S1 nuclease assay using oligonucleotide probe

Steve Hahn and Breeden Lab, 2001

These reactions must be performed using appropriate shielding from the ^{32}P labeled oligo. Dispose of all radioactive waste in an appropriate manner. In addition, use gloves and RNAse free solutions throughout.

1. Hybridize RNA and labeled oligonucleotide probe in 50 microliters.

Mix the following in an RNAse free 0.2 ml microcentrifuge tube:
15 micrograms total yeast RNA
50,000 – 100,000 cpm labeled oligonucleotide probe
5 microliter 10X hybrid buffer
0.2 microliter 25% Triton X-100
H2O to a final volume of 50 microliters

Place tubes in a thermal cycler with a heated lid to prevent sample evaporation. Heat to 94 degrees for 3 min. Reduce temperature to 55 degrees and incubate overnight (at least 10 hrs).
The optimum temperature for a particular application may have to be adjusted.

2. S1 nuclease digestion

Prepare S1 digestion mix (1 reaction):

0.33 M NaCl	148.5 microliters 1 M
66 mM NaOAc pH 4.6	29.7 microliters 1 M
2.2 mM ZnSO4	9.9 microliters 0.1 M
0.01% Triton X-100	0.18 microliters 25%
20 units S1 nuclease (Invitrogen)	0.2 microliters 100 U/microliter

H2O to a final volume of 450 microliters

If necessary, dilute the S1 nuclease with the S1 dilution buffer provided by the manufacturer.

Remove hybridization tubes from thermal cycler. Quick spin in centrifuge to bring all liquid to bottom of tube. Transfer the hybridization reactions to 1.5 ml eppendorf tubes. Add 450 microliters S1 digestion mix. Mix well, quick spin and incubate tubes at 37 degrees for 30 min.

3. Stop nuclease Digest and precipitate

After the 30 minute incubation, quickly stop the reactions and ethanol precipitate:

Add 30 microliters stop mix:

2.5 M NaOAc	25 microliter 3 M
40 mM EDTA	5 microliter 0.25 M
3 microgram tRNA	0.1 microliter 30 mg/ml

Add 1 ml 100% ethanol. Mix well and freeze on dry ice for at least 12 min. Spin in microfuge 10 min. Remove all liquid. Wash pellets with 100% ethanol. Briefly dry in speedvac. Resuspend in 8 microliters 2-fold diluted quickpoint sample buffer. Heat to 90 degrees 1 min and put on ice. Load to 8% quickpoint gel. Run till bromphenyl blue dye runs off bottom (~13 min @ 1200 volts).

10 X Hybridization Buffer
3 M NaCl
10 mM EDTA
380 mM HEPS pH 7.0

Oligonucleotide Design
For each RNA to be analyzed, the oligo should contain at least 35 residues (can be up to 70 residues or higher) that are complementary to the RNA coding strand. All oligonucleotides should be gel purified to give a clean signal. You can resolve fragments which are at least 5bp apart using the quickpoint gel system. If you don't care about looking at 5' ends, the oligo can be complementary to any part of the mRNA. It's better that the oligos are at least 50% GC with the 5' ends being GC(to prevent breathing that might lead to artifactual S1 cleavage). Also, add 4-6 bases at the 3' end of the oligo which are anti-complementary (i.e. purine-purine or pyrimidine-pyrimidine) to the mRNA. The reason for this is to distinguish undigested probe from true mRNA/DNA hybrid. If you care about the 5' initiation site, the oligo is basically the same except that the 3' end should be complementary to RNA beyond the initiation site (this allows discrimination between undigested probe and true hybrid). Hybridization reactions can contain at least 2-3 probes as long as the products can be resolved.

Notes:
For 15 micrograms of total yeast RNA, 20-30 units of S1 works best. This amount may have to be adjusted if different amounts of RNA are used. Breeden lab suggests 50 units S1 for 50 micrograms RNA.

The probe should be in excess over the specific RNA to give a signal proportional to the amount of specific RNA. To test this, do the reactions using different amounts of RNA (for example 1x, 2x, 4x RNA).

Last modified 6/25/01