Protocol

Southern Blotting

Michael R. Green and Joseph Sambrook

In Southern blotting, DNA is digested with one or more restriction enzymes, and the resulting fragments are separated according to size by electrophoresis through a standard agarose gel. The DNA is then denatured in situ and transferred from the gel to a solid support (usually a nylon or nitrocellulose membrane). The relative positions of the DNA fragments are preserved during their transfer to the membrane. The DNA is then fixed to the membrane and prepared for hybridization. Alternatively, DNA can be simultaneously transferred from the top and bottom surfaces of a single agarose gel to two membranes. This procedure is useful when the need arises to analyze the same set of restriction fragments with two different probes. Transfer of DNA fragments is rapid, but the efficiency is low because the agarose gel quickly becomes dehydrated as fluid is withdrawn from both sides. The method therefore works best when the target sequences are present in high concentration (e.g., when analyzing cloned DNAs [plasmids, bacteriophages, cosmids, PACs, or BACs] or less complex genomes [those of *Saccharomyces cerevisiae* or *Drosophila*]). Too little mammalian genomic DNA is transferred to allow signals from single-copy sequences to be detected in a reproducible or timely fashion.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

Agarose gel (0.7%) cast in electrophoresis buffer (0.5× TBE or 1× TAE) in the absence of a SYBR dye

For analysis of mammalian genomic DNA, most investigators use large gels (20 × 20 × 0.5 cm) containing 20 standard slots large enough to hold ~50–60 µL. This capacity allows the entire digestion reaction to be loaded without spillage. The gel may be cast and run in the usual way in buffers containing SYBR Gold, for example. However, a more accurate measurement of the size of DNA fragments may be obtained by staining the gel after electrophoresis with SYBR Gold. The inclusion of SYBR Gold in the gel matrix may cause distortion of the DNA bands and may retard the migration of the DNA fragments to varying degrees.

Alkaline transfer buffer (for alkaline transfer to nylon membranes) <R> or Denaturation solution (for neutral transfer) <R>, Neutral transfer buffer (either 10× SSC <R> or 10× SSPE <R>), and SSC (6×) <R>

Appropriate restriction endonucleases

Deionized H_2O

DNA size markers

From the Molecular Cloning collection, edited by Michael R. Green and Joseph Sambrook.

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Sets of size markers are available from many commercial manufacturers, or they can be prepared by digesting cloning vectors with appropriate restriction enzymes. We recommend using a 1-kb ladder (Life Technologies) in the lane closest to one side of the gel and a HindIII digest of bacteriophage λ DNA in the lane on the opposite side of the gel.

Markers radiolabeled with ^{35}S or ^{33}P are not recommended because they usually require exposure times that are different from those optimal for the target bands.

Electrophoresis buffer (1× TAE <R> or 0.5× TBE <R>)
Ethanol (optional; see Step 2)
Gel-loading buffer I <R> or IV <R> (6×, with sucrose)
Genomic DNA
HCl (0.2 N), for depurination of DNA (optional; see note to Step 6)
Neutralization buffer I (for transfer to uncharged membranes) <R> or Neutralization buffer II (for alkaline transfer to nylon membranes) <R>
SYBR Gold dye
TE (pH 8.0) <R>

Equipment

Cross-linking device (e.g., Stratalinker, Agilent; GS Gene Linker, Bio-Rad), microwave oven, or vacuum oven
Equipment for agarose gel electrophoresis (clean, dry horizontal electrophoresis apparatus with chamber and comb)
Fluorometer or NanoDrop instrument
Forceps
Glass baking dishes, large enough to accommodate the gel
Glass plate
Glass rod or pipette
Image capturing system (e.g., a CCD camera and a UV illuminator)
Large-bore yellow tips

Large-bore yellow tips can be purchased or generated rapidly by cutting off the end of standard yellow tips with scissors, a dog nail clipper, or a sharp razor blade. The cut off tips should be sterilized before use, either by autoclaving or by immersion in 70% alcohol for 2 min followed by drying in air.

