**SOP Sanger Clean by SPRI**

**Document Number: XX**

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**Purpose**

This SOP describes the procedure used to Clean-up unpurified PCR product before Sanger sequencing

**Scope**

**Regulatory References:** NA

**Responsibility of experimentalist:** understanding and performing this procedure as described; reporting any deviations or problems to area supervisor; adequately documenting the procedures and results.

**Responsibility of area manager or supervisor:** ensuring that the analyst performing this procedure is qualified; ensuring that the procedure is followed, and updating the procedure as necessary.

**Definitions/Abbreviations**

Elution Buffer : EB

Ethanol: EtOH

**Related Documents:** NA

**Required Equipment and Materials / Reagents**

* 70% Ethanol (Sigma-Aldrich; Cat# 24102)
* AmPure SPRI beads (Beckman Coulter, Cat# A63882)
* Elusion Buffer (Qiagen: Cat# 19086; or equivalent)
* PCR plate (VWR; Cat# 82006-704)
* 96 well magnet (ThermoFisher Scientific; Cat# AM10027)
* 200uL multichannel pipette
* 200uL filter pipette tips
* 10uL pipette
* 10uL filter pipette tips

**Precautions**

Personal protection equipment including sterile gloves and lab coat must be worn when executing this procedure.

**Procedure**

1. Aliquot out needed volume of SPRI beads (21.25uLs per reaction) and let come to room temperature for 10 minutes.
2. Aliquot 21.25uLs of beads into the 25uLs of reaction and mix well by pipetting
3. Let sit at room temperature for 7 minutes
4. Place plate or tube onto magnet for 2 minutes

Steps 5-9 are done on the magnet:

1. Carefully remove and dispose of supernatant

Note 1: do not remove beads with the supernatant

1. Wash the beads with 200uLs of 70% EtOH
2. Remove EtOH
3. Wash beads again with 200uLs of 70% EtOH
4. Remove all EtOH and move samples into laminar flow hood to dry for 15 minutes

Note 2: removing all the EtOH might require additional pipetting steps as it can be difficult to get it all out of the 96 well plates

1. Remove samples form the magnet and aliquot 30uLs of EB into each well and mix by pipetting

Note 3: if you are worried about the concentration of your reactions you can elute in 20uLs but you will most likely need to mix the plate by covering it with an aluminum foil plate cover and vortexing then spinning down very briefly

1. Let sit at room temperature for 7 minutes
2. Place samples back onto magnet for 2 minutes
3. Transfer supernatant to new plate/tubes

Note 4: It can be difficult to not suck up a lot of beads during this step. To avoid beads transfer 5uLs less than total volume.

**Version History: NA**

**Worksheets: NA**

**Appendix: NA**