Northern Blot (sRNA) -- LICOR

General Notes:

Practice RNase-Free technique:
-Use RNA-only pipet tips/tubes
-Use RNaseZap to clean counters and pipetmen
-Filter/sterilize solutions
-Use DEPC-H_2O or MQ-H_2O
-Keep RNA cold (4 ˚C or lower)

Needed Material:
-Mini-PROTEAN Tetra Cell
-Mini Trans-Blot Cell Assembly for Transfer
-Loading buffer (LBII (Ambion)- Glycerol, bromophenol blue, xylene cyanol, formamide), stored at -20 °C

- ***10 X TBE Stock (Use 1 X TBE dilution – F/S):***
  108 g Tris Base
  55 g Boric Acid
  40 mL 0.5 M EDTA (disodium salt), pH 8.0
  In 960 mL MQ H_2O

- 20 X SSC (1 X and 6 X dilutions – F/S):
1X: 1 mL 20X SSC
19 mL MQ H_2O

6X: 6 mL 20X SSC
14 mL MQ H_2O

-ULTRAhyb-Oligo (Ambion)*, stored at 4 °C, needs to be heated to go into solution prior to use*

-Odyssey Blocking Buffer (LiCor)

-Wash Buffers (does not need to be filter sterilized)
Low: 50 mL 20X SSC
2.5 mL 20% SDS
Bring to 500 mLs with MQ H_2O

High: 2.5 mL 20X SSC
2.5 mL 20% SDS
Bring to 500 mLs with MQ H_2O

Protocol (for detecting ~120 nt sRNA):

1. **Prepare a 10% TBE-Urea gel:**
   *To a beaker **add**:*
   a. 3.3 mL 30% acrylamide (protogel)
   b. 1.0 mL 10 X TBE
   c. 2.136 mL water (MQ)
   d. 4.2 g urea

   ***Cover with Parafilm and Incubate** at 37˚C or in hot bath (65˚C) until urea dissolves. Then add*
   e. 330 µL 1.6% APS (Needs to be less than 1 month old)
   f. 5 µL TEMED

   ***Swirl** to facilitate polymerization and then pour into gel casting frame.*
   *Allow gel to **solidify** in a Mini-PROTEAN Tetra cell casting frame/stand for 45'. Gel can be stored overnight, wet, at 4 ˚C.*

2. **Prepare RNA Samples:**
   a. **Prepare** RNA samples with equal volume of LBII, (try to load the same volume in each lane; adjust volume of RNA with DEPC-H_2O).
   b. **Heat** for 5' at 85 ˚C, **Pico-centrifuge**.
   c. Keep RNA cold (4 ˚C or lower).

3. **Pre-Run and Run Gel:**
   a. **Pre-Run gel** in 1 X TBE at 250 V for 20' in a Mini-PROTEAN Tetra cell tank.
   b. **Pipette** out gel debris from each lane before loading RNA samples.
   c. **Run** gel in 1 X TBE at 200 V for 45-60', (ensure dark blue loading dye runs off gel).

****If you are planning to use the Trans Blot Turbo, begin preparation of Riboprobe (see riboprobe protocol)****

4. **Transfer Gel to Membrane:**
   a. Traditional Method
      i. **Equilibrate** sponges, filter paper and membrane in 1x TBE.
      ii. **Prepare** gel sandwich (black, sponge, filter paper, 10% TBE-Urea gel, a 60 cm^2 membrane, filter paper, sponge, white).
      iii. **Transfer** (black to black, clear to red) in Mini Trans-Blot Cell in 1 X TBE at 200 mA for 60'. Add a stir bar and ice block to cell and place on a magnetic stirrer.
      ****Begin preparation of Riboprobe (see riboprobe protocol)****

   b. Trans Blot Turbo
      i. **Equilibrate** extra-thick filter paper and membrane in 1x TBE.
      ii. **Prepare** gel sandwich in the Trans-Blot Turbo cassette B (cassette bottom, extra-thick filter paper, membrane, TBE-Urea gel, extra-thick filter paper, cassette top)
      iii. **Transfer** using turbo command on main menu (third button from the left on the bottom row.) Select the number/size gel/s that you will be transferring with this program. This will take you to a screen showing the current conditions in the cassettes. Push the B:RUN button to start the transfer. Transfer will be finished in 7 minutes.

5. **Wash Membrane and cross-link:**
   Handle *membrane* ***gently****, avoid touching with fingers:*
   a. **Wash membrane** in 20 mL of 6 X SSC for 2' with rocking.
   b. UV **Cross link: "Autocross link"**.
   c. **Wash membrane** in 20 mL of 1 X SSC for 1' with rocking.

6. **Prehybridization and Hybridization:**
   a. **Pre-hybridize** (≥30') standard membrane size in 6 mL of ULTRAhyb-Oligo (Ambion) at 60-65 ˚C.
   b. Add biotinylated riboprobe specific for RNA (10 µL of a 20 µL in vitro transcription reaction is PLENTY!) and DNA probe for 5S (2.5-5 µL, ~ 250 ng, 800-5S).
   c. Allow to **hybridize** overnight at 60-65 ˚C.

Following day, wash membrane using Li-Cor detection method. See Li-Cor Northern protocol.