Neoprene stoppers
Nylon or nitrocellulose membrane

*See the section Membranes Used for Southern and Northern Hybridization in Introduction: **Analysis of DNA by Southern Blotting** (Green and Sambrook 2021a).*

Paper towels
Pipette
Plastic wrap (e.g., Saran Wrap) or parafilm
Power supply device capable of up to 500 V and 200 mA
Razor blade
Rotary platform shaker
Scalpel or paper cutter
Thick blotting paper (e.g., Whatman 3MM, Schleicher & Schuell GB004, or Sigma-Aldrich QuickDraw)
Transparent ruler with fluorescent markings

The ruler is used to measure the distance traveled by the marker DNAs. A ruler placed alongside the gel during photography allows the distances traveled from the loading wells by DNA markers of known size to be measured on the photographic image and plotted graphically. The sizes of radiolabeled bands detected by hybridization can then be estimated by interpolation.

Weight (400 g)

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METHOD

Digestion and Electrophoresis of the DNA

1. Digest an appropriate amount of genomic DNA with one or more restriction enzymes (see Discussion).

   Use large-bore yellow pipette tips to handle high-molecular-weight DNA.

2. If necessary, concentrate the DNA fragments at the end of the digestion by ethanol precipitation. Dissolve the DNAs in ~25 µL of TE (pH 8.0).

   Make sure that the ethanol is removed from the DNA solution before it is loaded on the gel; otherwise, the DNA will "crawl" out of the slot (see Troubleshooting).

3. Measure the concentrations of the digested DNAs by fluorometry or NanoDrop technology for quantifying DNA (see the section Quantifying DNA in Introduction: **Isolation and Quantification of DNA** [Green and Sambrook 2018]). Transfer the appropriate amount of each digest to a fresh microcentrifuge tube. Add 0.15 volume of 6× sucrose gel-loading buffer, and separate the fragments of DNA by electrophoresis through an agarose gel (for most genomic DNAs, a 0.7% gel cast in 0.5× TBE or 1× TAE may be used). Maintain a low voltage through the gel (about <1 V/cm) so that the migration rate of the DNA is slow.

   If the digested DNAs have been stored at 4°C, they should be heated for 2–3 min to 56°C before they are applied to the gel. This heating disrupts any base-pairing that may have occurred between protruding cohesive termini.

   See Troubleshooting.

4. After electrophoresis is complete, stain the gel with SYBR Gold, and record an image of the stained gel. Place a ruler alongside the gel so that the distance that any band of DNA has migrated can be read directly from the photographic image.

   If desired, the gel may be stored at this stage before the DNA is denatured and transferred to the membrane. Wrap the gel in Saran Wrap, and store it on a flat surface at 4°C. Because the bands of DNA diffuse during storage, the gel should not be put aside for more than 1 d before the DNA is transferred to a nitrocellulose filter or nylon membrane.

5. Denature the DNA and transfer it from the agarose gel to a nitrocellulose filter or to a neutral or charged-nylon membrane using one of the methods described below.

Preparation of the Gel for Transfer

6. Transfer the gel to a glass baking dish. Use a razor blade to trim away unused areas of the gel, including the section of gel above the wells. Be sure to leave enough of the wells attached to the gel so that the positions of the lanes can be marked on the membrane after transfer of DNA. Cut off a small triangular piece from the bottom left-hand corner of the gel to simplify orientation during the succeeding operations.

   It is best to cut off the lanes containing the molecular-weight markers because probes may contain sequences complementary to some of the marker bands. The resulting pattern of bands appearing on the autoradiogram is sometimes informative, but more often is puzzling.

   If the DNA fragments of interest are larger than ~15–20 kb, then transfer may be improved by nicking the DNA by brief depurination before denaturation (Wahl et al. 1979) (see Troubleshooting).

7. Denature the DNA by soaking in a denaturing (alkaline) solution as follows:

For Transfer to Uncharged Membranes

i. Soak the gel for 45 min at room temperature in 10 gel volumes of denaturation solution with constant gentle agitation (e.g., on a rotary platform).

ii. Rinse the gel briefly in deionized H_2O, and then neutralize it by soaking for 30 min at room temperature in 10 gel volumes of Neutralization buffer I with constant gentle agitation. Change the neutralization buffer and continue soaking the gel for a further 15 min.

