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Genomic DNA Extraction

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Genomic DNA extraction from *E. coli*

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1. Don't place any vials containing SDS into an ice tray as the SDS will precipitate
2. Use a laminar flow hood while working with any cultures in autoclaved reagents or cultures
3. Don't vortex any tubes
4. Dab with a paper towel before putting any new sample on NanoDrop
5. Look for a peak at 260 nm, and 260/280 and 260/230 ratios over ~1.8 to confirm a pure gDNA extract
6. NanoDrop may provide an overestimate of DNA concentration (due to the presence of other contaminants like RNA)

1. Cell Pellet
2. MilliQ Water
3. SDS (10%)
4. Proteinase K (20 mg/mL)
5. RNase A (10 mg/mL)
6. Phenol-Chloroform (1:1)
7. Isopropanol (100%)
8. Chilled 70% Ethanol
9. Sodium Acetate (3M, pH=5.2)

Phenol-Chloroform mixture contains some amount of Isoamyl Alcohol which allows the phase separation to appear distinct

1. Use gloves while handling Phenol-Chloroform
2. Do not leave Phenol-Chloroform flask open unnecessarily
3. Keep Phenol-Chloroform flask covered completely with aluminium foil, and store in a dark place

Preparing the cell pellet (E coli)

10m

1 Prepare cell pellet by centrifuging an overnight culture of E. coli at **4 °C** and **7000 rpm** for **00:10:00**

Extracting the genomic DNA (gDNA)

2 Gently resuspend the cell pellet in **500 µL** of autoclaved MilliQ water by mixing with a pipette and transfer into a **2 mL** microcentrifuge tube (MCT) in a laminar flow hood.

3 Add **75 µL** of SDS and **3 µL** of proteinase K (or how much ever volume is needed to achieve a final concentration of 100 ug/mL).

5m

4 Heat at **95 °C** for **00:05:00** to inactivate proteinase K.

10m

5 Let the tube cool to **Room temperature**

Add **5 µL** of RNase A (or how much ever volume is needed to achieve a final concentration of 200 ug/mL).

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Leave the tube at room temperature for **00:10:00** .

6 Add **1 mL** of Phenol-Chloroform mixture to the tube. 10m

Centrifuge at **10.000 rpm** for **00:10:00** at **4 °C**

The contents of the tube should phase separate into three layers: an aqueous layer on top, a viscous jelly-like layer in the middle, and a layer of chloroform at the bottom.

7 Collect the aqueous layer and the jelly-like layer using a cut tube and transfer them into a fresh MCT. 10m

Add **1 mL** of Phenol-Chloroform mixture to this tube.

Centrifuge again at **10.000 rpm** for **00:10:00** at **4 °C**

The contents of the tube should phase separate into three layers as before.

8 Collect only the aqueous layer at the top and transfer it into a fresh MCT.

Add **160 µL** of **3 Molarity (M)** Sodium Acetate to this tube.

Mix gently.

9 Add **1 mL** of Isopropanol to the tube and mix gently by inversion till white strands of DNA precipitate out 10m

Centrifuge at **5000 rpm** for **00:10:00** at **4 °C**

10 Discard the supernatant. 10m

Add **1 mL** of Chilled 70% Ethanol gently along the walls of the tube, without disturbing the DNA pellet.

Centrifuge at **5000 rpm** for **00:10:00** at **4 °C**

11 Air-dry the tube till there isn't any ethanol remaining.

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12 Resuspend the DNA pellet gently in **100 µL** of autoclaved MilliQ water by mixing with a pipette under a laminar flow hood.

Store the suspension in a **-30 °C** refrigerator.

Using NanoDrop Spectrophotometer to measure gDNA concentration

13 Load **1 µL** of autoclaved MilliQ water as blank on NanoDrop and load **1 µL** of gDNA suspension as the sample to measure its concentration.

Verifying gDNA presence using Gel Electrophoresis

14 Prepare a 1% agarose gel by adding **0.5 g** of agarose in **50 mL** of TAE buffer with **2 µL** of ethidium bromide.

15 Load **3 µL** of 1kb DNA Ladder into the first lane.

Load **1 µL** gDNA + **1 µL** dye into the second lane.

Run the gel.

Observe for a single band above the first band of the ladder, close to the well.

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