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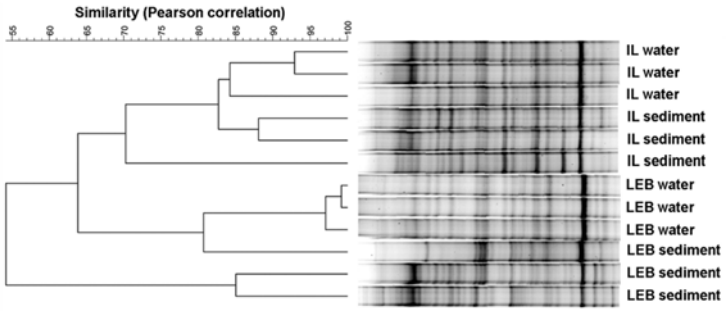
Denaturing Gradient Gel Electrophoresis (DGGE)

Background information

Denaturing gradient gel electrophoresis (DGGE) is a molecular fingerprinting method that separates polymerase chain reaction (PCR)-generated DNA products. The polymerase chain reaction of environmental DNA can generate templates of differing DNA sequence that represent many of the dominant microbial organisms. However, since PCR products from a given reaction are of similar size (bp), conventional separation by agarose gel electrophoresis results only in a single DNA band that is largely non-descriptive. DGGE can overcome this limitation by separating PCR products based on sequence differences that results in differential denaturing characteristics of the DNA. During DGGE, PCR products encounter increasingly higher concentrations of chemical denaturant as they migrate through a polyacrylamide gel. Upon reaching a threshold denaturant concentration, the weaker melting domains of the double-stranded PCR product will begin to denature at which time migration slows dramatically. Differing sequences of DNA (from different bacteria) will denature at different denaturant concentrations resulting in a pattern of bands. Each band theoretically representing a different bacterial population present in the community. Once generated, fingerprints can be uploaded into databases in which fingerprint similarity can be assessed to determine microbial structural differences between environments or among treatments (figure below). Furthermore, with the breadth of PCR primers available, DGGE can also be used to investigate broad phylogenies or specific target organisms such as pathogens or xenobiotics degraders.



Relatedness of DGGE fingerprints of *E. coli* populations (*uidA* gene) associated with triplicate cultures from Lake Erie Beach and Inland Lake water and sediment.

Materials

Plate sandwich materials
- 16 x 16 cm glass plate
- 16 x 14 cm glass plate
- 2 - 1 mm spacers
- 2 - plate clamps
- Pouring stand
- Foam gasket
- Well comb
- Spacing card

Gel solutions
- 40% Bis-Acrylamide: (37.5: 1, acrylamide: bisacrylamide)
- Deionized Formamide
- Urea
- 50 X TAE (20 mM Tris-acetate, pH 7.4, 10 mM sodium acetate, 0.5 mM Na-EDTA)
- 10% Ammonium Persulfate Solution (APS, 0,1 g in 1 ml of nanopure water. Make fresh when required or aliquot 0.5 ml into 1.5 ml microcentrifuge tubes and freeze until needed)
- TEMED (N,N,N',N' -tetramethylenediamine)
- Glycerol
- Nanopure water

Gradient wheel
GelStar nucleic acid stain
DCODE electrophoresis apparatus
Power supply

- Wear gloves throughout the entire protocol.
- Do not cross-contaminate your samples or the solutions. Be aware of your pipette tip.
- Work clean, either on fresh blue bench paper, in the hood, or on a freshly ethanol treated bench top.
- Acrylamide and formamide are toxic. Use caution when working with these materials.

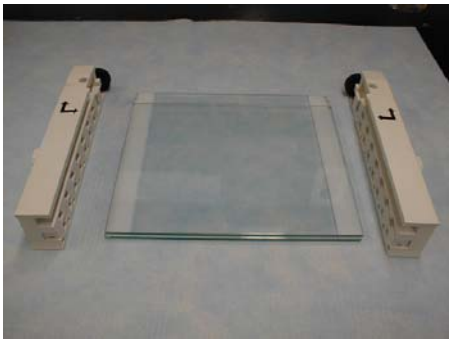
The protocol in brief

DGGE gels will be poured and run to separate similarly sized PCR products. You will create gels by combing two solutions containing acrylamide (structural material) and differing amounts of denaturants (urea and formamide) to form a gradient of denaturant in which double stranded DNA fragments of differing sequence will be denatured during electrophoresis. The gel will be stained and visualized to reveal band patterns that can be used to determine the similarity of sampled microbial communities.