For Transfer to Charged Nylon Membranes

i. Soak the gel for 15 min at room temperature in several volumes of alkaline transfer buffer with constant gentle agitation (e.g., on a rotary platform).

ii. Change the solution and continue to soak the gel for a further 20 min with gentle agitation.

If the gel floats to the surface of the liquid, weigh it down with several Pasteur pipettes.

Preparation of the Membrane for Transfer

8. Use a fresh scalpel or a paper cutter to cut a piece of nylon or nitrocellulose membrane ~1–2 mm larger than the gel in each dimension. Cut a corner from the membrane to match the corner cut from the gel. In addition, cut two sheets of thick blotting paper to the same size as the membrane. If doing a simultaneous transfer to two membranes, cut two pieces of membrane and four sheets of blotting paper.

Use appropriate gloves and blunt-ended forceps (e.g., Millipore forceps) to handle the membrane. A membrane that has been touched by oily hands will not wet!

To retain small fragments of DNA (<300 nt), use nitrocellulose membranes with a small pore size (0.2 µm) or nylon membranes.

9. Float the membrane(s) on the surface of a dish of deionized H_2O until it wets completely from beneath, and then immerse the membrane in the appropriate transfer buffer for at least 5 min. For simultaneous transfer to two membranes soak the membranes in 10× SSC and skip to Step 21.

The rate at which different batches of nitrocellulose membranes wet varies enormously. If the membrane is not saturated after floating for several minutes on H_2O, see Troubleshooting. Uneven wetting is not usually a problem with neutral or charged-nylon membranes.

Assembly of the Transfer Apparatus and Transfer of the DNA to a Single Membrane

Neutral transfer buffer (10× SSC or 10× SSPE) is used to transfer DNA to uncharged membranes. Alkaline transfer buffer is used to transfer DNA to charged nylon membranes.

10. While the DNA is denaturing, place a piece of thick blotting paper on a sheet of Plexiglas or a glass plate to form a support that is longer and wider than the gel. The ends of the blotting paper should drape over the edges of the plate. Place the support inside a large baking dish. The support can be placed on top of four neoprene stoppers to elevate it from the bottom of the dish.

11. Fill the dish with the appropriate transfer buffer until the level of the liquid reaches almost to the top of the support. When the blotting paper on the top of the support is thoroughly wet, smooth out all air bubbles with a glass rod or pipette.

12. Remove the gel from the solution in Step 7, and invert it so that its underside is now uppermost. Place the inverted gel on the support so that it is centered on the wet blotting paper.

Make sure that there are no air bubbles between the blotting paper and the gel.

13. Surround, but do not cover, the gel with Saran Wrap or Parafilm.

This protective mask serves as a barrier to prevent liquid from flowing directly from the reservoir to paper towels placed on top of the gel. If these towels are not precisely stacked, they tend to droop over the edge of the gel and may touch the wick. This type of short-circuiting is a major cause of inefficient transfer of DNA from the gel to the membrane.

14. Wet the top of the gel with the appropriate transfer buffer. Place the wet membrane on top of the gel so that the cut corners are aligned. To avoid bubbles, touch one corner of the membrane to the

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gel, and gently lower the membrane onto the gel. One edge of the membrane should extend just over the edge of the line of slots at the top of the gel.

Do not move the membrane once it has been applied to the surface of the gel. Make sure that there are no air bubbles between the membrane and the gel.

15. Wet the two pieces of thick blotting paper in the appropriate transfer buffer, and place them on top of the wet membrane. Roll a pipette across the surface of the membrane to smooth away any air bubbles.

16. Cut or fold a stack of paper towels (5–8 cm high) just smaller than the blotting papers. Place the towels on the blotting papers. Put a glass plate on top of the stack, and weigh it down with a 400-g weight.

