***STANDARD OPERATING PROCEDURE – E009***

**Southern Blotting and 32P Probe Hybridisation**

1. **Objectives**

The objective of this document is to establish standard operating procedures for Southern blotting and 32P probe hybridisation, 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 safety during Southern 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 contaminated.
* Heat-resistant gloves when handling hot items such as agarose gels 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 back.

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.

1. **Potential Hazards**

NOTE: The use of ethidium bromide is not recommended. Avoid using the ethidium bromide whenever feasible.

The process of Southern blotting and 32P probe hybridization presents various hazards that must be managed to maintain a safe working environment. This includes:

* **Chemical Hazards:** Southern blotting involves the use of hazardous chemicals, including ethidium bromide (EtBr), a mutagen that increases cancer risk upon skin contact or inhalation. All procedures with EtBr and other toxic and corrosive chemicals must be performed in a fume hood, and equipment/waste must not cross-contaminate non-EtBr workflows.
* **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.
* **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.
* **Physical and Mechanical Hazards:** UV transilluminators and cross-linkers emit UV-C radiation, which can cause skin burns and eye damage within seconds of unprotected exposure. Additionally, pressurized microwaving of agarose in sealed containers risks explosions, and fragile glassware (e.g., sample tubes) may cause cuts or punctures if mishandled.
* **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.

1. **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 start of work.

* MC01 Radiation Safety with Unsealed Radioactive Materials
* MC02 Radiation Safety with Sealed Radioactive Materials and Irradiating Apparatus
* 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.

1. **Procedures**

**IMPORTANT:** The safety data sheet (SDS) of all chemicals (e.g. EtBr, HCl, NaOH, sodium dodecyl sulfate, GelRed / GelGreen, and P-32 labelled nucleotides) should be read and understood by all users prior to the start of experiment.

* Gel Electrophoresis

1. Understand and know the location of eyewashes, spill kits, safety showers, fire blankets and fire extinguishers prior to starting work.
2. Buffer containing agarose gel in a conical flask should be heated in a microwave on medium heat, swirling gently until the agarose fully dissolves. The glass container containing agarose gel should be heated in short bursts to prevent overheating and overspilling of the agarose into the microwave.
3. Allow the agarose gel solution to cool down to approximately 65°C before adding the nucleic acid stain (e.g. EtBr and its alternatives GelRed / GelGreen).
4. Ensure the sealed gel cast tray has no leaks and test by pouring buffer into the tray and wait.
5. If there are no leaks, swirl the agarose gel should and pour the solution into the tray.
6. Place the comb in the gel and wait for the gel to set.
7. Transfer the set agarose gel to the tank. Buffer should be filled to the level so it just covers the gel. If EtBr is used as a DNA stain, it should be disposed of into the appropriate liquid waste container without contaminating other waste.
8. Add loading dye to samples and load samples into the gel. Cover with a lid and run the gel.
9. Turn off electrophoresis, remove the gel, and photograph it using a UV Imaging system.

**NOTE:** If you need to transport an EtBr-containing gel between different areas of the building, use a secondary container with a lockable lid.

1. For EtBr, pour the buffer into a liquid waste container specifically for EtBr. Other DNA stains that are non-mutagenic i.e. GelRed / GelGreen (alternatives to EtBr) solutions can be disposed of down the sink.

* Membrane Blotting and Hybridization

1. Prepare 1 L of fresh 0.25 M Hydrochloric acid in a plastic container. **(This step MUST be performed inside a fume hood with the fan ON and sash lowered (~ 20 cm vertical gap)).**
2. Add the gel to the acid solution, seal the container, and gently mix until the dye turns yellow (~ 15 minutes).
3. Afterwards, discard the acid solution into the Ethidium Bromide Liquid Waste container if using EtBr, or otherwise, down the sink, and then flush with copious amounts of cold water.
4. Wash the gel in 1 L of water for 15 minutes, which can be discarded down the sink.
5. Set up the blot apparatus and blot overnight using a 0.4 M NaOH solution.
6. Disassemble the blotting setup. Dispose of the wet blotting paper and gel. This can be disposed of in regular rubbish bins if the NaOH can be rinsed off.
7. Wash the membrane in 2X saline-sodium citrate (SSC) for 5 minutes and repeat the process once. Dispose the wash solution down the sink.

