**Purifying RNA using Denaturing Polyacrylamide Gels**

1. Pour gel with appropriate % acrylamide to separate RNA products of interest
	1. Use SequaGel UreaGel System, info online
	2. Can pour gel day before, and store with wet paper towels in saran wrap at 4C overnight.
2. Pre-run gel for ~45 minutes at 200V – optimal running temp is 50C.
3. Mix RNA with 2x loading dye, denature at 65C for 5 min, cool on ice
4. Flush urea from wells to facilitate sample loading
5. Load sample and run gel for appropriate length of time (40 -70 minutes)
6. Remove plates from buffer and let cool for about 10 minutes prior to disassembly
7. Stain gel with SYBR Gold or other appropriate nucleic acid dye
8. Cut out band of interest under UV
9. Put gel slice in 1.5ml Eppendorf tube
10. With 1ml pipette, take up 950ul of 0.3M NaCl (elution buffer), then draw up 50ul of air
11. In 1.5ml tube, crush gel slice with tip, expel elution buffer to unclog
12. Rotate at 37C overnight
13. Spin at top speed for 1 min at RT
14. Remove elution buffer, place in new 1.5ml tube – be careful not to remove any of the crushed gel slice
15. Add 15-25ul of linearized acrylamide co-precipitant
16. Vortex, split sample to three 1.5ml tubes
17. Add 1ml of 100% Ethanol to each, store at -80C for 1hour or -20C overnight
18. Spin at top speed in cold room for 30 minutes
19. Remove EtOH, add 1ml 80% EtOH, ice cold
20. Remove all traces of 80% EtOH by pipetting and sequential spins
21. Resuspend RNA in appropriate buffer (water, annealing buffer)
22. To anneal:
	1. 95 1 min
	2. 80 2 min
	3. 65 5 min
	4. 45 5 min
	5. Let cool slowly to room temp

**2X Loading Dye:**

95% Formamide

18 mM EDTA

0.025% SDS,

Xylene Cyanol

Bromophenol Blue

**10X TBE 1 liter:**

108g tris base

55 g boric acid

40ml 0.5M EDTA pH8

**1X Annealing buffer:**

50mM HEPES 7.5

100mM NaCl

5mM EDTA

**50 ml 1X annealing buffer:**

 5ml 500mM HEPES 7.5

 1ml 5M NaCl

 0.5ml 0.5M EDTA

 43.5ml H20