Promega

Product Application

DNA Size-Selection Before Oxford Nanopore Sequencing

Eliminate small (≤ 3-4kb) DNA fragments prior to sequencing using the ProNex® Size-Selective Purification System.

Kit: ProNex® Size-Selective Purification System (Cat.# NG2001)

Analyses:

- Pulsed-Field Gel Electrophoresis (PFGE)
- Oxford Nanopore Sequencing

Sample Types: Purified DNA

Materials Required:

- ProNex® Size-Selective Purification System (Cat.# NG2001)
- QuantiFluor® ONE dsDNA System (Cat.# E4871) or similar
- Quantus™ Fluorometer (Cat.# E5160) or similar
- Ligation Sequencing Kit (Oxford Nanopore Technologies®, Cat.# SQK-LSK109)
- NEBNext® Companion Module for Oxford Nanopore Technologies® Ligation Sequencing (New England Biolabs®, Cat.# E7180S)
- Flow Cell Priming Kit (Oxford Nanopore Technologies®, Cat.# EXP-FLP002)
- Spot-ON Flow Cell, R9 (Oxford Nanopore Technologies®, Cat.# FLO-MIN106D)
- MinION (Oxford Nanopore Technologies®, Cat.# MIN-101B)
- DynaMag™-2 (Invitrogen, Cat.# 12321D) or similar
- Thermal cycler

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM508, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Protocol:

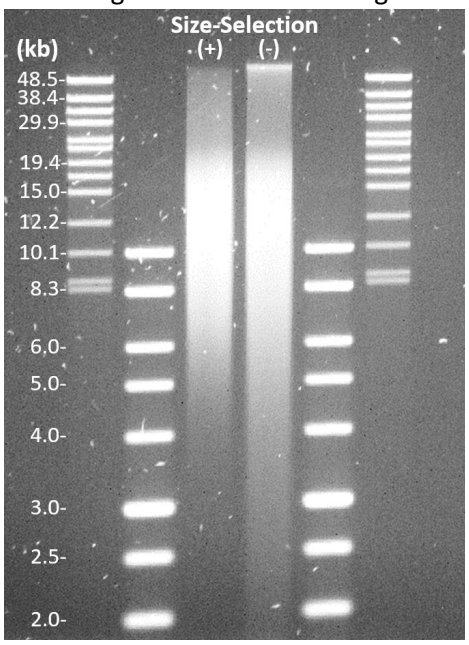
Naturally, DNA is lost during size-selection. The amount (mass) lost will depend on the percentage of sample DNA ≤ 3-4kb. For this reason, it is advised to input at least 2-3X the mass of required DNA.

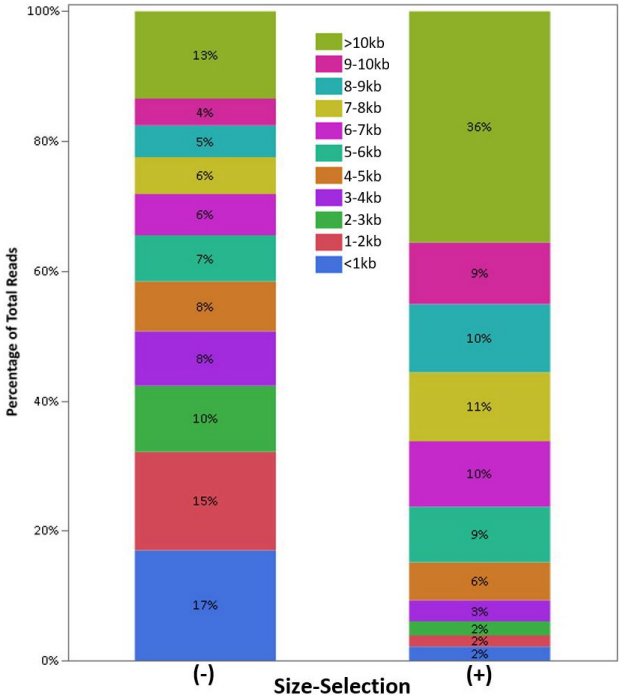
1. Follow the standard protocol for size-selection of DNA found in TM508, sections 5 and 6.A.
2. To eliminate DNA fragments ≤ 3-4kb, use a 0.9:1 ratio of ProNex® Chemistry to DNA sample. For instance, add 45µl of ProNex® Chemistry to a 50µl DNA sample.
3. After size-selection, continue with library preparation and sequencing according to the standard Oxford Nanopore Technologies® protocol "Genomic DNA by Ligation (SQK-LSK109)[1]". As an alternative to using AMPure XP beads for the cleanup steps, the ProNex® Size-Selective Purification System may also be used according to the AppNote "ProNex® Chemistry based clean-up in the Oxford Nanopore Ligation Sequencing Kit and Native Barcoding Expansion Kit[2]" following steps 1 and 3.

