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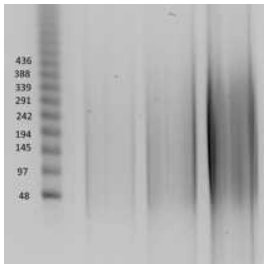
High molecular weight DNA extraction from plant nuclei isolation optimised for long-read sequencing



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Keywords: High molecular DNA plant nuclei Nanopore, high molecular weight dna extraction from plant, high molecular weight dna extraction, dna extraction, isolated plant nuclei, high molecular weight dna, lsk109 libraries for nanopore, nuclei isolation, dna, extraction, gb with read lenghts n50 value, nanopore, nuclei, read lenghts n50 value, lsk109 library, sequencing

Abstract

High molecular weight DNA was extracted from isolated plant nuclei and used to prepare RAD004 and LSK109 libraries for Nanopore sequencing. Typically, flow cells yielded 3-15 Gb with read lenghts N50 values ranging from 15 to 29 Kb and longest reads > 380 Kb.

Guidelines

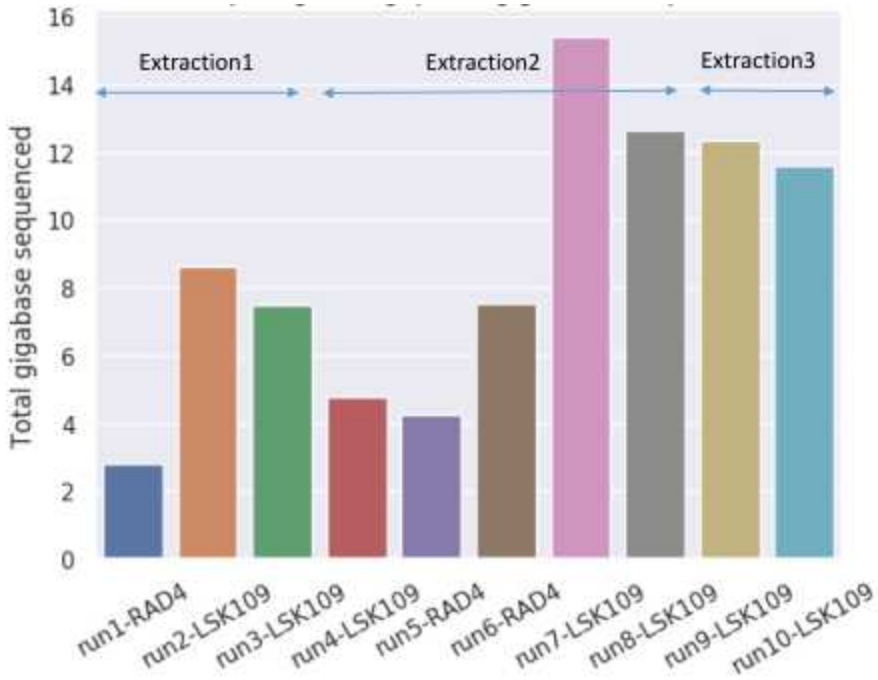
Pearl millet DNA extracts used for ONT-sequencing

	Pearl millet Extraction1	Pearl millet Extraction2	Pearl millet Extraction3
[cubit] ng/µl	58.9	305	289
[nanodrop] ng/µl	54	401	
260/280	1.84	1.88	
260/230	1.85	2.08	
PFGE estimated size in Kb	25-250	20-350	20-350

