Fingerprinting aquatic virus communities using pulsed field gel electrophoresis (PFGE)

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Abstract

The viral concentrate used for PFGE analysis must be molded into plugs, followed by lysis of the virus particles to release their DNA. It is possible to run solution-based preparation of viral DNA for PFGE (Steward 2001); however, large DNA molecules (>100 kb) are extremely sensitive to mechanical shearing in aqueous solution (Bouchez and Camilleri 1997). The consensus is that lysis inside viral plugs prevents mechanical shearing of the DNA, resulting in more discrete PFGE bands. Intact viral genomes are then separated by size by PFGE. After separation, the banding pattern is visualized by staining with a fluorescent DNA stain. This banding pattern provides a visual record of the genome size distribution that can be used for qualitative and quantitative comparisons between samples.

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Guidelines

Equipment and reagents:

* Pulsed field gel electrophoresis system. We recommend the CHEF-DR II (Bio-Rad);

* Casting stand, comes in 14 x 13 cm (small gel, 10 wells) or 21x 14 cm (large gel, 15 wells) frame and platform;

* Combination comb holder;

* Combs, 1.5 mm thick;

* Plug molds (Bio-Rad), each well holds 80 µL;

* Screened caps (Bio-Rad);

* DNA molecular weight standards, e.g., lambda ladder (available in blocks that have to be sliced into smaller pieces before use) and 5-kb ladder (e.g., Bio-Rad);

* Dilution or storage buffer: SM buffer (0.1 M NaCl, 8 mM $MgSO_4$ *$7H_2O$, 50 mM Tris-HCl, 0.005% (wt/vol) glycerin);

* Lysis buffer: must be freshly made (250 mM EDTA, pH 8.0, 1% SDS, 1 mg/mL Proteinase K); 5 mL lysis buffer is needed per sample;

* 1.5% PFGE-grade agarose (e.g., SeaKem GTG agarose; Cambrex) for preparing plugs dissolved in TE 10:1 (10 mM Tris, 1 mM EDTA, pH 8.0); the agarose can be stored at 4°C in between uses;

* Washing buffer: TE 10:1 (10 mM Tris, 1 mM EDTA, pH 8.0), 150 mL per sample;

* Storage buffer: TE 20:50 (20 mM Tris, 50 mM EDTA, pH 8.0), 20 mL per sample;

* Running buffer: 2 L of 1 x TBE (10 x TBE stock: 108 g Tris, 55 g boric acid, 40 mL 0.5 M EDTA pH 8.0);

* Gel buffer: 1 x TBE, 100 mL (small gel) or 150 mL (large gel);

* 1% PFGE-grade agarose for gel electrophoresis (e.g., SeaKem GTG agarose; Cambrex);

* Fluorescent DNA strain, e.g., SYBR I or SYBR gold (Molecular probes);

* UV transilluminator and gel documentation system

Molding of PFGE viral plugs: For long-term stability of viruses before PFGE and to avoid downstream interference with nucleic acid extraction or electrophoretic properties of PFGE, we recommend buffering of the virus concentrate in SM buffer, prepared via either ultracentrifugation or dialysis (Wommack et al. 2010, this volume). Samples can be stored for 2-3 days in SM buffer, but we recommend you use it immediately. Each of the agarose plugs should represent the same amount of sample volume; alternatively, number of VLPs.

