MSc III Sem – Biotechnology

Course – Principles of Genetic Engineering

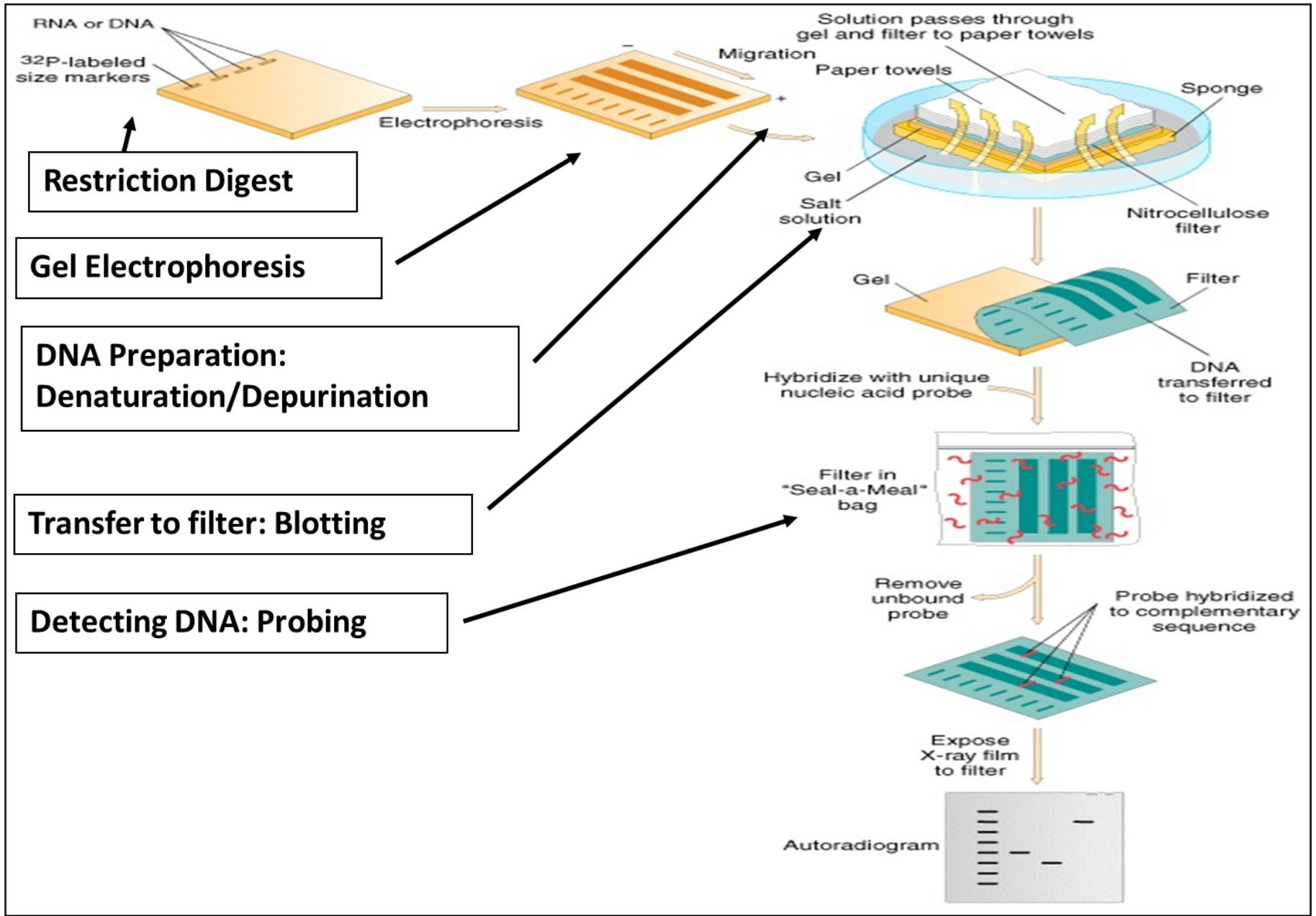
Nucleic Acid Hybridization

- **Hybridization** is basically a phenomenon in which single-stranded deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) molecules anneal to complementary DNA or RNA.
- A double-stranded DNA sequence is generally stable under physiological conditions, thus these conditions are changed in the laboratory (generally by raising the surrounding temperature) to let molecules separate into single strands.
- These strands are complementary to each other but may also be complementary to other sequences present in their surroundings.
- Lowering the surrounding temperature allows the single-stranded molecules to anneal or "hybridize" to each other.
- DNA replication and transcription of DNA into RNA both rely upon nucleotide hybridization, and based on them are two major techniques in molecular biology: ***Southern blotting and Northern blotting, or hybridization***.

