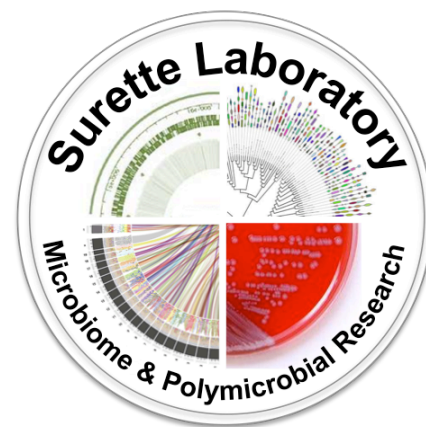

ISOLATION OF DNA FROM CLINICAL SAMPLES (GENOMIC PREP)



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BACKGROUND

- Isolation of DNA from a sample of interest is a precursor step to any type of sequencing project. Recently, sequencing of the 16S rRNA gene has been used extensively to profile various human microbiome sites during health and disease to study the impact of microbes at these locales.
- This protocol uses mechanical and enzymatic lysis in addition to phenol-chloroform extraction methods. The enclosed protocol includes steps beyond the standard which we have found to be instrumental to the isolation of DNA from a subset of Gram + organisms.

EQUIPMENT

- Bead based homogenizer (PowerLyzer, Medicorp Inc., #13155)
- Centrifuge
- Fume hood
- Vacuum manifold (*optional*) (EveryPrep Universal Vacuum Manifold, Life Technologies #K2111-01)
- Spectrophotometer (Nanodrop 2000c Spectrophotometers, Fisher Scientific, #ND-2000C).

REAGENT RECIPES

GES Recipe (per 100ml)

- Mix
 - 60g guanidine thiocyanate *This compound is corrosive; wear a mask.*
 - 20ml 0.5M EDTA (pH8)
 - 20ml sterile ddH₂O
- Heat to 65°C
- Cool to room temperature
- Add 1g N-lauroyl sarkosine

- Adjust to 100ml using sterile ddH₂O
- Filter sterile
- (Store at RT)

200mM Sodium Phosphate (monobasic) NaH₂PO₄ (per 200ml)

- Measure 4.8g of monobasic NaH₂PO₄ per 200ml
- Add sterile H₂O up to 150ml
- Measure the pH of the solution and normalize to pH of 8
- Adjust volume to 200ml, if necessary
- Filter sterile
- (Store at RT)

Lysozyme (10ml of 100mg/ml)

- Add 1g of lysozyme to a 15ml falcon tube
- Adjust ddH₂O to 10ml
- Mix, filter sterile, and aliquot out 1ml into 1.75ml eppendorf tubes
- (Store at -20°C)

Proteinase K (10ml)

- Measure 0.2g of Proteinase K
- Add:
 - 150µl 1M Tris
 - 1.5ml 0.1M Calcium acetate
 - 8ml ddH₂O
- Mix, filter sterilize, and aliquot out 1ml into 1.75ml eppendorf tubes
- (Store at -20°C)

PROTOCOL

Pre-work:

- 24 samples at a time is a manageable amount.
- Remove samples from the -80°C and thaw at room temperature.
- Label 1 set of 2ml screwcap, 1 set of 2ml eppendorfs, and 2 sets of 1.75ml eppendorfs.

1. Bead Beating

For sputum, plate pools, and liquid samples:

- Add 800µl of 200mM of monobasic NaPO₄ (pH=8) and 100µl of GES to a 2ml plastic screw top tube containing 0.2g of 0.1mm glass beads (Mo Bio Laboratories, #13118-50).
- Add 300µl of sample
 - For plate pools: this step may require the use of pipette tips whose tip has been cut back 0.5cm with scissors before autoclaving. This provides a bit more space for highly viscous samples.

- For sputum samples: the addition of 300µl should be completed by repeated passage through a 1ml tuberculin tip syringe with 18 gauge needle.
- Mechanically lyse cells using a bead beater for 3 min set to homogenize at 3000rpm using the PowerLyzer.

