***STANDARD OPERATING PROCEDURE – E010***

**Agarose Gel Electrophoresis**

1. **Objectives**

The objective of this document is to establish standard operating procedures for agarose gel electrophoresis, ensuring the safety of laboratory personnel by mitigating potential risks associated with hazardous materials, and injuries. In addition, this SOP aims to enhance the efficiency of experimental workflows.

1. **Personal Protective Equipment**

To ensure safety during agarose gel electrophoresis, 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 chemical splashes and UV light.
* Disposable nitrile gloves to prevent skin contact with hazardous chemicals.
* Heat-insulated gloves when handling hot agarose solutions.
* A UV-protective full-face shield when visualizing DNA bands under UV light.

1. **Potential Hazards**

Agarose gel electrophoresis presents various hazards that must be managed to maintain a safe working environment. These include:

* **Electrical Hazards:** Risk of severe electrical shock from leaking chambers, damaged electrode cables, or malfunctioning power supplies. Regularly inspect equipment and avoid using damaged components.
* **Thermal Hazards:** Spillage of superheated agarose can cause severe burns. Always handle hot solutions with heat-insulated gloves and allow them to cool before pouring.
* **Chemical Hazards:** Exposure to toxic substances can occur through skin contact or inhalation. Use safer alternatives such as SYBR Safe or GelRed.
* ***Note*:** Ethidium bromide is a potent mutagen and is not recommended for use as a dye during gel electrophoresis.
* **UV Light:** Prolonged exposure to UV light can cause skin burns and eye damage. Always use a UV-protective face shield and minimize exposure time.

1. **Procedures**

1. Agarose Gel Preparing:

* Ensure that all personnel have received training on agarose gel electrophoresis techniques and related safety protocols.
  + MC06 Biological Safety
  + MC03 Chemical Safety II / Hazardous Waste Management
  + MC07 Chemical Safety I / Chemical Safety for Laboratory Users
  + DC04 Electrical Safety
* Prepare the agarose powder accordingly. The concentration of agarose gels typically ranges from 0.7% - 2%, depending on the size of the DNA bands that need to be separated.
* Combine the agarose powder with 1X TAE or 1X / 0.5X TBE buffer
* Heat the mixture using a stirrer hot plate or microwave until the agarose is fully dissolved. Swirl if necessary to assist with dissolving the agarose.
  + **Note**: Ensure the container **is uncovered or has a loosely fitting lid** during heating.
  + The mixture may be heated at low power in short bursts of 10 to 20 seconds to ensure the overboiling of the solution will not occur.
  + Low-power heating over a longer period is preferred over high-power heating over a shorter period.
* Carefully handle the flask with heat-resistant gloves and allow the agarose to cool to **50-60°C** in a water bath.
  + **Note**: The solution should feel hot but not too hot to touch.

2. Gel Casting and Setup:

* Wear nitrile or latex gloves to prepare gel casting and the further steps of sample loading.
* Position the electrophoresis equipment on a clean, level surface in a well-ventilated area.
* Slowly pour the agarose solution into a casting tray that holds a comb.
* Ensure **no air bubbles** are trapped under or between the comb teeth and the gel.
  + **Note**: Use a pipette tip to gently dislodge any trapped bubbles, moving them away from the comb or towards the gel’s edges.
* After allowing the gel to solidify for about **20**-**30 minutes** at room temperature, carefully lift the comb straight up from the gel.
  + **Note**: The above steps are not necessary if using pre-cast gels.
* Transfer the agarose gel, still in its tray, into the electrophoresis chamber.
* Fill the chamber reservoir with **TAE** **or TBE buffer** until the agarose gel is completely submerged.

3. Sample Loading:

* Using a micropipette, carefully load the prepared DNA samples into the wells of the gel.
* Start by loading a **DNA ladder / marker** into the first well to serve as a size reference, followed by the experimental samples in the subsequent wells.
  + Ethidium bromide is not a dye recommended for RNA and DNA detection in gels.
  + If you are unsure of appropriate alternatives, please seek assistance from the HSEO team.
* Place a **lid** on the gel box to cover the gel after loading the samples.
  + **Note: Avoid overfilling** the wells to prevent cross-contamination.

4. Gel Running:

* Run the gel at a voltage between 5**0 to 200V** until the dye front migrates approximately 75-80% down the gel. The typical running time is about **1-1.5 hours**, depending on the gel concentration and voltage settings.
* Once the run is complete turn off the power, disconnect the electrodes from the power supply, and carefully remove the gel from the gel box.
* The DNA fragments may be visualized using a **UV transilluminator**. UV-blocking full face shield should be used when visualizing the fragments.
* The separated DNA fragments are commonly referred to as “**bands**” due to their distinct appearance in the gel.

5. Data Analysing:

* Capture an image of the gel using a gel documentation system or tablet / phone / camera to record the DNA bands for further analysis.
* Examine the banding pattern and compare the migration distances against the DNA ladder in the first lane.
* Turn off the UV illuminator when not in use.

6. Waste Disposal and Cleaning:

* Dispose of the agarose gel and contaminated buffer in accordance with established waste disposal guidelines.
* Uncontaminated buffers may be poured down the sink and flushed with copious amounts of tap water.
* Clean the gel casting tray, comb, and electrophoresis chamber with distilled / deionised (DI) water to ensure all residues are removed. Dry thoroughly.
* Clean the UV illuminator using a damp cloth and wipe dry to avoid contamination for future use.

**5) Incident Reporting**

* Any accidents that result in injuries must be reported to the Principal Investigator and/or the departmental safety officer (DSO) immediately.
* In the case of serious incidents, immediately inform the security unit by calling the 24-hour hotline at **23588999**.

**6) References**

* Dimauro, J. (2016). *SOP\_SMB001: Agarose gel electrophoresis.* Risk Assessment. The University of Sydney.
* Coleman, N. & Dimauro, J. (2014). *SOP SMB001.2 (JD NC 0314): Agarose gel electrophoresis.* The University of Sydney*.*
* Health, Safety and Environment Office, The Hong Kong University of Science and Technology (n.d.). *The Hazards of Electrophoresis.* Retrieved on June 30 2025. https://hseo.hkust.edu.hk/various-subjects/laboratory