Southern Blot: DNA-DNA

- A **Southern blot** is a method used in molecular biology for detection of a specific DNA sequence in DNA samples. Southern blotting combines transfer of electrophoresis-separated DNA fragments to a filter membrane and subsequent fragment detection by probe hybridization.
- The method is named after the British biologist Edwin Southern (1975).
- Other blotting methods (i.e., western blot, northern blot, eastern blot, southwestern blot) that employ similar principles, but using RNA or protein, have later been named in reference to Edwin Southern's name.
- Uses gel electrophoresis together with hybridization probes to characterize restriction fragments of genomic DNA (or DNA from other sources, such as plasmids).
- Identifies DNA with a specific base sequence.
- Can be done to detect specific genes present in cells.

General Scheme for Southern Blot

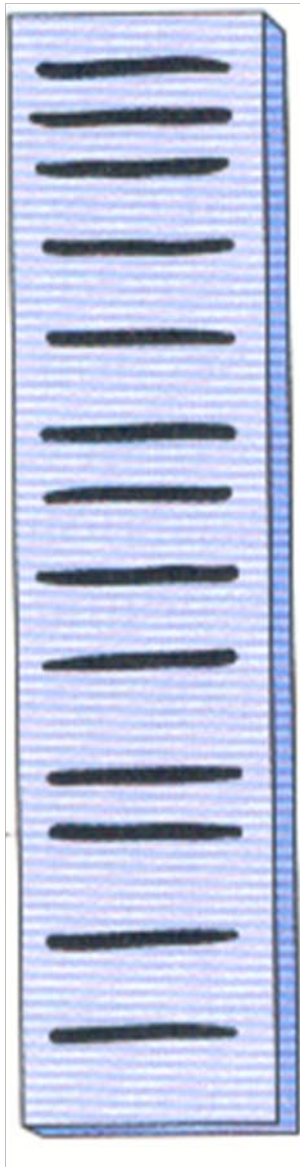


Southern Steps

1. DNA to be analyzed is digested to completion with a restriction endonuclease.
2. Electrophoresis to maximally separate restriction fragments in the expected size range.
3. Blot fragments onto a nitrocellulose membrane.
4. Hybridize with the ^{32}P probe.
5. Autoradiography.

