Protocol for Pulsed Field Gel Electrophoresis

PFGE Protocol (Griffith 2000)

A 15-20 liter natural seawater sample is passed through a glass fiber pre-filter (Gelman A/E) and a 0.22um pore size membrane (Durapore, Millipore). During this and each subsequent step, the sample should be kept as cold as possible. Concentrate to ca. 150-200ml using 30kD MWC Spiral Cartridge Concentrator(SCC). 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. Transfer the SCC retentate to Centriprep 30’s. These can initially be filled to about 2cm past the mark on their barrels to save handling time. Store balance of concentrate in refrigerator. Centrifuge in IEC-HN SE II at full speed for 15 minutes (in refrigerator if possible). Empty central reservoir without refilling and repeat centrifugation step. Empty and refill keeping volumes equal in order to maximize filtration area of Centripreps and balance rotor. Continue to spin and empty until no more water enters central chamber. Remove central insert from 2 of units, replace caps, and spin retentate chamber briefly to recover droplets from sides. Combine retentate into 2, and finally one Centriprep unit, by gentle pipetting. When all have been combined and spun, the final volume is ca. 500ul.

During the next set of steps, prepare the electrophoresis chamber. Make a 1% agarose gel by combining 1g Bio-Rad Molecular Grade Agarose with 99ml 0.5x TBE, and heating until completely clear of unmelted material. Assemble mold with backing plate in place and pour in liquid. Let set. Meanwhile, fill electrophoresis chamber with 2 liters 0.5x TBE. Turn on pump and chiller, and set temperature to 14 ºC. When gel is set, remove comb and sides of mold, and slide backing plate, with gel attached, out of mold. 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.

Transfer aliquots into Microcons (30 or 100kD MWC). 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. Suggested volumes from 20 liter starting volume are 50, 100, 150 and 200ul. 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). 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. When all samples have been reduced, add 50 ul 1:10 TE to each tube, taking care not to touch membrane with pipette tip, and spin again to reduce volume as described above. Perform this step twice more. Now add 20ul 0.5x TBE to membrane to elute viruses, again taking care not to touch membrane. Invert cartridge, place in fresh tube, and spin for 5 minutes at 1000 x g to recover. Place tubes containing recovered viruses in 60 ºC water bath for 10 minutes. Remove tubes and place immediately on ice for 2 minutes. Transfer to microfuge and spin briefly to recover condensation from walls of tubes. Prepare molecular weight markers by combining 100-200ng of marker stock to 0.5x TBE to a final volume of 20ul. Add 10ul PFGE loading buffer to each tube. Mix by simultaneously inverting several times slowly, while rolling between index finger and thumb.

Loading: Turn chiller and pump off. Load samples. Turn pump on, then turn chiller on. Close lid and check connections to make sure all is in order. Set voltage to 6V; set initial switch time to 1s; set final switch time to 10s; set run time to 18h; push start. After 10 minutes, check to make sure actual temperature is holding between 14 and 16 ºC, and that timer is running down.

PFGE Modifications (Schwalbach 2002)
- 1% Seakem Gold agarose gels are used. Gel is poured after it has cooled to below 45ºC.
- 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 10ul of TE each spin instead of one 5’ 1000 x g spin with 20ul 0.5x TBE
- NEB PFGE standards are occasionally used, though liquid lambda/5kb ladders are also used.
- Gels are destained in diH₂O for minimum of 30’
- Should use the majority of the 150 and 500m samples. (i.e. all of it)