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A Geno Technology, Inc. (USA) brand name

Southern Blotting

Teacher's Guidebook

(Cat. # BE-315)



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MATERIALS INCLUDED

This kit has enough materials and reagents for a single class demonstration.

- 1 vial DNA Ladder (1kb)
- 1 vial DNA Loading Buffer (6X)
- 1 bottle TAE Buffer (50X)
- 1 pack Agarose
- 1 pack Absorbent Paper
- 1 bottle Denaturation & Transfer Solution
- 1 Nylon Membrane
- 6 sheets Filter Paper
- 1 Plastic Support
- 1 Filter Paper Bridge
- 1 Southern Blot Tray
- 1 bottle Post Transfer Buffer
- 1 vial DNA Stain (500X)
- 1 Forceps

SPECIAL HANDLING INSTRUCTIONS

- Store DNA Ladder frozen (-20°C).
- All other reagents can be stored at room temperature.

The majority of reagents and components supplied in the *BioScience Excellence™* kits are non toxic and are safe to handle, however good laboratory procedures should be used at all times. This includes wearing lab coats, gloves and safety goggles.

For further details on reagents please review the Material Safety Data Sheets (MSDS).

The following items need to be used with particular caution.

Part #	Name	Hazard
D001	Denaturation & Transfer Solution	Corrosive
D161	DNA Stain (500X)	Flammable

ADDITIONAL EQUIPMENT REQUIRED

- Agarose electrophoresis equipment.
- Pure or distilled water
- Shaker
- Washing Trays 12cm X 12cm

TIME REQUIRED

- **Day 1:** 1-2 hours
- **Day 2:** 1-2 hours

AIMS

- Teach the principle of Southern blotting.
- Demonstrate transfer of nucleic acids to nylon membrane.
- Explain principle of hybridization.

BACKGROUND

Southern blotting is a method used to transfer DNA from an agarose gel to a membrane, where the DNA can be subsequently probed for a specific sequence. The technique was named after its inventor, Edward M. Southern, who developed the technique in Edinburgh, Scotland in the 1970s. As a result subsequent blotting techniques have used similar nomenclature, for example Northern blotting, the transfer of RNA; Western blotting, the transfer of proteins; and Southwestern blotting, for the characterization of proteins that bind DNA.

Basically the DNA mixture is separated by agarose electrophoresis that separates the DNA fragments by size, using an electric charge. On completion of electrophoresis, a nylon membrane, which binds DNA, is placed on the gel and by using capillary action the DNA migrates into the membrane. For nylon membranes, the DNA is cross-linked to the membrane by the action of ultraviolet light; nitrocellulose membranes use heat to fix the DNA to the membrane.

For the detection of specific DNA sequences, a hybridization probe is used. A hybridization probe is a short (100-500bp), single stranded nucleic acid that will bind to a complementary piece of DNA, or RNA in the case of Northern blots. Hybridization probes are labeled with a marker so that they can be detected after hybridization. The labels used include radioactivity, usually radioactive phosphorous, or fluorescent labels. The excess probe is washed away and the attached probes detected. The results show which DNA fragments contain the sequence of interest.

This kit teaches Southern Blot transfer of DNA to a Nylon membrane and the principles of hybridization probes.

TEACHER'S PRE EXPERIMENT SET UP

Preparation of agarose gel

You require one small agarose gel with approximate dimensions of 8 x 5cm (3 x 2in) that has at least 2 wells.



Wear heat protective gloves throughout the agarose melting and pouring procedure. Make 1-2 hours before the experiment.

1. **Prepare running buffer:** In a clean two liter container, add the entire contents of the 50X TAE buffer and add two liters of ultra pure water to make a 1X running buffer solution. Stir until thoroughly mixed.
2. **Prepare agarose:** In a clean, glass 1000ml container add the entire contents of the agarose pack and add 500ml of the running buffer from step 1.
3. Heat the solution in a microwave on full power, using 10 second bursts, or use a boiling waterbath. Check to see if all the agarose has dissolved. Continue until agarose has dissolved.



DO NOT BOIL. The agarose gets very hot, very quickly and can cause severe burns. Wear heat protective gloves throughout the melting and pouring procedure.

4. Once the agarose has cooled to the point it can be held comfortably in your hand, pour the agarose into the gel casting mould as per the manufacturer's instructions. You will need 2 wells that each hold 20 μ l, use an appropriate size comb.
5. Once the gel has set, remove the comb, transfer to the running apparatus and cover with the running buffer until ready to use.

Prepare the DNA Ladder

1. Add 25 μ l ultra pure water to the lyophilized 1kb DNA ladder; dissolve by gently pipetting up and down 5-6 times.
2. Add 5 μ l 6X DNA Loading Buffer, mix by gently pipetting up and down 5-6 times.

Prepare Dilute DNA Staining Solution

1. Add 100ml 1X TAE buffer to a 1-liter container and add 900ml deionized water to make a 0.1X TAE buffer. Stir until thoroughly mixed. The 0.1X TAE buffer is used to dilute the DNA Stain and also as the destaining solution.
2. To 500ml 0.1X TAE buffer add 1ml 500X DNA Stain. Stir until thoroughly mixed.