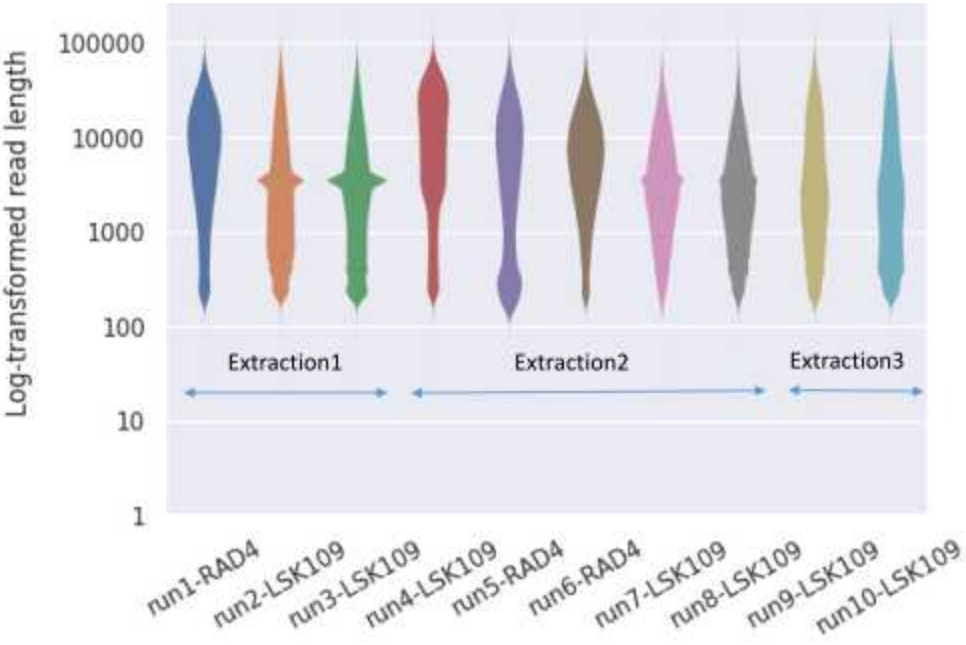
ONT sequencing results (Pennisetum glaucum).

	run1	run2	run3	run4	run5	run6	run7	run8	run9	run10
kit	RAD4 (1,5)	LSK109	LSK109	LSK109	RAD4 (2.0)	RAD4 (2.5)	LSK109	LSK109	LSK109	LSK109
Flow Cell ID	FAK49743	FAH71245	FAK46121	FAK49695	FAK45911	FAK49708	FAK46326	FAK45911	FAK60500	FAK57015
DNA	1	1	1	2	2	2	2	2	3	3
Starting DNA µg in Lib.	500.65	1650	1767	2745	1220	2290	3965	4000	3760	3760
Q DNA load	500.65	585	537	882	1220	2290	991.5	2067	2990	1379
Mean read length	10142.9	6035.4	5842.1	13861	8856.5	8827.3	6068.1	5058.2	7719.7	7735.8
Mean read quality	10.1	9.4	10.8	10.6	10.1	10.6	10.3	10	10.3	10.6
Median read length	5955	2107	2708	7487	3991	5318	2926	2321	2617	2190
Median read quality	10.6	9.8	11.1	11	10.5	11	10.7	10.3	10.6	11
Number of reads	276142	1428768	1280566	344462	479814	850664	2537834	2500427	1600302	1499957
Read length N50	19983	18393	14965	28983	20917	16330	13461	11556	21853	26100
Total bases	2800888747	8623147185	7481254762	4774600288	4249495402	7509067189	15399942443	12647699542	12353860944	11603427372

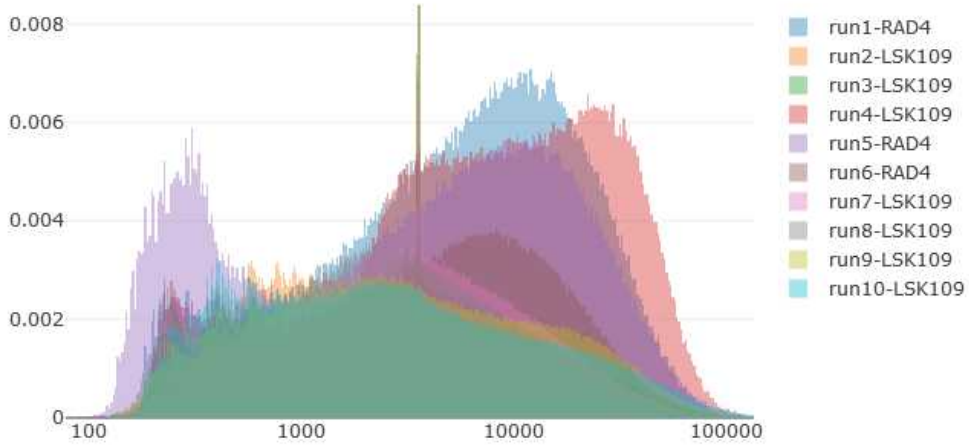
10 flowcells were loaded : 3 libraries prepared with the RAD4 kit and 7 librairies with the kit LSK109



Comparing throughput in gigabases sequenced



Comparing log length



Normalized histogram of log transformed read lengths.

Materials

Reagents and solutions

stock solution are identical or similar as in protocol:
www.genome.arizona.edu/modules/publisher/item.php?itemid=24

10x homogenization buffer (HB) stock (can be stored at 4°C):0.1 M Trizma base, 0.8 M KCl, 0.1 M EDTA, 10 mM spermidine, 10 mM spermine, final pH 9.35 adjusted with NaOH.

