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. PCR products from a given reaction may be of similar size (bp) and 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 will denature at different denaturant concentrations resulting in a unique pattern of bands. Each band theoretically representing a different DNA sequence.

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
   2.0M Tris – 121g
   28.6ml of glacial acetic acid
   50ml of 0.5M EDTA (pH 8.0) - 9.3g EDTA/50ml
   Adjust pH to 8.0
   autoclave
10% Ammonium Persulfate Solution (APS, 0.1 g in 1 ml of sterile water. Make fresh when required.)
TEMED (N,N,N',N' -tetramethylenediamine)
Sterile water
Gradient wheel
Ethidium bromide
DCODE electrophoresis apparatus
Power supply
CAUTION
• Wear gloves and goggles throughout the entire protocol.
• Do not cross-contaminate your samples or the solutions. Be aware of your pipette tip.
• Work clean, on fresh white bench paper.
• Acrylamide and formamide are toxic. Use caution when working with these materials.

Protocol
DGGE gels will be poured and run to separate similarly sized PCR products. You will create gels by combining 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 that differ in sequence will be denatured during electrophoresis. The gel will be stained with ethidium bromide and visualized to reveal band patterns that can be used to determine the similarity of samples.

A. Building the gel assembly

1. Using a Kimwipe, wipe the glass plates, spacers and combs. Do not use harsh abrasive cleaning materials to clean any of the equipment.

2. Assemble the gel sandwich (Figure 1) by placing the small glass plate on top of the large plate. Be sure to correctly place the 1 mm spacers along each edge of the plate assembly. The grooved side of the spacer should be facing up. 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. Breaches in the seal of the plate assembly with the bottom of the pouring stand will result in leaking gel solutions.

Figure 1 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 the pouring stand, insert the plate assembly, and clamp into place.

4. Attach the small delivery tubing to the syringes.

**B. Pouring the gel**

1 Run a 6% polyacrylamide denaturing gel containing a 40-80% gradient (2.8 M urea/16% formamide to 5.6 M urea/32% formamide).

Use the following table to prepare the stock solutions you will need.

<table>
<thead>
<tr>
<th>Component</th>
<th>40% (low)</th>
<th>80% (high)</th>
</tr>
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<tbody>
<tr>
<td>40% acrylamide/bis</td>
<td>15 mL</td>
<td>15 mL</td>
</tr>
<tr>
<td>50X TAE</td>
<td>2 mL</td>
<td>2 mL</td>
</tr>
<tr>
<td>formamide</td>
<td>16 mL</td>
<td>32 mL</td>
</tr>
<tr>
<td>urea</td>
<td>16.8 g</td>
<td>33.6 g</td>
</tr>
<tr>
<td>dH₂O</td>
<td>to 100 mL</td>
<td>to 100 mL</td>
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</tbody>
</table>

2. You will use two solutions (20 ml each) from the above stock: a “low-denaturant” concentration solution, and a “high-denaturant” concentration solution. Label one 50 mL blue conical centrifuge tube ‘low-density’ and a separate 50 mL blue conical tube as “high-density.” Add 20 mL of each stock (low and high density) into the correct 50 mL blue conical centrifuge tube. Add 400 µl of DCode dye solution to the high-density solution. The gradient wheel (Figure 2) will combine the high and low-density stocks during the pouring of the gel to create the gradient within the gel matrix.

3. Add 180 µl/20 mL of solution of 10% APS (0.1g/ 1.0 mL, made fresh) and 18 µl/20 mL of solution of TEMED into each tube and swirl gently to mix. These reagents begin the polymerization of the acrylamide. At this point, you will have approximately 10 minutes to pour the gel before the acrylamide begins to polymerize.

4. Draw each solution into the correctly labeled syringe (low and high density) that already contains the tubing attached. Make sure air is removed from syringes (by tapping on the side) before placing them into the gradient maker. Place each syringe into the proper location on the gradient wheel (Figure 2). Attach the “Y” connector to each tube. Attach an 18G-1 ½-inch needle to the end of the tubing. Be sure the gradient wheel is in the start position.
Figure 2 The gradient wheel. The two syringes hold the high denaturant-concentration solution (back syringe) and low denaturant-concentration solution (front syringe).

5. Place the delivery needle 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 just below the lower plate. It should take between two to three minutes to pour the gel.

6. Carefully, without disturbing the gel solution, place the comb at a slight angle between the plates. Be sure to avoid trapping any air bubbles as the comb is lowered into the solution. Let the gel polymerize for at least one hour to overnight (Figure 3).

Figure 3. Gel assembly with comb inserted.
C. Running the gel

1. Prepare approximately 7 liters of 1X TAE buffer and fill the buffer chamber; put about 0.5 L aside for later use. Preheat the buffer in the DCODE apparatus to 55°C.

2. Gently remove the comb. Using a small syringe and needle, flush each well at least twice with 1X TAE buffer to remove any unpolymerized acrylamide. Failure to do this might result in uneven well floors and unresolved bands.

3. Attach the gel plates to the core assembly. Wet the gaskets of the core assembly before attaching the gel plates. This will help to prevent leaks. Two sets of plates must be attached. If only one gel is to be run, the other set of plates can be assembled with no spacers or gel (Figure 4).

4. Place the core assembly into the chamber (red is on the right side of the chamber) and add enough 1X TAE buffer to the upper reservoir to cover the gel. Pre-run the gel at 55ºC at 90 volts for 30 minutes.

5. Remove the core assembly from the chamber (pour the buffer in the upper reservoir into a beaker) and remove the buffer from each well.

6. Add an equal volume of 2X Loading Dye to each sample. Load the entire PCR product into each well. Gently add enough 1X TAE buffer to each well to bring the volume to the top of each well. Gently add the buffer back to the upper reservoir and place the core assembly back into the chamber. Reset the temperature to 60ºC and pre-heat the gel for 10 minutes without any voltage.

7. Run the gel at 150 volts and 60ºC for 10 minutes before turning on the recirculation pump to minimize washing the sample from the wells. Run the gel at 150 volts and 60ºC for approximately 20-24 hours (Figure 5).
D. Staining the gel

1. When the electrophoresis complete, gently take apart the apparatus and remove the glass plates from the gel clamps. Using a plastic wedge, carefully separate the plates, leaving the gel exposed on the large plate. Use a razor blade to trim the well walls. Make a notch at the bottom of the gel to mark the first lane in the gel. Also, trim off any portion of the gel that does not contain sample. Place the gel in a small plastic container that already contains stain.

2. Stain the gel for approximately 1-2 hours in 5 µg/mL of ethidium bromide in 1X TAE. **WEAR GLOVES AND GOGGLES, ETHIDIUM BROMIDE IS A MUTAGEN.** Remove the ethidium bromide and place it back into a dark colored bottle (it can be used again). Destain the gel for 30 minutes in 1X TAE. Remove the destain (place the used destain in an ethidium bromide waste container) and add fresh 1X TAE.

3. View and photograph the gel using a UV transilluminator.