

## ***TMCF Protocols: Southern Blotting***

### **BUFFERS:**

#### 1) Prehybridization/hybridization buffer

1% SDS  
6X SSC  
10 % dextran sulfate  
100 µg salmon sperm DNA/ml (added with the radioactive probe)

**Note:** Store hybridization buffer at 4 degrees and warm to 65 degrees prior to use since the dextran sulfate tends to precipitate during storage.

#### 2) 20X SSC Stock Solution

Add 175.3 g NaCl and 88.2 g Sodium Citrate to 800 ml distilled water and pH to 7.0.  
Add water to 1000 ml and autoclave to sterilize.

### **PROTOCOL:**

Digest 10 µl (10 µg) of the sample DNA with the appropriate restriction enzyme.

#### Conditions:

10.0 µl	DNA
2.5 µl	10x restriction buffer
2.5 µl	0.1 M 2-mercaptoethanol
2.0 µl	enzyme
8.0 µl	<u>water</u>
25 µl final volume	

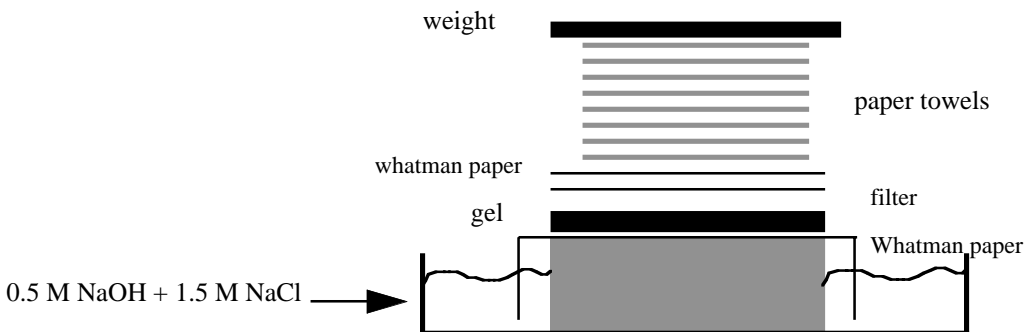
Mix well and digest **overnight** at the appropriate temperature for the enzyme of your choice. This is necessary to ensure proper digestion of the genomic DNA.

Spin down the digested samples and run on a 0.8 % TAE (or TBE) agarose gel. Be sure to include both a negative and a positive control and a size marker. Run the gel at **no greater than 60V** until proper separation of the desired bands is achieved. This generally takes from 6-18 hours depending on the size differential between the expected bands.

Take a picture of the stained gel. Be sure to use a UV ruler so that the size of the bands can be estimated. If desired, you can trim the gel to remove areas which will not contain the bands of interest.

**Optional:** Shake the gel in 0.2 M HCl for 10 minutes. This step hydrolyzes the DNA breaking it into smaller fragments which are transferred more quickly to the nitrocellulose membrane. However, too long an incubation will destroy the DNA completely, therefore it is not advised to perform this step unless the bands of interest are expected to be greater than 12-15 kb in size.

Shake the gel in 0.5 M NaOH/1.5 M NaCl for 30 minutes. Prewet the nylon filter according to the manufacturers instructions and set up the blot as per the diagram below. Transfer overnight.



Rinse the filter in 2X SSC buffer. Place between two sheets of Whatman paper and bake at 80 degrees under a vacuum for 1 hour, or crosslink with U.V. light.

Prehybridize your blot for at least 2 hours at 65 degrees, add radioactive probe and hybridize overnight. The amount of buffer that you add to your blot depends upon your hybridization set-up but should be enough to fully cover the blot.

Wash the filter:           1x 15 minutes in 2X SSC + 1% SDS, room temperature  
                                  2x 30 minutes in 0.1X SSC + 0.1 % SDS, 65 degrees

Dry the blot briefly between two sheets of Whatman paper, wrap the blot in saran wrap and expose to film overnight.