Stock250 ml
Trizma base 1M25 ml
KCl (74)14.8 g
EDTA 0.5 M50 ml
Spermidine (145.2)0.36 g
Spermine (202.3)0.5 g

1x S-HB (can be stored at 4°C):1x HB with1M sorbitol
Volume final500 ml
10x HB50 ml
Sorbitol (182.17)91.08 g

1x ST-HB (can be stored at 4°C): 1x HB containing 1M sorbitol and 20% Triton X-100
Stock100 ml
10x HB10 ml
Triton X-I0020 ml
Sorbitol18.21 g
QSP 100 ml using H2O

Wash Buffer (WB): Add b-mercaptoethanol just before use
Stock100 ml
1x ST-HB2.5 ml
b-mercaptoethanol 150 µl
QSP 100 ml using **1x S-HB**

MATAB Lysis Buffer (can be stored after autoclave):
Stock[Final]100 ml
Tris 1M (pH7,5)100 mM10 ml
NaCl 2.5 M1,4 M56 ml
EDTA 0,5 M (pH8)20 mM4 ml
PEG60001 g
H_2O up Qsp 100 ml

Add 2% MATAB just before use (2g. for 100 ml)

1 **Plant material sampling** 30m

Collect 0.7 g to 1 g of fresh tissue in a 50 ml tube containing liquid nitrogen.
Grind in a frozen mortar (stored at -80 °C) using a pestle. (Try to avoid mixing tissues with different developmental stages since it might affect DNA yield and quality)

2 **Homogenization of fresh tissue** 30m

Transfer powder into an ice-cold tube (50 ml) containing 25 mL ice-cold WB
Filter the homogenate into an ice-cold 50 ml centrifuge bottle through one layer of Scrynel Polyester filter of 25 µm mesh.

Collect the remaining nuclei by squeezing the homogenate with gloved hands.

Complete filtrate lysing using a DOUNCE first with loose fitting (A, 10 times) and, then, with a tighter one (B, 10 times)

3 **Nuclei extraction** 45m

Pellet the homogenate by centrifugation with a fixed-angle rotor at 2500 x g and 4 °C for 00:20:00 (to be ajusted depending on species)
Discard the supernatant and add 1 mL of ice-cold WB
Gently resuspend the pellet (you may use a small paint brush) and add up to 25 mL of ice-cold WB.
Centrifuge 00:15:00 at 2500 x g and 4 °C in a swinging bucket centrifuge.
Discard supernatant and resuspend pelleted nuclei in 5 mL MATAB lysis buffer supplemented with 17 µL spermine; 17 µL spermidine, and; 20 µL DTT.

4 **Nuclei lysis and DNA purification** 5h

After gentle homogenization, incubate at 65 °C for 01:00:00 .
Mix once with extreme precaution.
Add 10 µl of proteinase K (20 mg/ml; eg from Terra PCR Kit, Clonectec) and transfer 55 °C for 01:45:00 .
Pour gently onto a 15 ml tube.
Add 5 mL of 24 :1 chloroform/isoamylalcool.
Agitate horizontally at 5 rpm for 00:10:00 .

Centrifuge 00:20:00 at 5500 x g and 15 °C .
Pour the supernatant into a clean 15 ml tube.
Add 10 µL RNase A (Promega, 1mg/ml).
Mix gently by inversion and incubate for 00:30:00 at RT.
Add 5 mL of 24 :1 chloroform/isoamylalcool and agitate horizontally under chemical hood at 5 rpm for 00:10:00 .
Centrifuge 00:20:00 at 5500 x g and 15°C. Pour the supernatant into a clean 15 ml tube.

(2-3 chloroform extractions in total; if the second extraction is clean enough, the last one can be skipped)

5 DNA precipitation

45m

Add 500 µl of 3M NaOAc and 4 ml of isopropanol at room temperature.
Agitate horizontally at 5 rpm until DNA precipitates (medusa).
Glass hook DNA and transfer into 10 ml 70% EtOH.
Glass hook DNA and transfer into a 2ml-tube with 2 ml of 70% EtOH.
Centrifuge 10 min at 10000g and discard supernatant. •
Dry DNA at 45°C for 15 min.
Resuspend in 100µl of sterile H2O.

