Long-Read Low-Pass Sequencing for Next-Generation Breeding

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Long-read low-pass (LRLP) sequencing-based genotyping offers an abundance of benefits for plant and animal breeding that short-read sequencing cannot achieve including improved mapping resolution and identification of important large structural variants (SVs). LRLP sequencing has been held back by the lack of high-throughput protocols and reagents available. Recent developments by PacBio and SeqWell are making high-throughput DNA extraction, shearing and library preparation possible.

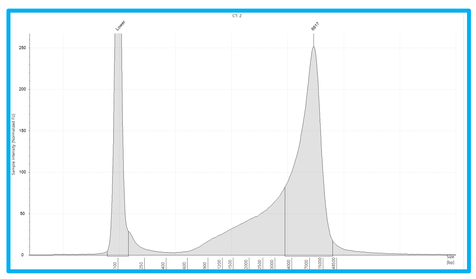
Comparison of Long-Read Low-Pass Sample Preparation Methods

DNA Extraction with PacBio Buffers and Protocol
Up to 96 at a time, 8 hours

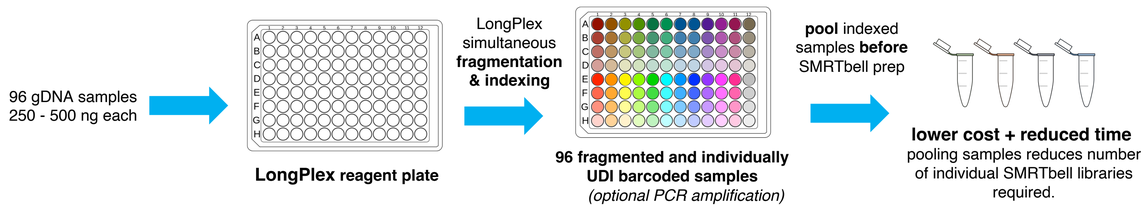
SRE
2 hours

SeqWell LongPlex Workflow

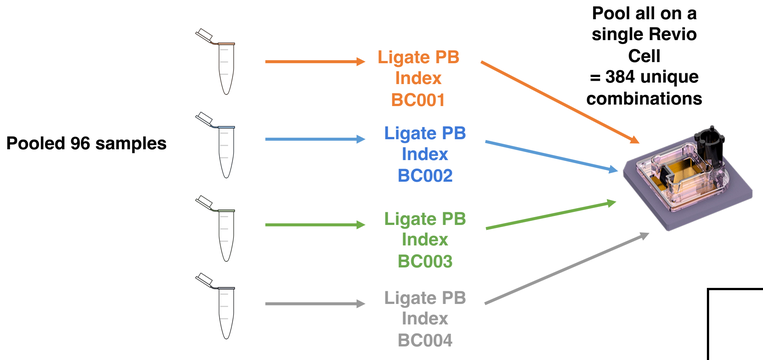
1. Fragments and barcodes 96 samples ~ 5 hours with amplification



Average size → 8 kb



2. SMRTBell Library Prep ~ 5 hours

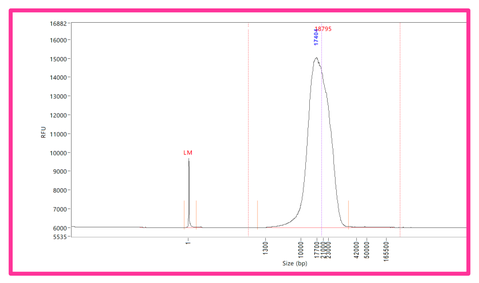


Best Use:
- Small genomes
- Ultra-low coverage

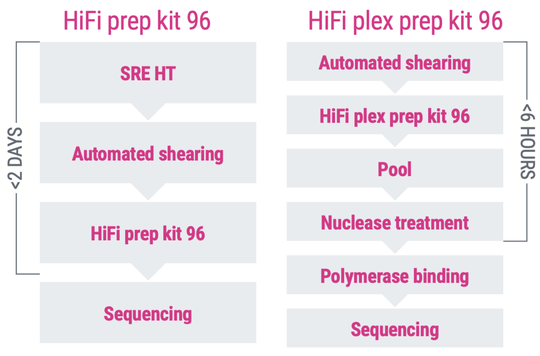
PacBio Workflow

1. Shear samples using Hamilton MicroLab Prep- 2 hours

Average size → 15-20 kb



2. HifiPlex Library Prep or HiFi Prep Kit with subsequent pooling ~ 5 hours



Best Use:
- Larger genomes
- More in-depth analysis

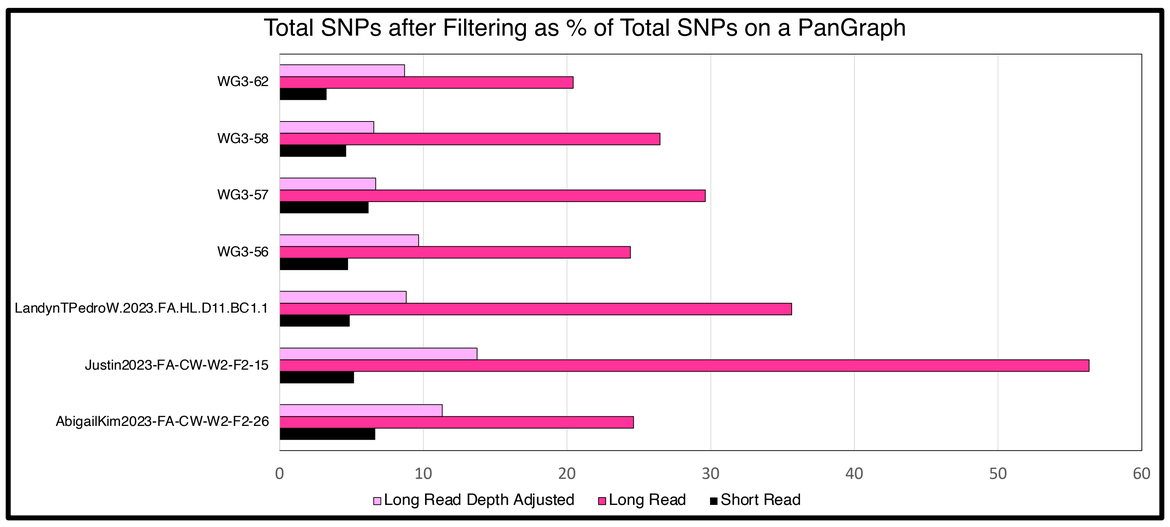
Pool up to 24 samples

Sequencing Results

	Average bps per sample	Avg Median Read Length per sample	Total bp (one revio cell)	Q-Score
LongPlex	10,526,225,753	5,504 bp	63,157,354,520	> 39
HifiPlex	10,716,691,634	1,4394 bp	75,016,841,438	> 38

Long vs Short Read SNPs- PanGraph

- Short and Long reads were both mapped to the same pangraph
- Long reads were down-sampled to match the depth of the short reads



See poster **#603** for more info on how LRLP captures variants

Thank you
SeqWell & PacBio for early access to reagents, protocols, support and materials.