NUCLEASE-S1 MAPPING OF RNA

Nuclease-S1 mapping is used to map the locations of the ends of RNA molecules and of any splice points within them in relation to specific sites (e.g., positions of restriction endonuclease cleavage) within the template DNA. The procedure is based on the observation of Casey and Davidson (1977) that hybridization conditions can be established that minimize the formation of DNA · DNA hybrids while promoting the formation of DNA · RNA hybrids. These hybrids are then digested with the single-strand-specific nuclease S1 and analyzed by gel electrophoresis (Berk and Sharp 1977).

Although more-refined techniques are available (e.g., cDNA copies of RNAs can be prepared by primer extension and sequenced) (Ghosh et al. 1978; Reddy et al. 1978), the coordinates determined by nuclease-S1 analysis are accurate enough for most purposes. Furthermore, the method is rapid, easy to perform, and extremely sensitive—the equivalent of as little as one molecule of RNA per cell can be detected.

The method given below describes the nuclease-S1 technique in its simplest and most-basic form. A more-detailed description, together with a thorough discussion of the pitfalls of nuclease-S1 mapping, is presented in an excellent review by Favaloro et al. (1980).

1. Mix in a sterile Eppendorf tube:

DNA	0.1–1.0 μg
RNA	0.5–500 μg

If necessary, add tRNA carrier to bring the total amount of RNA to 100–200 μg.

Note. The amount of DNA used in the hybridization reaction depends on its molecular weight. For fragments approximately 5 kb in length, 0.1 μg is required; for smaller fragments, proportionately less DNA should be used.

The amount of RNA required depends on the concentration of the sequences of interest. To detect sequences present in low amounts, up to 250 μg of RNA may be used per 50 μl of hybridization reaction. These quantities of RNA and DNA are suitable for hybridization in a volume of 50 μl. If reagents are in short supply, the hybridization reactions can be scaled down to 10 μl. For ease of manipulation in subsequent steps, it is advisable to keep the hybridization volume to 50 μl or less.

2. Precipitate the mixed DNA and RNA with ethanol (−70°C for 15 minutes).

3. Resuspend the pellet in 30 μl of hybridization buffer (see page 208). Pipette up and down many times to make sure that the pellet is dissolved.

Hybridization buffer

40 mM PIPES (pH 6.4)
1 mM EDTA (pH 8.0)
0.4 M NaCl
80% formamide

Notes

i. Use the disodium salt of PIPES.

ii. Use formamide deionized by passage through a mixed-bed resin (Bio-Rad AG 501-X8), recrystallized at 0°C, and stored in small aliquots in tightly capped tubes.

4. Immerse the tubes in a water bath at 72°C for 10–15 minutes to denature the DNA. (Higher temperatures [75–85°C] have been used successfully.)

5. Transfer the tubes *rapidly* to a water bath set at the desired hybridization temperature. Do not allow the tubes to cool below the hybridization temperature during transfer.

   The hybridization temperature, which depends on the G + C content of the DNA, is chosen so as to minimize the formation of DNA · DNA hybrids while allowing DNA · RNA hybrids to form. The following table gives the approximate hybridization temperatures for DNAs of different G + C content. It is advisable to carry out the hybridization reactions at different temperatures to find the optimal temperature for your RNA.

G + C	Hybridization temperature
41%	49°C
49%	52°C
58%	60°C

6. Hybridize for 3 hours. Open the lid of the hybridization tube, but keep the body of the tube submerged. Rapidly add 0.3 ml of ice-cold nuclease-S1 buffer (usually containing 100–1000 units/ml of nuclease S1). Mix well and incubate for the appropriate time and temperature (see note below).

Nuclease-S1 buffer

0.28 M NaCl
0.05 M sodium acetate (pH 4.6)
4.5 mM $ZnSO_4$
20 μg/ml carrier ssDNA

Note. A variety of temperatures and nuclease-S1 concentrations have been used with particular RNA · DNA hybrids. Incubation at 20°C will minimize the digestion of RNA across from DNA loops (this is critical for neutral gel analysis). Higher temperatures (37–45°C) may be required for complete digestion for denaturing gel analysis. In general, the nuclease-S1 digestion and the incubation temperature should be optimized for each RNA · DNA hybrid.

7. Chill to 0°C. Add 50 μl of 4.0 M ammonium acetate and 0.1 M EDTA to stop the reaction.

8. Extract once with phenol/chloroform. Add 20 μg of carrier tRNA. Precipitate with an equal volume of isopropanol (−70°C for 15 minutes).

9. Dissolve the precipitate in 40 μl of TE (pH 7.4).

10. Add 10 μl of loading buffer (50% glycerol and 0.2% bromocresol green) and mix well.

11. Load half of the sample on a neutral agarose gel. Adjust the remaining sample to 0.05 M NaOH and run on an alkaline agarose gel.

12. Following electrophoresis, transfer the DNA from both gels to nitrocellulose filters as described on pages 382ff. (The alkaline gel does not require soaking in denaturation solution).

13. Hybridize the filters to an appropriate ^{32}P-labeled DNA probe.