THE UNIVERSITY OF ADELAIDE AUSTRALIA

SAFE OPERATING PROCEDURE (AGAROSE GEL ELECTROPHORESIS)

LOCATION DETAILS

School/Branch: School Medical Sciences, Pharmacology N533

SAFE OPERATING PROCEDURE DETAILS

Task/activity (including specify particular equipment, substance)	Date prepared:
Agarose Gel Electrophoresis	19/8/2008

PREPARED BY Name, Position and Signature (insert names of the supervisor, HSR, HSO and operator involved)

Name	Position	Signature
Dr Janet Coller	FTT Fricker Research Fellow	

RISK ASSESSMENT

Has a risk assessment been completed and have all other environmental considerations been made?	See Risk Assessment dated:	Risk Rating:
Yes ☒ No ☐	20/8/2008	☒ Low ☐ Medium ☐ High ☐ Very High

SAFE OPERATING PROCEDURE DETAILS

Procedure (Include control measures listed in risk assessment within the procedure):

The following safety precautions must be adhered to:

- Laboratory coat, safety glasses and nitrile gloves must be worn at all times.
- Any spills must be cleaned up immediately with copious amounts of water followed by spraying with 70% ethanol located in the lab.
- All waste (tubes, pipette tips, gloves, paper towel from wiping down benches and equipment) must be disposed of in the yellow biohazard bag.
- All equipment must be checked for in date electrical safety tags, do not use if out of date and inform Dr J Coller immediately.

Procedure:

ALL SET-UP IS TO BE DONE UNDER STERILE CONDITIONS TO PREVENT CONTAMINATION OF SAMPLES, YOU MUST BE TRAINED PRIOR TO PEFORMING THIS PROCEDURE. NO GEL ELECTROPHORESIS IS TO BE PERFORMED OUTSIDE OF NORMAL BUSINESS HOURS.

1. Preparation of agarose gel solutions containing ethidium bromide:
   - Note: Ethidium bromide solution is highly toxic and hazardous, you must be familiar with the MSDS for this compound prior to using this. The stock solution must be housed at all times in the fume cupboard in N533. You must wear protective clothing (laboratory coat and nitrile gloves) whilst handling the stock solution and any agarose gel solutions or set gels containing this compound.
   - Using a clean spatula, weigh out required amount of agarose in the balance room and place in schott bottle already containing the required volume of 1xTBE buffer (see card in lab folder for making this solution).
   - Heat agarose solution *once the lid is loosened* in microwave located in Rm 533 for a maximum of 1min, making sure that the solution does not bubble over and out of the bottle. Explosions can occur if the lid is not loosened with this superheated solution.
   - Wearing heat proof gloves over nitrile gloves for protection against steam burns, secure lid and remove bottle from microwave, swirl to aid in dissolution of agarose in buffer. **DO NOT HANDLE HOT BOTTLE WITHOUT HEAT PROOF GLOVES AND ALWAYS ENSURE LID IS SECURE PRIOR TO MOVING BOTTLE.**
   - Repeat heating procedure until agarose solution becomes completely transparent at which time the agarose will be completely in solution.
   - Place bottle in container with cool water to allow solution and bottle to cool to a temperature at which you can handle without the heat proof gloves for at least

one minute. At this cooled temperature, agarose solution can be poured into gel trays safely.

- Add required volume of ethidium bromide to agarose solution once cooled. **THIS MUST BE DONE ONLY WHEN ANOTHER SENIOR MEMBER OF LAB IS PRESENT IN CASE OF ACCIDENT.**

2. Once agarose solution is made, heated and cooled it can be poured into gel trays. The ends of the trays need to be taped with masking tape to prevent the agarose from leaking out of the tray and to determine the length of the gel that is being prepared. Once taped, pour the cooled agarose solution into the gel tray.
3. Place the gel comb into the poured agarose at the top of the gel and remove any air bubbles from the solution with a yellow pipette tip. Allow the gel to set at room temperature.
4. Once the gel is set, remove the comb slowly by lifting from one edge of the tray across the width of the gel. Also remove the tape from the gel tray and place in yellow biohazard bag.
5. Place the gel tray in the gel electrophoresis tank slowly by lowering the top first, following through the length of the tray in order to prevent the 1xTBE buffer in the tank from spilling over the edges of the tank. PCR fragments are negatively charged and so will migrate down the gel from the negative = black to the positive = red end of the tank, so *need to put the gel tray in the tank with the lanes made by the comb at the black end of the tank*.
6. Once gel is in the tank the PCR samples can be loaded into the gel lanes. If samples have been stored at -20°C they will need to be thawed. Mix samples with the required volume of 2x gel loading buffer (see card in lab for making up this solution) on a piece of paraffin film that is taped down to the bench. Load samples slowly with pipette – if loaded too quickly, samples will float out of lanes. Also load a molecular

weight marker to enable size estimation of bands on gel. Place all waste in yellow biohazard bag.

7. Once all samples are loaded on gel, connect black and red leads from gel tank lid to the black and red connection on the BioRad power pack. Turn on power pack and set to required amps to run the samples the length of the gel.
8. Once samples have run the length of the gel turn off the power pack both at the switch and at the power point, and remove leads. Remove gel tank lid and gel tray from the tank, placing on paper towel to move to area for imaging and photography of fragments. For gel photography refer to separate SOP.
9. Replace lid on gel tank to prevent evaporation of buffer and wipe down all working surfaces with 70% ethanol.

Contact person for this SOP: Dr J Coller, Rm N513, ext 33906

Note: This Safe Operating Procedure must be reviewed :
a) after any accident, incident or near miss;
b) when training new staff;
c) if adopted by new work group;
d) if equipment, substances or processes change; or
e) within 5 years of date of issue.