

RNaseH analysis

(submitted by Bernhard Dichtl)

Set up samples

Approx. 15 µg total yeast RNA
1.5µl of 10 µM oligo (directed against mRNA or poly(A) or both)
4 µl H-Buffer
Fill up to 12 µl with water

Anneal oligos with RNA

Heat to 85°C for 5 min
Transfer to 37 °C heat block 30 min
Quick spin to collect entire sample at bottom of tube
Transfer back to 37 °C heat block

RNaseH digest

Add 2 µl RNaseH-mix to each sample, mix well by tapping the tube and incubate at 37°C for 60 min

RNA extraction and precipitation

Add 28 µl H₂O, 40 µl phenol/chloroform/IAA (25:24:1) to each sample.
Vortex 30 sec, spin 3 min
Transfer 38 µl of upper phase to fresh tube
Add 4 µl 3M NaOAc, 110 µl 100% EtOH, vortex
Spin 20 min at 4°C in table-top centrifuge, take off supernatant completely
Add 150 µl 70% EtOH, spin 5 min at 4°C in table-top centrifuge
Take off supernatant completely, dry RNA 3 min in speed-vac
Add 20 µl formamide RNA loading buffer, quick vortex, quick spin

PAA Northern gel

Heat up to 85°C for 5 min, quick spin to collect entire sample at bottom of tube
Load on 8% PAA/urea gel (32 ml PAA, 48 ml buffer), 20 x 20 cm, 1 mm thick
Run at 350 V const. (with metal plate, otherwise danger of 'smiling') till XC dye runs approx. 3-5 cm from gel bottom (dye migrates roughly with 160 nt RNA; run time about 2.5 hrs)
Marker: 5' end labeled HpaI digested pBR322 fragments (about 10 counts/sec on geiger)

Northern transfer

Northern transfer in wet-blot tank with 0.5 x TBE on Hybond N⁺ membrane with 250 mA const. o/n (about 16 hrs)

Reagents:

RNaseH-Mix (prepare for X+2 samples):

1 µl RNaseH (5 U/□l; NEB)
0.5 µl H-Buffer
0.5 µl RNaseOut (40 U/µl; Invitrogen)

H-Buffer: 100 mM KCl, 50 mM Tris-Cl 7.5, 5 mM MgCl₂, 1 mM DTT