Determining the hybridization temperature for S1 nuclease mapping*

Michael Dean

Program Resources, Inc., Frederick Cancer Research Facility, PO Box B, Frederick, MD 21701, USA
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The use of S1 nuclease to digest RNA:DNA hybrids has proven to be useful in a wide variety of applications. A major modification of the method was the use of double-stranded, end-labeled DNA probes (3). Because DNA:RNA hybrids are more stable than DNA:DNA hybrids in 80% formamide (4) conditions can be chosen in which the probe will remain denatured but can still hybridize to RNA.

With double stranded probes the only variable to be determined is the hybridization temperature, which must be above the T_m of the probe but below the T_m of the RNA:DNA hybrid. Although this can be accomplished by experimentally determining the T_m for each probe, most researchers prefer to avoid this step. If the DNA sequence of the probe is known, the T_m of the probe could be determined empirically. Based on data of Casey and Davidson (5) and experimental determination of the T_m for several probes of known sequence I have generated a plot useful for estimating the hybridization temperature of any probe of known sequence (Fig. 1). For example, a DNA fragment that is 50% G+C should be hybridized at 55°C.

This graph should only be used to estimate the hybridization temperature. In situations where a small amount of re-annealed probe will interfere, the assay should be optimized using several temperatures 3-4°C above and below the estimated one. Probes with stretches of high G+C content 50 bp or longer may require higher hybridization temperatures.

Figure 1. S1 hybridization temperature. The percent G+C content of the double-stranded probe is plotted against the temperature. --- RNA:DNA T_m , — hybridization temperature, ... DNA:DNA T_m .

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REFERENCES. 1. Berk, A.J., and Sharp, P.A. (1977) Cell 12, 721-732. 2. Dean, M., Levine, R.A., and Campisi, J. (1986) Mol. Cell. Biol. 6, 518-524. 3. Weaver, R.F., and Weissmann, C. (1979) Nucl. Acids Res. 7, 1175-1193. 4. Casey, J., and Davidson, N. (1977) Nucl. Acids Res. 4, 1539-1552.