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Analysing complex nucleic acid mixtures (DNA or RNA)

The total cellular DNA of an organism (genome) or the cellular content of RNA are complex mixtures of different nucleic acid sequences. Restriction digest of a complex genome can generate millions of specific restriction fragments and there can be several fragments of exactly the same size which will not be separated from each other by electrophoresis.

Techniques have been devised to identify specific nucleic acids in these complex mixtures

- **Southern blotting - DNA**
- **Northern blotting - RNA**

Technique devised by Ed Southern in 1975, is a commonly used method for the identification of DNA fragments that are complementary to a know DNA sequence. Allows a comparison between the genome of a particular organism and that of an available gene or gene fragment (probe). It can tell us whether an organism contains a particular gene (DNA fragment) or not

In Southern blotting

1. Chromosomal DNA is isolated from the organism of interest, and digested to completion with a restriction endonuclease enzyme.
2. The restriction fragments are then subjected to electrophoresis on an agarose gel, which separates the fragments on the basis of size.
3. DNA fragments in the gel are denatured (i.e. separated into single strands) using an alkaline solution.
4 .Transfer fragments from the gel onto nitrocellulose filter or nylon membrane.

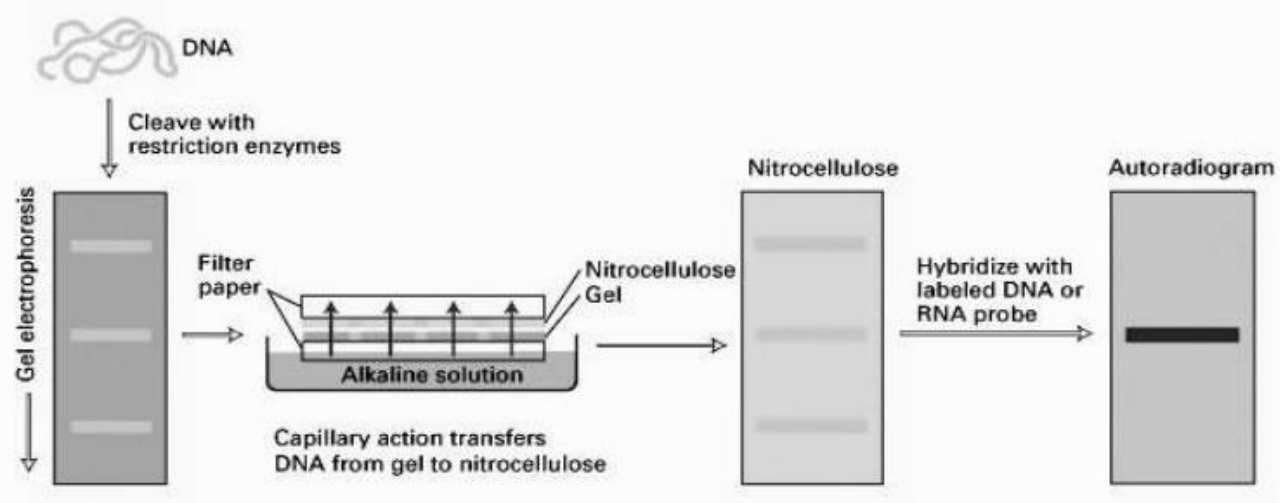

Fig 7-32, Lodish et al (4th ed.)

DNA is bound irreversibly to the filter/membrane by baking at high temperature (nitrocellulose) or crosslinking through exposure to UV light (nylon).