RESTRICTION OF GENOMIC DNA AND PREPARING A SOUTHERN BLOT

You will digest your genomic DNA samples with several combinations of restriction endonucleases, electrophorese those samples on an agarose gel, and make a Southern blot transfer of that gel to a nitrocellulose filter. The transfer will then be analyzed by hybridization to a probe that will detect fragments containing your transposon.

1. Label five microtubes (#1-5). Using a "master mix" method to ensure uniformity among samples (to be demonstrated), pipet into each microtube 20 μl of restriction mixture containing the following: 10 μg of genomic DNA, 2 μl appropriate restriction enzyme 10X mix, 1 μl (20+ units) of the following restriction endonucleases:

    - EcoRI (mix 4)
    - EcoRI + KpnI (mix 4)
    - SacI + KpnI (mix 4)
    - SacI (mix 2)
    - SacI + XhoI (mix 2)

2. Incubate your samples overnight at 37°C.

3. Pour a 1% agarose gel and allow to solidify at least 1 hr.

4. Add 5 μl of loading dye to each microtube and load the samples into the gel. Electrophorese at 10 mA for 10 min., then at 70 mA until the bromphenol blue dye has migrated about ¾ of the gel length.

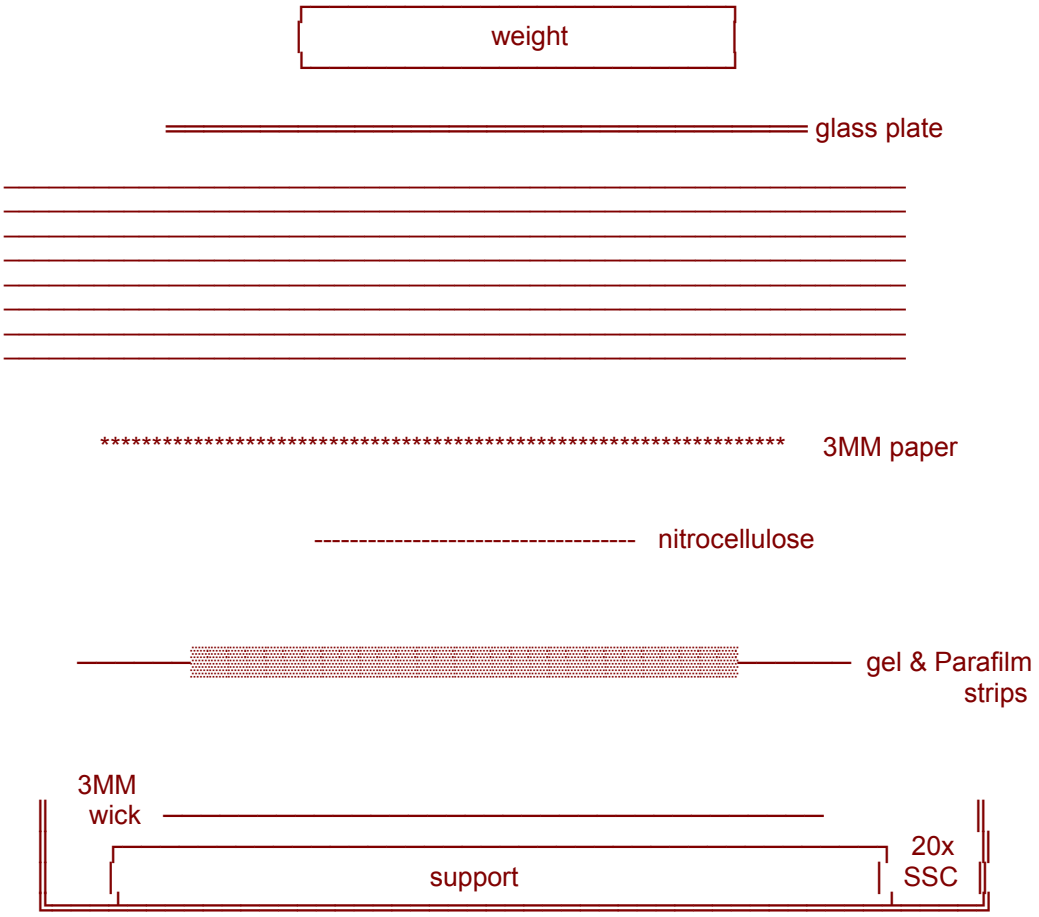
5. Stain your gel by bathing in ethidium bromide for 20 min. Obtain a photo image of the gel, including a ruler to note distances of migration.

6. Denature the DNA in the gel by bathing 30 min. in 1.5M NaCl +0.5M NaOH.

7. Neutralize the gel by bathing 30 min in 1.5M NaCl + 0.5M Tris-Cl, pH 7.5. During this step, prepare a nitrocellulose sheet and other materials for your Southern blot apparatus.

8. Assemble the gel/filter sandwich and blotting apparatus as demonstrated. Allow to percolate overnight.

9. Disassemble the apparatus, marking the locations of wells and corners of the gel on the nitrocellulose membrane with a ballpoint pen. Air dry the membrane for at least 1 hr, then bake at 80°C under vacuum for 2 hrs.



Southern blotting apparatus

****

20X TAE buffer:	0.8 M Tris	(96.8 g/l)
	0.04 M EDTA	(14.9 g/l)
	glacial acetic acid to pH 8.1	(~28 ml/l)
20X SSC (Saline Sodium Citrate):	3 M NaCl	(174 g/l)
	0.3 M sodium citrate	(88 g/l)
	pH 7 with citric acid	
Loading dye:	30% glycerol	
	1X TAE buffer	
	bromophenol blue (to taste)	