Methods of DNA Labelling.

⊕ Agarose gel electrophoresis :
separation of DNA & RNA molecules of diff length

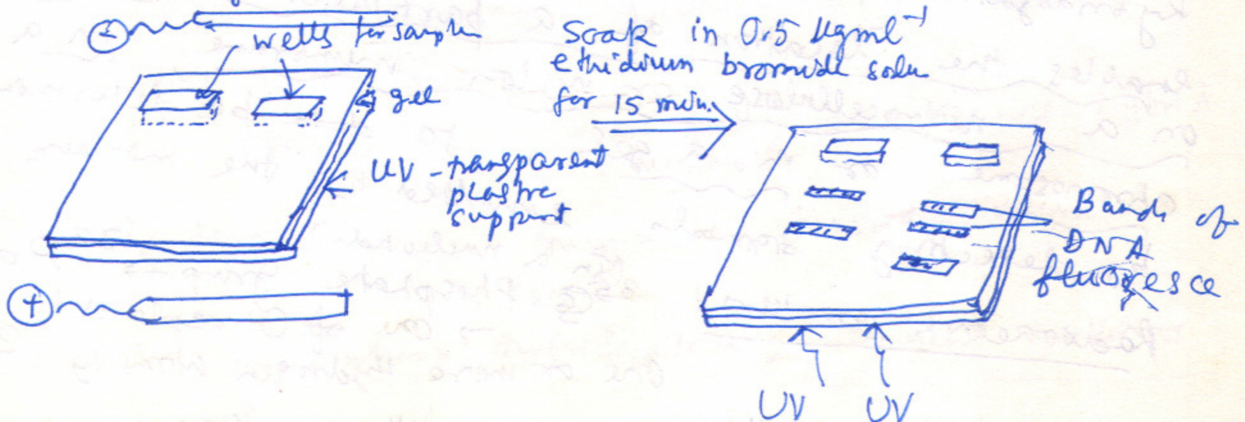
Gel electrophoresis is the standard method for separating DNA molecules of diff. lengths & can also be used to separate RNA molecules.

Electrophoresis is the movement of charged molecules in an electric field, usually carried out in aqueous soln, however in this case it is carried out in "gel", a network of pores through which the DNA molecules have to travel to reach the +ve electrode. Shorter molecules are less impeded by the pores than are longer molecules and so move through the gel more quickly. Molecules of diff. lengths therefore form bands in the gel.

Two type of gels → Agarose gel (Pores: 100 nm to 300 nm) depends on the concentration of gel. Polysaccharide
→ Polyacrylamide gel

Gel concentration determines the range of DNA fragmts to be separated.

0.3% gel → 5 – 50 Kb molecules
5% gel → 100 – 500 bp "



Polyacrylamide gel electrophoresis
(Separation of DNA molecules differing in length by just one nucleotide).

Polyacrylamide gels have smaller pore sizes than agarose gels and allow precise separation of molecules from 10-1500 bp.

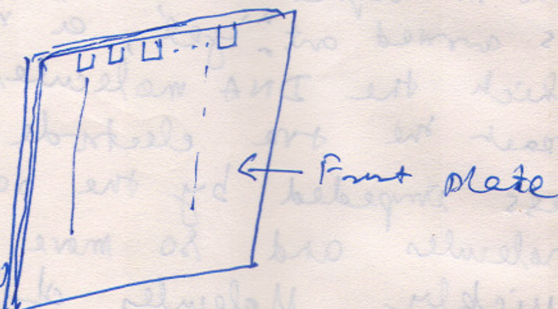
Concentration of Polyacrylamide + bis (another chemical) determines pore size.

e.g. 6% gel with 19:1 ratio ⇒ 100 - 750 nucleotide bases.

8% → 50 - 400

4% → 500 - 1500

(As DNA fragments are not visible, they are labelled at (end nucleotide) with radioactive / atom).



DNA labelling:

DNA labelling is a central part of many molecular biology procedures, including Southern Hybridization, fluorescent in situ hybridization (FISH) & DNA sequencing. It enables the location of a particular DNA molecule on a nitrocellulose or nylon membrane, in a chromosome or in a gel, to be determined by detecting signals emitted by the markers.