Step 1 – restriction digestion

Step 2: electrophoretic separation



Gel electrophoresis

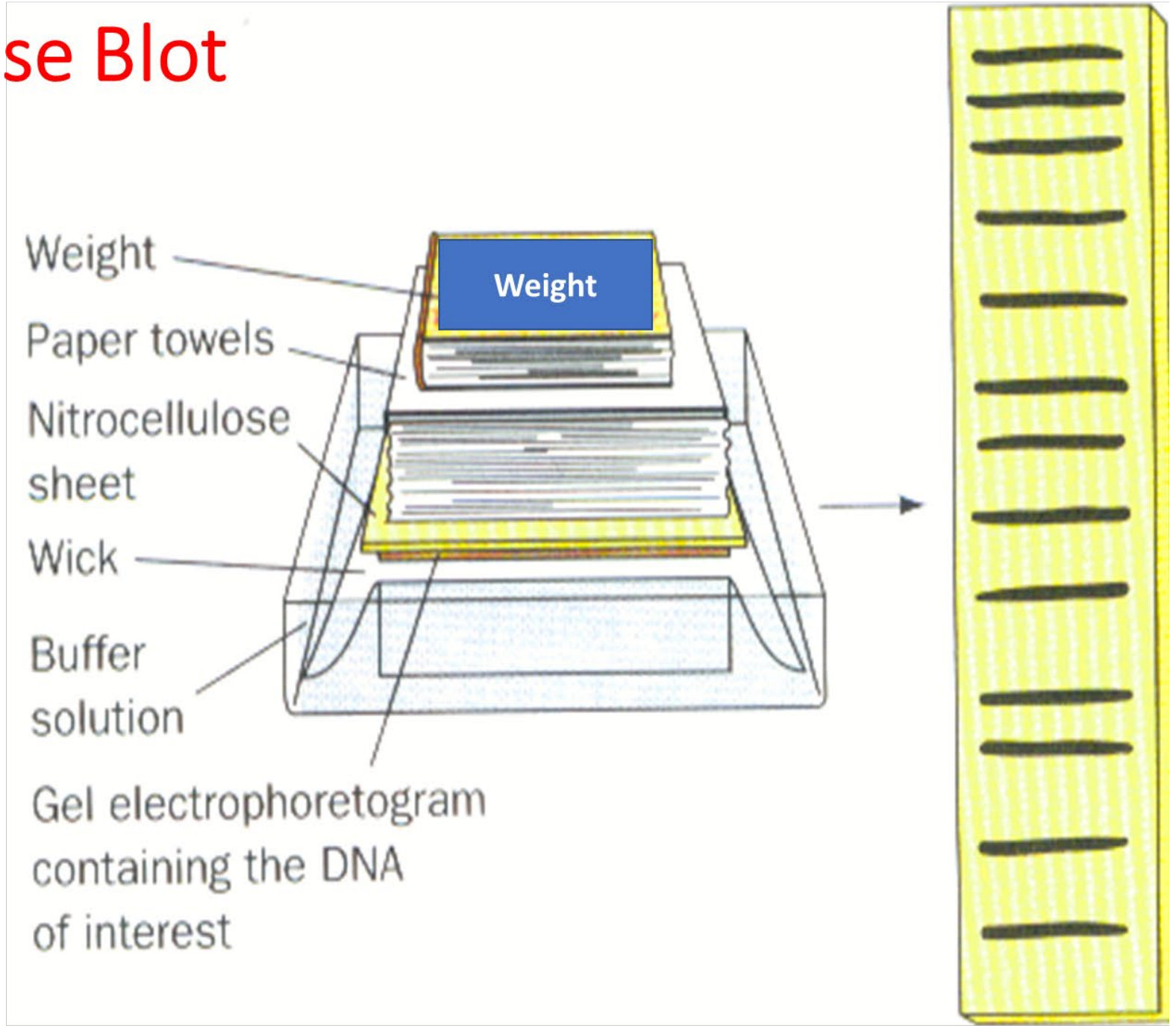
- Separates DNA fragments.