Results:

The ProNex® Size-Selective Purification System was used (according to the protocol provided here) to remove DNA fragments ≤ 3-4kb from a sample of hemp DNA purified using the Maxwell® RSC Purefood GMO and Authentication Kit[3]. DNA was prepared using the Oxford Nanopore Technologies® Ligation Sequencing Kit and sequenced using the MinION Sequencing Device, before and after size-selection. Mean sequence length, median read length, and N50 read length increased following size-selection.

QC Metric	Size-Selection	
	(-)	(+)
Time Sequenced	72h	~10h
Total Yield (Gb)	18.53	4.88
Reads Analyzed	3,649,962	536,577
Mean Quality Score	9.9	9.8
Median Quality Score	9.9	9.8
Mean Seq Length	5,078	9,100
Median Read Length	3,911	8,526
Maximum Read Length	215,204	122,799
N50 Read Length	8,222	10,416





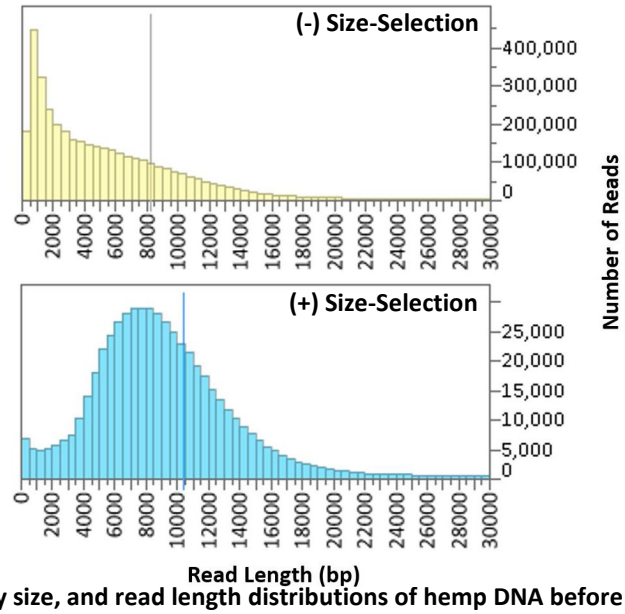


Figure 1. QC metrics, PFGE image, percentage of reads by size, and read length distributions of hemp DNA before and after size-selection using the ProNex® Size-Selective Purification System. DNA was size-selected according to the protocol provided here. DNA was sequenced before (-) and after (+) size-selection using the MinION Sequencing Device. **Top left:** Sequencing QC metrics. **Top right:** Pulsed-field gel electrophoresis (PFGE) image of hemp DNA. **Bottom left:** Percentage of sequencing reads by size. **Bottom right:** Sequencing read length distributions (N50 read length is indicated by a vertical line).

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The ProNex® Size-Selective Purification System was used (according to the protocol provided here) to remove DNA fragments ≤ 3-4kb from a sample of tomato leaf DNA purified using the Wizard® HMW DNA Extraction Kit. DNA was prepared using the Oxford Nanopore Technologies® Ligation Sequencing Kit and sequenced using the MinION Sequencing Device, before and after size-selection. Mean sequence length, median read length, and N50 read length increased following size-selection.

