



## Biology 475 - Molecular Biology Lab Southern Blotting/DNA Transfer



### Goals

To master denaturing and neutralization procedures, understanding the purpose  
To transfer DNA from a gel to paper, understanding why and what will happen next  
To learn about alternative methods for blotting from gel to paper

### Background

Southern, Northern, and Western blotting transfer gel-separated biological molecules to paper  
Southern = DNA; Northern = RNA; and Western = protein  
In all cases, gels are denatured in acid

### ACTIVITIES

#### Samples to Load/Analyze

Standard marker; your full-length PCR products from Yellowstone mat sample  
Extra Red Positive Control (to be provided) - in case your PCR did not work  
Negative Control (to be provided) - in case your PCR did not work

#### Review/Recall Agarose Gel Set-Up

Prepare 50 ml of 1% agarose per gel box in TAE buffer  
After boiling (heat on high and watch like a hawk), cool 10 minutes at 50°C before pouring  
Tape ends of a gel tray and place ONE 12-lane comb in upper holders  
Pour the cooled agarose into the tray; it will take 10-15 minutes to harden  
Place hardened gel/tray in box such that, when the power is on, the DNA will run to the positive end  
Cover the gel completely with 1X running TAE buffer. CAREFULLY pull the comb out of the gel  
Mix your samples with loading dye by adding 2 ul dye to the top walls of each tube and tapping down  
Your instructor will do a quick demonstration load of the standard markers (10 ul - very expensive!)  
Load your samples, setting the pipetteman to 15 ul.  
Run your gels at 75-100 volts for 30-60 minutes; make CERTAIN you record what you loaded where.

#### Solution Preparation During Gel Run

*Each person will be assigned one of the solutions used for blotting to mix. You will only be given final concentrations and formula weights. Come in with each completed for your pre-lab.*

Monica: 0.25 M denaturation solution (1.5M NaCl/0.5 NaOH)

Tyler: neutralization solution 1.5M NaCl/0.5 M Tris-Cl (adjust to pH 7 with HCl)

Jenn: 20X SSC (3 M NaCl, 0.3 M trisodium citrate/2H<sub>2</sub>O) (adjust to pH7 with HCl)

#### Gel Staining and CAREFUL Documentation

Using gloves, stain gels with ethidium bromide solution (a mutagenic DNA-binding stain)  
Solution = 10 ml ethidium bromide plus 200-300 ml TE (may be used)  
Photograph gels using UV light - visualized ethidium bromide/DNA  
Place a ruler next to the gel for reference later; retrieve pictures and interpret

*Ethidium bromide can not be disposed of in the sink or public waste bins; the back hood of NS201 contains the only departmental waste area for this material.*

## **Southern Blotting**

After running and photographing gel, shake it in 300-400 ml distilled water (1-2 minute rinse)

Transfer to 300-400 ml 0.25 M HCl, shaking for 30 minutes; Rinse in distilled water (1-2 minutes)

Transfer to 300-400 ml 0.25 M denaturation solution (1.5M NaCl/0.5 NaOH), shaking for 20 minutes

Rinse in 300-400 ml distilled water (1-2 minutes)

Transfer to 300-400 ml 0.25 M neutralization solution, shaking for 20 minutes

*Watch the colors of the dye to assess denaturation and neutralization. The dyes should change colors during the former and revert to "normal" during the latter.*

## **ROUGH set-up**

Wet nitrocellulose and soak in 20X SSC 5 minutes during final gel neutralization

Fill baking dish with 20X SSC – place glass platform over dish

Wet blotting sheets in 20X SSC and "hang over" platform to wick 20X SSC reservoir in dish

Carefully flatten wicking blotting paper and lay gel on platform – carefully flatten with glass rod

Stack in order on gel (all cut to size): nitrocellulose, 2 blotting papers, 3-4 inches paper towels

Place 2-4 g weight in center on top of paper towels, wick overnight

*Instructors will bake blots (2 hours, 85 degrees C) and place in pre-hybridization probe the night before your next lab.*