**SOP# 02.352.01**

**PCR product purification – Isopropanol**

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**Purpose**

This SOP describes an isopropanol procedure used to purify polymerase chain reaction products after amplification.

**Scope**

For exploratory purposes

**Regulatory References**

NA

**Responsibility**

* Responsibility of experimentalist – understanding and performing this procedure as described; reporting any deviations or problems to area supervisor; adequately documenting the procedures and results
* Area manager or supervisor – ensuring that the analyst performing this procedure is qualified; ensuring that the procedure is followed and update the procedure as necessary

**Definitions/Abbreviations**

PCR – polymerase chain reaction

uL - microliter

**Related Documents**

SOP# 03.004.01 – Laminar flow hood operation and maintenance

SOP# 02.351.01 – Full length 16S PCR preparation – 27F-1492R [Correct? Any others?]

**Required Equipment and Materials / Reagents**

* + 96 well PCR plate (VWR, catalog # 82006-704), any PCR clean 96 well plates may be used
  + PCR reactions, for example those generated using SOP# 02.351.01 – Full length 16S PCR preparation – 27F-1492R
  + Isopropanol (VWR Cat# 89370-086)
  + Aluminum foil plate covers – (VWR, catalog # 60941-076)
  + Paper towels
  + Laminar flow hood (SOP# 03.004.01 – Laminar flow hood operation and maintenance)
  + Centrifuge capable of holding 96 well plates, for example Eppendorf 5811F
  + Elution buffer (Qiagen, cat # 19086)

**Precautions**

* Personal protection equipment including gloves, lab glasses, and lab coat must be worn when executing this procedure
* Isopropanol is flammable and undiluted solutions must be handled in a chemical hood. Handle diluted solutions with care.

**Procedure**

1. Prepare Isopropanol solutions:
   1. 75% Isopropanol solution: 75% undiluted isopropanol, 25% milliq water, 100uLs needed per reaction well
   2. 70% Isopropanol solution: 70% undiluted isopropanol, 30% milliq water, 50uLs needed per reaction well
2. Using centrifuge spin plate down for 12 seconds to collect volume at bottom of well
3. Add 100uLs of 75% Isopropanol to each reaction well
4. Seal plate with aluminum foil cover and flip plate upside down twice to mix
5. Let sit at room temperature for 30 minutes
6. Centrifuge plate at 2800g for 30 minutes
7. Remove sealing foil
8. Lay a paper towel over the top of the plate
9. Gently invert plate so it is resting on top of the paper towel face down on the lab bench
10. **Gently** rub plate back and forth to drain isopropanol solution from wells
11. Invert plate again so it is top up
12. Add 50uLs 70% Isopropanol solution to each reaction well
13. Seal plate with aluminum foil cover and flip plate upside down twice to mix.
14. Centrifuge plate at 2000 x g for 10 minutes
15. Remove sealing foil
16. Lay a paper towel over the top of the plate
17. Gently invert plate so it is resting on top of the paper towel face down on the lab bench
18. Centrifuge plate upside down on top of paper towel for 1 minute at 700 x g
19. Invert plate so it is top up and place it in the laminar flow hood
20. Let plate air dry in laminar flow hood for 1 hour
21. Add 30uL of Elution buffer to each well
22. Cover plate with aluminum foil plate cover
23. Vortex and briefly spin plate down
24. Well now contain purified DNA and the plate can be stored at -20°C

**Version History**

This is the first version of this document

**Worksheets**

NA

**Appendix**

Lab notebook pages: Allison Perrotta page 28