Standard Operating Procedure

Procedure	*In vitro* transcription/nucleic acid labeling using ^{32}P
Department	
Location	
SOP Prepared By:	

Section 1: Purpose

In vitro transcription re-creates the cellular mechanism of the transcription process of DNA to RNA in a test tube. Furthermore, by using radioactively labeled nucleotides, the resulting RNA strand is radioactive and can serve as a probe in further tests.

Section 2: Personal Protective Equipment and Survey Equipment

PPE:

- Lab coat
- Nitrile gloves
- Heat resistant gloves
- Safety glasses
- Closed-toe shoes

Other Equipment:

- Geiger counter with pancake probe
- Personal chest dosimeter
- Personal finger dosimeter

Section 3: Radioactive Material

UTP, [α-^{32}P]
Supplier: Perkin Elmer
Starting activity: 10 uCi/uL
Typical use quantities:

- for commercial kit: 50 uCi per reaction
- for non-commercial kits/reagents made from scratch: variable, can be as low as 3 uCi per experiment/batch of samples
- Radioactive material exposure (RAM) time on benchtop: ~30 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.
- Reagents or buffers used in the procedure may present hazards.
    - If using a commercial in vitro transcription kit, the 10X transcription buffer may contain dithiothreitol (DTT) which can be harmful if swallowed or inhaled.

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:
    - Pipettes
    - Ice Bucket
    - Heat block/thermocycler
    - Centrifuge
- Prepare the workspace before conducting the actual procedure to maximize efficiency and keep radioactive exposure to a minimum.
- 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.
- Pulse centrifuge so the tubes contain the liquid mix at the bottom of the tube.
- 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 contamination by keeping a hot hand (holding tubes with radioactive material) and cold hand (pipetting, etc.) as much as possible.

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

Section 6: Procedure

**indicates step must be performed in a radioactive area and behind Plexiglas shielding.*

This procedure may be done from scratch or by using a commercial in vitro transcription kit (for example, Ambion's MAXIScript Kit) that comes with an enzyme (with an RNA polymerase from virus) mix, nucleotide solutions, transcription buffer, nuclease-free water, and loading buffer to be used if running a gel. Procedures may vary slightly but should try to keep exposure to radioactivity at a minimum. The general equipment used is the same.

Transcription reaction:

1) Obtain DNA template of interest using isolation and purification techniques.
2) Scale the transcription reaction and add in appropriate volumes of nuclease-free water, DNA template, 10x transcription buffer (containing DTT), and ribonucleotides (ATP, CTP, GTP, ~8X less concentrated UTP) in an Eppendorf tube. Mix well by pipetting up and down with the last pipette tip used.
    a) Depending on the volume of the reaction, adjustments to the amount of each reagent will have to be made. Normally, around 1-2 uCi of each reagent (excluding the nuclease-free water) is used for a 20 uL reaction.
    b) Keep the ribonucleotides on ice and the 10X transcription buffer at RT while working.
1) *Move to a RAM designated area with the enzyme mix on ice.
2) *Take out the [α-^{32}P] UTP and add the appropriate volume to the mix with a pipette. Mix well.
3) *Add the appropriate volume of the enzyme mix (containing the RNA polymerase and ribonuclease inhibitor to prevent RNA degradation) to the mix with a pipette. Mix well.
4) *Incubate the reaction mix on a heat block or thermocycler at 37 C for about 3 hours.
5) *(Optional) If necessary to research's application, add a small volume of TURBO DNase 1 to digest and degrade the DNA in the mix. Mix well with pipette.
6) *Incubate the tube at 37 C for about 15-20 minutes.
7) *(optional) add 1 uL of 0.5M EDTA to stop the reaction. This is important if the reaction will be heated (in which case DNase 1 will need to be inactivated).

Further Application

*Depending on research's application, the resulting RNA transcript can be purified and/or quantified using various detection methods (scintillation counter, gel electrophoresis and phosphorimaging, etc.)

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.
- Check the centrifuge for possible leakage. If contaminated, clean the rotor of the centrifuge - use an effective cleaner for radioactive material. Check again with Geiger counter, and keep cleaning until counts are at background level. Document the spill and cleanup procedure used with other radiation records and notify radiation_protection@harvard.edu.
- At any point you may call Radiation Safety Services for assistance.

Section 8: Transportation, Storage, and Disposal

- Store kit reagents in a -20 degree C freezer. Keep the ribonucleotides and enzyme mix on ice and the transcription buffer at room temperature. Nuclease-free water can be stored in at room temperature, a fridge, or freezer.
- Store [α-32P]-UTP stock in a locked 4 degrees C 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.
- When using a pipette, keep the lid of the solid RAM waste container slightly open to quickly discard of pipette tips.
- Liquids can be discarded in the sink as long as the amount of ^{32}P is below the daily sink disposal limit of 10 uCi.
- Check ice from the ice bucket for any radioactive contamination with a Geiger counter. If there is no contamination, ice may be disposed in a regular lab sink.
- Dispose of gels and membranes (if applicable) in the solid RAM waste container. If the gel still has some liquid, discard in a RAM waste container with sawdust.
- Radioactive waste should be tagged and separated by isotope.

Section 9: References

- Harvard EHS website: https://www.ehs.harvard.edu/services/radiation-protection
- Ambion MAXIScript Procedure: https://tools.thermofisher.com/content/sfs/manuals/fm_1308.pdf