***STANDARD OPERATING PROCEDURE – B009***

**Northern blotting and 32P probe hybridization**

1. **Objectives**

The objective of this document is to establish standard operating procedures for the northern blotting and 32P probe hybridization, ensuring the safety of laboratory personnel by mitigating potential risks associated with hazardous materials, and injuries. Additionally, this SOP aims to enhance the efficiency of experimental workflows.

1. **Personal Protective Equipment**

To ensure the safety of the northern blotting and 32P probe hybridization, appropriate personal protective equipment (**PPE**) must be worn. This includes:

* Long pants and closed-toe shoes to protect against spills and splashes.
* A long-sleeved, buttoned lab coat to minimize skin exposure.
* Safety glasses or goggles to protect against splashes or flying debris.
* Disposable nitrile or latex gloves to prevent direct contact with hazardous materials.
* Double nitrile gloves to prevent direct contact with radioactive materials. Change immediately if it is contaminated.
* Heat-resistant gloves when handling hot items such as agarose gel or heated solutions.
* A personal dosimeter must be worn by personnel working with radioactive materials.
* If the user has long hair, it should be tied up.

**3) Potential Hazards**

The northern blotting and 32P probe hybridization poses various hazards that must be managed to maintain a safe working environment. These include:

* **Radiation Hazard:** Exposure to radioactive materials such as 32P may pose significant health risks. All personnel working with radioactive substances must undergo medical surveillance prior to starting work. A personal dosimeter must be worn when working in the radioactive workroom.
* **Chemical Hazards:** Formaldehyde and formamide are toxic and volatile. Always handle these chemicals in a fume hood. Exposure to other chemicals may result in skin or respiratory irritation. The chemical safety data sheet (SDS) must be read and understood prior to the start of experiments.
  + **NOTE:** Ethidium bromide should be avoided whenever possible, and safer alternatives should be used.
* **Heating Hazards:** Heating agarose in a microwave can pose a burn risk if heat-resistant gloves are not worn when handling hot beakers or other vessels. High-temperature water baths and heat blocks should be used with caution to avoid burns.
* **Electrical Hazards:** Electrical shock may result from a leaking chamber of the gel tank, damaged or corroded electrical cables, or a faulty power supply, leading to serious electrical shock or electrocution.
* **Sharps Hazards:** Cuts or puncture wounds may occur from mishandling sharp instruments such as blades, tweezers, syringes, or scissors.
* **Environmental Hazards:** Improper disposal of hazardous chemicals or radioactive waste can lead to environmental contamination.

Laboratory workers with **pre-existing conditions,** including but not limited to allergies, immunocompromised states, chemical sensitivities, or those who are pregnant or planning pregnancies should notify their supervisors and medical specialists. Should any concerns be expressed by these workers, their job duties and activities should be reviewed.

**4) Training / Licenses**

Ensure all personnel have received proper training on their hazards and safe handling techniques. All users that will use radioactive isotopes must complete the relevant training and undergo medical surveillance and register as a radiation worker prior to the start of work.

* MC01 Radiation Safety with Unsealed Radioactive Materials
* MC06 Biological Safety
* MC03 Chemical Safety II / Hazardous Waste Management
* MC07 Chemical Safety I / Chemical Safety for Laboratory Users
* DC04 Electrical Safety

Ensure the licenses for the apparatus, users, and workers remain valid.

**5) Procedures**

1. Agarose Preparation

* Agarose gel should be heated in a microwave using a glass bottle with the lid loosely screwed to prevent pressure buildup.
* Heat the gel in short intervals, swirling between heating cycles until fully dissolved.
* Formaldehyde should be added to the cooled agarose solution inside a fume hood.
* Rinse glass bottles containing formaldehyde and agarose gel with water. Dispose of the rinse water as organic chemical waste; it must not be poured down the drain.

1. RNA Denaturing

* RNA denaturing mix which contains MOPS buffer, formamide, formaldehyde, and ethidium bromide should be prepared inside the fume hood.
* Ensure RNA sample tubes have their caps closed prior to heating the samples at 65°C for 10 minutes.
* High temperature water baths and heat blocks should be operated with caution.

1. Gel Preparation and Running

* Set agarose gel should be moved to a gel tank that is freshly prepared with 1X MOPS running buffer.
* Samples should be loaded and gel run until desired RNA separation is achieved.
* Transfer gel to a container, add MilliQ water, secure lid, and wash gel free of formaldehyde/formamide for 5 minutes.
  + **NOTE:** the MilliQ water that is used to wash the gel MUST NOT be poured down the sink or drains.
* Decant contaminated running buffer and gel wash into a **formaldehyde/formamide liquid waste** container. **DO NOT** pour down the sink or drains.

