Procedure: Northern blotting and \(^{32}\text{P}\) probe hybridisation

School/Department: School of Molecular Bioscience

SOP prepared by: Craig Jackson and Nick Coleman

Version: SMB022.3

Section 1 - Personal Protective Equipment

1. Lab coat or lab gown (buttoned up)
2. Proper enclosed shoes
3. Safety glasses at stages where corrosives are used
4. Nitrile gloves
5. Heat resistant gloves at stages where hot liquids handled

Section 2 – Potential Hazards + Safety precautions

1. Burns from hot agar
2. Needlestick injury
3. Electrical shock
4. Exposure to toxic chemicals (especially formaldehyde)
5. Exposure to radiation
6. Read the MSDS for all chemicals before starting work (see section 6 below)
7. Use barrier/filter pipette tips for ALL pipetting
8. Use fume hood when using hazardous volatile substances
9. Use appropriate Perspex shielding when handling \(^{32}\text{P}\) Isotope
10. Wear your TLD Radiation badge (personal dosimeter) when performing any task in the radioactive work room
11. See risk assessment “Northern Blotting” for full description of risks
12. Workers with pre-existing medical conditions (e.g. allergy, immunocompromised state, chemical sensitivity) and workers who are pregnant or expecting pregnancy must consult with their supervisor AND medical specialist AND the university’s WHS services before performing this procedure. If there are any serious concerns expressed by any of these individuals, this task must not be performed.

Section 3 – Procedure

Steps labelled ◆ must be performed in a fume hood - confirm fan is on and sash is lowered to the correct position during use. See risk assessment and SOP for fume hood. Steps labelled ★ must be performed in a registered Radiation room using appropriate Perspex shielding. See SOP and risk assessment for radioactive isotopes (P32/P33)

1. Heat 1.4% agarose in an appropriately sized open-ended vessel in a microwave on medium heat, swirling gently and regularly until the agarose has dissolved.
2. ◆ Allow hot agarose solution to cool to ~ 65\(^{\circ}\)C, transfer to the fume hood and add desired volume of formaldehyde, swirl gently, then pour into gel cast tray.
3. Immediately rinse vessel containing any unset gel residue and dispose into Formaldehyde/Formamide Liquid Waste container.
4. ★ Set up RNA denaturing mix (containing MOPS buffer, Formamide, Formaldehyde and Ethidium Bromide) and aliquot to RNA sample tubes
5. Heat RNA samples at 65 \(^{\circ}\)C water bath for 10 minutes – ensure caps are closed.
6. Place tubes on ice for 5 minutes, spin briefly to pellet samples, return to ice.
7. ◆ Transfer set agarose gel to tank, fill with fresh 1X MOPS running buffer.
8. ◆ Load samples and run gel at 65 volts until desired RNA separation is achieved.
9. ◆ Transfer gel to a container, add MilliQ water, secure lid, and wash gel free of formaldehyde/formamide for 5 minutes.
10. ◆ Decant contaminated running buffer and gel wash into Formaldehyde/Formamide Liquid Waste container.
11. Photograph gel via UV Imaging system, e.g. GelDoc.
12. ◆ Set up blot apparatus and blot overnight using 20X SSC blotting solution.
14. **UV-fix RNA to nylon filter via Crosslinker**
15. Wash RNA filter in 2X SSC, then load into Hybridisation vial. Discard SSC solution down the sink.
16. Pre-treat RNA filter with 20ml of hybridisation solution at 42°C for at least 3 hours or overnight.
17. **Pierce a small hole in the lid of the microfuge tube containing the 32P labelled DNA probe** with the sharp point of tweezers or syringe, then tape over hole with masking tape.
18. **Denature 32P labelled DNA probe in a heat block at 95°C for 5 minutes, spin down briefly, then add probe directly to hybridisation solution in vial.**
19. **Incubate overnight in hybridisation oven located in the radiation room.**
20. **Decant probe solution into 50ml Falcon tube, place in a Perspex box and store behind Perspex shielding of Radiation workstation.**
21. **Wash probed RNA filter with 2 x 100ml of 2X SSC at 42°C for at least 15 minutes each.** Decant wash volumes into the 32P- Radioactive Liquid Waste container.
22. **For the third wash use 25ml of 2X SSC at 42°C for 15 minutes. Decant into a 50ml Falcon tube and measure radioactivity using a (G-M) Geiger counter. If the counts are below 3 cps proceed to the next step. If not, repeat 25ml washes until radioactivity falls below 3 cps.**
23. **Continue washing probed RNA filter at appropriate SSC conc., temperature, and time until the desired cps count is achieved. Decant washes down the sink.**
24. **Wrap RNA filter in a thin plastic sleeve and seal.**
25. **Place sealed RNA filter into the Phosphor Imaging cassette.**
26. Store cassette in the dark, at room temperature for appropriate exposure time.

