Determining the hybridization temperature for SI nuclease mapping*

Michael Dean

Program Resources, Inc., Frederick Cancer Research Facility, PO Box B, Frederick, MD 21701, USA
Submitted June 26, 1987

The use of SI 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 Tm of the probe but below the Tm of the RNA:DNA hybrid. Although this can be accomplished by experimentally determining the Tm for each probe, most researchers prefer to avoid this step. If the DNA sequence of the probe is known, the Tm of the probe could be determined empirically. Based on data of Casey and Davidson (5) and experimental determination of the Tm 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. SI hybridization temperature. The percent G+C content of the double-stranded probe is plotted against the temperature. ----- RNA:DNA Tm, ---- hybridization temperature, DNA:DNA Tm.](image-url)

*Research sponsored, at least in part, by the National Cancer Institute, DHHS, under contract N01-C0-23910 with Program Resources, Inc. The contents of this publication do not necessarily reflect the views or policies of the DHHS, nor does mention of trade names, commercial products or organizations imply endorsement by the US Government.