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Pulse Field Gel Electrophoresis for Long Read Sequencing

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High molecular weight DNA extraction from all kingdoms
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Protocol status: In development
We are still developing and optimizing this protocol

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Abstract

Pulsefield gel electrophoresis is an easy and affordable method of quality control for high molecular weight DNA extractions, particularly for use in long-read Oxford Nanopore sequencing.

This method is currently used in the Borevitz Lab (ANU) and is adapted from the method used by Benjamin Schwessinger (ANU).

For more details on the PFGE hardware, refer to the manual at http://www.bio-rad.com/webroot/web/pdf/lsr/literature/M1703690.pdf

You may need to use different settings for different DNA size ranges - see pg. 25 of the manual ("5.2 Pulsed Field Conditions by DNA Size")

Guidelines

Remember to flush/ wash and dry the system (container, pump, cooler) as fungus and salts can accumulate.

Materials

MATERIALS

Agarose **Merck MilliporeSigma (Sigma-Aldrich)**

1x TBE buffer

Use ladders appropriate for the size range you expect.

Reagent setup

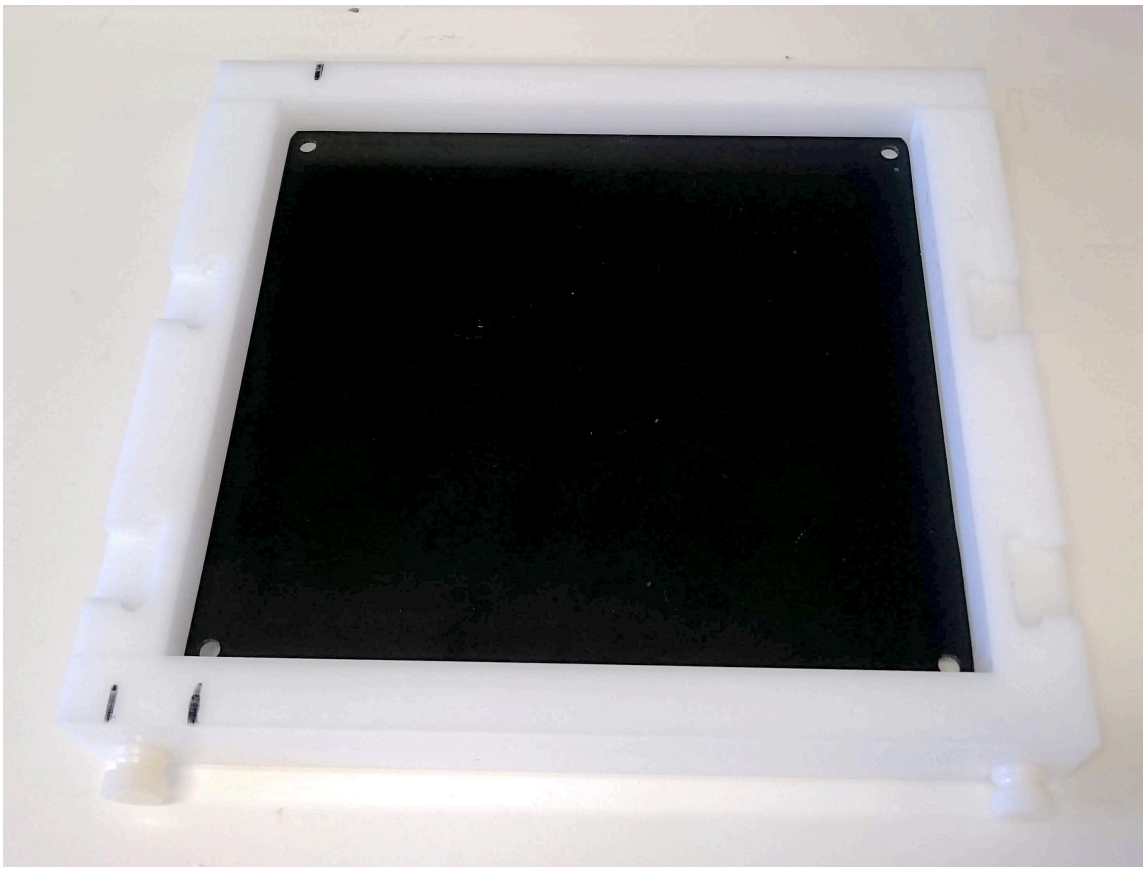
1 Prepare 2 L of 0.5x TBE buffer, along with 100 ml of 0.5x TBE buffer in a separate container.

