**DNA BLOT HYBRIDIZATION**

Reference: Sambrook et al. (1989). Molecular Cloning Manual #2.

**Solutions Needed:**

* **Depurination** Solution (0.25M HCl)

Measure 490 mL of double-distilled H2O (from the Millipore water system) into a 500-mL glass or plastic graduated cylinder. Carefully, pipet 10 mL of concentrated hydrochloric acid (HCl) solution using a 10-mL disposable pipet and dispense it into the double-distilled water. Pour the mixture into a 1-L Erlenmeyer flask. Seal the flask with a piece of parafilm. Swirl to mix the contents.

* **Denaturation** Solution (1.5 M NaCl, 0.5 M NaOH)
* **Neutralization** Solution (1 M TRIS, 1.5 M NaCl, pH 7.4)
* **20X SSC** (3 M NaCl, 0.3 M Sodium citrate, pH 7.0)

**Apparatus Needed:**

1. Gel Cutter
2. A roll of positively charged nylon membrane
3. Several pieces of 3MM Whatman filter paper
4. A roll of plastic wrap or parafilm
5. Three plastic containers (one for wetting the membrane, one for setting up the blot, one for gel treatments)
6. Two pairs of blunt-ended forceps
7. Two pieces of glass plates
8. Orbital shaker

**Transferring DNA from an Agarose Gel to a Positively Charged Nylon Membrane (Blotting via Capillary Transfer)**

Reference: Southern, E.M. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98: 503.

1. After taking the picture of the gel, trim away unused areas of the gel with a gel cutter. Cut off the top right‑hand corner of the gel (to the right of the right-most well) for identification.
2. Carefully, pour 250 mL of the **Depurination** solution into a plastic container (provided by your TA).

Caution: Do not let the Depurination solution get on your skin and clothes because it

causes mild skin irritation and creates holes on your clothes (after you do your laundry!).

If you accidentally get some solution on your hand/arm, calmly rinse your hand/arm with cold water for a few minutes.

1. Carefully, put the gel into the **Depurination** solution and soak it for 15 minutes, at room temperature, with gentle shaking (~50 rpm) on an orbital shaker.

Note: Do NOT soak the gel in this solution for more than 15 minutes because over-

depurination will result in a large piece of DNA broken down into many tiny fragments (with less than 100 bases in length) that would not be efficiently transferred onto the nylon membrane.

1. After 15 minutes, **carefully** pour off the depurination solution with one hand **gently** over the gel preventing it from slipping out of the container.
2. Rinse the gel with ~ 250 mL of deionized water, **TWICE**, to wash HCl off the gel.
3. Soak the gel in 250 mL of **Denaturation** solution with constant, gentle shaking for 15 minutes at room temperature.
4. (After 15 minutes), pour off the **Denaturation** solution using the same technique as in **step 4** above. Repeat **step 6** with another 250 mL of **Denaturation** solution.
5. Rinse the gel with ~ 250 mL of deionized water, **ONCE**, to wash **Denaturation** solution off the gel.
6. Soak the gel in 250 mL of **Neutralization** solution with constant, gentle shaking for 15 minutes at room temperature.
7. (After 15 minutes), pour off the **Neutralization** solution using the same technique as in step 4 above. Repeat **step 9** with another 250 mL of **Neutralization** solution.
8. Meanwhile, cut a piece of a positively charged nylon membrane to the same size as the size of the gel tray (or ~ 0.5 cm larger than a portion of the gel in both dimensions).

Note: Wear gloves and use blunt‑ended forceps to handle the filter.

1. Cut three pieces of 3MM Whatman paper to the size 1 cm larger than the size of the cut nylon membrane, and another long piece of 3MM Whatman paper with its width the same as the width as the small piece and its length of double or triple the length of the small piece (see Figure 1).

Note: the dimensions of small and large pieces of 3MM Whatman paper are variable,

depending on the size of the gel and the blotting setup.

1. Float the nylon membrane on the surface of a dish of distilled water for a minute or until it wets completely from beneath, and then replace the water with 20X SSC solution and leave the membrane in this solution for at least 5 minutes.
2. Assemble the transfer apparatus (see Figure 1) for the **capillary transfer** of DNA to the nylon membrane.
3. Put a piece of glass plate over the support sitting in the container containing 20X SSC solution.
4. Wet the long piece of 3MM Whatman paper in 20X SSC solution and place it on a glass plate.
5. Roll out any bubbles formed between the Whatman paper and the glass plate using a 5-mL disposable plastic pipet.

