[Pseudomonas DNA Extraction for MinION Sequencing](https://my.scinote.net/protocols/400990/edit_name_modal.json)

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Description: [**Extraction of DNA from Pseudomonas bacterial culture utilizing chemical lysis and phenol:chloroform separation**](https://my.scinote.net/protocols/400990/edit_description_modal.json)

Protocol steps

**1** [**Materials** | Published on *21.05.2018 11:47* by *Emily Reesey*](https://my.scinote.net/protocols/400990/edit#step-panel-906171512)

* TE buffer
* EB buffer
* Genomic DNA solution
* Fresh lysozyme solution
* 20% Sarkosyl solution
* 5M NaCl
* CTAB Solution
* 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** | Published on *21.05.2018 11:47* by *Emily Reesey*](https://my.scinote.net/protocols/400990/edit#step-panel-906171511)

1. Add sample to inventory if not already added.  Assign to SciNote project.
2. From a frozen stock, plate pseudomonas on MacConkey agar and grow overnight at 37C.
3. Select an individual colony and plate onto a new MacConkey plate. Grow overnight at 37C.
   * For broth cultures, innoculate 3mL media with an individual colony.  Grow overnight at 37C rotating.

**3** [**Lyse Bacteria** | Published on *21.05.2018 11:47* by *Emily Reesey*](https://my.scinote.net/protocols/400990/edit#step-panel-906171510)

1. Scrape plates with small sterile loop and add one loop-full of bacteia into tube with 200uL of genomic DNA solution. Pipette up and down to resuspend bacteria in solution.
   * For broth cultures, spin down culture.  Remove the media and the viscous non-pelleted debris above the pellet.  Resuspend the bacterial pellet in 200uL genomic DNA 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 incubate for 1hr at 37C.
4. Add 20uL of proteinase K (10mg/mL), mix well 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 the tube.
6. Add 100uL CTAB (heated to 65C) and gently mix by inverting the tube.  Incubate at 65C for 10 minutes.
7. Add 100uL TE buffer to sample.
8. Transfer to a 2mL phase lock tube.

**4** [**Phenol:Chloroform Extraction** | Published on *21.05.2018 11:47* by *Emily Reesey*](https://my.scinote.net/protocols/400990/edit#step-panel-906171514)

1. Add 600uL of phenol phenol:chloroform:isoamyl alcohol to phase lock tube.  Invert tube three times.
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 6. 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.  Invert tube three times.
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** | Published on *21.05.2018 11:47* by *Emily Reesey*](https://my.scinote.net/protocols/400990/edit#step-panel-906171513)

1. Add 50uL of 3M sodium acetate (pH 5.2) and 2-3 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.  Flick tube to dislodge pellet and any DNA on the side of the tube which did not pellet previously.
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.  DNA should form a clear or white viscous pellet at the bottom of the tube.
6. Resuspend DNA in 50-100uL of EB buffer and store at 4C overnight – do not freeze DNA.  Flick tube to resuspend DNA.  Do not pipette up and down as this can shear the 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.