For stool, biospies, and solid samples:

- Add 800µl of 200mM of monobasic NaPO₄ (pH=8) and 100µl of GES to a 2ml plastic screw top tube containing 0.2g of 2.8mm ceramic beads (Mo Bio Laboratories, #13114-50).
- Add 0.1-0.2g of sample
- Mechanically lyse the sample using a bead beater for 2 cycles of 3 min set to homogenize at 3000rpm with a 45s delay between cycles using the PowerLyzer.
- Add 0.2g of 0.1mm or 0.5mm glass beads (Mo Bio Laboratories, #13118-50, #13116-50) to the screw top tube with the sample and the 2.8mm ceramic beads.
- Mechanically lyse cells using a bead beater for 3 min set to homogenize at 3000rpm using the PowerLyzer.

2. Enzymatic Lysis (Part I)

- Add
 - 50µl lysozyme (100mg/ml) (Sigma-Aldrich, #L6876-10G)
 - 10µl RNase A (10mg/ml in H₂O) (Qiagen, #19101)
- Mix by vortexing.
- Incubate samples in a 37°C waterbath for 1-1.5 hours.

3. Enzymatic Lysis (Part II)

- Add
 - 25µl 25% SDS (diluted in ddH₂O, filter sterilized)
 - 25µl Proteinase K (Sigma-Aldrich, #P2308-1G)
 - 62.5µl 5M NaCl (sterilized)
- Mix by vortexing
- Incubate samples in a 65°C waterbath for 0.5-1.5 hours. *Preheat ddH₂O at this time for use in Step 5.*

4. Phenol-chloroform extraction

- Centrifuge screwcap tubes at 13,500g for 5min
- Add 900µl of 25:24:1 phenol-chloroform-isoamyl alcohol (Sigma-Aldrich, #P3803-400mL) to 2ml eppendorf tubes. *This step can be carried out as the screwcap tubes are being centrifuged.*
- Remove 900µl of supernatant and add to the 2ml eppendorf tubes, creating an equal volume solution of sample to phenol-chloroform.
- Mix by vortexing.
- Centrifuge eppendorf tubes at no more than 13,000g for 10min. *The phenol-chloroform weakens the plastic and eppendorfs can crack at higher speeds.*

5. Column Purify DNA

- Add 200µl of DNA binding buffer (DNA Clean and Concentrator-25, Cedarlane laboratories, #D4034) to 1.75ml eppendorf tubes. *This step can be carried out as the 2ml eppendorf tubes are being centrifuged.*
- Carefully transfer the top layer, avoiding taking up any of the interface, of the centrifuged sample to the DNA binding buffer in the 1.5ml eppendorf tubes; mix.
- Transfer this solution to a DNA column (DNA Clean and Concentrator-25, Cedarlane laboratories, #D4034) 600µl at a time.
 - Spin columns at a max speed of 12,000g or use a vacuum manifold to move the solution through the column; discard flow-through.
- Once sample has moved through the column, add 200µl of wash buffer (DNA Clean and Concentrator-25, Cedarlane laboratories, #D4034) to the column.
 - Spin columns at a max speed of 12,000g or use a vacuum manifold to move the solution through the column; discard flow-through.
- Repeat wash step once more.
- ****If using the vacuum manifold the tubes must be dried of residual wash buffer by a 1 minute spin at 13,500xg in the microcentrifuge.**
- Place the columns into a new, sterile 1.75ml eppendorf tube. Add 50µl of sterile DNase/RNase free ddH₂O preheated at 65°C to the center of each column.
- Incubate the columns at room temperature for 5min.
- Elute the DNA into the 1.75ml eppendorf tube by centrifuging the columns in the eppendorf tubes at a max speed of 12,000g for 1min.

6. Quantify DNA

- Quantify DNA using a spectrophotometer.

(Store DNA at -20°C).