2 Add 1g of Agarose (I/Ultrapure) to the 100 ml 0.5x TBE, to make a 1% agarose gel. Heat and mix until homogenous. Add 10 µl SYBR safe dye and mix again.

3 Transfer 200 ul of the 1% gel solution to a separate Eppendorf tube. Keep this tube heated at 60ºC on a heat block.

4 Assemble the casting tray, ensuring that the screws are tightened to make the setup watertight. Add the gel comb to the comb slot closest to the edge.

Casting tray components.



The assembled casting tray. Slots on the side are for the gel comb.

5 Pour the remaining molten gel into the casting tray. Allow to cool/ solidify without disruption.

Equipment setup

6 Pour the 2L of 0.5x TBE into the central tray of the PFGE container.

7 Switch on the power and pump switches on the main CHEF unit.

8 Switch on the power for the cooling module and set it to 14ºC.

9 Set the variable speed pump to 55. Wait until container current temperature is 14 degrees before adding gel.

Sample prep

10 For each sample, prepare a PCR tube with 4 µl of loading dye.

11 Add 300 ng of DNA (or as close to) for each sample to the prepared tube, and make up volume to 16 µl with the buffer the DNA is stored in.

Gel setup

12 The gel has 15 wells; it is generally advisable to use at least 2 sets of ladders on both sides of the samples (4 wells for ladders). Remove the well comb and add (less than 1 mm) any gel-stored ladders to the gel while it is still in the casting tray.

13 Add the warm 200 µl of agarose over the gel plug ladders. Wait for these to cool.

Starting the run

14 When the current temperature of the TBE buffer is around 14 ºC, switch off the pump and cooling system.

15 Unscrew the front and rear sides of the casting tray, and place the gel in the central slot of the electrophoresis cell.

16 Load the samples and any liquid ladders.

17 Set the program 1 and start the run.

18 Wait for around 10 minutes, and then verify that the gel is running (the dye should run into the gel at this point)

19 Switch the pump and cooling module back on. Set the pump to around 80 and the cooling module to 14ºC.

After run completion

20 After the program has finished, remove the gel and place it in a suitable tray. (Find the smallest container which fits the gel but is large enough to comfortably slip the gel with gel backplate in and out.)

21 Image the gel as usual.

22 Dispose of the gel and rinse and dry the gel backplate.

System cleanup

23 Acquire a large bucket or other liquid container (2L or more recommended). Place this below the output port of the electrophoresis cell. (marked "TUBE TO CHILLER")

24 Acquire a second large bucket (2-5L), and fill it with RO/DI water. Place this in a stable location within reach of the input tube to the cooling module.

25 Ensure that the CHEF program is inactive and that the pump and cooling module are switched off.

26 Disconnect the tube leading to the cooling module. The buffer should start draining into the container under the output port.

27 Place the tube leading to the cooling module into the clean water container, and turn the pump on.

28 As needed, drain the output container and fill the clean water container until about 6 L of water has been flushed through the system.

29 Place a drainage container under the rear input port (from the pump) of the electrophoresis cell. Disconnect the pump tube and let the remaining flush water drain from the electrophoresis cell.

30 Disconnect the tube between the cooling module and pump.

31 Leave the tubes disconnected and the cell lid ajar to dry. If not in use for an extended period, return in a week to reconnect tubes and cell lid to prevent dust build up.