11) BLOTTING TECHNIQUES

- Blotting is of three types
  (1) Southern Blotting — For DNA analysis
  (2) Northern Blotting — " RNA "
  (3) Western Blotting — " protein "

(1) Southern Blotting

Inventor : E. M. Southern (Molecular biologist)
Name of this blotting is given on the name of its discoverer.

Basis : DNA : DNA hybridization

Technical Procedure :

- A sample of DNA is treated with various restriction enzymes (RE) to get the fragments of different sizes. These fragments are subjected to electrophoresis using polyacrylamid or agarose gel.
- Now DNA sample is subjected to mechanical shearing or restriction endonuclease digestion in order to generate fragments further.
  - Agarose gel = few 100 to 20 Kb size.
  - Polyacryl amide + agarose gel = smaller fragment
  - Agarose gel + electric field inversion = large fragments 1000 - 2000 kb.
- Gel provide a complex network of polymeric molecule through which DNA fragment migrate depending upon their sizes under the electric field, since DNA molecule are negatively charged.
- Smaller molecule of DNA migrate relatively faster than larger molecule. Marker DNA fragments of known sizes run in separate lane; this permit an accurate determination of size of an unknown DNA by interpolation.
- The gels were stained with intercalacting dye ethidium bromide, which give visible fluorescence on illumination of gel with ultraviolet light; as little as 0.05 μg of DNA in one band can be detected by using this dye.

This approach also separates the closed circular (supercoiled), nicked (relaxed) and linear configuration of a single DNA molecule.

Uses:

1) To detect and identify DNA fragment in a sample that are complementary to a given DNA sequence.
2) To demonstrate presence of gene in transgenics
3) To detect and study RFLP (restriction fragment length polymorphism.
4.) DNA fingerprinting

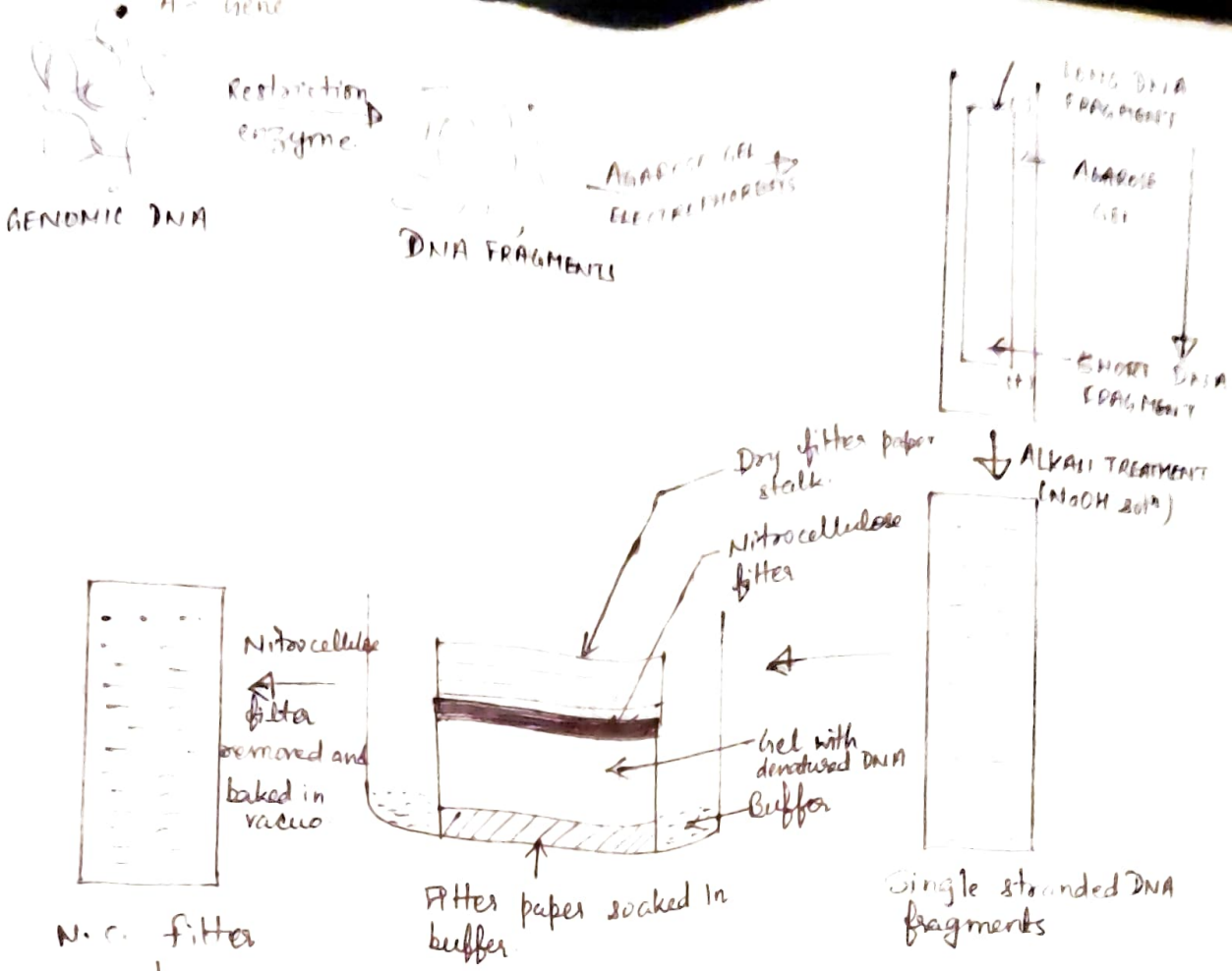
Steps of Blotting:

1. Restriction fragments of DNA present in agarose gel (after electrophoresis) are denatured into single stranded form by alkali treatment.
2. Now these are transferred to nitrocellulose membrane by placing gel on top of buffer ~~solution~~ saturated filter paper, then laying nitrocellulose membrane on top of gel and finally placing stalk of some dry filter papers on top of this membrane. The buffer move due to capillary action from bottom filter paper to that on top through gel which carry denatured DNA with it.
3. Nitrocellulose paper ~~not~~ now removed from blotting stalk and DNA is permanently immobilised on membrane by baking it at 80° C in vacuo.

The distinct bands on photographic film are produced by hybridisation reaction of selected probe with one or few fragment present in gel.

→ Some new membrane material eg. nylon membrane have been developed which have following advantageous feature.

(a) ~~They~~ They are physically robust in comparision to nitrocellulose filter membrane.
(b) Both DNA and RNA become cross linked to them ~~by~~ on a brief exposure to ultraviolet (UV) light.
(c) Save time needed for baking of nitrocellulose membrane.
(d) Nylon membranes are reusable.



SOUTHERN BLOTTING (TRANSFER OF DNA FROM GEL TO NITROCELLULOSE PAPER)