Soak gel in 0.5 M NaOH

- Converts dsDNA to ssDNA

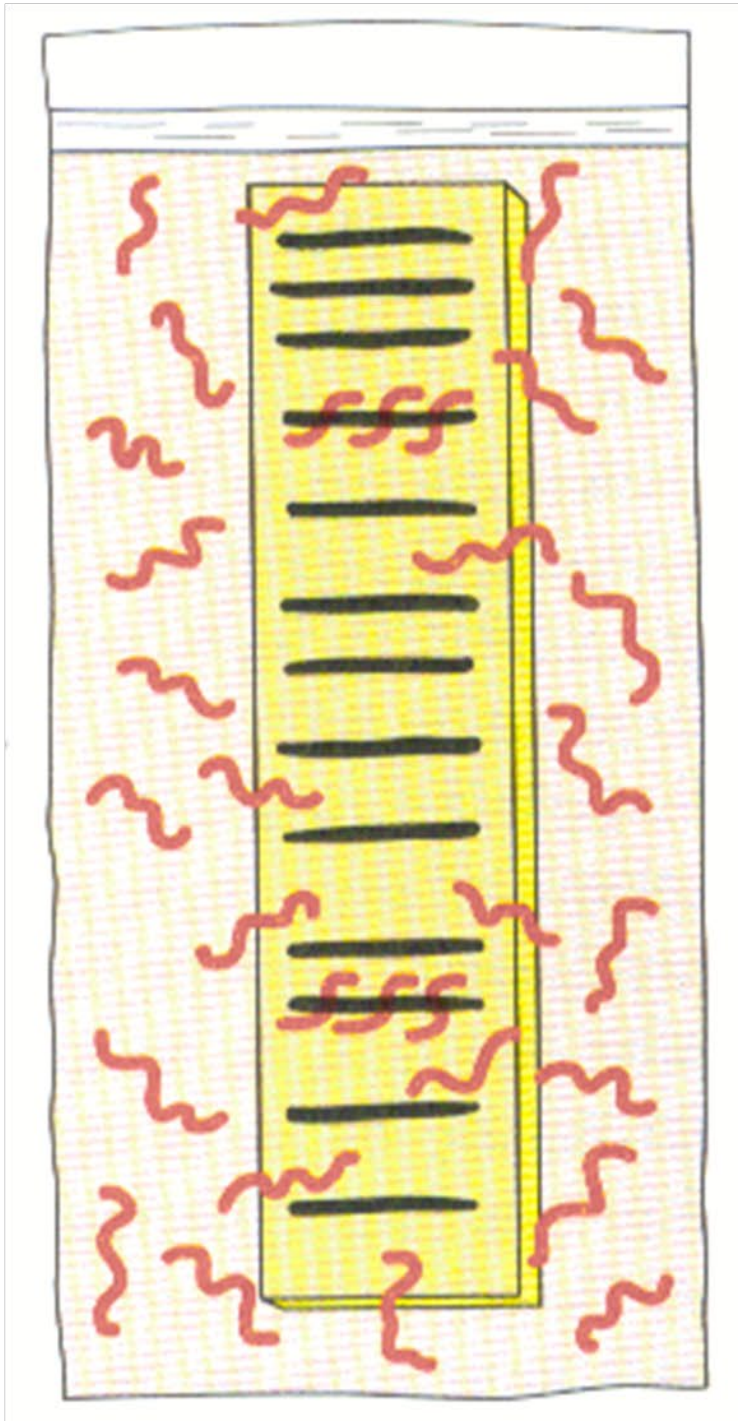
Step 3. Nitrocellulose Blot

- Cover gel with nitrocellulose paper then
- Cover nitrocellulose paper with thick layer of paper towels.
- Compress apparatus with heavy weight.
- ssDNA binds to nitrocellulose at same position it had on the gel.
- Vacuum dry nitrocellulose at 80°C to permanently fix DNA in place or cross link (via covalent bonds) the DNA to the membrane.



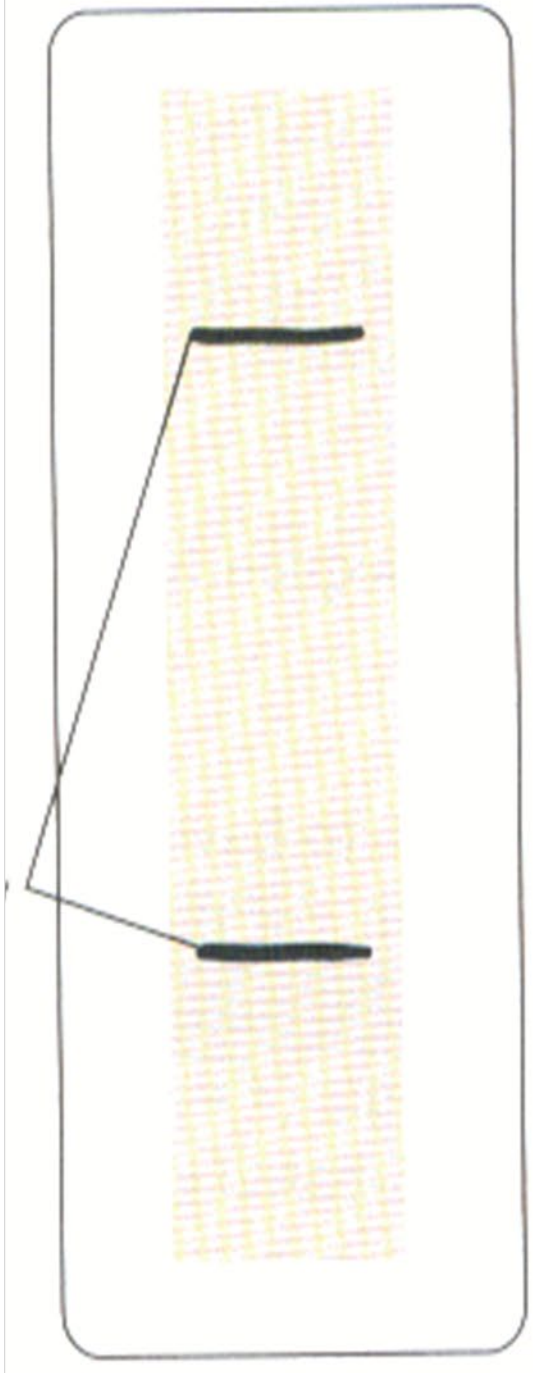
Step 4. Hybridization

- Incubate nitrocellulose sheet with a minimal quantity of solution containing ^{32}P-labeled ssDNA probe.
- Probe sequence is complementary to the DNA of interest.
- Incubate for several hours at suitable renaturation temperature that will permit probe to anneal to its target sequence(s).
- Wash & dry nitrocellulose sheet.