MATERIALS FOR DEMONSTRATION

- 1 Agarose gel in 1X Running Buffer
- 1 vial DNA Ladder
- 1 bottle Denaturation & Transfer Solution
- 1 Nylon Membrane
- 6 sheets Filter Paper
- 1 Filter Paper Bridge
- 1 Plastic Support
- 1 Southern Blot Tray
- 1 bottle Post Transfer Buffer
- 1 Weigh Boat
- 1 bottle DNA Staining Solution
- 1 Forceps

DEMONSTRATION PROCEDURE

The loading and migration of the DNA fragments normally takes 30-60 minutes and can be started before the class arrives. The demonstration of Southern blot analysis begins at step 4.

1. Load 10 and 20 μ l DNA Ladder into two wells, leaving an empty well between the samples.
2. Once the samples are all loaded apply a current and migrate at 12-15V/cm. For an 8cm long gel run at 96-120 volts.
3. Once the blue dye front has migrated $\frac{1}{2}$ - $\frac{3}{4}$ the length of the gel, turn off the power and carefully transfer the gel to the lid of the Southern Blot Tray.



CAUTION: Agarose gels are very fragile, handle with extreme care.

4. Add the entire contents of the Denaturation & Transfer Solution bottle.
This buffer contains sodium hydroxide, a strong alkali, and sodium chloride that denatures the DNA. Denaturation facilitates transfer and also prepares the DNA for probe access for hybridization. If large DNA is to be transferred, the gel is soaked in hydrochloric acid before this step. This depurinates the DNA and aids in the transfer of large DNA strands.
5. Incubate at room temperature for 15-30 minutes.
6. During this incubation, add some deionized/ distilled water to the Southern Blot tray. Using the forceps, transfer the nylon membrane to the tray. Incubate until completely soaked through.
7. With the forceps transfer the nylon membrane to the tray lid with the gel and Denaturation & Transfer Solution. Incubate for a minimum of 5 minutes.



Ensure the nylon membrane does not come into contact with the agarose gel.

8. After incubation is complete, assemble the Southern blot (Figure 1).

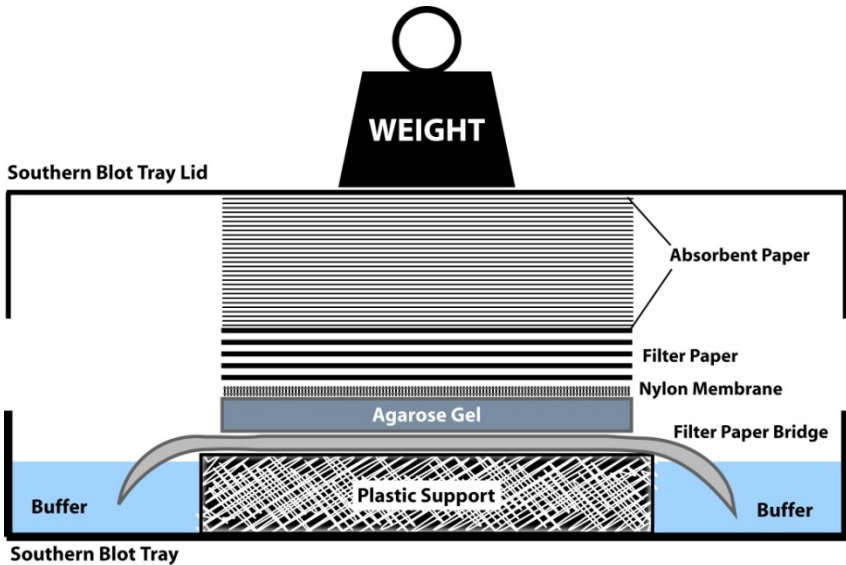



Figure 1: Assembly of Southern Blot.

9. Firstly discard the water from the bottom of the Southern blot tray. Place the plastic support in the center of the tray. Briefly, soak the Filter Paper Bridge (the long sheet of filter paper) in the Denaturation & Transfer Solution containing the agarose gel.
10. Lay the Filter Paper Bridge across the Plastic support so the ends hang down into the tray.
11. Carefully move the agarose gel onto the Filter Paper Bridge.

 **CAUTION:** Agarose gels are very fragile, handle with extreme care.
12. Using the forceps lay the Nylon Membrane on top of the gel, ensuring no air bubbles are present.
13. Pour the Denaturation & Transfer Solution into the bottom of the tray, so that it reaches just below the top of the plastic support (see figure 1).
14. Next place the six dry sheets of Filter paper on top of the Nylon Membrane.
15. Remove the Absorbent Paper from the bag and keeping the paper folded in half place the Absorbent Paper on top of the filter paper.

16. Place the lid on the Southern Blot Tray and carefully apply pressure to lock the lid onto the base.
17. Place a 500gm weight, such as a 1-inch thick book or 250ml bottle on top of the entire assembly.
18. Leave overnight at room temperature.
19. The following day, disassemble the Southern Blot assembly and using the forceps transfer the Nylon membrane to the Southern blot tray lid. Add the entire contents of the Post Transfer Buffer to neutralize the membrane. Incubate with gentle shaking at room temperature for 15 minutes.
The Post Transfer Buffer is a neutral pH buffered saline solution.
20. With the forceps remove the Nylon Membrane from the buffer, allowing all excess liquid to drain away and transfer to the clean washing tray.
21. Add the DNA stain on top of the membrane and incubate for 5-15 minutes at room temperature with gentle shaking.
22. Rinse with distilled or deionized water and then destain with destaining solution until the blue background disappears and the DNA bands are visible (1-2 hours).
23. Once destained sufficiently, the membrane can be removed from the tray and placed on a paper towel to dry.

DISCUSSION

Discuss the principles of hybridization probes and the uses of Southern Blot Analysis.

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