Note: the area where bubbles formed will be lack of DNA on the nylon membrane.

1. Pour small amount of 20X SSC solution at the center of the Whatman paper.
2. Carefully, place the gel **upside down**, starting at the pool of the 20X SSC solution on the Whatman paper. Again, roll out any bubbles formed between the gel and the long piece of 3MM Whatman paper as in the **step c**.
3. Place the wet nylon membrane on top of the gel using two pairs of blunt-end forceps. Perform **step c** to roll out any bubbles.
4. Cut out four pieces of plastic wrap (or parafilm longer than the blot). Place one piece at ~0.5 cm on each of the four edges of the nylon membrane, and stretch them over the sides of the plastic container. These plastic pieces separate the paper towels from the long piece of 3MM Whatman paper. Thus, 20X SSC solution will go from the container through the gel and the small pieces of 3MM Whatman paper towards the paper towels. As the solution travels, it will bring DNA from the gel onto the nylon membrane. This action is **capillary transfer** of DNA to the nylon membrane.
5. Wet each of three pieces of 3MM Whatman paper in a container containing 20X SSC solution in which the nylon membrane was soaked. Place the wet 3MM Whatman on top of the nylon membrane. Again roll out any bubbles formed between the nylon membrane and 3MM Whatman paper. Repeat for the second and third pieces of 3MM Whatman paper.
6. Place a stack (~3-inch thick) of paper towels on top of the last 3MM Whatman paper. The paper towels will absorb water from the 3MM Whatman paper, resulting in water being pulled up from the reservoir. This is **capillary** action.
7. Place a glass plate on top of the paper towels and then a weight.

**GEL**

**Stack of Paper towels**

**Nylon membrane**

**20X SSC**

**solution**

**Plastic wrap or parafilm**

**Small Pieces of 3MM Whatman**

**paper**

**~ 500 g Weight**

support

**Long Piece of 3MM Whatman paper**

**Glass plate**

**Figure 1:** Capillary transfer of DNA from an agarose gel to a nylon membrane. 20X SSC solution is drawn from a reservoir and passes through the gel into a stack of paper towels. The DNA is eluted from the gel by the moving stream of 20X SSC solution and is deposited on the nylon membrane. A weight on the top of the paper towels helps to establish a tight connection between the layers of material used in the transfer system. Plastic wrap or parafilm helps to

prevent the paper towels from touching the long piece of 3MM Whatman paper below the gel. If

such touching occurs, 20X SSC solution is drawn directly from the reservoir to the paper towels. Thus, there would little or no capillary transfer of DNA to the nylon membrane.

1. Record the orientation of the gel in regards to the filter below. Let the DNA transfer occur for at least 16 hours, record the time the transfer began.
2. Remove the weight, glass plate, paper towels, and three pieces of 3MM Whatman paper on top of the gel.
3. Mark the positions of gel slots on the filter with a pencil.

Note: DNA and the pencil mark are on different sides of the nylon membrane.

1. Remove the membrane from the gel and place it on a piece of plastic wrap slightly larger than the blot.
2. Expose the blot to a ultraviolet (UV) box for 5 min (DNA side faces the UV source) to crosslink the DNA to the membrane. This step fixes DNA onto the membrane.
3. Soak the blot (DNA side faces up) in a 6 X SSC solution for 5 min. Lightly scrub the membrane to remove agarose debris adhered to it. Blot the excess liquid off the membrane by placing it sandwiched between several layers of Kimwipes tissues.
4. If the blot is NOT subjected to prehybridization immediately, bake it under vacuum at 80oC in a vacuum oven for 1.5 hours to dry up the membrane as well as to fix DNA on it.

Note: Before baking the blot, place it sandwiched between two layers of Kimwipes

tissues that are inside a folded 3MM Whatman paper (this piece of paper is about 5-10

cm larger than the membrane). (TAs will demonstrate how to use the vacuum oven).

1. After baking the blot (membrane) under vacuum, place it in a heat‑sealable bag (e.g., Sears Seal‑A‑Meal).
2. Seal the bag containing the blot and store it in a drawer until prehybridization step is carried out.
3. If the blot is subjected to prehybridizing immediately right after step 21, put the blots in a heat‑sealable bag. Do not seal the bag until a pre-hybridization solution is added.

