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Sanger Tree of Life HMW DNA Fragmentation: Diagenode Megaruptor®3 for LI PacBio

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Adam Bates[1], Iszy Clayton-Lucey[2], Caroline Howard[2]

[1]Wellcome Sanger Institute; [2]Tree of Life, Wellcome Sanger Institute, Hinxton, Cambridgeshire, CB10 1SA

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We use this protocol and it's working

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Abstract

This protocol describes the fragmentation of HMW DNA from one of the MagAttract v.2, Plant MagAttract v.3, or Plant MagAttract v.4 Sanger Tree of Life HMW DNA extraction protocols, using the Diagenode Megaruptor®3. This process is highly effective for the clean-up and shorter fragment removal from DNA extracts from all of the taxonomic groups covered by the Tree of Life Programme, with DNA sheared into an average fragment size range of 12–20 kb. However, highly concentrated or viscous samples are challenging. The output of this protocol is sheared DNA which can be directed towards Fragmented DNA clean up, with either the Manual or Automated SPRI protocols. This protocol was adapted from Sanger Tree of Life HMW DNA Fragmentation: Diagenode Megaruptor®3 for PacBio HiFi in order to process samples resulting from the Sanger Tree of Life HMW DNA Extraction: Automated MagAttract v.2, Automated Plant MagAttract v.3 and Automated Plant MagAttract v.4 protocols.

Acronyms
HMW: high molecular weight
SPRI: solid-phase reversible immobilisation
LI: low-input
HiFi: high fidelity

Guidelines

- The DNA sheared with this protocol must be intended for LI PacBio sequencing.
- For this protocol, we recommend shearing no more than 3,900 ng of undiluted DNA in 100–130 µL.
- This protocol is for the shearing of DNA into fragment sizes of 12–20 kb, with ideal fragment sizes of 15–18 kb.

Troubleshooting

- **Highly concentrated or viscous samples**
  Highly concentrated samples and/or very high molecular weight genomic DNA tend to aggregate and clump, clogging the hydropore. For highly viscous DNA solutions it is recommended to dilute samples 2- or 4-fold using EB buffer prior to shearing in the Megaruptor. If necessary, split the sample into two, but make sure to re-use the same Hydropore-Syringe in different runs for the same sample.
- **Purity**
  If any detergents or suspended particles are visible in the sample, it is advised to centrifuge samples to remove these impurities from the DNA in solution. Centrifuge the samples at room temperature for 15 minutes at 16,000 x *g*. Transfer the supernatant containing the DNA sample into a new tube, leaving the pellet undisturbed.
- **Hydropore-Syringe not drawing up sample**
  If the sample does not move through the Hydropore-Syringe, open the cassette (the run will momentarily stop) and take out the sample. Use a combination of the points above - dilution, splitting the sample, centrifugation – it might be necessary to do them multiple times for difficult samples.
- **Sample loss**
  It is normal that 5–15 µL of the sample can be lost due to pipetting errors, plastic retention and dead space inside the hydropore. See the following notes on ‘maximising sample retrieval’ with indications on how to recover maximum sample volume from hydropore syringe.

Maximising Sample Retrieval:

DNA samples tend to stick to the hydropore tubing. The volume of sample retained on the tubing surface is positively correlated with total sample volume.

Tip 1: Unplug the hydropore from the hydrotube slowly by tilting the syringe (hold the hydropore). Pull the tubing slowly away from the liquid surface: this will avoid random splashing of the sample and pulling the tubing slowly from the liquid surface will provide enough time for the surface retention forces of liquid to ‘’catch’’ a maximum of the liquid retained on the surface of the tubing. If large droplets remain on the tubing, use a P10 pipette to remove them and add them back to the tube.

Tip 2: Press the syringe plunger as much as possible in order to recover the remaining volume of sample inside the hydropore tubing. Slowly lower the hydropore tubing until it contacts the surface of the sample, and the drop will transfer to the sample.

Tip 3: Look inside the black top where the tubing meets the black plastic, sometimes there is a small droplet here which can be recovered using a p10 or p20 pipette.