A. Building the gel assembly

1. Using lint-free tissues, wash glass plates, spacers and combs thoroughly with isopropanol. Do not use soap or harsh abrasive cleaning materials to clean any of the equipment. If the materials are cleaned diligently, there is no need to use any detergents. A simple water rinsing will suffice followed by isopropanol cleaning.

2. Assemble the gel sandwich by placing the small glass plate on top of the large plate, being sure to correctly place a 1 mm spacer along each edge of the plate assembly. Attach the plate clamps (tight enough to hold everything together) and place the entire assembly into the rear slot of the pouring stand. Loosen the clamps slightly and use the spacing card to assure the proper spacer alignment. Tighten the plate clamps (snug, as if you were trying to prevent "leakage") and remove the plate assembly from the pouring stand. Inspect the plate assembly to ensure that the two glass plates and the spacers form a flush surface across bottom of the assembly. If the surface is not flush, re-set the plate assembly, as breaches in the seal of the plate assembly with the bottom of the pouring stand will result in leaking gel solutions.



Components of the gel sandwich; gel clamps and spacers between two glass plates.

3. Place a foam gasket into one of the two front slots of pouring stand, insert the plate assembly, and clamp into place. Place the well comb firmly in between the plates and draw a line on the glass plate where the bottom of the wells is located. This will be a future reference point when pouring the gel.

4. Set up the gradient former by inserting 30 ml syringes into a "closed" gradient wheel. Secure the syringes and 'back-out" the syringe plungers by reversing the gradient wheel. If the syringes do not move smoothly, replace them with new syringes. Note the resulting volume measured on each syringe (between 12 – 13 ml), which will be the amount of solution that the system will deliver to create the new gel.

5. Attach the small (10 cm) delivery tubing to the syringes.

B. Pouring the gel

Use the following table to determine the appropriate composition of the denaturing gradient gel.

Table 1. DGGE gel composition. (Concentrations in bold are variable for different denaturing concentrations).

Reagent	Final Conc.	mls reagent for denaturant concentration											
		cap	20%	25%	30%	35%	40%	45%	50%	55%	60%	65%	70%
Water	N/A	**3.6**	**9.5**	**9.0**	**8.5**	**8.0**	**7.5**	**7.0**	**6.5**	**6.0**	**5.5**	**5.0**	**4.5**
40% acrylamide:bis (37:1)	8%	1.3	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
50 X TAE buffer	1 X	0.1	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Urea	Variable	**0**	**1.3**	**1.6**	**1.9**	**2.2**	**2.5**	**2.8**	**3.1**	**3.4**	**3.7**	**4.0**	**4.3**
Formamide	Variable	**0**	**1.2**	**1.5**	**1.8**	**2.1**	**2.4**	**2.7**	**3.0**	**3.3**	**3.6**	**3.9**	**4.2**
Glycerol	2%	0	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3

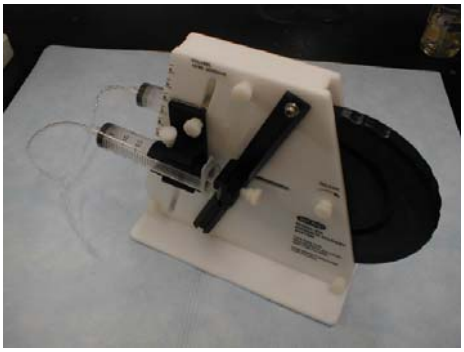
Table 2. Volume of polymerization reagents for denaturing gels.

Polymerizing reagent	Volume to add (ml)	
	Low and high gel solutions	Cap
10% APS	81	40
TEMED	4.5	2.5

6. You will make two solutions of 15 ml volume each; a “low” denaturant concentration solution, and a “high” denaturant concentration solution. For example, if you wish to make a 40–55% gradient, then you would make a 40% (low) solution and a 55% (high) solution based on the reagent volumes in Table 1. The gradient wheel will combine these during the pouring of the gel to create the gradient within the gel matrix.

7. Add APS and TEMED (Table 2) into each solution and swirl gently to mix. These reagents begin the polymerization of the acrylamide. At this point you will have approximately 15 minutes to pour the gel.

8. Draw each solution into the syringes. For our gels, the low denaturant syringe is on the front side of the gradient maker and the high denaturant is in on the backside. Make sure air is removed from syringes (by tapping on the side) before placing them into the gradient maker. Attach the “Y” connector to each tube and attach the final long (15 cm) tube to the “Y” connector.