1. Gel Imaging and Blotting

* When the gel has finished running, it should be visualized using an imaging system (e.g., GelDoc).
* Set up blot apparatus and blot overnight using 20X saline sodium citrate (SSC) blotting solution.
* **NOTE:** Wet blotting paper and gel should not be discarded as general waste and placed in **formaldehyde/formamide solid waste** containers.
* A crosslinker can be used for UV-fixing the RNA to a Nylon filter. A crosslinker system is recommended over a hand-held UV illuminator system as this reduces the amount of UV exposure to the user.

1. RNA-Nylon Filter Washing

* The RNA-nylon filter should be washed in 2X SSC and then loaded into the hybridization vial.
  + **NOTE:** SSC solution should be discarded down the sink.
* RNA filter should be pre-treated with 20 ml of hybridization solution at 42°C for at least 3 hours or overnight.

**The following steps should only be completed in a room that is prepared for radiation work:**

1. Probe Preparation and Application

* The lid of the microfuge tube should be pierced with tweezers or syringes which contains 32P-labeled DNA probe and masking tape placed over the hole.
* 32P-labeled DNA probe should be denatured in a heat block at 95°C for 5 minutes and then spun down briefly with the hybridization solution added.
* Incubate overnight in a hybridization oven.

1. Final Washing and Storage

* The following day, the probe solution should be decanted into a 50 ml Falcon tube and placed inside a Perspex box and shielded by Perspex shielding.
* All subsequent washes should be decanted into a 50 mL Falcon tube. The solution used for washing should be SSC, at 42°C and for a minimum of 15 minutes.
* Measure the wash solution with the Geiger counter should be measured using a Geiger (GM) counter and if it is above 3 counts per second (cps), discard it in the 32P radioactive liquid waste container.
* Continue washing until desired counts per second (cps) is achieved. If it reaches below 3 cps, SSC used to rinse the RNA nylon filter may be poured down the sink.

1. Imaging

* RNA nylon filter should be wrapped in a thin plastic sleeve and sealed
* Sealed RNA Nylon-filter should be placed in the Phosphor Imaging cassette for imaging.
* Cassettes should be stored in the dark and brought to room temperature when ready for exposure.

**6) Disposal**

* Formamide, formaldehyde and ethidium bromide waste must be disposed of as hazardous chemical waste and dedicated waste disposal containers.
* If possible, ethidium bromide waste should be separated from formaldehyde / formamide.
* Each isotope has its own individual container; liquid and solid waste are separated in the radiation work room.
* Different isotopes should not be mixed together unless there is no way to avoid this.

**7) Spills / or Incident Reporting**

* Promptly notify the Principal Investigator (PI) or departmental safety officer (DSO) of any accidents, spills, or equipment malfunctions.
* All spills should be **cleaned up immediately**.
* **Small chemical spills**: Clean up using tissue paper and discard as a Solid with Toxic Chemicals waste.
* **Large chemical spills**: Spill kit containing chemical absorption mats may be used. Used mats should be tied up in a bag, labelled with name, location, and estimated volume and discarded as solid waste.
* **Radioactive spills:** A tissue padheld by long tweezers soaked in Decon 90 should be used to decontaminate radioactive spills. Refer to “SOP for cleanup of radioactive spills”. A Geiger counter may be used to monitor the progress of cleanup to ensure surfaces are free from contamination.
* For serious incidents, contact the Security Unit immediately by calling the 24-hour hotline on **2358 8999**.

**8) References**

* Coleman, N. (2016). *SOP\_SMB022: Northern blotting and 32P probe hybridisation.* Risk Assessment. The University of Sydney.
* Jackson, C. & Coleman, N. (2014). *SOP SMB022.2 (CJ NC 0714): Northern blotting and 32P probe hybridisation.* Standard Operating Procedure. The University of Sydney.
* Safety and Environmental Protection Manual *- Chapter 7: General Laboratory Safety | Health, Safety and Environment Office - the Hong Kong University of Science and Technology*
* Safety and Environmental Protection Manual *- Chapter 10: Radiation Safety | Health, Safety and Environment Office - the Hong Kong University of Science and Technology*
* HKUST Emergency Procedures – *Hong Kong University of Science and Technology*