Standard Operating Procedure

Procedure	Northern Blotting using ^{32}P
Department	
Location	
SOP Prepared By:	

Section 1: Purpose
Northern blotting is a standard method for the detection and quantification of RNA from a cell. This is done by isolating and purifying RNA and using a radioactively-labeled DNA or RNA probe to hybridize to and detect the RNA. Using this technique, temporal and spatial location of RNA expression can be found[8]. Unlike RT-PCR (real-time PCR), which quantifies the amount of RNA or DNA at various times, Northern blotting can be used not only to quantify but also determine the size of the RNA. It is also useful to perform Northern blotting when studying transfer RNA (tRNA), which appears as the two lowest bands on a gel[1]. The probes used in Northern blotting do not have to be radioactive, but radioactive probes still have the greatest sensitivity. Quantifying mRNA of tRNA can provide insight into gene expression in cells that are exposed to different environments.
Section 2: Personal Protective Equipment and Survey Equipment
PPE: • Nitrile Gloves • Lab coat or lab gown • Proper enclosed shoes • Safety glasses Other Equipment: • Geiger counter • Personal chest dosimeter
Section 3: Radioactive Material
^{32}P, specific type varies according to what procedure is followed for probe design Supplier: Perkin Elmer Starting activity: 10 mCi/mL Typical use quantities: no more than 10uCi per experiment • Radioactive material (RAM) benchtop exposure time: ~10-20 minutes **Activity used per experiment:** _____

RAM handling time: _____
Frequency of experiment: _____

Section 4: Potential Hazards

- ^{32}P is a high-energy beta emitter and has a half-life of 14.29 days. ^{32}P can present a substantial skin and eye dose hazard.
- Acid phenol may be used in RNA isolation and presents oral, dermal, and inhalation toxicity. It can penetrate latex gloves, but the effects are not immediate.
- The agarose may get hot while microwaving.
- Formaldehyde may present a hazard if using a formaldehyde agarose gel for gel electrophoresis (common for RNA gels).
- Emission of UV light in the UV crosslinker.
- The rotating oven can get hot (60-80 degrees C).

Any other hazards (potential for airborne release of radioactive material, chemical hazards, etc.)?

Section 5: Safety Precautions

The following precautions should be taken while handling ^{32}P:

- Designate area for handling ^{32}P and clearly label all containers and equipment. Equipment used in procedure:
    - Pipette
    - Gel electrophoresis
    - Glass tray
    - UV Cross Linker
    - Cylindrical glass tube with O-ring cap
    - Northern blot oven
    - Phosphorimager
- Any samples, stock, or equipment containing ^{32}P should be used behind Plexiglas shielding.
- Use filtering pipette tips to prevent contamination of pipette.
- Line all RAM countertops with absorbent sheets.
- Survey all areas and equipment where RAM is used with a Geiger counter before and after the procedure.
- Keep Geiger counter on when working with ^{32}P directly.
- Survey hands, body, and face with Geiger counter after conducting the procedure.
- Minimize exposure by keeping a hot hand (holding tubes with radioactive material) and cold hand (pipetting, etc.) as much as possible.
- Conduct all non-radioactive steps of the procedure before moving to the RAM corner.
- Minimize the time of contact with ^{32}P by setting up bench space before starting procedure.
- Acid phenol should be used in an open and well-ventilated area. Tubes should be tightly capped during all steps. Follow guidelines in the MSDS for phenol if accidents occur[5].
- Use rubber pads or heat resistant gloves to remove agarose from the microwave.

- Prepare the formaldehyde agarose gel in a fume hood. Follow guidelines in the MSDS for formaldehyde[6].
- The UV Crosslinker should be completely sealed when on, so there is no exposure to the UV light.
- The rotating oven should be shut tightly when on. There is no radioactive exposure when the oven is completely shut.

Consult the MSDS for reagents used in the procedure and follow safety instructions accordingly.

Section 6: Procedure

**indicates step must be performed in a designated RAM area and behind shielding.*
+ indicates a hazardous chemical might be used.

A. RNA Isolation from cells:

There are multiple ways of isolating RNA. Acid phenol is sensitive and produces high output. Qiagen has an RNeasy Mini Kit that does not use acid phenol.
It is essential that RNase free tubes and RNase free water are used to prevent RNA degradation.

1) +Isolate the RNA of interest by cell or tissue extraction using the appropriate buffers and/or column purification (multiple centrifugations of special tubes with a filter to wash and isolate RNA)

Steps B - E, G-H: Ambion offers two kits, NorthernMax™ Kit[7] and NorthernMax™-Gly[8] Kit, that contain the necessary ingredients for RNA gel electrophoresis, pre-hybridization and hybridization, and suggested methods to visualize the blot. Labs may choose to use these kits, which come with detailed protocols, or perform a similar procedure using the equipment already available in the lab. Below is a procedure that is commonly used in the labs across the university and is applicable to other assays.

B. Gel electrophoresis

2) +Prepare appropriate gel and buffer for running RNA samples.
3) +Prepare RNA samples to load and run on gel.

C. Transfer of Gel to Membrane

4) Make a buffer reservoir with appropriate concentration of SSC in a glass tray. The buffer reservoir should be made as follows from bottom to top: (specifics are included in kits)
    a) Prewet Whatman paper
    b) Gel
    c) Pre-wet nylon membrane
    d) Prewet Whatman paper
    e) Dry Whatman paper
    f) A stack of paper towels
    g) Glass plate
    h) Weight
5) Allow the transfer to sit overnight for 18-24 hours.
6) Rinse the membrane accordingly and allow to dry at room temperature.