    The objective is to set up a flow of liquid from the reservoir through the gel and the membrane so that fragments of denatured DNA are eluted from the gel and are deposited on the membrane. The weight on the top of the gel should be heavy enough to ensure good contact between the various components of the blot but light enough to prevent compressing the gel. Compression will squeeze liquid from the interstices of the gel, leaving a dehydrated matrix that greatly retards the movement of DNA and drastically reduces the efficiency of transfer from the gel to the membrane.

    Intact, rather than cut or folded, paper towels can also be used in this setup, but only if the protective mask of Saran Wrap or Parafilm efficiently prevents seepage of buffer.

    To prevent evaporation, some investigators wrap the entire transfer setup in Saran Wrap. This is unnecessary.

17. Allow the transfer of DNA to proceed for 8–24 h. Replace the paper towels as they become wet. Try to prevent the entire stack of towels from becoming wet with buffer.

18. Remove the paper towels and the blotting papers above the gel. Turn the gel and the attached membrane over and lay them, gel side up, on a dry sheet of blotting paper. Mark the positions of the gel slots on the membrane with a very soft lead pencil or a ballpoint pen.

19. Peel the gel from the membrane and discard the gel.

    Instead of discarding the gel, it can be stained (45 min) with SYBR Gold and visualized to gauge the success of the DNA transfer. Note that the intensity of fluorescence will be quite low because any DNA remaining in the gel will have been denatured.

20. Proceed to Step 25.

Simultaneous Transfer of DNA to Two Membranes

This transfer is considered to be a neutral transfer because 10× SSC is used to wet the membrane.

21. Roll a moistened pipette over each layer as it is assembled to ensure that no air bubbles are trapped, especially between the membranes and the gel sides. Place one of the membranes on two pieces of dampened blotting paper. Lay the gel on top of the membrane, aligning the cut corner of the gel with the cut corner of the membrane. Without delay, place the second membrane on the other side of the gel, followed by two sheets of dampened blotting paper.

22. Transfer the entire sandwich of blotting papers, membranes, and gel onto a 2–4-in. stack of paper towels. Cover the sandwich with a second stack of paper towels. Put a glass plate on top of the entire stack, and weigh it down with a 400-g weight.

23. After 2–4 h, remove the paper towels and blotting papers. Transfer the gel and membrane sandwich to a dry sheet of blotting paper, and mark the approximate positions of the gel slots with a very soft lead pencil or a ballpoint pen.

24. Proceed to Step 25.

Fixation of the DNA to the Membrane

The sequence of steps from immobilization of DNA to the membrane to subsequent hybridization depends on the type of membrane, the method of transfer, and the method of fixation (see Table 1). Because alkaline transfer results in

TABLE 1. Fixing DNA to the membrane for hybridization

Type of membrane	Type of transfer	Method of fixation	Sequence of steps
Positively charged nylon	Alkaline transfer	Alkaline transfer	1. Soak membrane in Neutralization buffer II. 2. Proceed to prehybridization.
Uncharged nylon or positively charged nylon	Neutral transfer	UV irradiation (please see Step 26 for details)	1. Soak membrane in 6× SSC. 2. Fix the DNA by UV irradiation. 3. Proceed to prehybridization.
Uncharged nylon or positively charged nylon	Neutral transfer	Baking in vacuum oven or microwave oven (please see Step 26 for details)	1. Soak membrane in 6× SSC. 2. Bake the membrane. 3. Proceed to prehybridization.

covalent attachment of DNA to positively charged nylon membranes, there is no need to fix the DNA to the membrane before hybridization. DNA transferred to uncharged nylon membranes in neutral transfer buffer should be fixed to the membrane by baking under vacuum or heating in a microwave oven, or cross-linked to the membrane by UV irradiation.

25. Soak the membrane in one of the following solutions as appropriate:

For Neutral Transfer

6× SSC for 5 min at room temperature

For Alkaline Transfer

Neutralization buffer II for 15 min at room temperature

This rinse removes any pieces of agarose sticking to the membrane and, in the latter case, also neutralizes the membrane.

