

Biology 475 - Molecular Biology Lab

Population Genetics - DGGE



Goals – Big Picture and Recall

PCR amplifies many copies of a target gene (in our case: the 16S rRNA gene)

Our product contains many organisms (i.e. mixed population of bacterial 16S genes, all of which vary)

Today, you will learn how to use TWO kinds of gels to analyze PCR products in different ways

One = agarose (by size); another = denaturing gradient gel electrophoresis (DGGE, by sequence)

Recall Simple Agarose

Uses agarose matrix to separate DNA based primarily on size – smaller faster; bigger slower

LOW resolution in terms of showing genetic differences - only about 50-100 base pairs

Given that your PCR products are the same length, all ran together – forming a single band

Compare/Contrast With Complex DGGE

Uses acrylamide and a gradient of melting chemicals to separate DNA based on genetic composition

Similar to melting temperature of DNA – higher %GC content, higher melting temperature

Melting can be achieved through the use of chemicals – like acid or base

By creating a chemical gradient, a given %GC DNA will melt and physically stop at a specific place

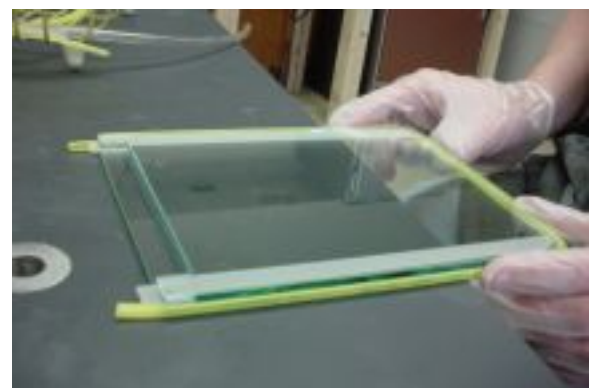
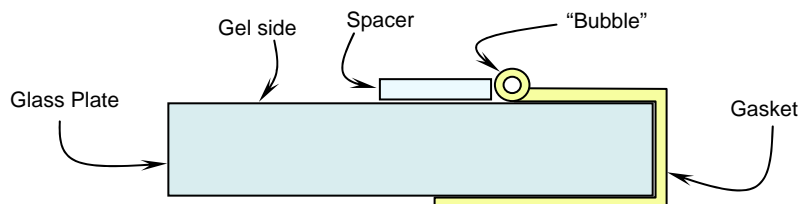
So if you are working with a population, each member will stop at a different place in the gel

THUS: each band represents a different members of your population; more bands = more diversity

Setting up the DGGE gel (takes longer, doing first)

Make sure plates are VERY clean; focus on one side of the plates only (remember which side this is) and wash twice with soap, then rinse with ddH₂O. Dry inner faces with 95% EtOH and chemwipes.

Take plate with rounded corners (back) and wrap with yellow gasket starting at the rounded corners with notched part of gasket. Make sure “bubble” side faces side receiving the gel (see diagram).



Lay spacers next to the gasket, and gently lay front plate down, making sure that the bottoms and the sides are lined up.

Hang the bottom of the stack over the end of the bench by about 2 inches. Use two clamps spaced evenly to secure the bottom and stand the plates up (fit plates as far as they fit on the clamps).

Use three more clamps on each side to finish setting up the plates.

Pouring the Gel

After retrieving ice, locate two dedicated 15 ml conical tubes: HIGH and LOW
Into each tube add 12 ml of the corresponding stock solution (HIGH or LOW) and place on ice
To HIGH, add 200 ul of Dcode dye and mix (this allows for visual interpretation of gradient)
Get TEMED out from the fridge and place on ice
Prepare APS by mixing 0.1 g with 1ml of H₂O in a microfuge tube and place on bench (not ice)
Set up the gravity pouring station (see pictures below because this can't be explained in words)

When the apparatus is set up, be prepared to move VERY quickly

To each gradient add 10 ul of TEMED and 100 ul of APS and cap and mix
Add the low gradient to the left side of the gradient maker (the side without the exit hole)
Ever so briefly, open then close the valve between the two to get all of the air out of the valve
Use a transfer pipette to put any excess back
Add the high gradient to the right side and set it spinning
Very carefully open both valves at the same time and sit back and watch the gradient being formed
When full, put needle in beaker and add ddH₂O to both sides of the gradient maker to wash
Carefully place the comb in the top of the gel - teeth first and at an angle to prevent air bubbles
Carefully wrap the top of the gel with plastic wrap, limiting air above gel
Use additional clamps around the comb and allow to polymerize for 30 minutes



Setting Up the Gel

Remove plastic wrap and squirt a little bit of 1x TAE on the comb to facilitate removal
Carefully and slowly remove comb and rinse wells with 1X TAE
Remove side clamps and lay gel on the bench so bottom clamps hang over bench and remove these
Slowly pull gel wrap gasket from ONLY bottom of the plates (stop 1 cm from start of rounding part)
Place gel in gel holder (front plate towards center) and use 4 clamps to clamp in place
Add another gel to the other side, or just a glass plate if you are only loading one gel)
Submerge gel holder in buffer tank and attach tube to inlet, letting upper gel chamber fill with buffer

Sample Loading and Running Gel

Mix a 20-30 ul solution - half sample and half 2X loading dye - in a separate tube
Use rounded gel loading tips to add 20-30 ul of sample to each well
Attach black anode to gel holder terminal, close lid, and turn on power 60 Volts overnight)
Gels will be stained and visualized tomorrow; will be done for you because it is extremely difficult