Hybridization methods/ Nucleic acid hybridization (NAH)

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- Detection of specific viral nucleic acid by hybridization by use of labeled viral DNA and RNA probes
- Rapid diagnosis

- Principle: In NAH ssDNA will hybridize by Hydrogen bonded base pairing to another ssDNA (or RNA) of complementary base sequence

- Heating: Two strands of target DNA molecule separated
- Cooling: Hybridize with a labeled ssDNA or RNA probe

- Stringency:
- Depends on conditions set for annealing
- Temperature
- Ionic strength

Stringency

- Low: a number of mismatched base pairs tolerated
- High: Heteroduplex is unstable

Specificity

- Nature of probes
- Probe corresonds in length to whole viral genome/ single gene/ shorter sequence represent either variable or conserved region
- Probe can be type specific or versatile
- Probes are produced by chemical synthesis or by cloning in a bacterial plasmid or bacteriophage

Labels

- Radioactive isotopes- 32P and 35S used to label probes
- Signal read by counting in spectrometer or autoradiography

- Now non radioactive labels are used
- Fluorescein and peroxidase- produce a signal directly
- Biotin and digoxigenin act indirectly by binding to another labeled compound (emit signals)

Dot-blot (filter hybridization methods)

- Most popular
- Two phase systems
- Filter hybridization
- Simplest format
- Dot-blot hybridization
- DNA or RNA extracted
- Denatured and spotted onto charged nylon or nitrocellulose membrane
- Binding occurs after baking
- Now ssDNA or RNA probe hybridized to target nucleic acid in situ on the membrane
- Wash unbound probe

- Signal generated by bound probe is measured
- By autoradiography or formation of coloured precipitation

- Sensitivity can be improved by using RNA as a probe
- False positives reduced by treatment of filters with RNase before counting

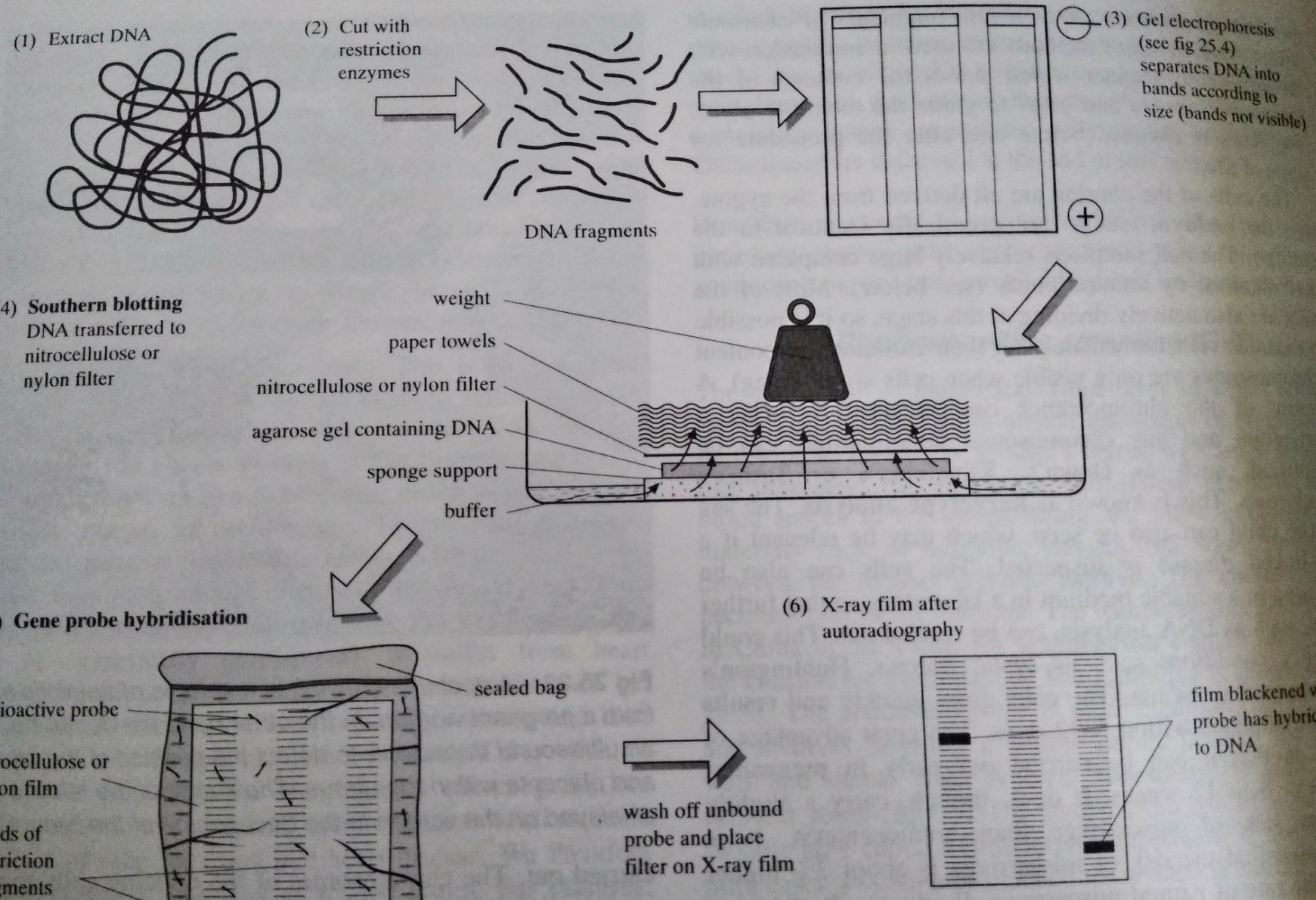
In situ hybridization methods

- Widely used by pathologist
- Screen animals with persistent infections
- Study viral induced cancers for evidence of integrated or nonintegrated copies of viral genome
- Frozen sections on slides are probed
- Intracellular location of viral nucleic acid sequences is revealed
- By autoradiography or immunoperoxidase cytochemistry

Southern blot hybridization methods

- DNA extraction
- Cleave DNA into fragments by the action of Restriction endonuclease
- Depending on the location and number of restriction sites DNA fragments of various sizes and number will be generated
- Fragments separate by agarose gel electrophoresis or polyacrylamide gel electrophoresis
- Stain with ethidium bromide
- To reveal position of fragments

- Now depurinate and denature DNA
- By treatment of gel successively with acidic and basic solutions
- Transfer DNA onto a nylon or nitrocellulose membrane
- Electrophoresis/Diffusion or other means "blotting" used to transfer
- Fragments are revealed by hybridization of a labeled probe and detection
- Northern blotting-RNA performed by similar way


(1) Extract DNA
(2) Cut with restriction enzymes
DNA fragments
(3) Gel electrophoresis (see fig 25.4) separates DNA into bands according to size (bands not visible)
(4) Southern blotting
DNA transferred to nitrocellulose or nylon filter
weight
paper towels
nitrocellulose or nylon filter
agarose gel containing DNA
sponge support
buffer
(5) Gene probe hybridisation
radioactive probe
nitrocellulose or nylon film
bands of restriction fragments (not visible)
sealed bag
wash off unbound probe and place filter on X-ray film
(6) X-ray film after autoradiography
film blackened where probe has hybridised to DNA

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