Radioactive markers: (in a nucleotide) Phosphate group → ^{32}P or ^{33}P
→ one of O's replaced by ^{35}S
One or more Hydrogen atoms by ^{3}H

→ Signal detection: "autoradiography" in X-ray plates,

or radiation sensitive phosphorescent screen (phosphorimaging).

^{32}P → high emission but low resolution (Scattering) due to

^{35}S / ^{3}H → low " " high "

1980's → radioactive markers are replaced by non-radioactive fluorescent DNA labels. These labels combine high sensitivity with high resolution & are ideal for southern hybridization.

(*) Southern Hybridization:

Southern Blot (by Edwin Southern)

(A) Transfer of DNA from gel to Nylon membrane (Blotting) ⇒ Procedure of tran.

(B) To carry out, the membrane is placed in a glass bottle, gently rotated for several hours (for complete hybridization) & membrane is washed ~~away~~ to remove any unhybridized probe. The signal from the label → to detection.

Under highly stringent hybridization conditions, a stable hybrid occurs only if the oligonucleotide is able to form a completely base-paired structure with the target DNA. If there is a single mismatch then the hybrid does not form.

To achieve this level of stringency, the incubation temperature must be just below the melting temp. Tm.

At temp. > Tm, even fully base-paired hybrid is unstable.

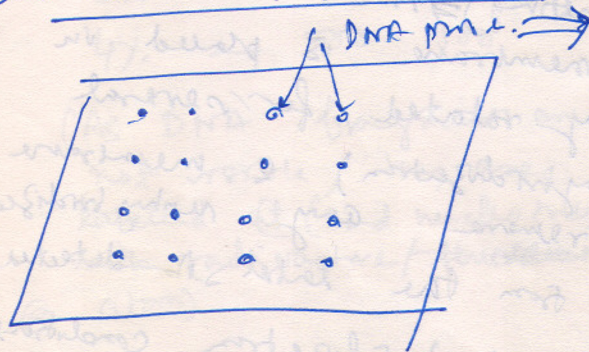
5°C < temp < Tm, mismatched hybrids might be stable.

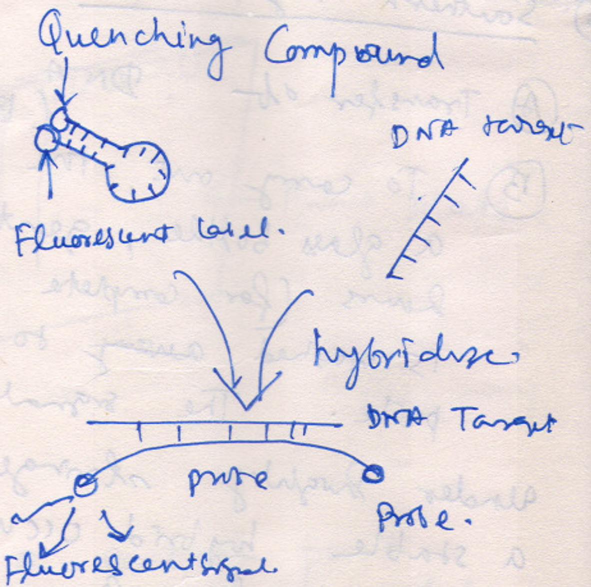
However, in Southern Hybridization, stringent conditions may not be imposed (JM).

Northern blot is the procedure of transfer of RNA (from gel) to a nylon membrane & then they are hybridized with a labeled DNA probe.

Western blot : a mixture of proteins is first fractionated by 2-D gel electrophoresis & then transferred to a sheet of nitrocellulose filter which is then incubated with a preparation of antibodies labeled either radioactively or fluorescently.

DNA μ-Array / Chip:





The oligonucleotide probe has two end-labels. One of these is fluorescent dye and the other is a quenching compound. The two ends of the oligonucleotide base-pair to one another, so the fluorescent signal is quenched. When the probe hybridizes to its target DNA, the ends of the molecule become separated, enabling the fluorescent dye to emit its signal. The two labels are called "molecular beacons".