**B. Prehybridizing the Blots**

## Thaw the following components that are stored in a -20oC freezer in room 2836A:

## a dark brown bottle of 100% formamide in a 55oC water bath. Once thawed out, the bottle can be kept at room temperature during preparation of the pre-hybridization and hybridization solutions.

* a 50-mL Falcon centrifugetube of 50X Denhardts solution in a 42oC water bath.

Caution: do NOT warm the Denhardts stock solution **warmer** than 42oC because Bovine

Serum Albumin (or BSA, one of three components of the Denhardts solution) will come

out of the solution as white precipitates and will not be redissolved in the Denhardts

solution.

* a 14-mL tube of 10 mg/mL sssDNA in either a 42oC or 55oC water bath. Once thawed out, the tube can be kept on ice.

## Note: If you anticipate of setting up more hybridization within a month, you can store the bottle of formamide and the tube of 50X Denhardts solution in a refrigerator.

## Prepare pre-hybridization and hybridization solutions in two 50-mL Falcon centrifuge tubes by adding components shown in Table 1 to each tube.

## Note: Pre-hybridization and hybridization solutions only differ by the lack of NaPPi in the

## pre-hybridization solution.

* Follow the component list in **Table 1**.
* Make sure the stock concentrations you are using match to those shown in **Table 1**.
* Add denatured **s**heared **s**almon **s**perm DNA (or component vii) into the pre-hybridization/hybridization solution **immediately before** the solution is added to a hybridization bag containing the blot.
* Prepare a denatured sssDNA solution as follows:
  + Heat 150-200 mL of deionized water to boiling in a 250-mL round deep dish using a hot plate.
  + Pipet 0.6 mL of 10 mg/mL sssDNA into a sterile 1.5-mL microcentrifuge tube.
  + Put this tube on a small rack floating on the boiling water and keeping the tube in the boiling water for 5 minutes.
  + After 5 minutes of boiling, **immediately** transfer the tube containing denatured sssDNA on ice for at least two minutes before adding it to the pre-hybridization or hybridization solution. This is called **"quenching"** step to prevent reannealing of denatured complement strands of sheared salmon sperm DNA fragments.

**Table 1**. Preparation of Pre-hybridization and Hybridization Solutions.

|  |  |  |  |
| --- | --- | --- | --- |
| **Stock Concentration of Components** | **Pre-Hybridization** | **Hybridization** | Final Concentration |
| i. 100% Formamide (Ultrapure Grade) | 15.0 mL | 15.0 mL | 50% |
| ii. 50X Denhardts Solution | 3.0 mL | 3.0 mL | 5X |
| iii. 20X SSC | 7.5 mL | 7.5 mL | 5X |
| iv. 0.5M Phosphate Buffer, pH 7.0 | 3.0 mL | 3.0 mL | 0.05M |
| v. 20% SDS | 0.3 mL | 0.3 mL | 0.2% |
| vi. 10% NaPPi | N/A | 0.03 mL | 0.01% |
| vii. 10 mg/mL sssDNA**\*** | 0.6 mL | 0.6 mL | 0.2 mg/mL |
| viii. Sterile double-distilled water | 0.6 mL | 0.57 mL | N/A |
| **Total volume** | **30.0 mL** | **30.0 mL** |  |

**\*:**10 mg/mL sssDNAis added into the pre-hybridization/hybridization solution **immediately before** the solution is added to a hybridization bag containing the blot.

1. Warm the pre-hybridization solution in a 42ºC water bath for at least 30 minutes. Store the tube of hybridization solution in **either** the 42ºC water bath (if hybridization will be set up several hours later) **or** the fridge until needed (if hybridization will be set up on the next day).
2. Add the warmed pre‑hybridization solution to the seal-a-meal bag containing the blot.

Note: the volume of the pre-hybridization or hybridization depends on the size of the blots and is calculated as 0.2 mL of solution for every centimeter square (cm2) of the blot (length x width). For example, if the blot size is 150 cm2 (= 10 cm x 15 cm), then the volume of pre-hybridization and hybridization solution would be

30 mL (= 0.2 mL/cm2 x 150 cm2).

1. Squeeze out as much air bubbles as possible from the bag.
2. Seal the open end of the bag with the heat sealer.
3. Put the bag on a plastic container or a Pyrex glass dish.
4. Incubate the bag for at least 1-2 hours at 42ºC in an air incubator with ~100-rpm shaking.