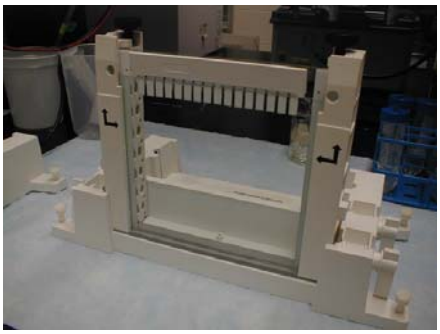


The gradient wheel is a much-maligned apparatus, but in the right hands can generate highly reproducible gradients in acrylamide gels. The two syringes hold the high denaturant-concentration solution (back syringe) and low denaturant-concentration solution (front syringe)

9. Place the delivery tube in between the two plates near the center of the top edge of the plate assembly. Slowly ***and consistently*** turn the wheel until the gel is poured to the level of the line drawn in step 3. It should take between two to three minutes to pour the gel.

10. Carefully, without disturbing the gel solution, add approximately 2 ml of 1X TAE buffer to the gel solution to form a layer on top of the gel solutions approximately 5 mm thick. This layer will help the top boundary of the gel to be smooth.

11. Let the gel polymerize for about 30 minutes. Then, remove the 1X TEA buffer with a syringe and with the same syringe add approximately 3 ml of "cap" solution (Table 1, 2) to the top of the polymerized gel. Carefully place the comb at top at a slight angle between the plates. Be sure to avoid trapping any air bubbles as the comb is lowered into the cap solution. Let polymerize at least two hours to overnight.

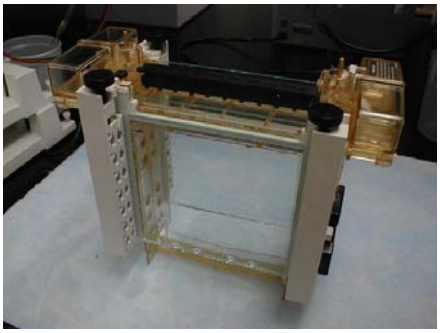


Gel assembly with comb inserted.

C. Running the gel

12. Prepare approximately 7 liters of 1X TAE and fill the buffer chamber, put about 0.5 I aside for later use. Preheat the buffer in the DCODE apparatus to 65°C; this will take about 2 hours.

13. When the temperature is about 50°C interrupt heating and attach the gel plates to the core assembly. Two sets of plates must be attached. Therefore, if only one gel is to be run, the other set of plates will be an assembly of two plates with no spacers or gel, assembled as described in step 2.



Gels attached to the core assembly.

Place the core assembly in the buffer chamber and the fill top reservoir with remaining buffer.

14. Flush each well with buffer to remove any unpolymerized acrylamide. Failure to do this might result in uneven well floors and unresolved bands. Continue heating until 65°C is reached. Do not add plate assembly to buffer that is too hot (>55^{o} C). This will cause the plates to crack.

15. Flush each well with buffer again and load approximately 40-50 μl of PCR product containing loading dye to each well. The volume loaded depends on the success of the PCR and the number of expected products. Soil samples produce many products, therefore the maximum volume should be loaded. Conversely, when running single isolate PCR products, a few microliters will suffice. Reset the temperature to 60°C and run at 20 V for 10 minutes, then run at 200 V for 5 hours (1000 V·h). Let the apparatus run at 200 V for 10 minutes before turning on the recirculation pump to minimize washing the sample from the wells.

The DGGE apparatus in action.

D. Staining the gel

16. When the electrophoresis is complete, take apart the apparatus and remove the glass plates from the gel clamps. Carefully separate the plates, leaving the gel exposed on the large plate. Use the edge of the small plate to trim the well walls, but be sure to leave the left-most wall slightly higher than the others for use as a reference. Also, trim off any portions of the gel that do not contain used lanes. For easy manipulation, the gel should be transferred to, stained on, and transported on plastic wrap.

17. Stain the gel for 15 minutes in 50 ml of GelStar nucleic acid stain (BioWhittaker) diluted 1:10,000 in 1X TAE. Remember, GelStar binds to nucleic acids therefore it is important to minimize contact with skin, so wear gloves (powder-free). Use a container that is slightly larger than the gel. The container should be plastic and not glass.

18. Slide the gel off of the plastic wrap onto a UV transilluminator and view the gel.

Further reading

Ercolini, D. 2004. PCR-DGGE fingerprinting: Novel strategies for detection of microbes in food. J. Microbiol. Meth. 56:297-314.

Muyzer, G. and K. Smalla. 1998. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. Antonie van Leeuwenhoek 73:127-141.