* Probe Hybridization

**The subsequent steps must be performed in a verified radiation work room using Perspex shielding. See SOP for radioactive isotopes (32P / 33P).**

**NOTE:** If you are using a non-radioactive probe, the subsequent steps **DO NOT** need to be completed in a radioactive work room or with shielding.

1. Load the membrane into a hybridization vial containing 20 mL of hybridization solution. Place the vial in a hybridization oven and incubate at 65°C for at least 3 hours.
2. A small hole should be pierced in the lid of the microfuge tube containing the 32P-labelled DNA probe with tweezers, then masking tape used to tape over the hole.
3. Denature the 32P-labelled DNA probe in a heat block at 95°C for 5 minutes. Spin down briefly, then add the probe directly to the hybridization solution in the vial.
4. Hybridize the 32P probe to the membrane overnight at 65°C.
5. The probe solution should be decanted into a 50 mL Falcon tube and placed in a Perspex box and stored behind Perspex shielding.
6. The probed membrane should be washed twice with 100 mL of 2X SSC at 65°C for at least 15 minutes each. After washing, the SSC should be discarded into the 32P-Radioactive Liquid Waste container or poured down the sink for non-radioactive probes.
7. For the third wash, use 25 mL of 2X SSC at 65°C for 15 minutes. Dispose of the wash into a 50ml Falcon tube and measure radioactivity using a Geiger counter.
8. Continue washing the probed membrane at the appropriate SSC concentration, temperature, and time until the desired cps (counts per second) is achieved.
   * **NOTE:** Dispose down the sink if it is 3 counts per second (cps) or less. Should it be higher, continue to wash with SSC.
9. Wrap the membrane in a thin plastic sleeve and seal it to prevent it from drying out.
10. The sealed membrane can then be placed inside an imaging cassette with film.
11. Store it in the dark at room temperature for the required exposure time.

* Waste Disposal
* EtBr waste should be disposed of in designated liquid and solid waste disposal containers.
* Radioactive waste must be disposed of in dedicated liquid or solid waste Perspex containers (thickness: 10 mm) located in the radiation work room. **DO NOT** mix isotopes unless there is no way to avoid this.
* Full Radioactive waste containers need to be stored in a Perspex box until the radioactivity has decayed to below 100Bq/g before disposal. At this point, solid waste can be disposed of (consult HSE officer), while the liquid waste can be decanted down the sink.
  + **NOTE: ONLY WASTE BELOW 100 Bq PER GRAM CAN BE DISPOSED OF DOWN SINK.**
* Never dispose wastes in near-full or full Chemical waste containers. Seal these containers and label for disposal.

1. **Spills or Incident Reporting**

* If any spills occur, clean it up immediately.
* For radioactive spills, use long tweezers to hold a pad of tissue paper soaked in DECON 90 or other suitable decontaminant to clean the surfaces. Use a Geiger counter to monitor progress and to confirm all surfaces are free of contamination.
* All significant spills involving radioactive or highly toxic substances (e.g. over 1 L) must be reported to PI and/or the laboratory safety officer. Near misses (hazardous situations not leading to an incident) should also be reported.
* Any accidents that result in injuries must be reported to the PI and/or the departmental safety officer (DSO) immediately. If still feeling unwell after having received first aid, then seek medical attention as soon as possible.
* In case of serious incidents, immediately inform the Security Unit by calling the 24-hour hotline on **2358 8999**.

1. **References**

* Coleman, N. (2016). *SOP\_SMB022: Northern blotting and P32 probe hybridisation.* Risk Assessment. The University of Sydney.
* Coleman, N. & Jackson, C. (2014). *SOP SMB022.2 (CJ NC 0714): Northern blotting and 32P probe hybridisation.* The University of Sydney*.*
* Health, Safety and Environment Office, The Hong Kong University of Science and Technology (n.d.). *Laboratory Safety.* Retrieved on June 30, 2025, from https://hseo.hkust.edu.hk/various-subjects/laboratory