26. Immobilize the DNA that has been transferred to uncharged membranes.

Because alkaline transfer results in covalent attachment of DNA to positively charged nylon membranes, there is no need for additional steps to fix the DNA to the membrane.

To Fix by Baking in a Vacuum Oven

i. Remove the membrane from the 6× SSC, and allow excess fluid to drain away. Place the membrane flat on a paper towel to dry for at least 30 min at room temperature.

ii. Sandwich the membrane between two sheets of dry blotting paper. Bake for 30 min to 2 h at 80°C in a vacuum oven.

Overbaking can cause nitrocellulose membranes to become brittle. If the gel was not completely neutralized before the DNA was transferred, nitrocellulose membranes will turn yellow or brown during baking and chip very easily. The background of nonspecific hybridization also increases dramatically.

To Fix by Baking in a Microwave Oven

i. Place the damp membrane on a dry piece of blotting paper.

ii. Heat the membrane for 2–3 min at full power in a microwave oven (750–900 W).

Baking nitrocellulose membranes in a microwave oven attenuates the signal in Southern hybridizations and is not recommended (Angeletti et al. 1995).

To Cross-Link by UV Irradiation

i. Place the damp membrane on a dry piece of blotting paper.

ii. Irradiate at 254 nm to cross-link the DNA to the membrane.

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Immobilization of nucleic acids by UV irradiation can greatly enhance the hybridization signal obtained with some brands of positively charged nylon membranes. However, for maximum effect, it is important to make sure that the membrane is not overirradiated. The aim is to form cross-links between a small fraction of the thymine residues in the DNA and positively charged amine groups on the surface of the membrane (Church and Gilbert 1984). Overirradiation results in the covalent attachment of a high proportion of the thymines, with consequent decrease in hybridization signal. Make sure that the side of the membrane carrying the DNA faces the UV light source. Most manufacturers advise that damp membranes be exposed to a total of 1.5 J/cm^2 and that dry membranes be exposed to 0.15 J/cm^2. However, we recommend performing a series of preliminary experiments to determine empirically the amount of irradiation required to produce the maximum hybridization signal.

27. Proceed directly to hybridization of immobilized DNA to a probe (Protocol: **Southern Hybridization of Radiolabeled Probes to Nucleic Acids Immobilized on Membranes** [Green and Sambrook 2021b]).

    Any membranes not used immediately in hybridization reactions should be thoroughly dried, wrapped loosely in aluminum foil or blotting paper, and stored at room temperature, preferably under vacuum.

TROUBLESHOOTING

Problem (Step 2): The DNA "crawls" out of the slot of the gel.

Solution: Significant quantities of ethanol likely remain in the DNA sample. Heating the solution of dissolved DNA in an open tube for 10 min to 70°C is usually sufficient to drive off most of the ethanol.

Problem (Step 3): The DNA solution will not sink to the bottom of the well.

Solution: This floating occurs when very-high-molecular-weight DNA is present at the end of the digest (e.g., when the digest is incomplete or when mammalian DNA has been digested with enzymes like NotI that generate very large fragments of DNA). To minimize the problem, make sure that the DNA is homogeneously dispersed, and load the samples very slowly into the wells of the gel. After loading, allow the gel to stand for a few minutes so that the DNA can diffuse evenly throughout the wells.

Problem (Step 6): There is poor transfer of large (~15–20 kb) DNA fragments.

Solution: If the DNA fragments of interest are larger than ~15–20 kb, then transfer may be improved by nicking the DNA by brief depurination before denaturation (Wahl et al. 1979). Depurination lays the phosphate-sugar backbone of DNA open to subsequent cleavage by hydroxyl ions. After Step 6, soak the gel in several volumes of 0.2 N HCl until the bromophenol blue turns yellow and the xylene cyanol turns yellow/green. Immediately place the 0.2 N HCl in a hazardous-waste container, and then rinse the gel several times with deionized H_2O. Because depurination depends on diffusion of H^+ ions into the gel, the DNA molecules at different levels within the agarose are depurinated at different rates and to different extents. The reaction is therefore difficult both to control and to reproduce and, if performed too enthusiastically, can result in excessive fragmentation of DNA and a reduction in the strength of the hybridization signal. Depurination is best avoided when the size of the target fragments is <15 kb. However, depurination/nicking is advisable, if not essential for Southern analysis of higher-molecular-weight DNAs.

