**SOP Extraction Alkaline PEG (ALP) lysis buffer**

**Document Number: XX**

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**Purpose**

This SOP describes the procedure used to extract DNA from isolate

**Scope**

Extraction of DNA is the first step before Full length 16S PCR

**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**

Alkaline polyethylene glycol-based: PEG

Alkaline phosphatase : ALP

**Related Documents:** NA

**Required Equipment and Materials / Reagents**

- PEG200 (Sigma Aldrich: Cat# 88440-25ML-F; or equivalent)

- 1M KOH

- PCR plate or tube (VWR: Cat# 82006-704; or equivalent)

- Aluminum plate covers (VWR: Cat# 60941-076; or equivalent)

- PCR plate cover (VWR: Cat# 82018-846; or equivalent)

- Bottle-top vacuum filter system, pore size 0.2 μm- 1L (Sigma, Cat# CLS430515)

- Thermalcycler

- Vortexer, microcentrifuge

**Precautions**

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

Steps 3-6 should be performed in the Biosafety cabinet

**Procedure**

1. Make your alkaline PEG (ALP) lysis buffer:
   1. Combine 60 g PEG 200 with 1.86mL 1M KOH and 39 mL water.

Note 1: NaOH can substitute for KOH in the reagent

Note 2: PEG 200 is measured by mass rather than volume because of the viscosity of the liquid

* 1. Confirm pH is between 13.3-13.5. Adjust with additional KOH if needed
  2. Filter sterilize this buffer and store at room temperature

1. Thaw aliquot of isolate stock saved for Sanger, vortex and spin down when thawed

Note: Steps 3-6 should be performed in the Biosafety cabinet

1. In a PCR plate or tube, aliquot 20uLs of ALP buffer
2. Add 2uLs of isolate stock to ALP buffer and mix by pipetting
3. Seal plate with PCR plate cover
4. Seal source plate with aluminum foil plate cover and freeze at -80 in case need again
5. Run the PCR plate in the thermalcycler on the boil protocol (90C for 10mins)
6. When thermalcycler is finished remove plate and spin the plate/tubes down well to pellet cell debris.
7. Your extracted DNA will be in the supernatent

Note 3: you will not see this pellet so be careful not to shake the plate or mix while pipetting

Note 4: This plate can be stored at 4C for short term storage, frozen at -20C for longer storage, or used right away in a PCR

**Version History: NA**

**Worksheets: NA**

**Appendix: NA**