Purification of Genomic DNA from Bacteria

The Epicentre MasterPure Kit isolates genomic DNA while removing other bacterial componens such as cell envelope, RNA and proteins. The DNA is precipitated with isopropanol, an uncharged organic solvent that interacts poorly with the charged DNA molecules.

Part A. Removal of cell components

1. From stock, obtain for all your group samples 4 ml of Tissue/Cell Lysis Solution containing Proteinase K (50 mg/ml diluted 1:300).

2. For each overnight bacterial culture: Spin 0.5 ml bacterial culture in microfuge for 10 min. Remove supernatant and save the pellet. It's ok at this step to leave a small drop of liquid with the pellet.

3. Add 300 µl Tissue/Cell Lysis Solution/Proteinase K to pellet. Mix with pipet tip to make sure pellet is resuspended. (The lysis solution contains a detergent that dissolves cell membranes.)

4. Incubate at 65°C for 15 min. During this period, vortex every 5 min. (The Proteinase K cleaves all bacterial proteins.)

5. Incubate samples at 37°C for 2 min. Add 1 µl of 5 µg/µl RNase A; mix briefly, without bubbling. Incubate at 37°C for 30 min. (The RNase A cleaves all bacterial RNA.)

6. Place samples on ice for 3 min.

Part B. Precipitate DNA.

1. From stock, obtain for all your group samples 2 ml MPC Protein Precipitation Reagent.

2. For each DNA sample on ice: Add 175 µl of MPC Reagent. Vortex for 10 sec. (Protein and other cell debris now precipitate.)

3. Spin in microfuge for 10 min. **Save SUPERNATANT.** Transfer supernatant to a clean microfuge tube. (The supernatant contains DNA.)

4. Add 500 µl ice-cold isopropanol to the supernatant. Invert tube 30 times. Avoid shaking or bubbling. (The DNA is now coming out of solution.)

5. Spin tube in microfuge in cold room for 10 min. Use pipet to pull off supernatant and discard.

6. Add 100 µl of 75% ethanol to pellet (to remove isopropanol mixture). Spin briefly in microfuge. Repeat adding 100 µl of 75% ethanol and spin. Use pipet to remove ethanol completely, leaving small white DNA pellet.

7. Let tubes sit 30 min open to evaporate ethanol. Add 35 µl of EB buffer (10 mM Tris pH 8.5). Freeze DNA at -20°.