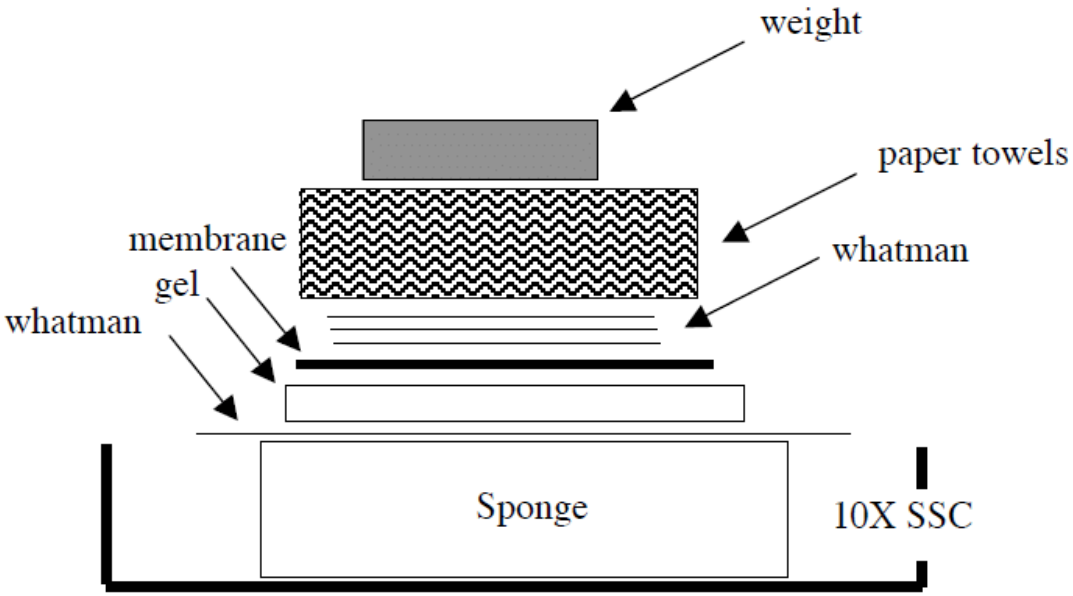

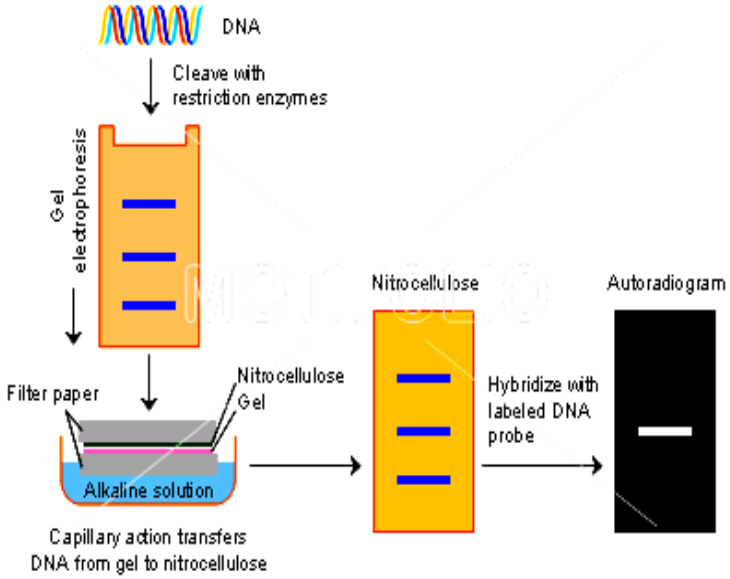

Southern Blotting

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Introduction

- A **Southern blot** is a method routinely used for detection of a specific DNA sequence in DNA samples.
- Southern blotting involves transfer of electrophoresis-separated DNA fragments to a filter membrane and subsequent fragment detection by hybridization with a suitable probe.
- The method is named after its inventor, the British biologist E. M. Southern. 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 Southern's name.
- The amount of DNA needed for this technique is dependent on the size and specific activity of the probe. Short probes tend to be more specific. Under optimal conditions, one can expect to detect 0.1 pg of the DNA.

