Protocol for Pulsed Field Gel Electrophoresis

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Abstract

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Guidelines

PFGE Protocol is from Griffith 2000

PFGE Modifications (Schwalbach 2002):

- 1% Seakem Gold agarose gels are used. Gel is poured after it has cooled to below 45°C.
- Instead of using 1:10 TE for the 3 microcon rinses, regular TE (pH 7.6) is used.
- For the final collection of viruses from microcons use two 240 x g spins with 10 µl of TE each spin instead of one 5 minute 1000 x g spin with 20 µl 0.5x TBE.
- NEB PFGE standards are occasionally used, though liquid lambda/5 kb ladders are also used.
- Gels are destained in diH_2O for minimum of 30 minutes.
- Should use the majority of the 150 and 500 m samples. (i.e. all of it)

Protocol

Step 1.
A 15-20 liter natural seawater sample is passed through a glass fiber pre-filter (Gelman A/E) and a 0.22 µm pore size membrane (Durapore, Millipore).

NOTES
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During this and each subsequent step, the sample should be kept as cold as possible.

Step 2.
Concentrate to ca. 150-200 ml using 30 kD MWC Spiral Cartridge Concentrator (SCC).

NOTES
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If a large volume has been concentrated, there is no need to flush the unit, as this increases (numerical) yield of virus by < 5%, and increases the time needed to complete the next step significantly.

Step 3.
Transfer the SCC retentate to Centriprep 30’s.

NOTES
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These can initially be filled to about 2 cm past the mark on their barrels to save handling time.

Step 4.
Store balance of concentrate in refrigerator.

Step 5.
Centrifuge in IEC-HN SE II at full speed for 15 minutes (in refrigerator if possible).

DURATION
00:15:00

Step 6.
Empty central reservoir without refilling and repeat centrifugation step.

Step 7.
Empty and refill keeping volumes equal in order to maximize filtration area of Centripreps and balance rotor.

Step 8.
Continue to spin and empty until no more water enters central chamber.

Step 9.
Remove central insert from 2 of units, replace caps, and spin retentate chamber briefly to recover droplets from sides.

Step 10.
Combine retentate into 2, and finally one Centriprep unit, by gentle pipetting.

Step 11.
When all have been combined and spun, the final volume is ca. **500 µl**.

Preparing Electrophoresis Chamber

Step 12.
Make a 1% agarose gel by combining 1 g Bio-Rad Molecular Grade Agarose with 99ml 0.5x TBE, and heating until completely clear of unmelted material.

Preparing Electrophoresis Chamber

Step 13.
Assemble mold with backing plate in place and pour in liquid.

Preparing Electrophoresis Chamber

Step 14.
Let set.

Preparing Electrophoresis Chamber

Step 15.
Meanwhile, fill electrophoresis chamber with 2 liters 0.5x TBE.

Preparing Electrophoresis Chamber

Step 16.
Turn on pump and chiller, and set temperature to 14°C.

Preparing Electrophoresis Chamber

Step 17.
When gel is set, remove comb and sides of mold, and **slide backing plate, with gel attached**, out of mold.

Preparing Electrophoresis Chamber

Step 18.
Place gel with backing plate into receiver in electrophoresis chamber, taking care not to dislodge gel from plate, with wells in rear and let chill.

Step 19.
Transfer aliquots into Microcons (30 or 100 kD MWC).

NOTES
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To obtain full detail of banding, a range of aliquots should be used until the sampling location has been well characterized. This range can then be narrowed.
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Suggested volumes from 20 liter starting volume are 50, 100, 150 and 200 µl.

Step 20.
Place each aliquot in a separate Microcon an spin at 1000 x g for 20 minutes (about 4000 RPM in Eppendorf Microfuge) to near dryness (most of the Microcon membrane is dry, and only a small ring of liquid remains around edge).

DURATION
00:20:00

Step 21.
As they reach the proper volume, smaller volume aliquots can be removed and stored in the refrigerator until the larger have been reduced to the proper volume.

Step 22.
When all samples have been reduced, add 50 µl 1:10 TE to each tube, taking care not to touch membrane with pipette tip, and spin again to reduce volume as described above.

Step 23.
Repeat step 22.

Step 24.
Repeat step 22 again.

Step 25.
Now add 20 µl 0.5x TBE to membrane to elute viruses, again taking care not to touch membrane.

Step 26.
Invert cartridge, place in fresh tube, and spin for 5 minutes at 1000 x g to recover.

DURATION
00:05:00

Step 27.
Place tubes containing recovered viruses in 60°C water bath for 10 minutes.

DURATION
00:10:00

Step 28.
Remove tubes and place immediately on ice for 2 minutes.

DURATION
00:02:00

Step 29.
Transfer to microfuge and spin briefly to recover condensation from walls of tubes.

Step 30.
Prepare molecular weight markers by combining 100-200 ng of marker stock to 0.5x TBE to a final volume of 20 µl.

Step 31.
Add 10 µl PFGE loading buffer to each tube.

Step 32.
Mix by simultaneously inverting several times slowly, while rolling between index finger and thumb.

Loading

Step 33.
Turn chiller and pump off.

Loading

Step 34.
Load samples.

Loading

Step 35.
Turn pump on, then turn chiller on.

Loading

Step 36.
Close lid and check connections to make sure all is in order.

Loading

Step 37.
Set voltage to 6V.

Loading

Step 38.
Set initial switch time to 1s.

Loading

Step 39.
Set final switch time to 10s.

Loading

Step 40.
Set run time to 18h.

Loading

Step 41.
Push start.

Loading

Step 42.
After 10 minutes, check to make sure actual temperature is holding between 14 and 16°C, and that timer is running down.

DURATION
00:10:00