Problem (Step 9): The nitrocellulose membrane does not wet evenly.

Solution: If the membrane is not saturated after floating for several minutes on H_2O, it should be replaced with a new membrane because the transfer of DNA to an unevenly wetted membrane is unreliable. The original membrane should be either discarded or autoclaved for 5 min between

pieces of 3MM paper that are saturated with 2× SSC. This treatment usually results in complete wetting. The autoclaved membrane, sandwiched between the autoclaved 3MM papers saturated with 2× SSC, may be stored at 4°C in a sealed plastic bag until needed.

DISCUSSION

Cardinal points to remember when setting up restriction digests of genomic DNA for standard Southern analysis are as follows.

- The amount of DNA digested must be sufficient to generate a signal. For Southern analysis of mammalian genomic DNA, ~10 µg of DNA must be loaded into each slot of the gel when probes of standard length (>500 bp) and high specific activity (>10^9 cpm/µg) are used to detect single-copy sequences. Proportionately lower amounts of DNA may be used when the preparation of DNA contains higher molar concentrations of the sequences of interest.
- The restriction enzymes used are likely to be informative. For example, there is little point in digesting DNA whose median size is 50 kb with a restriction enzyme that cleaves on average every 100 kb. As a general rule, the median size of the DNA before digestion should be at least three times greater than the median size of fragments generated during the digestion.
- The amount of DNA loaded into each lane of the gel is known with accuracy. This does not necessarily mean that restriction digests must contain equal amounts of DNA. Measuring small volumes of extremely viscous preparations of high-molecular-weight genomic DNA is difficult, and inaccuracies lead to overloading or underloading of lanes in the gel. If it is essential to analyze the same amount of DNA from multiple samples (e.g., when comparing genomic DNAs isolated from normal individuals and those affected with a genetic disease, or when attempting to determine the copy number of a gene), then it is best to digest a sufficiency of each of the DNAs without worrying too much regarding whether each digest contains the same amount of DNA. The exact concentration of DNA in each sample can be measured by fluorometry after digestion is complete before loading the agarose gel (see the section Quantifying DNA in Introduction: **Isolation and Quantification of DNA** [Green and Sambrook 2018]).

  In many cases, the volumes of restriction digests are defined by the concentration of DNA in the preparations under analysis. The concentrations of DNA in preparations of high-molecular-weight mammalian genomic DNA are sometimes so low that it becomes necessary to perform restriction digests in large volumes. There is no reason why restriction digests of a comparative series of genomic DNAs need be performed in equal volumes. As long as all digests are complete, the volume of each restriction digest is immaterial. After digestion, the fragments of DNA may be concentrated by precipitation with ethanol, measured by fluorometry, and then applied to the gel in a small volume of gel-loading buffer.
- The digests are complete. The chief problem encountered during digestion of high-molecular-weight DNA is unevenness of digestion caused by variations in the local concentrations of DNA. Clumps of DNA are relatively inaccessible to restriction enzymes and can be digested only from the exterior of the aggregate. To ensure homogeneous dispersion of the DNA, do the following.

1. If possible, set up the reactions in a total volume of at least 45 µL. Before adding the restriction enzyme, store reactions for several hours at 4°C after dilution of the DNA and addition of 10× restriction enzyme buffer.
2. Gently stir the DNA solution from time to time using a sealed glass capillary.
3. After addition of the restriction enzyme (5 units/µg of DNA), gently stir the solution for 2–3 min at 4°C before warming the reaction to the appropriate temperature.
4. After digestion for 15–30 min, add a second aliquot of restriction enzyme (5 units/µg of DNA), and stir the reaction as described above.

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5. Incubate the reaction at the appropriate temperature for 8–12 h.