6 **Clean up DNA** 15m

Removing contaminants (spermidine, spermine) is mandatory for DNA library preparation using both RAD004 and LSK109 procedures.
Purification was performed using 1X vol/vol AMPure, followed by two 70% ethanol precipation.
DNA was resuspended in 50µl DNAse-free water, and the volume was adjusted to reach a DNA concentration of 300-400 ng/µl.

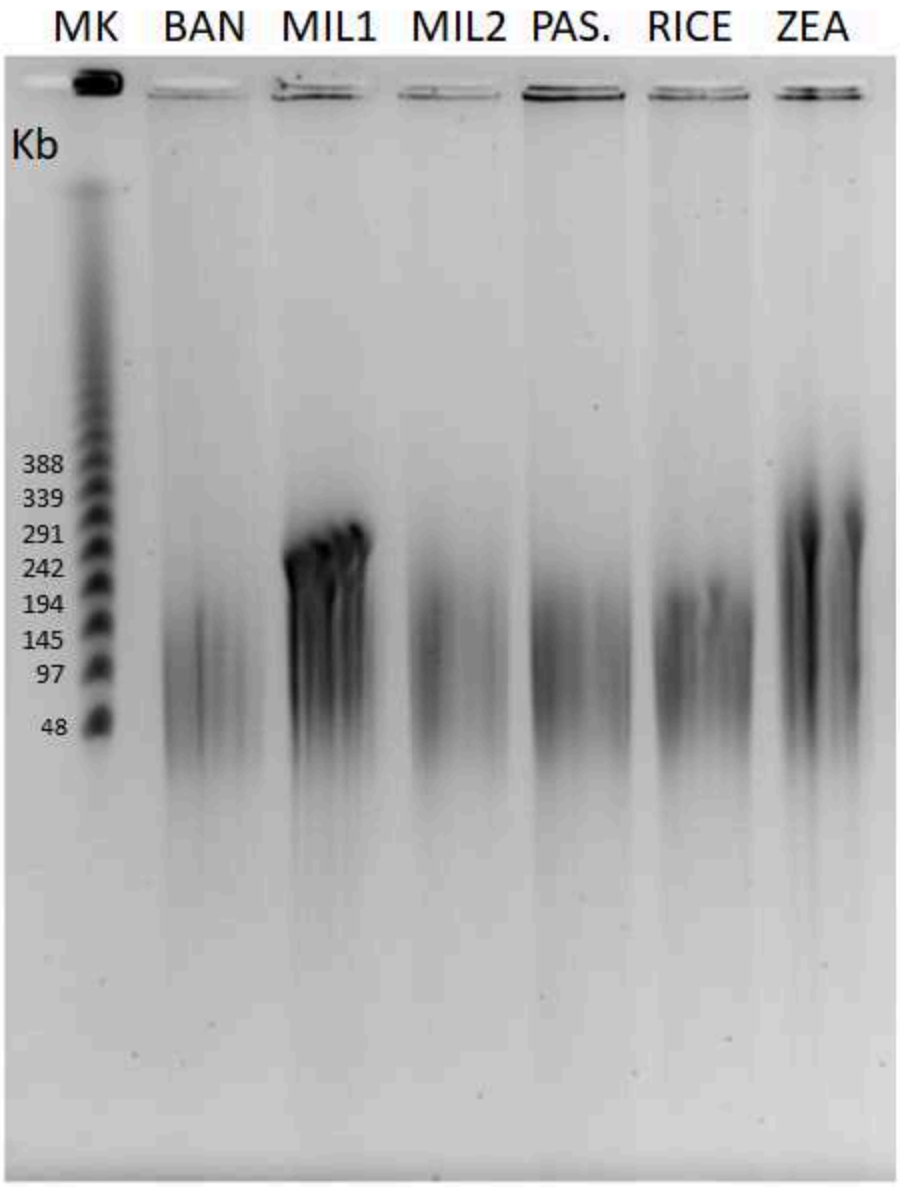
7 **DNA quantification** 15m

DNA was quantified using NanoDrop and Qubit (expected Nano/Qubit ratio < 2).

8 **Check HMW using Pulsed-Field Gel Electrophoresis**

Finally, molecular weight of extracted DNA was assessed by PFGE by loading 20 µl of DNA (1.5 to 5.5 µg of DNA, 5µl of 6x loading buffer) into 1% agarose gel and using the following parameters for migration:

Pulse time : initial = 5, final = 117
Run time = 20.5 h
V/cm = 5
Angle = 120
Temp = 14°
mA end of run = 255


MK
BAN
MIL1
MIL2
PAS.
RICE
ZEA
Kb
388
339
291
242
194
145
97
48