Step 5. Autoradiography

- Place nitrocellulose sheet over X-ray film.
- X-ray film darkens where the fragments are complementary to the radioactive probes.



Southern Application:

Diagnosis & detection of genetic diseases.

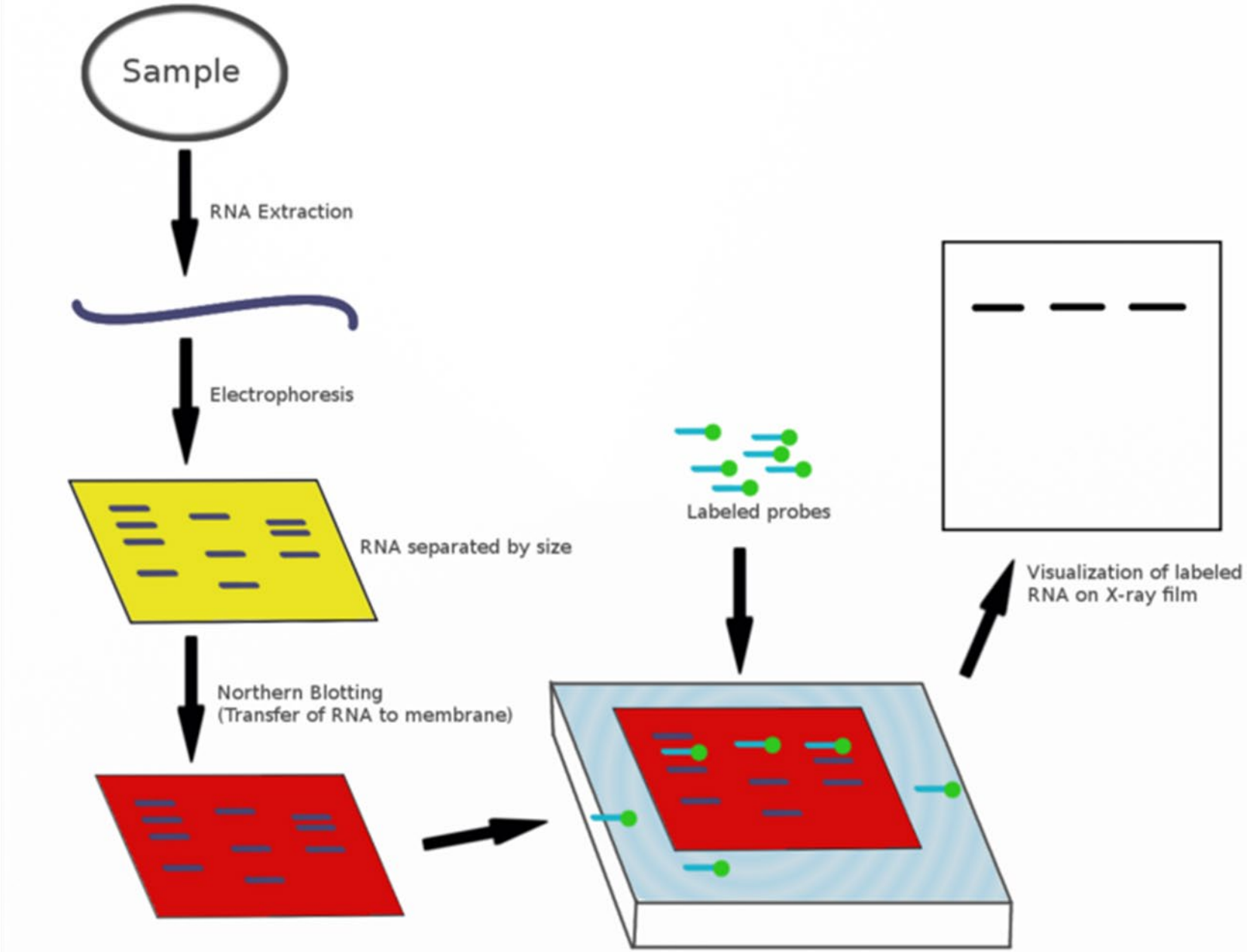
- Used to diagnose sickle cell-anemia.
- A→T base change in the β subunit of Hb

  Glu→ Val.
- Development of a 19 residue oligonucleotide probe complementary to sickle-cell gene's mutated segment.
- Probe hybridizes to DNA from homozygotes of sickle-cell anemia but not from normal individuals.