It is important to include controls to show whether digestion with the restriction enzyme(s) is complete and whether transfer and hybridization of the DNA have worked efficiently. This goal can be accomplished by setting up a series of digests containing high-molecular-weight genomic DNA and a very small amount of a plasmid carrying a sequence complementary to the probe (e.g., 10 µg of mammalian DNA and 10^{-5}, 10^{-6}, and 10^{-7} µg of plasmid). During digestion, the plasmid will be cleaved into a series of bands that may be invisible when the gel is examined by staining with SYBR Gold. However, fragments of the correct size should be detected by subsequent hybridization to the probe. To reduce the chance of accidental contamination and to minimize the possibility that the hybridization signal from the controls will obscure that from the test samples, the controls should be loaded into wells that lie toward one side of the gel, well away from the test samples of mammalian DNA.

For further information on Southern blotting, see Introduction: **Analysis of DNA by Southern Blotting** (Green and Sambrook 2021a).

RECIPES

6× Gel-Loading Buffer I

0.25% (w/v) xylene cyanol FF
40% (w/v) sucrose in H_2O
0.25% (w/v) bromophenol blue

Store at 4°C.

6× Gel-Loading Buffer IV

0.25% (w/v) bromophenol blue
40% (w/v) sucrose in H_2O

Store at 4°C.

Alkaline Transfer Buffer

0.4 N NaOH
1 M NaCl

For akaline transfer of DNA to nylon membranes.

Denaturation Solution

0.5 M NaOH
1.5 M NaCl

For neutral transfer, double-stranded DNA targets only.

Neutralization Buffer I

1 M Tris (pH 7.4)
1.5 M NaCl

Neutralization Buffer II

0.5 M Tris-Cl (pH 7.2)
1 M NaCl

For alkaline transfer of DNA to nylon membranes.

SSC

For a 20× solution: Dissolve 175.3 g of NaCl and 88.2 g of sodium citrate in 800 mL of H_2O. Adjust the pH to 7.0 with a few drops of a 14 N solution of HCl. Adjust the volume to 1 L with H_2O. Dispense into aliquots. Sterilize by autoclaving. The final concentrations of the ingredients are 3.0 M NaCl and 0.3 M sodium citrate.

SSPE

For 20× solution: Dissolve 175.3 g of NaCl, 27.6 g of $NaH_2PO_4 \cdot H_2O$, and 7.4 g of EDTA in 800 mL of H_2O. Adjust the pH to 7.4 with NaOH (~6.5 mL of a 10 N solution). Adjust the volume to 1 L with H_2O. Dispense into aliquots. Sterilize by autoclaving. The final concentrations of the ingredients are 3.0 M NaCl, 0.2 M NaH_2PO_4, and 0.02 M EDTA.

TAE

Prepare a 50× stock solution in 1 L of H_2O:
- 242 g of Tris base
- 57.1 mL of acetic acid (glacial)
- 100 mL of 0.5 M EDTA (pH 8.0)

The 1× working solution is 40 mM Tris-acetate/1 mM EDTA.

TBE Buffer

Prepare a 5× stock solution in 1 L of H_2O:
- 54 g of Tris base
- 27.5 g of boric acid
- 20 mL of 0.5 M EDTA (pH 8.0)

The 0.5× working solution is 45 mM Tris-borate/1 mM EDTA.
TBE is usually made and stored as a 5× or 10× stock solution. The pH of the concentrated stock buffer should be ~8.3. Dilute the concentrated stock buffer just before use and make the gel solution and the electrophoresis buffer from the same concentrated stock solution. Some investigators prefer to use more concentrated stock solutions of TBE (10× as opposed to 5×). However, 5× stock solution is more stable because the solutes do not precipitate during storage. Passing the 5× or 10× buffer stocks through a 0.22-µm filter can prevent or delay formation of precipitates.

TE Buffer

Reagent	Quantity (for 100 mL)	Final concentration
EDTA (0.5 M, pH 8.0)	0.2 mL	1 mM
Tris-Cl (1 M, pH 8.0)	1 mL	10 mM
H_2O	to 100 mL	

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