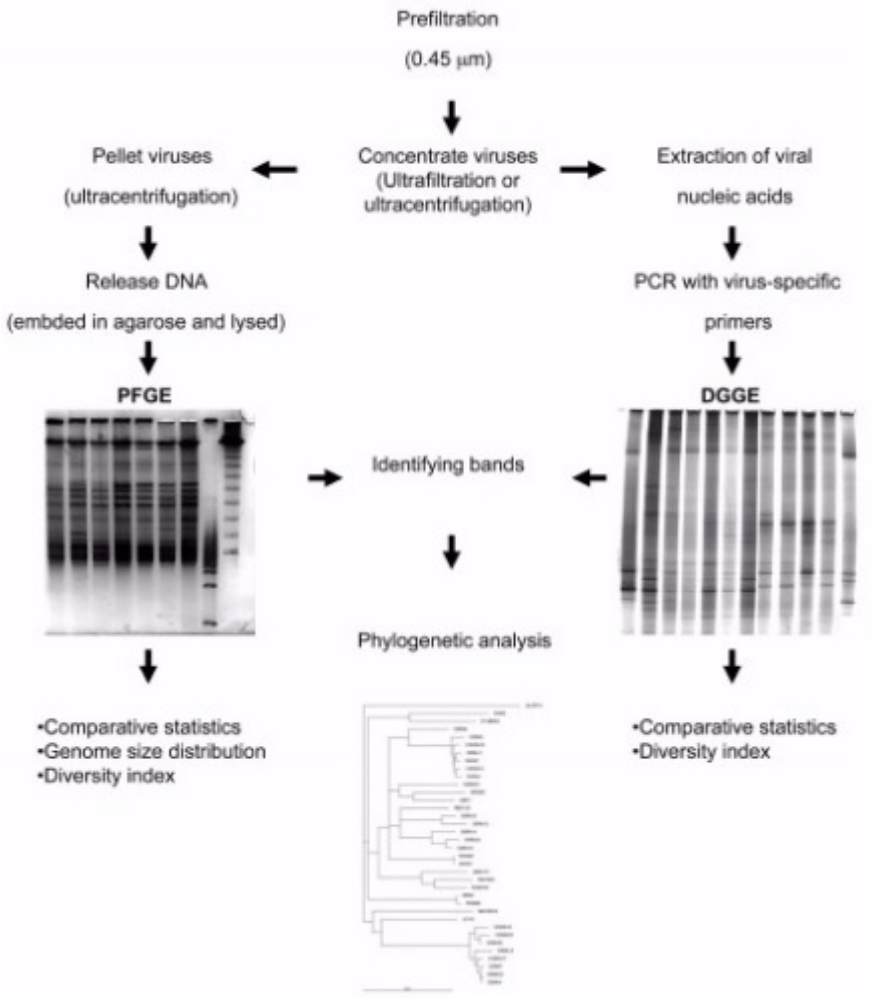

Fig. 1. Flowchart of methods involved in figerprinting aquatic virus communities.

Analysis of PFGE and DGGE fingerprints:

DGGE and PFGE fingerprints can be analyzed using a variety of commercially available gel analysis software products (e.g., GelCompar II, Applied Maths; BioNumerics 5.1, Applied Maths; Quantity One, Bio-Rad). A common method to analyze DGGE/PFGE fingerprints involves creating a binary matrix representing the bands occurring in a set of DGGE/PFGE patterns. The presence or absence of bands in a sample is simply scored in a binary manner as 1 (present) or 0 (absent), relative to all of the bands detected in a set of DGGE/PFGE patterns. The binary data can then be presented in a dendrogram where the differences in fingerprint patters are represented in a graphical format or as a dendrogram using a distance-based cluster analysis techniques such as unweighted pairwise grouping with mathematical averages (UPGMA). Another possibility is to use multidimensional scaling (MDS) to reduce a complex fingerprint pattern to a point in a two-dimensional space (Van Hannen et al. 1999a). It is important to note that these types of analyses depend on consistency when detecting bands, and subjective determination of presence or absence should be avoided. Luckily, most commercially available gel analysis software programs allow researchers to use automated band detection parameters (e.g., band width and intensity), or even set their own thresholds for each parameter. As an alternative to comparisons based on presence or absence, the overall pattern of gel lanes can be compared directly using densitometry profiles (i.e., the pixel intensities at discrete positions in the gel). This type of analysis is based on pairwise correlations of profiles and can be used to avoid biases associated with band detection or loading unequal amounts of DNA. For densitometry

profile analyses, each lane profile is compared to the others, and the resulting matrix of correlation values correspond to lane similarities. The correlation values can then be transformed to dissimilarity values (1 – similarity) that can be used for cluster analysis via UPGMA.

Quantitative analysis of DGGE and PFGE gels is also possible. For DGGE, only semiquantitative analysis is possible, since individual band intensities cannot be used to infer target abundances in natural samples because differences in band intensity can arise from variable amplification efficiencies for different targets, and/or differences in background DNA. Thus fingerprint patterns can be compared, but individual band intensities should not be used to infer target abundance in natural samples. For PFGE, quantitative analysis involves measuring the relative fluorescence of each band. Based on this information, it is possible to obtain values for richness and abundance that can be used to calculate diversity indices. This type of analysis can be conducted using the commercial software noted above. Of course, it should be noted that both DGGE and PFGE are subject to a number of confounding errors. These sources of error should be carefully considered when deciding what types of analyses or comparisons should be conducted and not the least when the outcome of the analysis is interpreted.

Protocol

Molding of PFGE viral plugs

Step 1.

Prepare the 1.5% agarose and the lysis buffer.