QC Metric	Size-Selection	
	(-)	(+)
Total Yield (Gb)	11.09	16.0
Reads Analyzed	8,350,643	3,507,993
Mean Quality Score	10.5	9.9
Mean Seq Length	1328	4561
Median Read Length	570	2855
Maximum Read Length	291,918	220,916
N50 Read Length	2,878	7,433

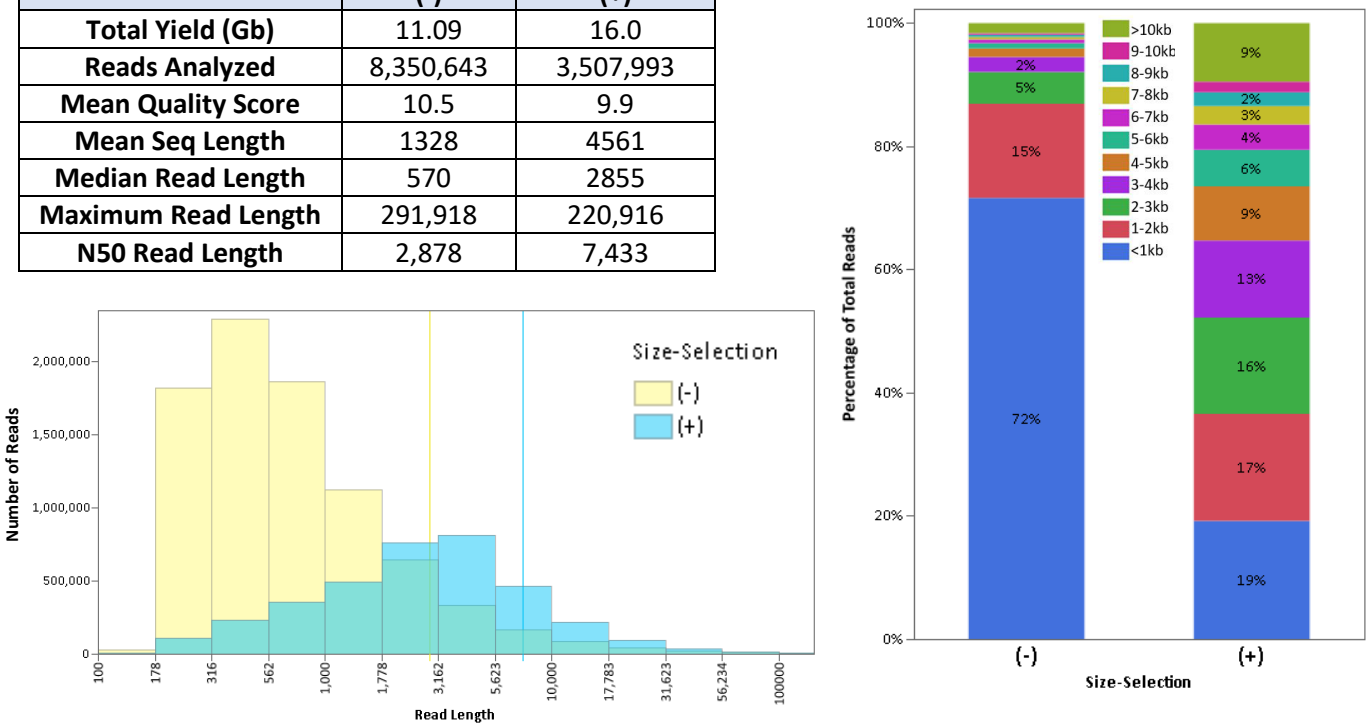

Figure 2. QC metrics, percentage of reads by size, and read length distributions of tomato leaf DNA before and after size-selection using the ProNex® Size-Selective Purification System. DNA was size-selected according to the protocol provided here. DNA was sequenced before (-) and after (+) size-selection using the MinION Sequencing Device. **Top left:** Sequencing QC metrics. **Right:** Percentage of sequencing reads by size. **Bottom left:** Sequencing read length distributions (N50 read length is indicated by a vertical line).

References:

1. Oxford Nanopore Genomic DNA by Ligation (SQK-LSK109) Protocol. Version: GDE_9063_v109_revT_14Aug2019. Last update: 04/03/2020.

2. Applications Note PA411: ProNex® Chemistry based clean-up in the Oxford Nanopore Ligation Sequencing Kit and Native Barcoding Expansion Kit.

3. Applications Note PA188: Automated DNA Purification from Hemp.