Ultra-high throughput multi-omic analysis for agrigenomics on PacBio Revio system

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Introduction

Climate change and the rapidly growing global population are increasingly straining world food production.

Long read sequencing is being used in large-scale agricultural initiatives to help improve crop yields and combat pests and disease to meet increased agricultural demand.

The PacBio Revio system will enable researchers to meet the throughput demands for agrigenomics while maintaining best-in-class quality.

- **High-throughput** → 1000s of plant genomes per year
- **Low-cost** → ~$10/Gb
- **High-quality** → >90% of bases ≥Q30

To complement the potential of Revio, a high-throughput end to end workflow was developed and validated.

Plant DNA extraction challenges

Extraction of high-quality DNA from plants is often time consuming and difficult due to:

- Challenges with tissue and cell wall disruption
- Presence of nucleases, polysaccharides, and secondary metabolites
- Variable sample age and quality

Manual protocols typically utilize liquid nitrogen disruption, nuclei isolation, and CTAB precipitation which are not amenable to high-throughput applications.

High-throughput workflow

Corteva and PacBio collaborated to develop an end-to-end workflow for generating and processing thousands of HiFi libraries per year for plants.

	Traditional: nuclei isolation	High-throughput: direct tissue
Tissue requirement	0.5-5 g	20-200 mg
gDNA yield	2-20 µg	2-20 µg
Time requirement	6 h	<2 h
# samples in parallel	1	96+

Corteva/PacBio high-throughput workflow

Tissue disruption
DNA extraction
DNA shearing
Library prep
Size selection
Sequencing
Analysis

High-throughput tissue disruption

A high-throughput tissue disruption method was developed to enable processing of samples in a 96-well plate format.

- Protocols were developed for the SPEX Geno/Grinder, MP Biomedicals FastPrep-96, and SPEX MiniG
- All three instruments performed equivalently
- Able to disrupt 96 plant samples <10 min
- The same equipment can be utilized for DNA shearing

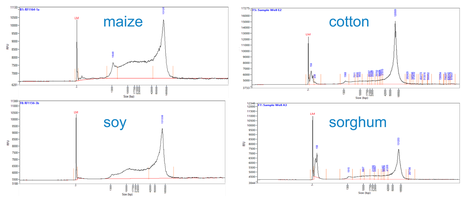
High-throughput DNA extraction

DNA extraction was automated on the Kingfisher DNA purification systems with PacBio developed DNA extraction reagents and protocols.

Sample type	Input mass (mg)	DNA yield (µg)	DNA mode size (bp)
Maize	40	3.0-4.3	120,333
Soy	40	4.2-4.6	129,366
Pepper	50	3.7-4.4	151,137
Wheat	50	13.4-20.3	163,106
Cotton	40	0.6-3.6	120,509
Sorghum	40	0.9-2.0	131,253
Sunflower	40	0.9-2.1	127,224

Leaf tissue from several different plant types was disrupted using the SPEX Geno/Grinder and extracted using this protocol.

- DNA mode size as measured by Femto Pulse is >100 kb for all plants – example electropherograms are shown below



A plate of 96 maize samples was extracted to demonstrate the fully automated HT workflow. Input was ~40 mg of tissue per well.

- Average DNA recovery was 2.0 ± 0.8 µg
- >10% of samples yielded >1 µg of DNA
- Most of the lower yielding samples were a result of lower tissue input

DNA recovery (µg)

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.4*	2.1	4.4	3.0	0.9	1.0	1.9	2.5	1.9	2.8	0.7**	2.5
B	2.2	1.2	1.2	1.8	2.3	1.5	1.9	1.0	0.7*	1.8	3.0	1.5
C	3.0	2.0	1.3	2.7	2.0	0.5**	1.3	1.0	1.9	2.4	2.0	1.6
D	2.4	3.2	2.7	1.7	2.1	0.8**	1.4	1.9	1.2	1.6	1.9	1.6
E	2.8	2.7	2.4	2.6	1.9	1.2	2.6	2.4	1.0	2.1	2.0	1.9
F	2.8	2.1	2.2	3.0	2.3	1.5	2.3	3.0	0.8**	1.5	2.0	1.2
G	2.1	0.9	1.0	2.1	1.7	1.3	1.4	3.0	3.6	1.6	2.3	2.3
H	0.6*	2.6	2.3	3.4	2.7	3.3	2.9	2.8	3.9	0.1**	3.7	2.3

