Protocol 36.0 July 1, 2000

Strand Separating Gel

1. Starting material must be salt-free, purified ds RNA (ie. gel-purified ds sat).

2. I've loaded up to 200 ng/well (in 4 μl) of a PROTEAN II mini-gel aparatus with good success. 500 ng/well resulted in lower yields of ss RNA, ie some of it reannealed.

3. The gel: For minigel, (5 ml) use 1 ml 35% acryl (80:1 A:Bis), 0.5 ml 10 X TBE II, 3.1 ml H_2O 35 μl 10% APS, 5 μl TEMED. Pre-run for 30 min at 60 V constant current, in 1 X TBE II.

4. Mix RNA with an equal volume of 2 X dye mix. Boil 5 min.

5. Place RNA on ice and load immediately. Run the gel at 120 V until the dyes just begin to seperate, then at 60 V until the XC runs off (about 2.5 hours). Stain with Toluidine Blue as normal. For CMV-sat, the strands seperate about 1.5 cm, with any ds running way ahead, near the XC. The upper band is (-) sense, the lower is (+). Of course this may be reversed with different RNAs.

The theory of this procedure is that you denature the RNA, and then load it immediately. Since the gel is non-denaturing, the single strands renature with themselves (rather than each other) and hence run differently. Consequently, the rapid loading, and the higher voltage for the first minute or so, to get them seperated before they can get together again. This is a modification of the old DNA strand separating gel protocol.

2X dye Mix: Store at -20°C

60% DMSO
2 mM EDTA
0.2% BPB and XC