Method

- **DNA Digestion by Restriction Endonuclease:** A suitable restriction endonuclease is used to cleave high-molecular-weight DNA into smaller fragments.
- **Electrophoresis:** The DNA fragments are then run by electrophoresis on an agarose gel to separate them by size.

Method: Depurination

- **Depurination (Optional):** If some of the DNA fragments are larger than 15 kb, then prior to blotting, the gel may be treated with an acid, such as dilute HCl, which depurinates the DNA fragments, breaking the DNA into smaller pieces, thus allowing more efficient transfer from the gel to membrane.
- A depurination step is optional. Fragments greater than 15 kb are hard to transfer to the blotting membrane. Depurination with HCl (about 0.2M HCl for 15 minutes) takes the purines out, cutting the DNA into smaller fragments. However, it is very important to neutralize the acid after this step.
- **DNA Denaturation:** The DNA gel is placed into an alkaline solution (typically NaOH solution) to denature the double-stranded DNA. The denaturation in an alkaline environment may improve binding of the negatively charged DNA to a positively charged membrane, separating it into single DNA strands for later hybridization to the probe and destroys any residual RNA that may still be present in association with the DNA. The choice of alkaline over neutral transfer methods, however, is often empirical and may result in equivalent results. However, it is very important to neutralize the base after this step.

Method: Transfer

- **Transfer of DNA to Membrane:** Traditionally a sheet of nitrocellulose (or, alternatively, nylon or a positively charged nylon membrane) membrane is placed on top of (or below, depending on the direction of the transfer) the gel.
- Pressure is applied evenly to the gel (either using suction, or by placing a stack of paper towels and a weight on top of the membrane and gel), to ensure good and even contact between gel and membrane.
- 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.
- DNA is transferred from the gel on to the membrane. Ionic interactions bind the DNA to the membrane because of the negative charge of the DNA and positive charge of the membrane.

Method: Transfer (cont.)

- Transfer is usually done by capillary action, which takes several hours. Capillary action transfer draws the buffer up by capillary action through the gel and into the membrane, which will bind ssDNA.
- However, one may also use a vacuum blot apparatus instead of capillary action. In this procedure, a vacuum sucks SSC through the membrane. This works similarly to capillary action, except more SSC goes through the gel and membrane, so it is faster (about an hour). SSC provides the high salt level that is needed to transfer DNA.
- **Fixation of DNA on membrane:** After transfer, the membrane is baked in a vacuum or regular oven at 80°C for 2 hours (standard conditions; nitrocellulose or nylon membrane) or exposed to ultraviolet radiation (nylon membrane) to permanently attach the transferred DNA to the membrane. UV light cross links the transferred DNA to the membrane via covalent bonds.

Method: Pre-hybridization

- A prehybridization step is required before hybridization to block non-specific sites, since single-stranded probe is not supposed to bind just anywhere on the membrane. To prehybridize, non-specific ssDNA such as somicated salmon sperm DNA is added.

