SHORT NOTES

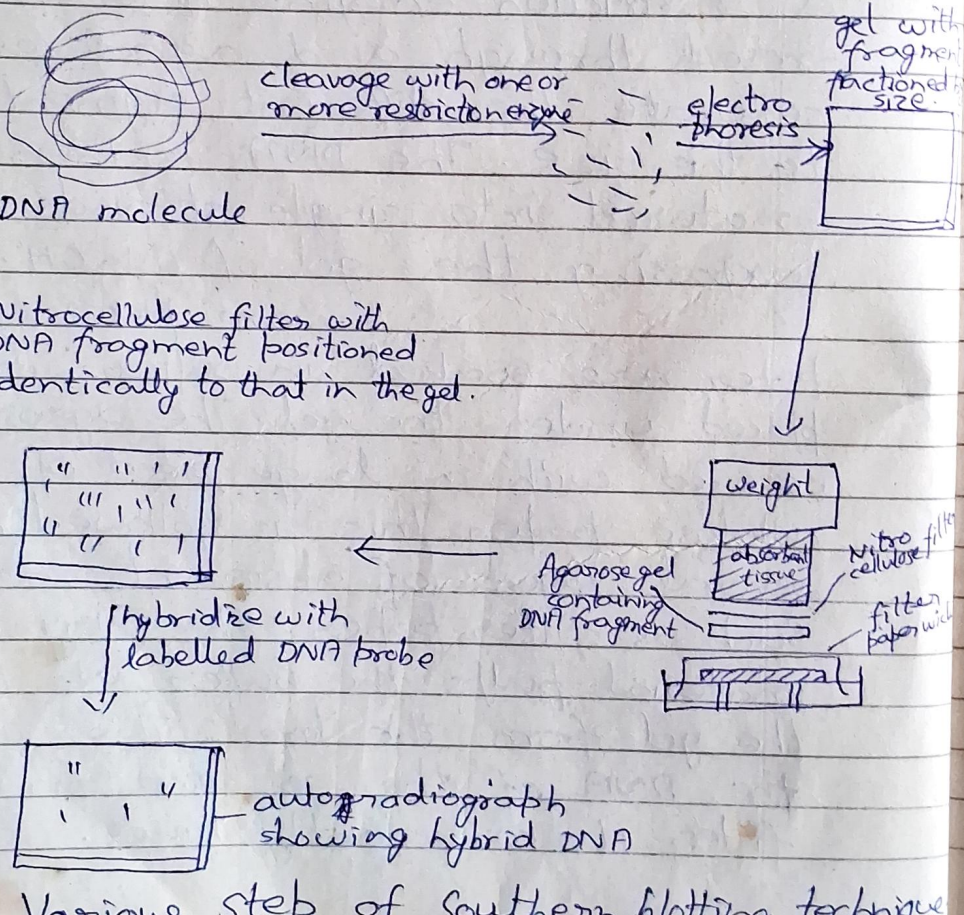
SOUTHERN AND NORTHERN BLOTTING OF DNA

These are the methods of isolation of genes. The Southern blotting technique was divised by E.M. Southern in 1975 where as Northern blotting was formulated by Alwine and co-worker 1979.

SOUTHERN BLOTTING TECHNIQUE → The technique consist of cutting a DNA molecule into seperate fragments by a restriction enzyme. It is electrophoresed through and agrose gel which seperate the different segment according to the size. The DNA is then denatured into single strands by exposing the gel of NaOH.

Some pieces of filter paper are soaked in buffer. They are placed under the gel. The agrose gel is covered with a large piece of nitrocellulose paper. This is followed by placing several layers of absorbent material. Such as filter paper. This dry absorbent material pulps the buffer up through the gel from the layer. This washes the DNA of the gel and on the filter it covalently bines.

The position of DNA molecule on the filter paper are identical to their position on gel. The nitro cellulose filter containing the DNA is first dried and then exposed to a sat solution of P32 labelled mRNA called molecular probe. The radioactive mRNA forms a hybrid with the single stranded DNA that contains complementary sequences. The nitrocellulose filter is then removed and placed in contact with photographic field. The specific restriction fragments are easily identified.



Various step of Southern blotting technique

NORTHERN BLOTTING TECHNIQUE→ This technique for isolation and identification of particular genes consist of RNA band blot transfering from the gel into chemically reactive paper.

The paper is called Amino benzyl oxymethyl paper.

In this technique the DNA molecule is also cut into small fragment by a restriction enzyme. They are electrophosphoresed through an agrose gel. The reactive DNA probes are hybridized with cut frag-ments. The hybridized band can be located easily by radioactive probes.