Materials

- Buffer EB (Qiagen Cat. no. 19086)
- Nuclease-free water
- Diagenode Megaruptor® 3 shearing kit – contains Hydro Tubes and Hydropore-Syringes (Cat. no. E07010003)

Equipment:

- Pipettes for 0.5 to 1000 µL and filtered tips
- Wide-bore tips (200 µL, filtered if available)
- Diagenode Megaruptor® 3 (Cat. no. B06010003)
- Mini centrifuge (Cat. no SS-6050)
- Eppendorf™ Centrifuge 5425/5425 R (Cat. no. 5405000263)

Protocol PDF: Sanger Tree of Life HMW DNA Fra... 94KB

Safety warnings

- The operator must wear a lab coat, powder-free nitrile gloves and safety specs to perform the laboratory procedures in this protocol.
- Waste needs to be collected in a suitable container (e.g. plastic screw-top jar or Biobin) and disposed of in accordance with local regulations.

Laboratory protocol

1 Label the required number of Diagenode Hydro Tube for each DNA sample that will be sheared; ensure to label the tubes both on the lid and on the side.

2 Prior to transferring the DNA sample from its original tube, first mix the DNA sample by pipetting carefully with wide-bore pipette tip.

3 Transfer the desired amount of each DNA sample to their corresponding labelled Diagenode Hydro Tube shearing tube. The volume of DNA to be sheared must be 100–130 µL and at a concentration of 3 ng/µL–39 ng/µL, ideally 30 ng/µL.

4 Ensure that all the tubes have an equal volume by using EB buffer or nuclease free water to normalise the volumes.

5 Replace the Hydro Tube lids and briefly centrifuge the tubes using a mini-centrifuge.

6 Before attaching the Hydropore-Syringe to the top of the tube, check that each syringe is screwed together and ensure that all joints/screw tops are tight and that the plunger is pushed all the way down.

7 Remove the Hydro Tube lids and fit a Hydropore-Syringe to the top of the tube by pushing firmly (note: it does not screw on). Retain the lid of the Hydro Tube.

8 Switch on the Diagenode Megaruptor®3 and initialise using the on-screen instructions: follow the prompt to remove the loading cassette, lifting up and away from the base unit, wait approximately 3 seconds for the arms to set the machine to ‘zero’ position, and then replace the cassette on the base unit.

9 Once initialised, use the touch screen to set up the run; select “Protocols” and then “Go & Shear''. Set the parameters as follows:

1. Concentration: select the highest concentration in the sample set.
   Note: Concentrations in the same run should not vary more than ± 25 ng/µL, and samples with concentrations below 50 ng/µL should not be combined on the same run as those above ng/µL. The Megaruptor should not be used to shear samples with concentrations higher than 150 ng/µL – these samples should be diluted.
2. Volume: as measured
3. Speed: 31

10 Load the Hydropore-Syringes into the cassette when prompted by touch screen. The Hydropore-Syringes must be balanced, i.e. loaded symmetrically across the 8 positions, with an empty balance Hydropore-Syringe used where there is an uneven number of

samples. When loading onto the instrument, orientate the Hydropore-Syringe so that the clear side faces out to allow the DNA solution to be observed moving up and down.

11 After starting the program, visually confirm that all syringes are drawing up liquid. If a sample is not moving through the hydropore once the “shearing” step is reached there may be an issue. See the ‘Troubleshooting’ section in the Guidelines section for details on how to deal with any difficult samples.

12 The run takes approximately 45 mins. Be aware the displayed countdown on the machine is not accurate.

13 Once the program is complete, remove each Hydropore-Syringe from the instrument.

14 Remove the Hydropore-Syringe from the Hydro Tube, pressing down on the plunger during removal to ensure the collection of any residual liquid/sample within the hydropore. Refer to the ‘Maximising Sample Retrieval’ for tips on how to minimise sample loss within the Hydropore-Syringe.

15 Discard the Hydropore-Syringes as biological waste and replace the retained lids onto the Hydro Tubes.

16 Store the sheared DNA at 4 °C until further processing.

Protocol references

Procedure & checklist - Preparing whole genome and metagenome libraries using SMRTbell prep kit 3.0 (pacb.com)