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DNA SIZE SELECTION METHOD FOR LONG READ SEQUENCING

BENEFITS

- SIMPLE: modifies one step of the current protocol without major adjustment to liquid handling steps, ideal for automation.
- SENSITIVE: allows removal of fragments smaller than 7.5 kb and also tolerant to low inputs (i.e. <10ng/μL).
- COST-EFFECTIVE: does not require use of expensive reagents.
- VALIDATED: on PacBio sequencers and protocols.
- FLEXIBLE: tolerant to a wide range of input amounts.

PROBLEM

High-quality and complete reference genome assemblies are fundamental for the application of genomics to biology, disease, and biodiversity conservation. Long-read sequencing (LRS) technologies are essential for maximizing genome quality, however, to generate sufficient coverage for genome assembly, it is critical to maximise yields of high-quality long-read data derived from often limited amounts of DNA.

Current available technologies often don't allow the highest consensus accuracy and uniform coverage for reference quality genomes in humans, plants, animals and microbes. Often, post-size selection yield may be insufficient for onwards progression to sequencing. Similarly, other, bead-based, commercial methods for size selection have cutoffs (100-1000bp) which are of limited effectiveness for LRS.

SOLUTION

A LRS method that allows simple selection of fragments larger than 7.5 kb in length from a fragmented nucleic acid sample using paramagnetic Solid Phase Reversible Immobilization (SPRI) beads in a binding buffer.

The protocol accommodates the desire for a technology for size selection >10kb that is tolerant to a wide range of input amounts, does not add complexity to laboratory workflows, is amenable to automation and reliably recovers high yields of on-target library fragments.

APPLICATIONS

LRS and any protocols where size selection of the DNA fragments requires removal of fragments smaller than 7.5kb. Our method has already been optimised for PacBio protocols by replacing one step of the current workflow.

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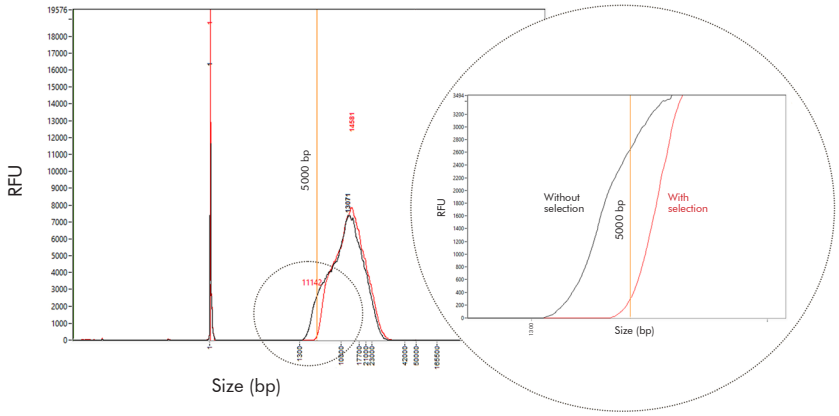
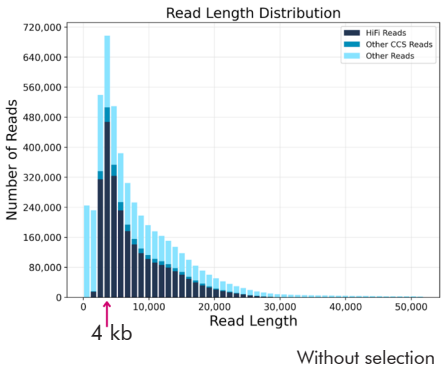
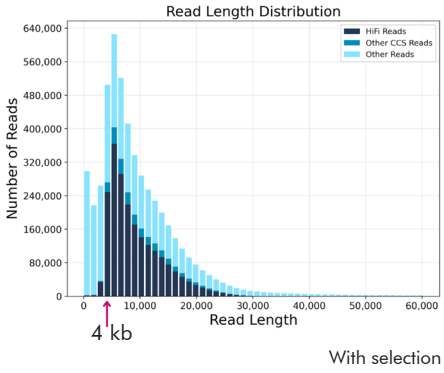
Size analysis of sheared gDNA from ladybird taken through Pacbio TPK 2.0 kit library construction without and with size selection.



Read length distribution analysis following sequencing of ladybird gDNA nucleic acid library generated without and with our size selection method.



Without selection



With selection

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INTELLECTUAL PROPERTY

Invention attributed to Dr Naomi Park. Priority patent applications filed in UK. The Wellcome Sanger Institute is offering license to the IP.