

Probes and Southern Blot Hybridization (A very detailed video)

Probe Preparation

Concepts of Labeling

Hybridization: Annealing of complementary DNA. Probes are labeled DNA that allows for visualization (either via radioactivity or color change.)

3 probe types:

1. T4 polynucleotide Kinase: 5' -OH is replaced with * P from *ATP (AKA end labeling.)
2. Radioactivity in the nucleotide themselves and....

3. Nick Translation Reaction: Similar to # 2 in that radioactivity is in the nucleotides.

Nicks are created and exonuclease activity will remove a few bases and then add them back, creating a wide range of probes complementary to target DNA.

The probe “generation cocktail” consists of typical components of PCR reaction except that one of the nucleotides is labeled, and DNAase is present at low concentration to nick DNA which allows for exonuclease and follow up polymerase activity.

The temperature is closely followed to optimize polymerase and nicking to create oligonucleotides.

Purify Probe

Probe is added to TRIS, Salmon Sperm (blocking agent, explained later) and spermine (to encourage DNA ppt.)
Spin, dry and resuspend.

Percent incorporation: equals radioactivity in sample after ppt / divided by radioactivity before ppt.

Prepare Filters and Gel: Do not touch filter with bare hands:

- Nitrocellulose: 500 bp lower limit, less background (non specific binding)
- Nylon: 50 bp lower limit, strong binding, but more background.
- Positively charged nylon membrane, similar to above, but requires no cross linking.

Background: Non specific DNA binding to nylon membrane.

Cross Linking: Use of UV light and baking to bind DNA to membrane. Must be optimized to ensure adequate linkage without damage to DNA.

Transfer and Immobilization of DNA

All material handled with gloves and cut to size (see Cold Spring Demo for more detail if necessary.)

Soak gels in 0.4 NaOH (takes DSDNA to SSDNA)

Transfer sandwich: sponge sitting in buffer, filter paper, gel (with wells cut off to avoid bubbles.), nylon membrane, filter paper, paper towels in a big stack

Duration of transfer is crucial as too long, will give lots of background (non specific transfer/binding) and too short will not allow for enough target DNA to bind.

To immobilize the DNA (see cross linking above.)

A few other steps, and the prehybridize:

Prehybridization: Involves covering remaining surfaces of membrane with non-target DNA (otherwise probe may bind with nylon (as it is DNA as well!))

Prehybridization solution: 10 % PEG, 250 μ /ml salmon sperm DNA, 7 % SDS, 50 mM NaH₂PO₄ (pH control), and water.

This process is optimized as well. Placed in bag, burped and incubated.

Hybridization:

- Boil probe and quench in ice (makes SS probe)
- Hybridize as in prehybridization (remember probe) and let anneal
- At or above 63 °C results in high stringency
- Below 63 °C results in less specific binding and formamide can be added to increase stringency at lower temperatures.

Wash Filter: To remove any unhybridized probe.

Low salt will increase stringency. High salt will lessen stringency.

Blot dry but keep slightly moist

Expose to X ray plate for 2-3 days or...Colorimetric techniques (use of less problematic dyes)

Results: Can detect polymorphisms in DNA (i.e. RFLP and VNTRs.) Pieces of DNA are excised from genome and are used to make probe. Then exposed to genome again as a probe, and specificity decides if bonding will occur