**C. Preparing Radioactively Labeled DNA Probes Using Prime It II Random Prime Labeling Kit (Stratagene)**

Caution: When working with radioactive material, **concentrate** on what you are doing and follow the following tips:

* Work behind a plexi-glass shield.
* Wear goggles to protect your eyes from radiation source.
* Wear a lab coat to protect you and your clothes from getting radioactive materials on in case of accidental spill.
* Check the working area before and after your work with a portable **Geiger** counter.
* Wear gloves and change them when they are contaminated with radioactive material.
* Constantly check your gloves after handling tubes, vial, etc. containing radioactive materials with a portable **Geiger** counter.
* Check all the pipetman that you just used with a portable **Geiger** counter. Decontaminate the pipetman with a mild detergents solution.
* Wash your hands before touching your hair, your clothes, etc.

Note: The reagents and amounts of the Random prime reaction used to make your probe are shown in **Table 2**. Fill out the Table 2 before setting up the reaction.

**Table 2:** Randomly Primed DNA Probe Reaction.

|  |  |
| --- | --- |
| Components | **Volume** |
| i. 25-50ng DNA template | L |
| ii. 5X Random primers solution | 10 L |
| iii. Sterile double-distilled H20 | L |
| iv. 5X dCTP buffer | 10L |
| v. - 32P dCTP | 5 L |
| vi. Klenow fragment  (Large fragment of DNA polymerase I) | 1 L |
| **Total volume** | **50** L |

1. Thaw out tubes of components ii and iv that are stored in a -20oC freezer (room 2836A). Once thawed out, keep these tubes on ice.
2. Add components i - iii to a 1.5-mL microcentrifuge tube. Close the lid tightly.
3. Boil the components in the tube for 5 minutes in a round deep dish containing boiling water on a hot plate.
4. After 5 minutes, **immediately**, quench the tube on ice for at least 2 minutes.
5. Spin down water condensation on the lid of the tube in a microcentrifuge at room temperature for ~30 seconds. Put the tube back on ice.
6. Add components iv-vi into the microcentrifuge tube and mix the contents thoroughly by pipetting up and down several times.
7. Incubate at 37ºC for 10 minutes to generate randomly primed probes.
8. Meanwhile, prepare a Sephadex-100 column to purify labeled DNA probes from unincorporated - 32P dCTP nucleotides as follows:

* Get a 1cc or 1 mL syringe from a "General Use" drawer.
* Remove a cotton filter from a 1-mL disposable pipet using a pointed-end forceps.
* Stick the loose end of the cotton into the syringe.
* Use the plunger to push the cotton piece to the bottom of the syringe. Slowly, withdraw the plunger.
* Wet the cotton piece with TNE buffer using a disposable transfer pipet.
* Use a disposable transfer pipet to add resin solution (G100 Sephadex) until resin itself is above the 1.0-cc mark on the syringe. Note: Do not allow bubbles to form in the resin column.

1. After 10 minutes of incubation at 37ºC, add an equal volume (50 L) of 2X Nick translation dye (containing bromophenol blue and dextran blue dyes) to the labeling reaction.
2. Mix the contents well by pipetting up and down several times. Then add the whole mixture (~100 L) to the syringe/resin.
3. Wait until the dyes completely enterring the resin. Add ~100 L of TNE buffer at a time.
4. The dextran-blue dye (faster migrating dye) co-migrates with the probes. **Collect this fraction**. Stop the collection when clear liquid starts to come out.

Note: The purple (slower migration dye) co-migrates with very short fragments and

unincorporated - 32P dCTP.

1. When done, use a new tube to stop the flow and discard the syringe/resin in a plastic bag sitting in a 1-L plastic beaker dedicated as a radioactive dry waste container.
2. Use a P-1000 pipetman to measure the final volume of the probe solution as well as to mix the probe solution.

Volume: L

1. (Optional) Spin the tube containing probe solution in a microcentrifuge briefly.
2. Determine total cpm of probe in the labeling reactions using Beckman Scintillation counter LS 6500 as follows:

a. Dispense 2 mL of scintillation cocktail into a 20-mL glass scintillation vial.

b. Pipet 1L of the labeling reaction into the cocktail. Swirl to mix. Cap the vial.

c. Look inside of the chamber of the scintillation counter for a white rack with a black

tag **#2** (for **USER No. 2)**.

