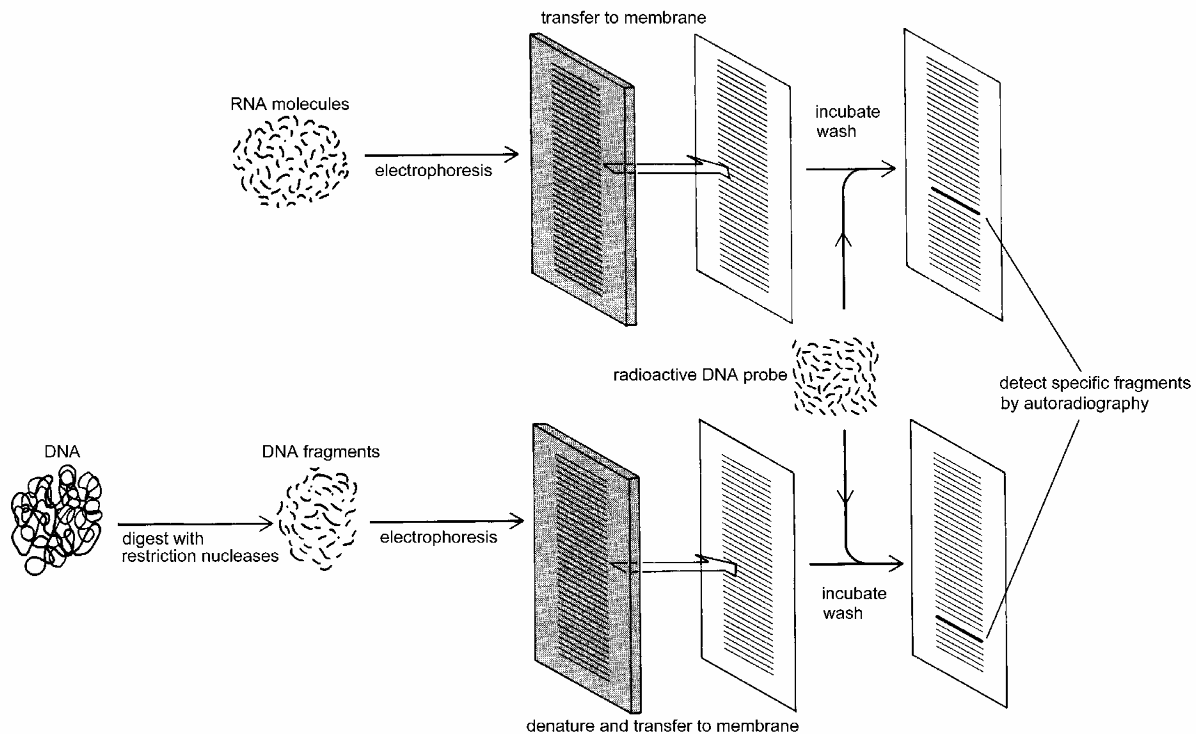
Nucleic Acid Blotting

General principles

- DNA can be denatured into single stranded molecules
- Duplexes (i.e., double-stranded molecules) can be reformed in vitro from complementary nucleic acids.
- Hybridization with a homologous 'probe' can be used to detect specific nucleic acid molecules or fragments.
- Commonly carried out in a 'blotting' format. (Nucleic acids are transferred to a membrane following electrophoresis.)
  - Southern Blotting (digested DNA)
  - Northern Blotting (RNA)
  - Dot/Slot Blots (no electrophoresis)



Generic Blotting Protocol

1. Digest DNA or isolate RNA
2. Electrophoresis
   - denaturing gels for RNA
3. Depurinate (optional)
   - treatment with dilute HCl will cause random breaks and promote a more efficient transfer of very large DNA molecules
4. Denature dsDNA
   - treat with alkali (NaOH) to separate strands
5. Transfer to membrane
   - capillary action (original method, slow)
   - vacuum apparatus (rapid)
6. Fix nucleic acid to membrane
   - heat (80°0
   - UV crosslink
7. Prehybridize
   - incubate with non-specific DNA to 'block' free sites on the membrane
8. Incubate with probe
   - generally carried out at lower stringency
9. Wash
   - generally carried out at higher stringency
10. Detect (autoradiography or develop with substrates)

Factors affecting hybridization
- temperature
- ionic strength (Na^+ concentration)
- chaotropic agents (% formamide)
- probe length
- probe mismatch
- % GC

