Northern Blot (*mtlA* mRNA, ~2 kb)

General Notes

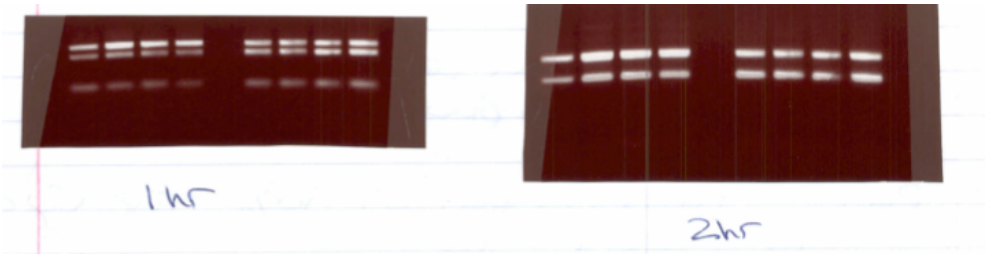
Ref: J. M. Liu Post-doc, experiment 63

Always practice RNase-Free technique when working with RNA
- Use RNA-only pipet tips and tubes
- Wipe off counter tops and pipetmen with RNaseZap
- Filter-sterilize solutions prior to use
- Use either DEPC-H_2O or MQ-H_2O
- Keep RNA cold (4 °C or lower)

Protocol:

Running out total RNA

1. Prepare a 0.8% (w/v) agarose gel with 1 x TBE and ethidium bromide (EtBr)
2. Prepare RNA samples:
   a. ~5 µg RNA + 6x DNA loading buffer (load 0.5-1 vol loading buffer)
   b. Load directly onto gel
3. Run gel in 0.5x TBE, 120 V, 2 h
4. Visualize RNA using transilluminator (see below for an example)



Transfer RNA to nylon membrane (see protocol from NorthernMax kit (Ambion))

After 1.5-2 h transfer, set-up hybridization:

1. Rinse membrane with 0.5x TBE (handle membrane carefully, with gloves or forcep)
2. Crosslink 3x
3. Prehybridize membrane with ~ 6 mL UltraHyb Oligo (Ambion), 42-50 °C, for at least 30 minutes in hybridization oven.
4. Add ~600 ng probe directly to hybridization buffer
5. Hybridize overnight in oven. Make sure that buffer is not dripping out of tube and that membrane is well-covered by the buffer.

Detection of probe (see protocol in BrightStar BioDetect kit (Ambion))