Molding of PFGE viral plugs

Step 2.

Incubate the agarose at 80°C until further use.

Molding of PFGE viral plugs

Step 3.

Dispense 5 mL of the freshly made lysis buffer into 50-mL Falcon tubes, one for each sample.

Molding of PFGE viral plugs

Step 4.

Combine equal volumes (200 µL) of virus concentrate and molten 1.5% agarose.

NOTES

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Avoid bubbles in the plugs.

Molding of PFGE viral plugs

Step 5.

Mix briefly.

Molding of PFGE viral plugs

Step 6.

Dispense the mixture into plug molds with a pipette.

Molding of PFGE viral plugs

Step 7.

Place the plug molds in the freezer (–20°C) for at least 2 min to set.

DURATION

00:02:00

Molding of PFGE viral plugs

Step 8.

Remove the tape from the bottom of the plug molds and push the plugs out from the molds into 5 mL lysis buffer.

NOTES

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Make sure that the entire plugs are submerged in the lysis solution.

Molding of PFGE viral plugs

Step 9.

Incubate the plugs in lysis buffer overnight in the dark at 30°C.

DURATION

18:00:00

Molding of PFGE viral plugs

Step 10.

The next day, decant the lysis buffer using a plastic sieve (screened cap) that can be attached at the top of the Falcon tube.

NOTES

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Be sure that no plugs are stuck in the cap before moving on to decant the next sample.

Molding of PFGE viral plugs

Step 11.

Wash the plugs three times, 30 min each, in TE buffer 10:1 at room temperature.

DURATION

00:30:00

Molding of PFGE viral plugs

Step 12.

The plugs can be stored at 4°C in TE 20:50 for several month before further processing; nevertheless, we recommend running the samples as soon as possible, because degradation of the viral DNA will occur and result in less discrete bands.

Gel preparation

Step 13.

Set up the gel rig.

NOTES

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Be sure that the comb sits evenly along its entire length.

Gel preparation

Step 14.

Prepare a 1% agarose gel in 1× TBE buffer.

Gel preparation

Step 15.

Melt until the agarose is completely dissolved.

Gel preparation

Step 16.

Place the warm agarose at 60°C for 10 min before pouring into the gel rig and allowing it to cool.

DURATION
00:10:00

NOTES
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Avoid air bubbles in the gel.

Gel preparation

Step 17.
Keep 5 mL agarose at 60°C for later use to seal the wells.

Gel preparation

Step 18.
When the agarose is set, pour 50 mL of 1× TBE running buffer on the top of the gel and place it in the refrigerator for at least 20 min or overnight.

DURATION
00:20:00

Gel preparation

Step 19.
Place molecular weight standards (slices of 5 mm) on either side of the gel.

Gel preparation

Step 20.
Place the samples between the markers using a sterile loop.

NOTES
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Be sure that no air bubbles are trapped in the well.

Gel preparation

Step 21.
Overlay the wells with leftover molten 1% agarose.

Gel preparation

Step 22.
Remove the gel from the pouring rig and remove any extraneous agarose from the bottom and edges.

Electrophoresis

Step 23.
Prepare the 1× TBE (running buffer) and place at 14°C until further use.

Electrophoresis

Step 24.
Place the gel into the electrophoresis chamber and carefully pour the cooled 1× TBE running buffer into the chamber.

Electrophoresis

Step 25.
Run the gel at 6 V cm^{-1} with pulse ramps from 20 to 40 s (for example) at 14°C for 22 h.

DURATION
22:00:00

NOTES
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Size markers should be encompassed to facilitate size determination for all the different PFGE viral

bands. A number of size markers for PFGE are commercially available. These conditions result in runs that make a good starting point for further analysis.

Separate different viral genome size classes

Step 26.

To separate different viral genome size classes, each sample could be run three times:

DURATION

20:00:00

PROTOCOL

. Separate different viral genome sizes

CONTACT: Declan Schroeder

Step 26.1.

(1) 1–5 s switch time with 20 h run time for separation of small genome sizes (0–130 kb).

DURATION

20:00:00

Step 26.2.

(2) 8–30 s switch time with 20 h run time for separation of medium genome sizes (130–300 kb).

DURATION

20:00:00

Step 26.3.

(3) 20–40 s switch time with 22 h run time for separation of large genome sizes (300–600 kb).

DURATION

22:00:00

Step 26.4.

Stain the gel for 30 min or overnight in fluorescent stain (according to manufacturer’s instructions) and view on a UV transilluminator.

DURATION

00:30:00