

Small RNA Northern Blots

Small RNA Northern blots are actually quite easy, once you are set up for it. In getting set up for it, I recommend performing a control blot in which DNA oligos are loaded onto the gel (instead of RNA). See my note on controls below.

Ingredients

To begin, make sure to get the right ingredients: small RNA Northern blotting can be especially sensitive to the membrane and hyb conditions, and that is why you will want to begin with a control blot.

For a hybridization buffer, I prefer to use Ambion's ExpressHyb, although homemade preparations (5% SDS, 200 mM Na₂PO₄ pH 7.0) work well also.

I prefer to use Hybond N+ membrane (Amersham) because it has proven consistently successful over multiple batches. Other membranes, such as GenescreenPlus (NEN) also work, just be sure to run the below control with every new membrane batch. U6-driven small RNAs and some microRNAs (such as let-7) are really abundant, and can be visualized quite readily with just a few hours exposure.

The Blot:

Using ~17 cm wide, ~30 cm long glass plates, pour a 1.5 mM thick, 10% DNA sequencing PAGE gel. I prefer to use a 10-12 tooth comb, which will allow 60 ug or more RNA to be loaded per lane.

I typically pre-run the gels until the temperature is ~50° C, at around a constant 40 mA or so, depending on your power supply. Run the gel until the BPB hits the bottom of the gel, as your small RNA should be about midway between the XC and BPB dyes.

On a semi-dry transfer apparatus, transfer the RNAs from your gel to the membrane. I use a BioRad transblot apparatus run at 250 mA for about 3 hours. After the transfer, crosslink the membrane at 1200 □joules.

During the transfer, prepare your probe by 5'-end labeling a 25-50 pmoles DNA oligo in a ~1 hour kinase reaction. Remove free ³²P by ETOH ppt or desalting column.

After a ~30 minute equilibration of your membrane in the hyb buffer, add fresh hyb buffer (containing your labeled probe) and hybridize between 4 hours and overnight at 37° C. I usually do this in 50 ml disposable conical tubes, and my buffer volume is 5 ml.

For the washes, I suggest gradually stepping in the stringency. Try starting with 3 washes 2x SSC, 0.1% SDS at RT, exposing, and if that is not clean enough, try reducing to 0.5X, 0.1% SDS, or lower SSC concentrations. If background is a problem in a 1-2 day exposure, something is wrong, so troubleshoot your buffer, probe, and membrane. Boil the membranes in 0.1% SDS to reprobe (make sure to check efficiency of stripping with an exposure!).

Now for the controls (VERY important!):

Obtain a 21 nt sense and antisense DNA oligo pair for the positive controls. Choose a sequence that will correspond to the small RNA that you are trying to detect.

For your first control gel, you will want to load decreasing amounts of one of your DNA oligos (you will use the other one as a probe). Try starting off by loading 1 pmol, and step down across your wells to 10 fmoles. If your conditions are right, you should be able to visualize 10 fmoles or lower with an overnight exposure.

If you want to test your probe labeling and transfer efficiency, you might consider loading your radiolabeled probe on the gel and performing the transfer as usual. Try loading 1 pmol, and again stepping down across your wells to 10 fmoles, then do an overnight exposure. If transfer was efficient, your 10 fmol band should be clearly visible on the autorad (providing your probe has a high specific activity). Be sure to follow your counts throughout the process.

Also, remember that every Northern blot should have an internal loading control. Try reprobing your blot for either let-7 or 5S RNA.