

Northern Gel Protocol

- 1] **Wash** the gel rig, comb, caster, etc. with Hot 0.5% SDS for 10 minutes
- 2] **Wash** the gel rig, etc. with DEPC water and allow to air dry in hood
- 3] **Prepare** 1% Gel by:
 - Add** 0.80gm Agarose
 - Add** 8ml 10X FA Gel Buffer
 - Add** 69.6ml DEPC water
 - Cover** with saran and poke hole to vent
 - Heat** microwave until all agarose is dissolved
 - Cool** for a few minutes at room temp
 - Place** in 65C water bath with light shaking for 10-15 minutes
 - Add** 2.4ml Formaldehyde
- 4] **Set** up gel caster with comb in it
- 5] **Pour** gel in casting tray and let sit for at least 15min
- 6] **Place** gel with tray in the gel rig
- 7] **Fill** gel rig with FA Gel Running Buffer so that it barely covers the gel
- 8] **Equilibrate** gel by letting it sit in buffer for 30min
- 9] **Prepare** Sample and Ladder:
 - Add** ~20ug total RNA (use 2-3ul of ladder)
 - Add** 15-20ul RNA Sample Buffer
 - Add** 3-5ul RNA Loading Buffer
 - Mix** by vortex
 - Heat** at 65C for 10min
 - Quench** on ice for 2 minutes
 - Spin** to collect everything
- 10] **Load** samples onto gel
- 11] **Run** gel at ~60-70V
- 12] **Stop** running when bromophenol blue front is a few centimeters from end of gel
- 13] **Soak** gel in DEPC water for 10min with gentle shaking
- 14] **Take** picture with **ruler** and proceed to blotting protocol