* Note: User No. 2 is a program set by the Goldberg lab for reading 32P-dNTP nucleotides. If the rack does not have a black USER tag on it, get the tag from a drawer below the scintillation counter.

d. Put the vial on the first available slot (or slot #1) of the white rack. Then, put the rack on the right side of the scintillation's chamber.

Note: There is a RED rack with "**HALT**" tag on the right side of the chamber. This rack serves to tell the scintillation counter to stop after reading all the vials in the WHITE rack. Slide this RED rack toward you. Then place your WHITE rack in front of the RED rack.

e. Press "MAIN MENU" button on the panel board of the scintillation counter.

* You will see "AUTOMATIC COUNTING" program of the MAIN MENU being highlighted.
* Press the "SELECT" button to select the "AUTOMATIC COUNTING" program.
* Presss "START" button to initiate the counting. The scintillation counter will read the sample. Areadout of samples will be automatically printed out on the printer.

f. Calculate the total cpm of labeled probes and probe specific activity as shown below:

Sample Calculations:

**Total cpm of probe** = (**100,000 cpm/L** of probe added) (350 L of probe solution) = 35,000,000 cpm of probe **Specific Activity** = (35,000,000 cpm of probe/50 ng of DNA template) (1000 ng/g) = 700,000,000 cpm of probe/g of DNA template or = 7 x 108 cpm of probe/g of DNA template

1. Determine a volume of the probes to be added to the hybridization solution. Note: Normally, we want to add 1 x106 cpm of probe to every mL of hybridization solution. Sample Calculations: Volume of hybridization solution to be used = **30 mL**The amount of probe needed = (30 mL)(1 x106 cpm of probe/mL of hybridization ) = 3 x107 cpm of probeHave probe's concentration = **100,000 cpm/L** or 1 x105 cpm/L Then, volume of probe needed = (3 x107 cpm of probe)/ (1 x105 cpm/L)

= **300 L**

**D. Hybridizing the Blots with the Denatured Radioactive Labeled Probes**

1. Boil the labeled probe and sheared salmon sperm DNA solution (sssDNA) in separate 1.5-mL microcentrifuge tubes for five minutes.
2. Meanwhile, warm the hybridization solution to 42oC in a water bath.
3. Immediately, quench the tubes on ice for at least two minutes.
4. Spin tubes in a microcentrifuge for 30 seconds.
5. Cut a corner out of the pre-hybridization bag. Pour out the pre-hybridization solution into a sink. Squeeze out almost all of the solution in the bag.
6. Add denatured sssDNA to the hybridization solution, then pipet this solution mix into the seal-a-meal bag containing the blot using a 10-mL or 25-mL disposable pipet.
7. Behind a plexi-glass shield, add the probe to the hybridization bag.
8. Seal the cut corner of the hybridization bag. Note: Leave space in the bag for trapping bubbles.
9. Eliminate bubbles by pushing them away from the blots. Seal the bag to separate bubbles from the blot.
10. Hybridize the blot at 42oC in the air incubator or water bath for at least 16 hours.
11. Record time of starting hybridization.

**Washing the Blots**

#### Prepare washing solutions in two 1-L Erlenmeyer flasks as shown below:

|  |  |  |  |
| --- | --- | --- | --- |
| **Final Concentration** | **Low Stringency** | **High Stringency** | **Stocks** |
|  | **(2X SSC/0.1% SDS)** | **(0.2X SSC/0.1% SDS)** |  |
| 2X SSC | 70 mL | N/A | 20X |
| 0.1% SDS | 7 mL | 7 mL | 10% |
| 0.2X SSC | N/A | 7 mL | 20X |
| ddH20 | up to 700 mL | up to 700 mL |  |
| **Total Volume** | 700 mL | 700 mL |  |

