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 150 kb 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 molecule 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 (>30 mb) 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 modules on the right side of the gel column.

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

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.

Example 1: Isolation of 200kb E.coli genomic fragment (cont.)
Experimental overview:
1) E.coli superplasms were loaded into HLS cassette.
2) Genomic DNA was electrophoretically purified in HLS cassette.
3) Genomic DNA was digested in cassette with a pool of Cas9 (NEB) assembled with gRNAs (SageHLS System) (+/- 130 kb ROI (4-2% of genome)).
4) DNA released by Cas9 digestion was size selected & electroeluted in HLS cassette, and analyzed by PI gel electrophoresis.

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

gRNAs for cut sites surrounding a 200 kb 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 10 ug). The elution products were evaluated by qPCR (ABI Taqman kits, RNaseP gene as control).

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 (Ion 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 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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