Method: Hybridization

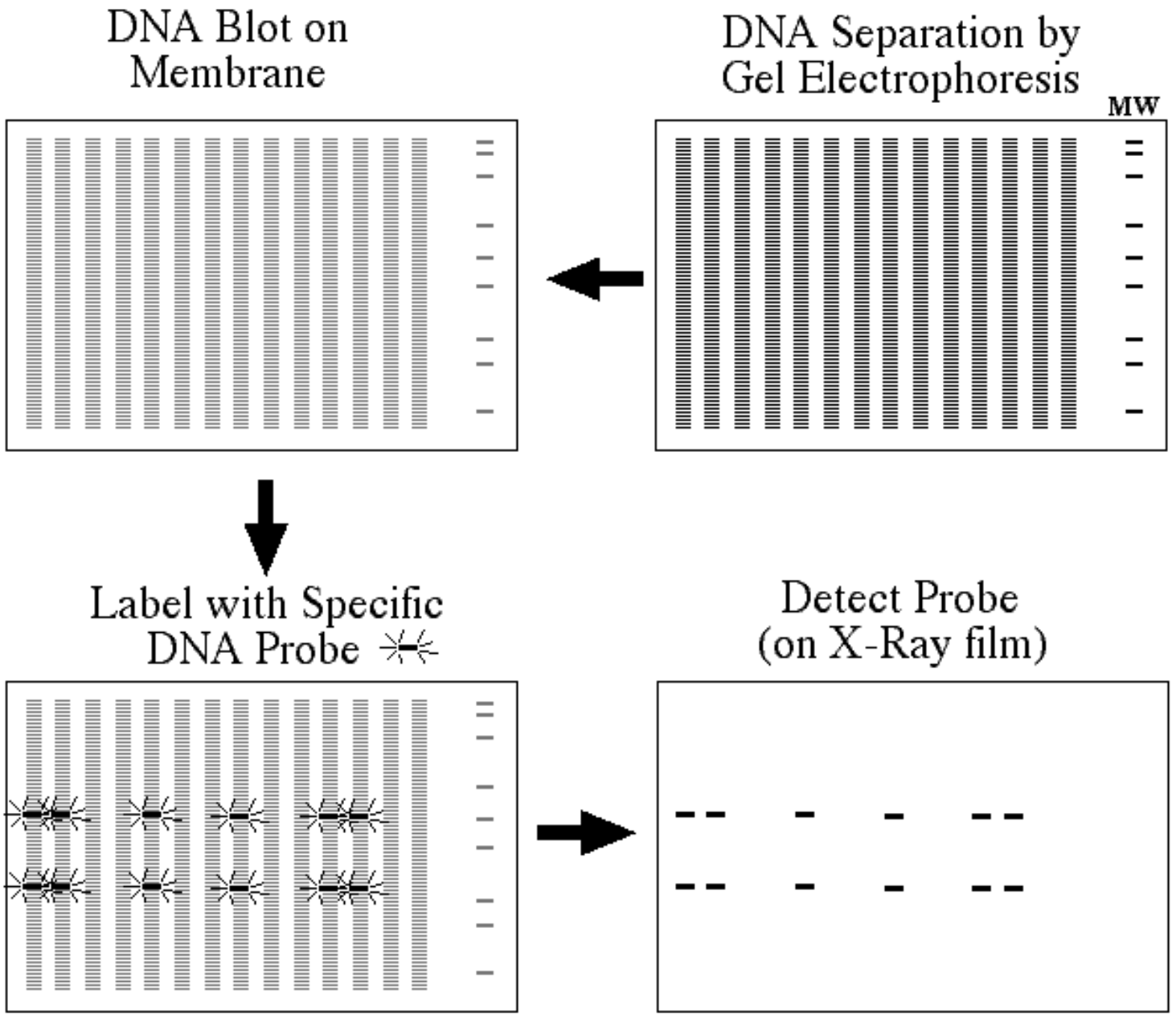
- **Hybridization with Probe:** The membrane is then exposed to a hybridization probe—a single DNA fragment with a specific sequence whose presence in the target DNA is to be determined. The probe DNA is labelled so that it can be detected, usually by incorporating radioactivity or tagging the molecule with a fluorescent or chromogenic dye. In some cases, the hybridization probe may be made from RNA, rather than DNA. To ensure the specificity of the binding of the probe to the sample DNA, most common hybridization methods use salmon or herring sperm DNA for blocking of the membrane surface and target DNA, deionized formamide, and detergents such as SDS to reduce non-specific binding of the probe.

- Hybridization of the probe to a specific DNA fragment on the filter membrane indicates that this fragment contains DNA sequence that is complementary to the probe.

- Modification of the hybridization conditions (for example, increasing the hybridization temperature or decreasing salt concentration) may be used to increase specificity and decrease hybridization of the probe to sequences that are less than 100% similar.

Method: Detection

- After hybridization, excess probe is washed from the membrane (typically using SSC buffer), and the pattern of hybridization is visualized on X-ray film by autoradiography in case of a radioactive or fluorescent probe, or by development of colour on the membrane if a chromogenic detection method is used.
- In some cases biotin/streptavidin detection is done by colorimetric methods, and bioluminescent visualization uses luminesence.