Northern Blot: RNA-DNA*(RNA*)

- The northern blot technique was developed in 1977 by James Alwine, David Kemp, and George Stark. Northern blotting takes its name from its similarity to the first blotting technique, the Southern blot.
- The major difference is that RNA, rather than DNA, is analyzed in this process.
- Northern blotting it is possible to observe cellular control over structure and function by determining the particular gene expression rates during differentiation and morphogenesis, as well as in abnormal or diseased conditions.
- Northern blotting involves the use of electrophoresis to separate RNA samples by size, and detection with a hybridization probe complementary to part of or the entire target sequence. The term 'northern blot' actually refers specifically to the transfer of RNA from the electrophoresis gel to the blotting membrane.
- However, the entire process is commonly referred to as northern blotting.

Flow diagram outlining the general procedure for RNA detection by northern blotting



Northern blotting steps

1. Isolate RNA & treat with formaldehyde.

   [No need to digest RNA with restriction enzymes. Use formaldehyde to break H-bonds and denature RNA because single-stranded RNA will form intramolecular base pairs and "fold" on itself.]

2. Electrophorese RNA in denaturing agarose gel (has formaldehyde). Visualize RNA in gel using Ethidium bromide stain and photograph.
3. Transfer single-stranded RNA to nitrocellulose or nylon membrane. Covalently link RNA to membrane.
4. Incubate membrane (RNA immobilized on membrane) with labeled DNA or RNA probe with target sequence.
5. Development.

Step 1

Isolate RNA:

-To detect rare mRNA, isolate the poly A^+ mRNA.

-RNA is both biologically and chemically more labile than DNA. Thus eliminate RNases.

Step 2

Electrophoresis:

-Performed in formaldehyde agarose gel to prevent RNA from folding on itself.

-Stain with EtBr to visualize the RNA bands.

Step 3

-Transfer single-stranded RNA to nitrocellulose or nylon membrane:

Traditionally, a nitrocellulose membrane is used, although nylon or a positively charged nylon membrane may be used.

Nitrocellulose typically has a binding capacity of about 100µg/cm, while nylon has a binding capacity of about 500 µg/cm. Many scientists feel nylon is better since it binds more and is less fragile.

-Covalently link RNA to membrane:

UV cross linking is more effective in binding RNA to the membrane than baking at 80°C.

Step 4 & 5

-Prehybridize before hybridization:

Blocks non-specific sites to prevent the single-stranded probe from binding just anywhere on the membrane.

-Incubate membrane with labeled DNA or RNA probe with target sequence:

Probe could be ^{32}P, biotin/streptavidin or a bioluminescent probe.

-Autoradiography:

Place membrane over X-ray film.

X-ray film darkens where the fragments are complementary to the radioactive probes.

Agarose gel & Membrane & Autoradiography

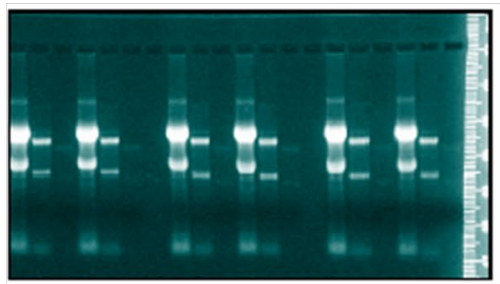


Figure 1. Ethidium-bromide-stained RNA after electrophoresis in a formaldehyde-agarose gel. Six series of lanes were loaded with 10 g, 1 g, and 0.1 g of total RNA. Band intensity confirmed equivalent loading to each series on the gel.

