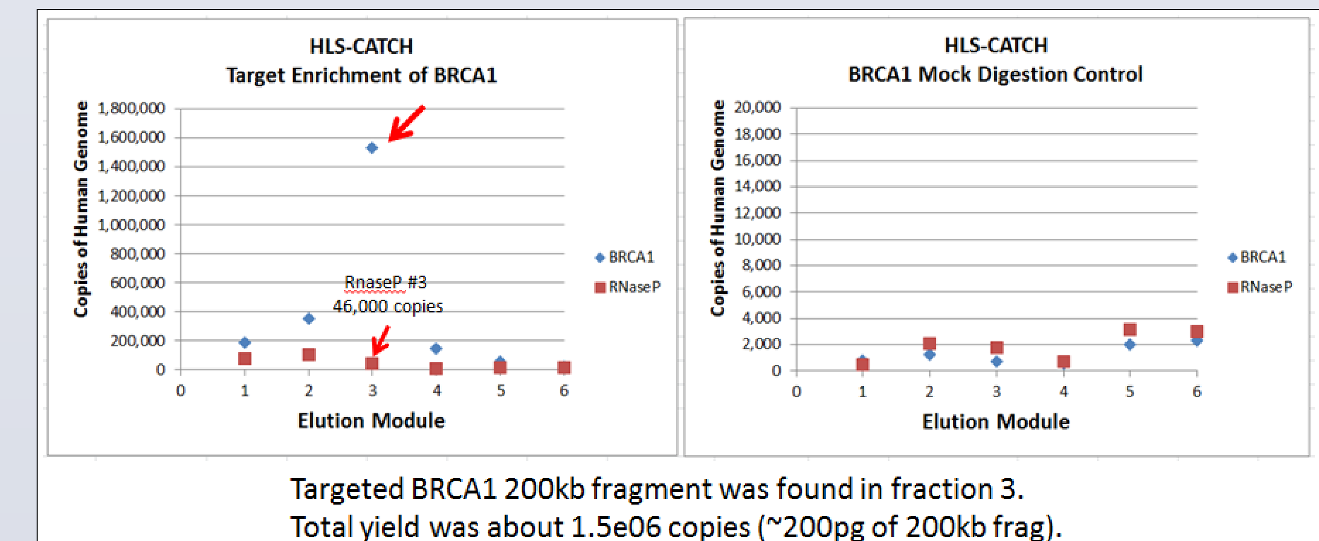
gRNAs for cut sites surrounding a 200kb region containing the human BRCA1 gene were designed and tested. A pool of five effective gRNAs were used in an HLS-CATCH experiment to excise the BRCA1 fragment from 1.5e06 Raji cells (input gDNA content about 10ug). The elution products were evaluated by qPCR (ABI Taqman kits, RNaseP gene as control).

hg38 map of BRCA1 region on Chr17, showing region targeted by gRNAs.



chr17:43,018,400 — Left gRNA sites: 43,018,466 and 43,018,501

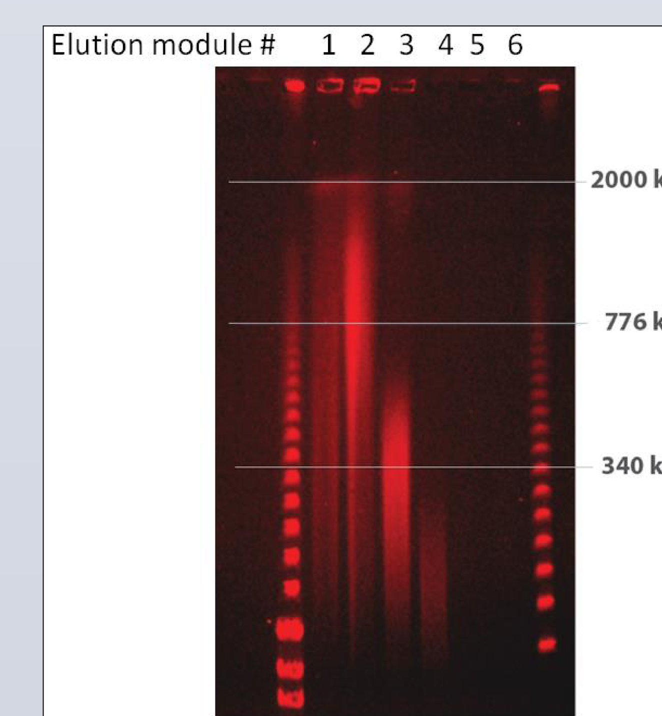
chr17:43,216,900 — Right gRNA sites: 43,216,880; 43,216,881; 43,216,877



Targeted BRCA1 200kb fragment was found in fraction 3.
Total yield was about 1.5e06 copies (~200pg of 200kb frag).

Conclusion

These data demonstrate that HLS-CATCH represents an efficient, specific method for targeted isolation of large genomic DNA fragments. It is likely to find applications in targeted optical mapping (Genomic Vision, Bionano Genomics), targeted long-read single molecule sequencing (PacBio, Oxford Nanopore, Genia); as well as targeted analyses of long-range phasing by linked-read sequencing (10X Genomics, seqWell LongBow [AGBT poster #303]), and digital droplet PCR (Drop-Phase). Previous work on using HLS for HMW DNA extraction (see right), demonstrates that the system can produce DNA up to low single mb's in size. **By extension, we think it will be possible to use HLS-CATCH for targeted recovery of mb-sized genomic targets.**



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