Southern Blotting

This method is used to check for a specific DNA sequence in a DNA sample.

Range of Separation in Gels Depending on Agarose Concentrations

Amounts of Agarose in gel (% [w/v])	Efficient Range of Separation of linear DNA molecules (kb)
0.3	5-60
0.6	1-20
0.7	0.8-10
0.9	0.5-7
1.2	0.4-6
1.5	0.2-3
2.0	0.1-2

Commonly used Electrophoresis Buffers

Buffer	Working Solution	Conc. Stock (per liter)
Tris acetate (TAE)	1 x : 0.04 M Tris acetate 0.001 M EDTA	50 x :242 g Tris base 57.1 mL glacial acetic acid 100 mL 0.5 M EDTA (pH 8)
Tris phosphate (TPE)	1 x :0.09 M Tris phosphate 0.002 M EDTA	10 x :108 g Tris base 15.5 mL 85% phosphoric acid(1.679 g/mL) 40 mL 0.5 M EDTA (p H 8.0)
Tris borate (TBE)	0.5 x :0.045 M Tris borate 0.001 M EDTA	5 x :.54 g Tris base 27.5 g boric acid 20 mL 0.5 M EDTA (pH 8.0)
Alkaline	1 x :50 MN NaOH 1 mM EDTA	1 x :.5 mL 10 N NaOH 2 mL 0.5 M EDTA (pH 8.0)

Preparation of an Agarose Gel

1, Seal the edges of a clean, dry, glass plate with autoclave tape to form a mold. Set the mold on a horizontal section of the bench.

2. Prepare a sufficient amount of the electrophoresis buffer to fill the electrophoresis tank and to prepare the gel. Add the correct amount of powdered agarose according to the first table.

3. Loosely plug the neck of the Erlenmeyer flask with Kimwipes. When using a glass bottle, make sure the cap is loose. Heat the mixture in a boiling water bath or microwave until the agarose dissolves.
4. Cool the solution to 60°C, and add ethidium bromide (handle with caution!) to a final concentration of 0.5 ug/mL and mix.
5. Using a Pasteur pipette, seal the edges of the mold with a small quantity of the agarose solution. Allow the seal to set. Position the comb 0.5-1.0 mm above the plate so that a complete well will be formed upon addition of the remaining agarose solution.
6. Pour the solution into the well and check that there are no air bubbles. After the gel is completely set, carefully remove the comb and autoclave tape and mount the gel in the electrophoresis tank.
7. Add just enough electrophoresis buffer to cover the gel to a depth of about 1 mm.
8. Mix the samples of DNA with the desired gel-loading buffer. Slowly load the mixture into the slots of the submerged gel using a disposable micropipette.

Gel Loading Buffers

Buffer Type	**6X Buffer**	**Storage Temp**
1	0.25% bromophenol blue 0.25% xylene cyanol FF 40% (w/v) sucrose in water	4°C
2	0.25% bromophenol blue 0.25% xylene cyanol FF 15% Ficoll (Type 400) in water	Room temp.
3	0.25% bromophenol blue 0.25% xylene cyanol FF 30% glycerol in water	4°C
4	0.25% bromophenol blue 40% (w/v) sucrose in water	4°C
5	*Alkaline Loading Buffer* 300 mN NaOH	4°C

	6 mM EDTA 18% Ficoll in water 0.15% bromocresol green 0.25% xylene cyanol FF	

9. Close the lid of the gel tank and attach the electrical leads so that the DNA will migrate towards the anoda (red). Apply a voltage of 1-5 V/cm. If the leads were attached properly, bubbles should be generated and the bromophenol will start to migrate.
10. Turn off the gel once the DNA has migrated. If ethidium bromide was used, view the gel under ultraviolet light.