**Pseudomonas DNA Extraction for MinION Sequencing**

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Description: **Extraction of DNA from pseudomonas bacterial culture utilizing chemical lysis and phenol:chloroform separation.**

**1 Materials**

* TE buffer
* EB buffer
* Genomic DNA solution
* Fresh lysozyme solution
* 20% Sarkosyl solution
* 5M NaCl
* 10mg/mL RNase A
* 10mg/mL proteinase K
* Phenol:chloroform:isoamyl alcohol: 25:24:1
* Chloroform:isoamyl alcohol, 24:1
* 3 M sodium acetate
* 70% and 100% ethanol
* Phase-lock light gel tubes

**2 Bacterial Culture**

1. Record sample info in results.
2. From a frozen stock, plate pseudomonas on MacConkey agar and grow overnight.
3. Select an individual colony and plate onto a new MacConkey plate. Grow overnight.

**3 Lyse Bacteria**

1. Scrape plates with sterile loop and add into tube with 200uL of genomic DNA solution.  Pipette up and down to resuspend bacteria in solution.
2. Add 50uL lysozyme solution (50mg/mL) and incubate for 1hr at 37C
3. Add 100uL of 20% Sarkosyl solution and 15uL RNase A (10mg/mL) and for 1hr at 37C.
4. Add 20uL of proteinase K (10mg/mL) and incubate at 56C for 1-2hr. If solution does not clear (cells are not lysed), continue incubation at 37C overnight.
5. Add 100uL of 5M NaCl and gently mix by inverting tube. Spin at high speed for 10 minutes.
6. Remove the supernatant to a new tube and use TE buffer (~100uL) to bring the volume up to 600uL. Transfer to a 2mL phase lock tube.

**4 Phenol:Chloroform Extraction**

1. Add 600uL of phenol phenol:chloroform:isoamyl alcohol to phase lock tube.
2. Gently mix on rotator for 5 minutes at 20rpm to form an emulsion – do not vortex.
3. Spin at high speed for 10 minutes.
4. Remove aqueous phase to new tube phase lock tube.
5. Repeat steps 1-4. Avoid pipetting any precipitate on top of phase lock gel. Should it resuspend, spin the phase lock tube for an additional 5 minutes.  If it cannot be avoided, remove the precipitate with a pipette and then extract the remaining supernatant.
6. Add 600uL of chloroform:isoamyl alchocol (24:1) to extracted aqueous phase from step 5.
7. Gently mix on rotator for 5 minutes, then spin at high speed for 10 minutes.
8. Remove aqueous phase to new tube.

**5 Precipitate DNA**

Note: due to volume, aqueous phase from the previous step may need to be split into two tubes and the DNA precipitated separately.  After resuspending the DNA in EB buffer can be pooled together if desired.

1. Add 50uL of 3M sodium acetate (pH 5.2) and 3 volumes of cold 100% ethanol.
2. Spin at full speed for 5 minutes.
3. Decant off supernatant then wash pellet with 500uL of 70% EtOH.
4. Spin at full speed for 5 minutes.
5. Remove ethanol and dry pellet in 55C dry block for 5 minutes – do not over dry the pellet.
6. Resuspend DNA in 50-100uL of EB buffer and store at 4C overnight – do not freeze DNA.
7. Heat DNA at 65C for 10 minutes and then leave at room temperature overnight.
8. Quantify DNA on Qubit and Nanodrop; record in results.
   * If the concentration is very high (>1ug/uL), create a 1:10 diluted stock of DNA for quanitfication.
9. DNA can be stored at 4C.  Prior to library prep, repeat step 7 the day before to reduce DNA viscosity.