### Section 4 – Disposal / Spills / Incidents

1. Formamide, Formaldehyde, and Ethidium Bromide waste (either liquid or solid) must be disposed of as hazardous chemical waste into dedicated waste disposal containers. If possible, separate ethidium waste from formaldehyde/formamide. (this may not be possible for above procedure)
2. Radioactive waste is placed in either the Radioactive Liquid or Solid Waste Perspex containers stored in the Radiation room. NB: Each isotope has its own set of waste containers. Do not mix isotopes unless there is no way to avoid this.
3. All spills must be cleaned up immediately.
4. For small chemical spills use tissue paper to wipe up spill. For large chemical spills, use chemical absorption mats kept in the Hazardous Substances Spill kit, bag and tie waste, and label with details of the hazardous substance, estimate volume, your name and laboratory. Transport to Room 225 for disposal. Decontaminate spill area with moistened tissue paper and dispose of waste to appropriate solid waste container.
5. Full chemical waste containers are sealed, labelled, and transported to Room 225 for disposal.
6. For radioactive spills use long tweezers to hold a pad of tissue paper soaked in DECON90 to decontaminate surfaces. Use (G-M) Geiger counter to monitor progress and to confirm all surfaces are free of contamination.
7. Full Radioactive waste containers need to be stored in a Perspex box until the radioactivity has decayed to below 100Bq/g before disposal. Solid waste is transported to Room 225 for disposal, while the liquid waste can be decanted down the sink. **NOTE: ONLY WASTES BELOW 100 Bq/g CAN BE DISPOSED OF DOWN SINK!**
8. All large spills of radioactive or highly toxic substances (e.g. >1L) should be reported to the school safety officer, and an incident report generated.
9. All incidents and injuries should be reported to the relevant supervisor, safety officer and to the online RiskWare safety management system.

### Section 5 – Certification / Training

1. Radiation lab must be kept in registration, and details of this registration must be posted in a prominent place e.g. on the outside of the door.
2. All users of radiation must be licenced or in the case of students, supervised by someone with a licence to use radioactivity
3. All users of radiation must have completed a radiation safety training course.
Section 6 – Relevant Material safety data sheets

1. Ethidium Bromide
2. Formaldehyde
3. Formamide
4. P32 – ATP (or other P32 labelled chemical, as appropriate)
5. SDS – Sodium Dodecyl Sulphate

Section 7 - References

1. Risk Assessments and SOPs: “Agarose Gel Electrophoresis”, “Northern Blotting & 32P Probe Hybridisation”, UV Light Sources”, “Radioactive Isotopes” and “Chemical Waste”.

SOP Consultation, Training and Approval

Print names and enter signatures and dates to certify that the persons named in this section have been consulted/trained in relation to the development and implementation of this Standard Operating Procedure. WHS Representative (WHS Committee) certifies that consultation has taken place.

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Name Authorising (Printed): DIANNE FISHER

Signature: ........................................Date: 2/3/15 ........................................

WHS Committee Representative Name (Printed): MARKUS HOFER

Signature: .............................................Date: 2/3/15 ........................................