DNA Extraction&Purification Protocol

[Plasmid Propagation & Extraction]

We use EasyPure® Plasmid MiniPrep Kit by TransGen to extract plasmid from *E. coli* cells.

The plasmid propagation and extraction protocol

Step1. Take colony sample into 5 mL tube with LB to culture overnight about 10-12 h.

Step2. Centrifuge the cultured *E. coli* solution at 10000 g for 1 min to collect cells.

Step3. Add 250 μL solution RB (Resuspension Buffer with RNase A stored at 4 °C) in each tube and vortex to resuspend the cells.

Step4. Add 250 μL solution LB (Lye Buffer) and flip over the tube 4-6 times to split the cells.

Step5. Add 350 μL solution NB (Neutralization Buffer) to neutralize the mix and settle the cell debris.

Step6. Centrifuge at 12000 g for 5 min to separate the sediment and supernatant and transfer solution in to new clean purification columns.

Step7. Add 650 μL WB (Wash Buffer with Ethanol) in each columns and centrifuge at 12000 g for 1 min to clean the tube.

Step8. Centrifuge tubes again at 12000 g for 2 min to flow residual liquid and change new clean 1.5 mL tubes.

Step9. Put in the air for 15 min to volatilize ethanol.

Step10. Add 30 μL ddH_2O(65 °C pre-heat)and centrifuge at 10000 g for 1 min to elute plasmid.

[DNA Electrophoresis & Gel Extraction]

We use agarose by Biowest and TAE buffer (Tris-HCl, EDTA, Acetic acid) to make 1.0% agarose gel and add 1:10000 Gelstain by Transgen nucleic acid dye. We used DNA markers and loading buffer from Transgen and Dongsheng Biotech.

We use DNA agarose gel extraction kit by Axygen to recycle DNA from gel.

The DNA agarose gel extraction protocol

Step1. Cut the gel contained target DNA and add 500 μL Buffer DE-A to keep in metal bath at 75 °C for about 10 min to melt the gel.

Step2. Add 150 μL Buffer DE-B and mix. If the DNA segment is shorter than
400 bp, add 150 μL extra isopropanol.

Step3. Transfer the mixed solution into new clean recycle columns to centrifuge at 12000 g for 1 min.

Step4. Add 500 μL Buffer W1 to centrifuge at 12000 g for 1 min.

Step5. Add 700 μL Buffer W2 to centrifuge at 12000 g for 1 min
Step6. Centrifuge tubes again at 12000 g for 2 min to flow residual liquid and change new clean 1.5 mL tubes.
Step7. Put in the air for 15 min to volatilize ethanol.
Step8. Add 30 μL ddH_2O(65 °C pre-heat)and centrifuge at 10000 g

[Genome Extraction]

We use TIANamp Bacteria DNA Kit by TIANGEN BIOTECH to extract genome from *E. coli* cells.
The genome extracrion protocol
Step1. Take 1-5 mL cultured *E. coli* solution into a tube and centrifuge the solution at 11500 g for 1 min to collect cells.
Step2. Add 200 μL solution GA in each tube and vortex to resuspend the cells.(For G- bacteria like *E. coli.* Extra treatment may be needed for G+ bacteria to break cell wall.)
Step3. Add 20 μL solution Protease K.
Step4. Add 220 μL solution GB, vortex for 15 s and keep in metal bath at 70°C for about 10 min.
Step5. Add 220 μL ethanol, vortex for 15 s and briefly centrifuge to remove the droplet from the inner wall of the tube.
Step6. Transfer both solution and sedimentation in to new clean purification columns and centrifuge at 13400 g for 30 s.
Step7. Add 500 μL solution GD and centrifuge at 13400 g for 30 s.
Step8. Add 600 μL solution PW and centrifuge at 13400 g for 30 s.
Step9. Repeat Step8.
Step10. Centrifuge tubes again at 13400 g for 2 min, put in the air for several minutes to volatilize ethanol and change new clean 1.5 mL tubes.
Step11. Add 50-200 μL solution TE, keep in room temperature 2-5 min and centrifuge at 13400 g for 2 min to elute DNA.