

HYBRIDIZATIONS USING OLIGONUCLEOTIDE PROBES

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Hybridizations normally use 0.5-2 ng of labelled oligonucleotide per ml of hybridization solution. The following is based on a 20 ml hybridization, using ~20 ng of a 25 base oligonucleotide. This should represent about 10^6 Cerenkov cpm/ml.

Preparation of probe

A. Mix together in a screw-cap tube:

30 ng oligonucleotide*
3 μ l 10X T4 polynucleotide kinase buffer
26 pmol (80 μ Ci) Gamma-32P-ATP (>6000 Ci/mmol)
25 units T4 polynucleotide kinase
H₂O to 30 μ l

**Label must be in excess compared to oligonucleotide. For a 25 nt oligo, 26 pmol equals 220 ng. So the 30 ng in the recipe represents an excess of label.*

B. Incubate 30 min at 37C. Stop reaction by adding 1.5 μ l of 0.5 M EDTA

C. Separate oligos from unincorporated label as follows:

1. Run through a gel filtration column using the standard lab method, being careful to collect all of the fractions before the bromophenol blue dye (the oligos will take longer to exit the column than standard random-primed DNA).

(or)

2. Add 25 μ g tRNA, ammonium acetate to 2.5 M, and 9 volumes of 100% ethanol. Incubate at -80 for 60 min, spin in a microfuge 20 min in the cold, and wash with 70% ethanol.

3. Check counts on the Geiger counter for a rough estimate, or count 1 μ l in the scintillation counter.

Hybridization

- A. Prehybridize filters in high SDS hybridization solution as in the standard method.
- B. Add the labelled oligo and incubate for 14-48 hours at the temperatures indicated below:

17-mer oligo: 37C	<i>aiming for about T_m-25, based on</i>
20-mer: 42C	$T_m = 16.6 \log M + 0.41(\%GC) + 81.5$
23-mer: 48C	
25-mer: 52C	

- C. Wash as follows:

- 5 to 15 min in 6X SSPE, 0.05% sodium pyrophosphate (NO SDS), room temperature
- repeat the above for a total of 4 washes
- wash in pre-warmed 6X SSPE, 0.05% sodium pyrophosphate at the temperatures indicated below, for 15 min:

17-mer oligo: 48C
20-mer: 55C
23-mer: 58C
25-mer: 63C

The final wash should be about T_m-10 . At 6X SSPE, a 25-mer with 60% GC will have a T_m of about 76C).