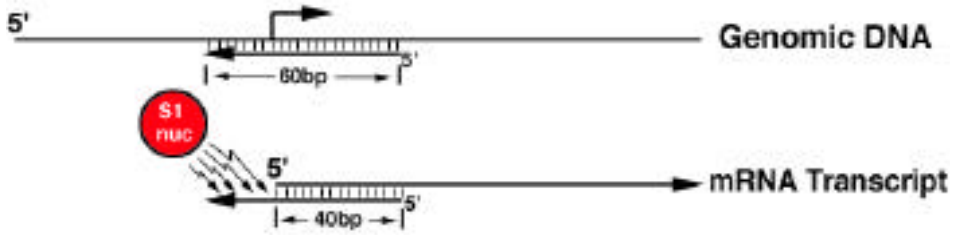
S1 Nuclease Analysis

Experimental design considerations for promoter studies

- Used to quantitate transcript levels by assessing the number of 5' ends of the RNA of interest.
- 60 bp oligonucleotide should be designed that span the promoter initiation site in an anti-sense direction [the 5' 40bp of this oligo should hybridize to the mRNA of interest while the remaining 20bp should be complimentary to the 20bp sequence immediately upstream from transcription initiation site (which will not be contained in the mRNA) – this allows the detection of a specific cleaved product upon digestion with S1 nuclease following hybridization with RNA].



- Method typically works well using total RNA (typically prepared using tri-reagent).

Protocol

I. Kinase Oligonucleotide

1ul	Oligonucleotide (100 ng/ul)
1.5ul	Polynucleotide Kinase Buffer (10X)
1ul	Polynucleotide Kinase
6ul	[gamma-^{32}P]ATP
5.5ul	H_2O
15ul	Total

- 37^{o}C, 1 HR.
- Add 135ul TE
- Run over G25 Spin Column

II. Hybridization

- ETOH Precipitate 50ug Total RNA/sample.
- Wash with 800ul 70% EtOH.
- Dry briefly and suspend in 15ul 2X Hyb. Buff [suspend by mixing with finger a few times followed by letting soln sit for a few minutes (repeat 3-4 times)] .
- Add 15ul Formamid and 3ul of labeled oligonucleotide.
- Heat to 68^{o}C for 10 min.
- Transfer directly to 37^{o}C water bath and incubate overnight with glass plate (or cover) over water bath (covering the water bath prevents evaporation of sample which condenses on top of eppy tube)

III. S1 RXN.

- Make up S1 digestion mix (500 units/ml; 0.5ul S1/1ml of 1X S1 Buffer [BRL S1 nuclease, 20000u/20ul, CAT# B001SA]).
- Add 300ul S1 RXN. mix per sample.
- Mix by vortexing on low speed for a couple seconds and spin briefly (10 sec on high speed).
- Incubate 30 min. at 37^{o}C.

- Phenol/$CHCl_3$ extract(300ul).
- Add1ml ETOH.
- Spin 30 min on high.
- Wash with 900ul 70% ETOH.
- Dry Briefly.
- Suspend in 10ul Sequencing Stop Buffer.
- Heat @ 68^{o}C for 10 min and load 4ul onto 8% sequencing gel [as a control, load a small amount of the original undigested probe in a separate lane (for control, dilute 1ul of original probe in 100ul TE, mix, and transfer 1ul of this solution into a new tube with 8ul of sequencing loading dye – load 4ul of this onto gel)].

Buffers for S1 Nuclease Analysis

10X Polynucleotide Kinase Buffer (-ATP)	10ml
0.5 M Tris (pH 8.0)	2.5ml 2M Tris (pH 8.0)
70 mM $MgCl_2$	700ul $MgCl_2$
10 mM DTT	100ul 1M DTT
H_2O	6.7ml H_2O

2X Hyb Buffer	10 ml
40 mM Pipes, 6.4	800 ul 1M Pipes, 6.4
1 mM EDTA	40 ul 0.5M EDTA
0.4 M NaCl	1.6 ml 5M NaCl
0.1% SDS	200ul of 10% SDS
H_2O	7.36 ml H_2O

S1 Buffer	50 ml
0.28 M NaCl	2.8 ml 5M NaCl
50 mM NaAc, 4.6	2.6 ml 1M NaAc, 4.6
4.5 mM $ZnSO_4$	2.25 ml 0.1 M $ZnSO_4$
H_2O	42.4 ml H_2O