1. Warm the **high** stringency wash solution to 60oC in a water bath.
2. Keep the low stringency wash solution at room temperature.
3. Pour ~ 350 mL of the low stringency wash solution into a Pyrex glass dish.
4. Write on a 50-mL Falcon centrifuge tube "Name of the hybridization solution", "your initial", and "Date".
5. Remove the hybridization bags from the 42oC incubator or water bath incubator.
6. If the water bath incubator is used, change the temperature from 42oC to 60oC.
7. Behind a plexi-glass shield, cut a corner of the hybridization bag.
8. Carefully, pour the hybridization solution into the labeled 50-mL Falcon centrifuge tube.
9. Store the hybridization solution in a 50 mL Falcon centrifuge tube in a -20oC freezer.
10. Cut the hybridization bag. Remove the blots from the bag. Immediately, place the blots one-by-one into a Pyrex glass dish containing 350 mL of **low** stringency wash solution.
11. Place the Pyrex dish on an orbital shaker and turn the speed dial to ~ 50-75 rpm. Wash the blots at room temp for 15 minutes.
12. After 15 minutes of washing, use a blunt-end forceps to hold the blots and pour the washing solution into a liquid waste radioactive container behind a plexi-glass shield.

Note: Do NOT let the blots dry!

1. Repeat steps 11-13 for the second wash with a fresh 350 mL of **low** stringency wash solution.
2. While washing the blots in the **low** stringency wash solution, prepare another Pyrex dish containing ~ 350 mL of **high** stringency wash solution. Leave this dish in a 60oC water bath incubator.
3. After 15 minutes of the second **low** stringency wash, transfer the blots into the Pyrex dish containing 350 mL of **high** stringency wash solution at 60oC using blunt-end forceps. Wash blots in the high stringency wash at 60oC for 30 minutes.
4. While washing the blots, pour off the low stringency wash solution into the liquid waste radioactive container. Rinse the dish with small amount of water using squirt bottle. Use the Geiger counter to monitor the amount of labeled probes that remained on the dish. Wash the dish with a 7x Detergent solution and rinse it with water until radioactive lable cannot be detected with the Geiger counter.

**Note:** All washing solutions are disposed in the liquid waste radioactive container.

1. Repeat the **high** stringency wash with a fresh 350 mL of high stringency wash solution.
2. While washing, cut out a piece of 3MM Whatman paper to a size of 8 inch x 10 inch. Wrap the paper with a piece of plastic wrap. (TA will demonstrate).
3. After washing the blots, briefly blot the blots on two or three layers of Kimwipe tissues to remove excess liquid. But DO NOT LET THE BLOTS DRY!
4. Place the blots (DNA side facing away from you) on the plastic-wrapped 3MM Whatman paper prepared in step 19.
5. Blot off any excess liquid at the edges of the blots with Kimwipes.
6. Place another piece of plastic wrap over the blots.
7. Determine the cpm on each filter using a Geiger counter.

# Cpm detected on the blot:

**F. Exposing the Blots to X-Ray Film**

Note: The X-ray film is very sensitive to light. Therefore, the setup steps of exposing the blots to X-ray film will be performed in a darkroom under a dimmed light with a special red filter over the lamp. The darkroom is on the first level of this building. You will work with TAs.

1. In the lab, place the wrapped blots facing an intensifying screen in a vinyl X-ray cassette.
2. Bring the cassette, 2 pieces of small plexi-glass plates, 4 clamps, and a box of X-ray film to the darkroom on the first floor. Note: we are on the second floor of the building.
3. In the darkroom, remove a piece of X-ray film from the box.
4. Bend an upper right corner of the film to mark the orientation of the film relative to the blots.
5. Put X-ray film between the blots and the intensifying screen in the cassette.
6. Close the cassette.
7. Put the plexi-glass plates on both sides of the cassette.
8. Clamp the plates with clamps.
9. Expose the blot to X-Ray film in a -80oC freezer from several hours to a few days depending on how hot the blot is.

Record exposure conditions:

**G. Developing the Exposed X-ray Film (Autoradiography)**

1. Remove the cassette from the -80oC freezer and leave it in the lab until the cassette warms up to room temperature (~ 15 - 30 minutes).
2. Take the cassette to the darkroom.
3. Develop the exposed X-ray film (autoradiogram) using the Kodak Film Developer.
4. Align the autoradiogram to the blots. Mark well positions on the blots to the autoradiogram. Write on the autoradiogram positions of the 1-kB ladder, super pool number, etc.
5. Re-expose the blots to X-ray film for longer exposure.

**H.** **Analyzing the Results**

Tips:

* Look for the hybridized band on the positive control lane (super pool #31).
* Look for any hybridized bands at positions either the same level (means same length) or lower level (means shorter length) than the positive control.
* Example: if the size of the hybridized band of the positive control is 3 kb, then look for any hybridized band(s) in 30 super pools with size smaller than 3 kb.