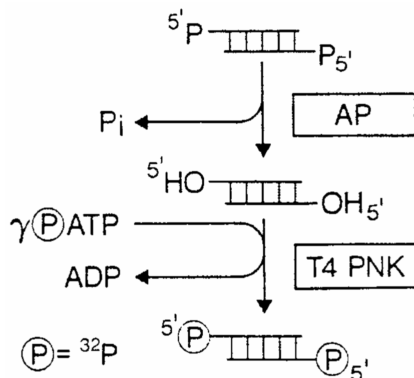
Stringency and Melting Temperature

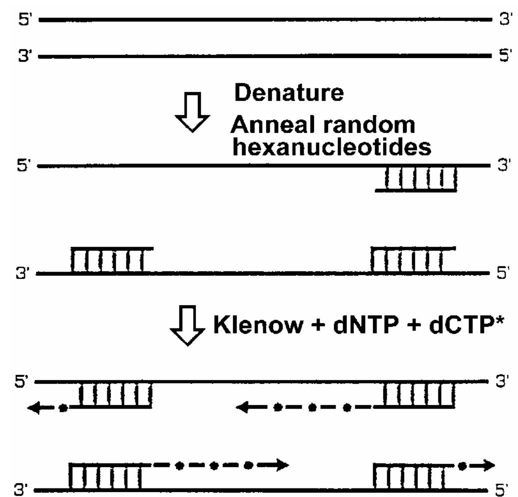
- Melting temperature (T_m) is the temperature at which a particular DNA will separate into single strands
- Stringency refers to the relative conditions of the hybridization as compared to the T_m.
- It is related to the homology between probe and target.
- Effective T_m is estimated from formulas:
  - cloned DNA fragment
    $T_m = 81.5^o + 16.6\log[Na^+] + 0.41(\%GC) - 0.65(\%formamide) - 1.4(\%mismatch)$
  - synthetic oligonucleotide
    $T_m = 2(A + T) + 4(C + G)$

high	$T_m - 15^o$
moderate	$T_m - 25^o$
low	$T_m - 35^o$

Labeling DNA probes

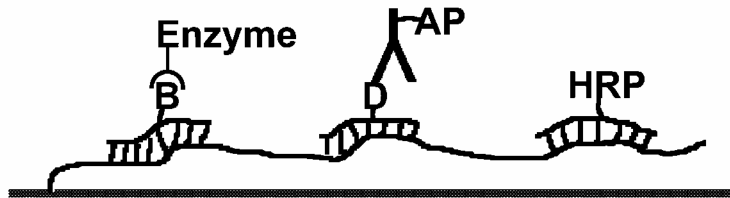
- random priming (cloned DNA fragments)
- T4 polynucleotide kinase (synthetic oligonucleotides)





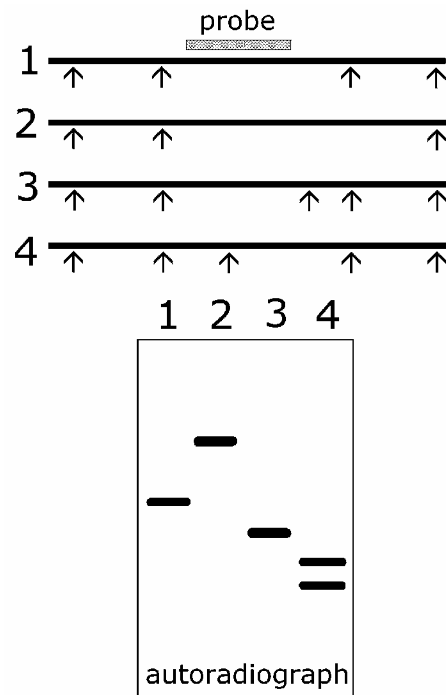
Non-radioactive probes (enzyme-linked detection)

- Biotin-Avidin (conjugated with enzyme)
- Digoxigenein-11-dUTP + enzyme conjugated antibody
- Direct cross-linking of enzyme (HRP)
- Often detected with substrates that produce light



Restriction Fragment Length Polymorphisms (RFLP)

- single nucleotide mutations can result in the loss or gain of restriction sites
- results in different sized DNA fragments associated with that locus
- diagnosis of genetic diseases
- polymorphisms can be used as genetic 'fingerprints' to distinguish species, strains, individuals, etc



RNA Applications (Gene Expression)

- Northern blots
  - sizes of transcripts
  - information on gene expression
  - one gene at a time
- DNA microarray (gene chips)
  - different DNA ‘probes’ are fixed onto solid surface (glass) in array
  - fluorescent labeled target cDNA (mRNA) incubated with chip
  - analyze 1000’s of genes simultaneously
    - identification of specific sequences (transcripts)
    - expression levels (mRNA abundance)
- in situ hybridization
  - radioactive or fluorescent probes used to localized mRNA to particular cells within tissue