

SAFE OPERATING PROCEDURE (Polymerase Chain Reactions)


LOCATION DETAILS

School/Branch: School of Medical Sciences, Pharmacology N533

SAFE OPERATING PROCEDURE DETAILS

Task/activity (including specify particular equipment, substance) Polymerase Chain Reactions (PCR) - using eppendorf centrifuges & thermal cyclers	Date prepared: 19/8/2008
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PREPARED BY Name, Position and Signature (insert names of the supervisor, HSR, HSO and operator involved)

Name	Dr Janet Coller	Position	FTT Fricker Research Fellow	Signature	
	Gordon A. Crabb		Lab Manager, HSO		

RISK ASSESSMENT

Has a risk assessment been completed and have all other environmental considerations been made? Yes <input checked="" type="checkbox"/> No <input type="checkbox"/>	See Risk Assessment dated: 20/8/2008	Risk Rating:	<input checked="" type="checkbox"/> Low <input type="checkbox"/> Medium <input type="checkbox"/> High <input type="checkbox"/> Very High
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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 gloves must be worn at all times
- Human genomic DNA samples and PCR samples pose a biohazard risk and must not be carried through the office between the clinical pharmacogenomics lab and the clinical pharmacokinetics lab where the PCR set-up area is located.
- Any spills must be cleaned up immediately with copious amounts of 70% ethanol located within both labs.
- All waste (tubes, pipette tips, gloves, paper towel from wiping down benches) 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- All equipment must be checked for in date electrical safety tags, do not use if out of date and inform Dr J Coller immediately.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 PERFORMING THIS PROCEDURE.

1. Remove from -20°C freezer in N522 all constituents of PCR reaction mix required for assay and thaw at room temperature, except the DNA Taq Polymerase enzyme which must be kept on ice at all times.
Failure to keep on ice or at -20°C will result in absence of activity and hence unsuccessful PCRs.
2. Place PCR set-up rack in esky containing ice as *PCRs must be prepared on ice.*
3. Obtain human genomic DNA samples required for analysis from the cold room, N530a.
4. Prepare any required dilutions of stock concentrations of PCR constituents or human genomic DNA in eppendorf tubes with autoclaved water.
5. Once PCR set-up rack is cold and all constituents are thawed, place constituents in set-up rack, close esky and carry into the Clinical Pharmacokinetics lab, Rm N532.
6. Label 0.2 or 0.5ml PCR tubes with texta to identify samples on the side of the tubes. *Do not label the lids as the texta marks the lid of the thermal cyclers.*
7. According to calculated volumes, make up the PCR master mix in an eppendorf tube in the PCR set-up area, vortex mix, carry back on ice to N533, and then pulse spin in eppendorf centrifuge (refer to centrifuge "Microfuge SOP").
8. Carry back to PCR set-up area in N532 and aliquot the required volume of PCR master mix into PCR tubes, close all lids of tubes and esky and carry back to Rm N533.
9. Add required volume of human genomic DNA samples to tubes already containing PCR master mix.
Pipette tips must be changed for each sample in order to prevent cross contamination.
10. Once lids are closed, vortex mix PCR tubes gently and pulse spin in eppendorf centrifuge (refer to centrifuge "Microfuge SOP").
11. Place in thermal cycler and start PCR following the *SOP for thermal cyclers* in N533 (refer to "Thermal Cyclers SOP").
12. Place all waste from containers on lab benches in the yellow biohazard bag and wipe down all lab benches used with 70% ethanol and paper towel, including the PCR set-up area. *Ensure that Perspex bench cover in this area is replaced over the set-up area.*

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Return all PCR constituents to the -20°C freezer in N522 and the human genomic DNA samples to the cold room, N530a.

Contact person for this SOP: Dr J Coller, Rm N515, 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.