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Size-selection of sheared nematode DNA (~20 kb length) in a SPRI clean-up

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We use this protocol and it's working

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Abstract

This protocol describes the SPRI clean-up with size selection of sheared nematode DNA. After this SPRI clean-up the amount of low-molecular weight DNA (> 10 kb) will be significantly reduced. After QC the DNA can be used for low-input HiFi library preparation.

Guidelines

Make sure to pipette accurately for the sample and the beads.
Make sure to keep the supernatant after the initial bead incubation until you are sure, you got your DNA back.

Materials

- Magnetic rack for 1.5 and 2.0 ml tubes
- ProNex Size-Selective Chemistry (magnetic beads) (Promega) Cat. No. NG2003
- Wash Buffer (Promega) Cat. No. NG1051 (Component of ProNex® Size-Selective Purification System (Cat.# NG2001, NG2002, NG2003)
- Buffer EB (Qiagen) Cat. No. 19086
- 1000 µl, 200 µl, 10µl tips low-bind
- 200 µl bore tips low-bind
- Eppendorf DNA LoBind Tubes 1.5 ml Cat. No. 022431021
- Eppendorf DNA LoBind Tubes 2.0 ml Cat. No. 022431048
- Eppendorf™ ThermoMixer™ C Cat. No. 15158953
- Eppendorf Smart Block 2.0 ml Cat. No. 5362000035
- Mini centrifuge for 1.5 ml and 2.0 ml tubes

For QC:

- Qubit™ 4 Fluorometer (Cat. No. Q33238)
- Qubit Assay tubes (Catalog number Q32856)
- Qubit reagents (for example Qubit 1X dsDNA HS Assay Kit Cat. No. Q33231)
- NanoDrop One (Cat. No. ND-ONE-W)
- Femto Pulse System (Agilent) (Cat. No. M5330AA)
- FemtoPulse reagents (Agilent): Genomic DNA 165 kb Kit (Cat. No. FP-1002-0275)

Safety warnings

- Please wear lab coat and nitrile gloves.
- Make sure to comply with the safety regulation in your laboratory.
- Please collect the waste in a suitable container and dispose of the waste in accordance with local regulations.
- On rare occasions it happens that the beads do not bind the DNA. I always keep the supernatant after the initial incubation step. If this happens the protocol can simply be repeated with the supernatant.

Before start

Make sure you added the required volume of ethanol to the Wash Buffer (Promega).
Take the ProNex beads (Promega) out of the fridge and warm them up to room temperature for 1 hr.
Make sure to know the exact volume of your DNA sample before you start the protocol.
Warm up the ThermoMixer to 37 degrees Celsius.

SPRI clean-up with ProNex beads

42m

1 Put your DNA solution in a 2 mL LoBind tube. Make sure you know the volume exactly! 1m

2 Vortex the ProNex beads for 1 to 3 min.
(We have the beads aliquoted in 2 mL tubes for use.) 3m

3 Add 0.81x ProNex beads
It is important to be accurate! If DNA is limited you can use a 0.9x, but it will leave more low-molecular weight DNA behind. 1m

4 Mix well with bore tip (at least 20x) and incubate for 10 min at room temperature. 12m

5 Short spin the tube and put it on the magnetic rack. 30s

6 Leave it for 2 min. 2m

7 Take off supernatant.
Keep supernatant in case the beads did not bind the DNA! 30s

8 Add 2mL wash buffer and let it stand for 30 seconds. (The tube remains on the magnetic rack for the washing steps.) 30s

9 Take off the wash buffer and add again 2 mL wash buffer. 1m

10 Leave it for 30 seconds. Then take off the wash buffer. 30s

11 Close the tube and short spin the tube 30s

12 Put the tube back on the magnetic rack and take off the rest of the wash buffer. 30s

13 Wait 1 to 2 min (not longer) and add 50 µL Elution Buffer. Remove the tubes from the magnetic rack. 2m

14 Pipette mix with 200 µL bore tip thoroughly (at least 20x) and put the tube in the ThermoMixer at 37 degrees Celsius with 300 rpm for 15 min. 15m

15 Take the sample tubes back to the magnetic rack.

16 Wait 2 min until the liquid is completely clean and pipette DNA elution slowly to new 1.5 mL LoBind tube 2m
It might be necessary to repeat this step if magnetic beads get taken over and are still in the solution. Make sure the solution is free from beads.

17 Having a clean DNA solution, you can perform the QC:
Nanodrop, Qubit, FemtoPulse

18 QC:
- We measure the concentration using the Qubit.
- We measure concentration and purity of the DNA elution with the NanoDrop.
- We run the DNA on the FemtoPulse from Agilent to see the final fragment size distribution before library preparation.

If the QC is good, we proceed to low-input HiFi library preparation

Protocol references

Large DNA Size Selection with the ...