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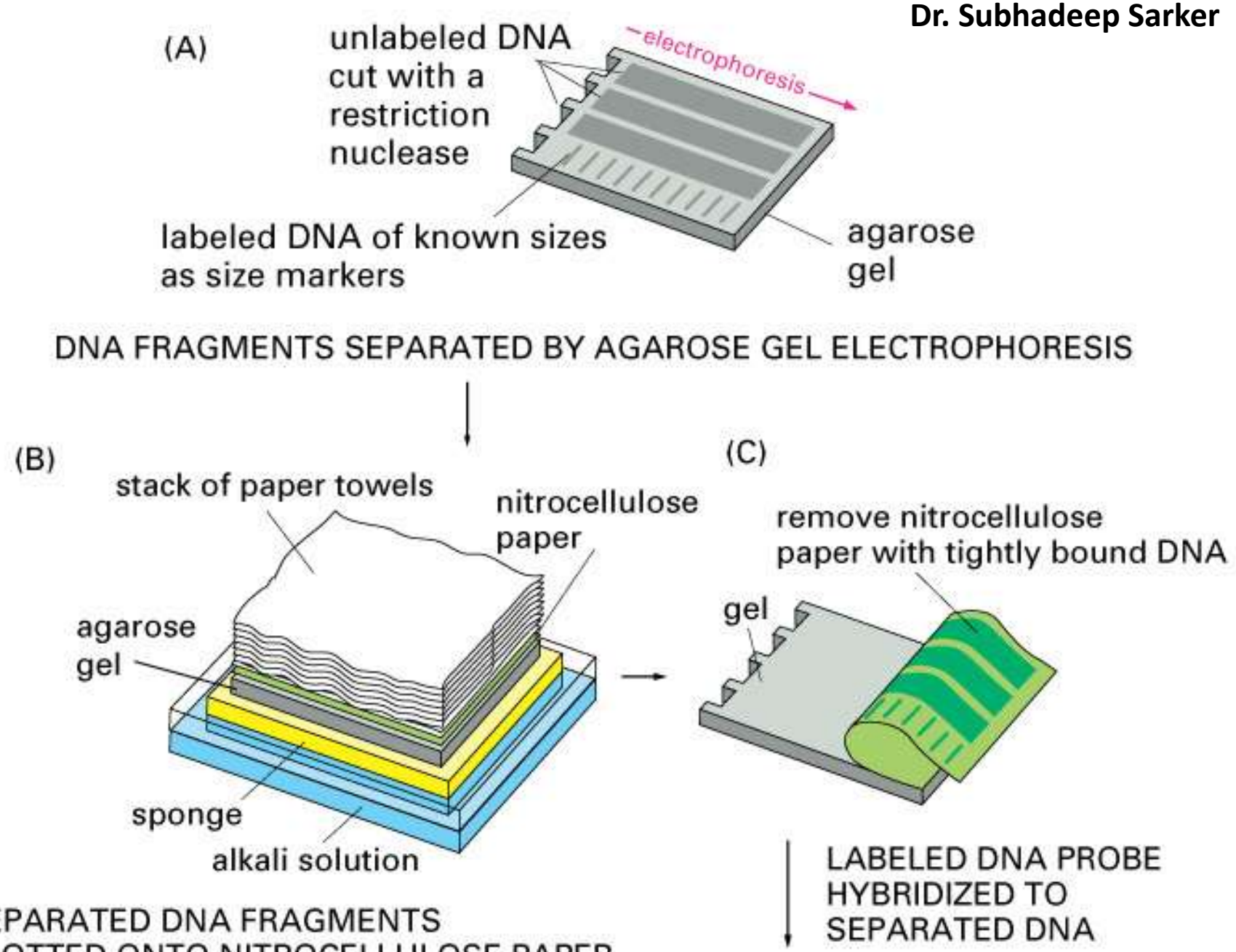

Southern Blot for determining gene copy number

- Southern blots performed with restriction enzyme-digested genomic DNA may be used to determine the number of sequences (*e.g.*, gene copies) in a genome.
- A probe that hybridizes only to a single DNA segment that has not been cut by the restriction enzyme will produce a single band on a Southern blot, whereas multiple bands will likely be observed when the probe hybridizes to several highly similar sequences (e.g., those that may be the result of sequence duplication).

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