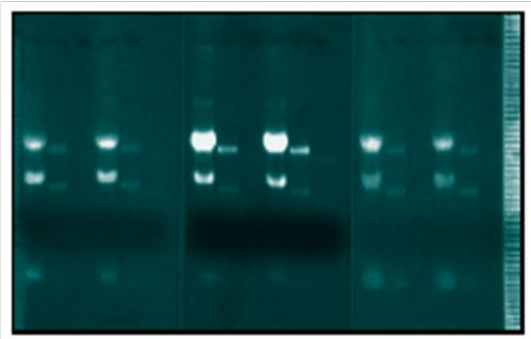


Figure 2. Ethidium-bromide-stained RNA after alkaline transfer to nylon membranes, visualized with a UV trans-illuminator. Biodyne B membrane shows higher

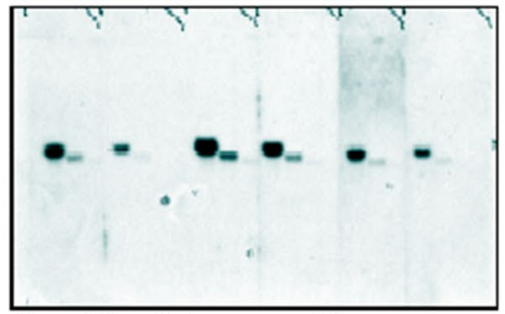


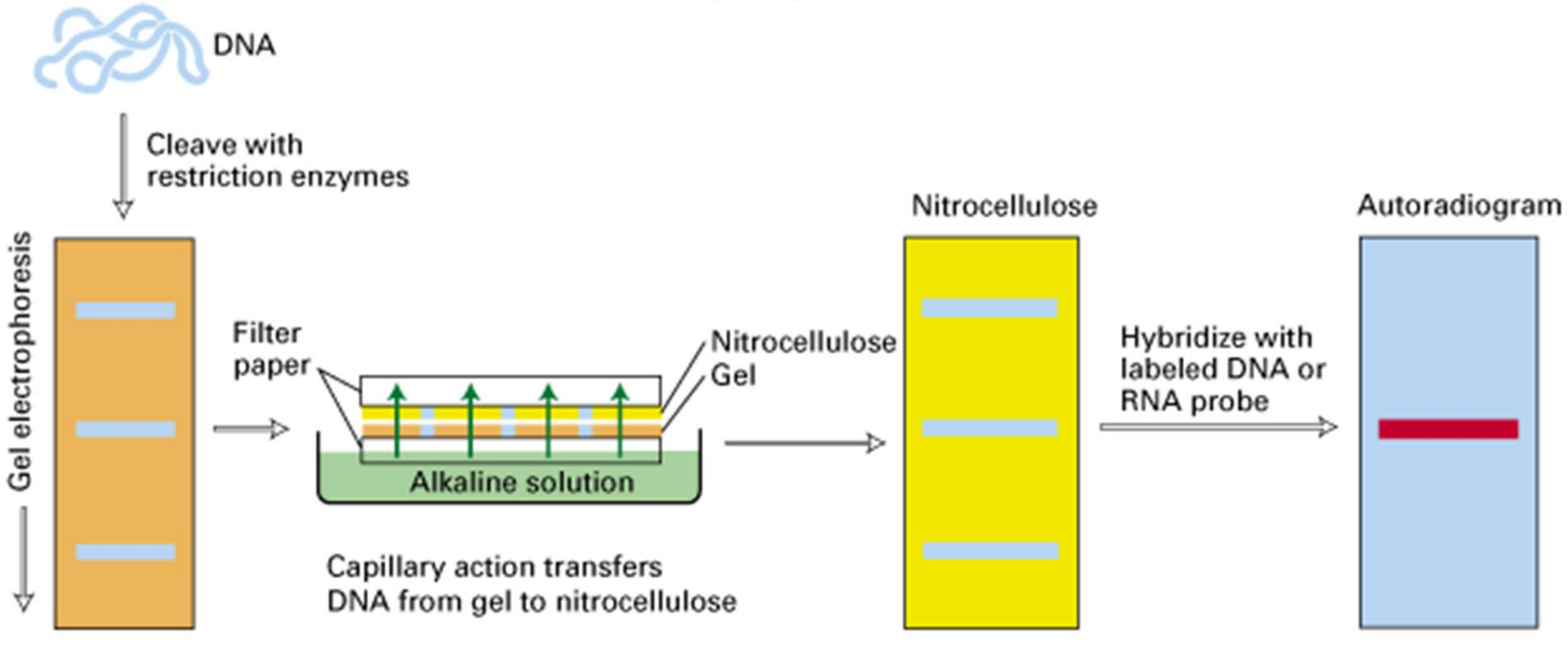
Figure 4. Phosphorimager scan (3-hour screen exposure) after hybridization with ^{32}P-labeled GAPDH probe; high-stringency wash (62°C). Signal is significantly higher on the Biodyne B membrane than on the competitor nylon membranes. 0.1 g RNA produced faint signal only on the Biodyne B membrane following UV fixation.

