

Isolation of Plasmid DNA from MiniPreps – derived from Mfg protocol

Materials:

AMRESCO Cyclo-Prep Plasmid DNA Purification Kit (Cat#. K179)

Solution 1 Re-suspension Buffer

Solution 2 Lysis Buffer

Solution 3 Neutralization Buffer

Wash Solution with Ethanol

Cyclo-Prep Spin Columns

Collection Tubes

Micro-centrifuge tubes (1.5 ml, autoclaved)

Centrifuge Tube 50l (waste collection tube)

TE Buffer 1x (Amresco) (Pre-heated to 70 deg. C)

Procedure:

0. Freeze 500 ul of each mini-prep bacterial culture by mixing with 500 ul sterile Glycerol and storing at -80 deg. C.
1. Transfer remaining bacterial culture into 1-3 micro-centrifuge tubes. Pellet the bacteria by spinning at $\geq 14,000g$ for 1 minute. Remove and discard supernatant into the waste tube.
(Place the TE Buffer tube in a 70 deg. C heater.)
2. Add **200 ul Solution 1** (Resuspension Buffer) to a tube from each group of tubes (i.e. 1a) then re-suspend and combine pellets from each group (i.e. 1a, 1b and 1c) by pipetting up and down until a single cell suspension is made. Discard empty tubes.
3. Add 200 ul **Solution 2** (Lysis Buffer) to each tube (i.e. 1a, 2a, 3a, 4a & 5a) mix thoroughly by inverting tubes 5-10 times.
4. Add 200 ul **Solution 3** (Neutralizing Buffer) to each tube (i.e. 1a, 2a, 3a, 4a & 5a) mix thoroughly by inverting tubes 5-10 times. (A white precipitate will form)
5. Pellet the precipitate by spinning at $\geq 14,000g$ for 5 Minutes. (Precipitate will pellet on the bottom or form a line on the side of the tube.)
6. Place a **Cyclo-Prep Spin Column** in **Collection Tube** for each sample tube; then, carefully transfer the supernatants from the sample tubes to the Spin Columns, leaving the precipitate.
7. Spin the columns at $\geq 14,000g$ for 30 seconds and discard the filtrate into the waste tube, re-using the collection tube.
8. Add 700 ul **Wash Solution** to each Spin Column, Spin at $\geq 14,000g$ for 30 seconds. Discard the wash solution flow-through into the waste tube, re-using the Collection Tube.
9. Add 350 ul **Wash Solution** to each Spin Column, Spin at $\geq 14,000g$ for 30 seconds. Discard the wash solution flow-through into the waste tube, re-using the Collection Tube.
10. Spin the empty Spin Column at $\geq 14,000g$ for 3 minutes to remove residual ethanol. Discard the collection tube containing ethanol and transfer the spin column to a new LABELED 1.5 ml micro-centrifuge tube.
11. Add 50 ul of HEATED **TE Buffer** to each Spin Column and elute DNA by spinning at $\geq 14,000g$ for 1 minute.
12. Re-apply the TE Buffer with DNA to the Spin Column and spin at $\geq 14,000g$ for 1 minute. (Discard Spin Column)
13. Assess sample DNA concentration using the TE Buffer for a blank, then store the DNA at -20 deg. C or set up analytical enzyme digestion.