*low yield due to bullet tube failure
**low yield due to lower tissue input

High-throughput DNA shearing

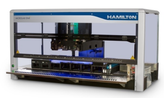
Two high-throughput shearing methods were developed.

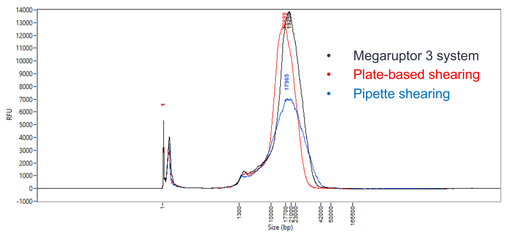
Plate-based shearing

- Protocols developed for FastPrep-96, Geno/Grinder, and MiniG
- Process 96 samples in <10 min
- Same equipment as used for tissue disruption

Pipette shearing

- Protocols developed for Hamilton NGS Star and Hamilton NIMBUS but are easily adaptable to other liquid handlers
- Process 96 samples in 15 min
- Requires only standard 300 µL pipette tips
- Can be incorporated into automated library prep



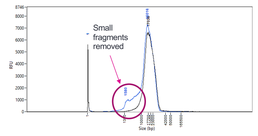


Maize DNA sheared using the two high-throughput shearing methods produced similar size distributions as DNA sheared with the Megaruptor 3 system.

Automated library preparation and size-selection

Library preparation, utilizing the SMRTbell prep kit 3.0, was automated onto a Hamilton NGS star.

- A new SMRTbell size selection wash was developed to improve removal of small fragments



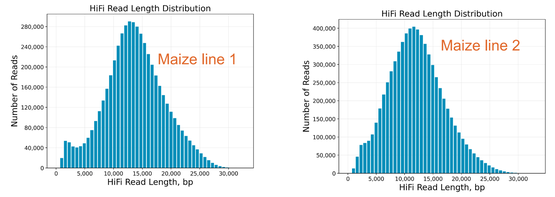
Sequencing on PacBio Revio system

Two maize lines were processed using the Corteva/PacBio high-throughput sample prep workflow and sequenced on the PacBio Revio system.

Methods

- Disruption on SPEX Geno/Grinder
- Extraction on KingFisher Apex
- DNA shearing on Hamilton NIMBUS
- Library preparation with SMRTbell prep kit 3.0
- SMRTbell size-selection wash
- 24 hour sequencing movie

Sample	Mean HiFi read length (bp)	HiFi yield (Gb)
Maize line 1	12,064	86.8
Maize line 2	13,662	70.9



Comparable HiFi read length distributions were obtained for the two maize lines.

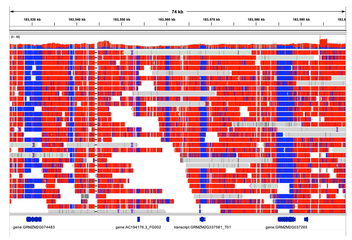
Sample	Assembly size (Gb)	Contig N50 (Mb)	Contig N90 (Mb)
Maize line 2	2.23	162.6	16.6

Assembly was performed on maize line 2 using hifiasm.

Methylation analysis

Revio performs 5 base sequencing (A, C, G, T + 5mC) by default with on-instrument basecalling and Google DeepConsensus. A representative region of the genome is shown.

- Red indicates hypermethylation
- Blue indicates hypomethylation
- Expressed genes indicated by blue, unmethylated regions



High-throughput capabilities

The Corteva/PacBio high-throughput workflow described has been validated by simultaneous processing of 96 plant samples with multiplexed sequencing on Revio. (Data not show)

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