

## **Satellite RNA purification**

1. Prepare a 6% acrylamide gel in TBE, with 7 M urea. Two mini-protean II gels are sufficient to purify satellite from about 80  $\mu\text{g}$  of viral RNA. Use a prep style comb for most efficient results.

2. Viral RNA should be resuspended in 0.1 mM EDTA and stored at  $-20^{\circ}\text{C}$ . Mix the viral RNA at about 1 mg/ml with an equal volume of formamide dye (99% deionized Formamide; 0.1 mM EDTA; 0.025% bromophenol blue and xylene cyanol).

NOTE: you do not need to heat the samples before running, the urea and formamide will partially denature the RNA without heating, and it will run as a nice sharp band.

3. Load the gel with 40  $\mu\text{g}$  viral RNA. Run at 250 volts until the bromophenol blue dye runs completely off the bottom and the xylene cyanol is a few centimeters from the bottom (about 30 to 45 minutes).

4. Disassemble the gel apparatus. Leave the gel on one glass plate and place on a tray that can catch run off (we usually use an empty pipette tip box). Stain the gel by flooding with about 5 ml of Toluidine blue dye for 1 minute. Destain by flooding with milli-Q water until the bands appear. It is easiest to use a light box to visualize the bands while cutting them out, but minimize light exposure to prevent damage. Excise the satellite band with a fresh scalpel blade, it will be about halfway down the gel. The viral RNA bands will be unseparated, and near the top of the gel. RNA 4 may separate somewhat from the others, but the satellite will be the lowest band in the gel.

5. Place the excised bands in a baked 15 ml COREX tube, and add 2.5 ml of elution buffer per gel (0.5 M  $\text{NH}_4\text{OAc}$ ; 0.1% SDS; 1 mM EDTA). Place in a dark place overnight.

6. Remove the eluate being careful not to take any of the gel. The eluate can be passed through a plug of siliconized glass wool to remove excess acrylamide. Extract the eluate with 1:1 phenol:chloroform. Add 1/10th volume 3M NaOAc and ethanol precipitate. The precipitate should be spun in a swinging bucket rotor if at all possible to minimize loss of the rather small pellet. Resuspend thoroughly in 0.5 ml of NAE (0.3 M NaOAc pH 6; 0.1 mM EDTA), and re-precipitate in an eppendorf tube. The pellets may look bigger than expected, this is due to some residual acrylamide.

7. Yield should be about 10% of viral RNA.

NOTE: This RNA should be treated as an infectious pathogen!