Northern Blotting Application

Northern blots are particularly useful for determining the conditions under which specific genes are being expressed, including which tissues in a complex organism express which of its genes at the mRNA level.

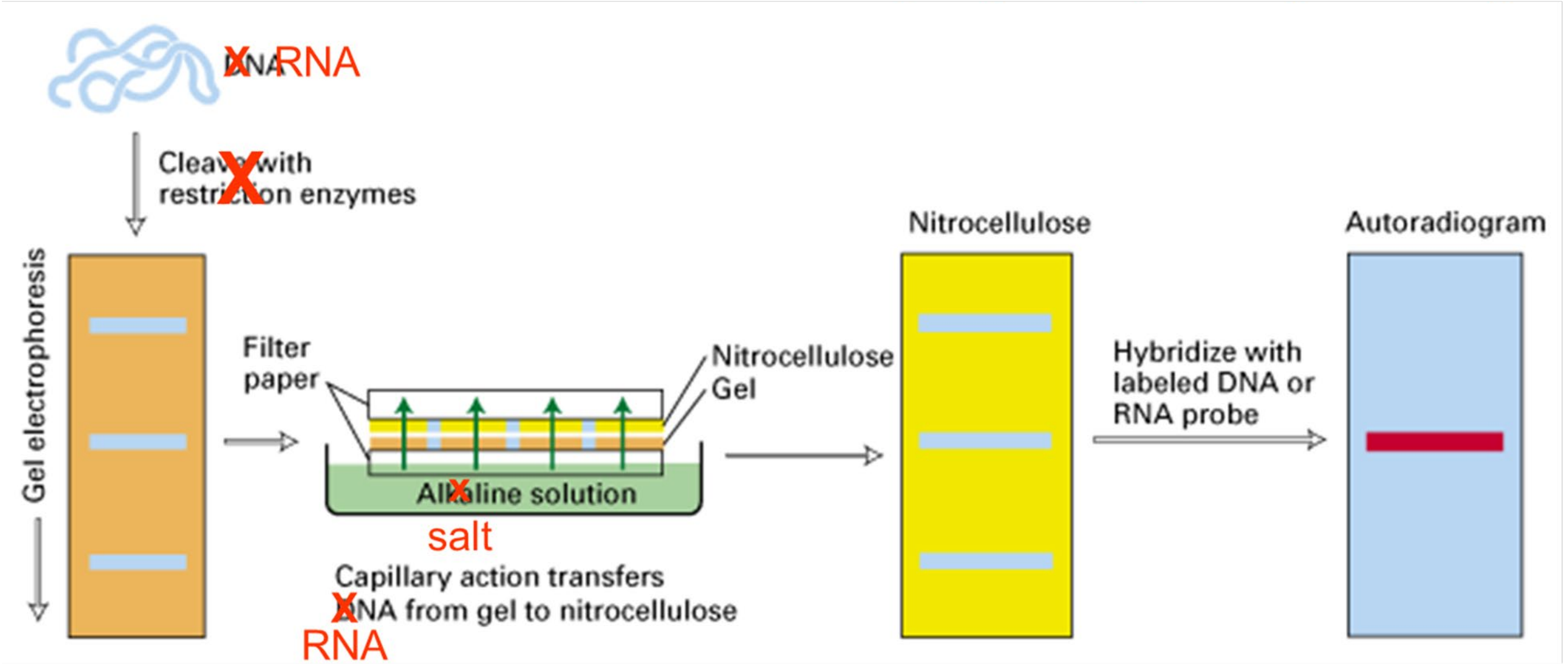
Characterization: Southern blot hybridization

-transfer of DNA from a gel to a membrane (e.g., nitrocellulose, nylon)
-developed by Edwin Southern



Characterization: Norther blot hybridization

-transfer of RNA from a gel to a membrane (e.g., nitrocellulose, nylon)
-reveals mRNA size (and approximate protein size), tissue- and organ-



Summary

Southern

- DNA on membrane.
- Digest DNA.
- Convert dsDNA to ssDNA.
- Probe with DNA or RNA.

Northern

- RNA on membrane.
- No need to digest DNA.
- Denature “folded” RNA with formaldehyde.
- Probe with DNA or RNA.

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