D. UV Cross Linking

7) Place the membrane in a UV cross linker to covalently bind the RNA to the membrane to increase hybridization signals during detection.

E. Pre-hybridization

8) Carefully insert the membrane into a cylindrical glass hybridization tube. (Add H20 to the tube to ensure that the membrane sticks to the sides of tube without any bubbles, and pour out.)
9) Add pre-hybridization buffer to the tube and close tube with a plastic O ring cap.
10) Incubate tube in a designated **rotating oven** at around 60 degrees C for several hours.

F. Preparation of radioactively labeled probe:

There are many ways of designing a radioactive probe. The method with the best output is using asymmetric PCR to amplify the probe sequence and using ULTRAhyb (Ultrasensitive Hybridization Buffer) when performing the actual Northern blot assay. Common kits include Ambion's MAXIscript™ Kit (in vitro transcription), Prime-a-Gene labeling kit or DECAprime kit for a DNA probe. Follow kit instructions, which can be found online.

11) *Move to a designated RAM area.
12) *Prepare a radioactively labeled DNA or RNA probe with ^{32}P. (described in nucleotide labeling SOP)

G. Hybridization of Probe to Membrane

13) *Prepare the hybridization solution containing the radioactively labeled probe.
14) *Add the hybridization solution in the glass tube with the membrane, and tightly seal the container with a plastic O-ring cap.
15) *Place the tube into the designated oven, close door, and incubate overnight at ~42-65 degrees C.
16) *Pour off hybridization solution to a designated and labeled tube (hybridization solution can be reused for a few weeks).
17) *Using a long pincet, take out the membrane and place onto a glass casserole dish (with a lid).
18) *Wash membrane with pre-heated wash buffers (about 3 times) at 42 degrees C by adding the buffer in the tube and placing the tube back in the oven for about 15-20 minutes each.
    a) Wash buffers usually are with an appropriate concentration of 0.1% sodium dodecyl sulfate (SDS) and 2X saline-sodium citrate (SSC).
19) *Check the membrane with a Geiger counter. If the membrane is still very hot, perform more washes with appropriate concentration of SSC and SDS until radioactivity appears localized to specific area.

H. Phosphorimaging

20) *Wrap the (dry) membrane with saran wrap or sandwich between thin plastic films.
21) *Place membrane in a cassette that contains a phosphor screen. Close the cassette to allow the radiation from the membrane to produce an image on the screen. The time it takes will vary.
22) Take the screen from the cassette and place on the phosphorimager and analyze readout with scanning software.

23) When finished, blank screen by exposing to visible light or a light box.

Section 7: Spills/Incidents/Clean Ups

- For major spills or any personal contamination, contact Radiation Safety Services for proper instructions and guidance. Try and contain the spill and check yourself and the area for radioactivity.
- For small spills onto lined countertops, carefully discard of the absorbent lining into the solid RAM waste box. Check the countertop with Geiger counter afterwards. Document the spill and cleanup procedure (https://www.ehs.harvard.edu/node/7589) used with other radiation records and notify radiation_protection@harvard.edu.
- For extra caution, place the SDS PAGE box in a secondary containment (casserole dish) to avoid buffer spills onto countertop.
- For possible leakage of radioactivity in the cassette with phosphor screen, clean with an effective cleaner and check with Geiger counter. (Leakage will occur only if the membrane is not completely dry or wrapped.)
- Normally, there should be no spills or leakage in the designated rotating oven and would only occur if the cap of the cylindrical glass tube is not properly closed. If there is a spill, clean with an effective RAM cleaner and check for remnants with the Geiger counter.
- The pincet used to take out the membrane from the glass tube should be checked with a Geiger counter afterwards, then washed with an effective cleaner.
- At any point you may call Radiation Safety Services for assistance.

Section 8: Transportation, Storage, and Disposal

- Store ^{32}P stock in a locked 4 degrees Celsius fridge in a locked acrylic box. Keep key/passcode in a safe place.
- If transportation of samples containing ^{32}P is necessary, place samples in an acrylic container as a secondary containment.
- To store RNA samples after RNA isolation, keep purified RNA samples in RNase-free water, as an ethanol precipitate, or in formamide in -20 C freezer. Samples in formamide can be stored in 4 C.
- Wash buffers in the hybridization step can be discarded in the sink as long as the amount of ^{32}P is below the daily sink disposal limit of 10 uCi.
- Phosphorimaging cassette is never hot on the outside and should not be hot on the inside when empty. Normally, the membrane is dry and/or wrapped in saran wrap, which contains the radioactivity.
- Phosphorimager is labeled with a radioactive sticker but should never be contaminated, as the phosphor screen contains no radioactive material.
- Radioactive waste should be tagged and separated by isotope.

Section 9: References

- Harvard EHS website: https://www.ehs.harvard.edu/services/radiation-protection
- Northern Blotting**:** http://www.ambion.com/techlib/basics/northerns/
- Southern and Northern Blotting: http://www.sigmaaldrich.com/technical-documents/articles/biology/southern-and-northern-blotting.html
- MSDS Acid Phenol: https://tools.thermofisher.com/content/sfs/MSDS/2011/AM9720_MTR-NALT_EN.pdf
- MSDS Formaldehyde:https://www.fishersci.com/shop/msdsproxy?storeId=10652&productName=SF10020
- Ambion NorthernMax Kit Manual: https://tools.thermofisher.com/content/sfs/manuals/cms_055600.pdf
- Ambon NorthernMax Gly Kit Manual: https://tools.thermofisher.com/content/sfs/manuals/cms_055601.pdf
- Phosphorimaging: http://www.ispybio.com/search/protocols/CPMB.Ch.App3.Techniques.pdf page 8-9