

Plasmid extraction protocol

A. Theory :

Extract plasmid from the vector which may be bacterium, yeast, or other

B. Materials :

1. Column
2. Plasmid extraction kit

C. Laboratory procedures :

1. Culture medium centrifuge
 - 6000rpm
 - 30 mins
 - 4 degrees Celsius
 - Balance with ddH₂O
2. Add solution 1
 - Each tube 400 μ l
 - Shake
 - Add to eppendorf
3. Add solution 2
 - 400 μ l
 - Reverse up and down 40 times
 - Rest for 5 minutes
4. Add solution 3
 - Repeat the same steps as step C but add solution 3
5. Centrifugation : 13000 turns, 10 minutes
6. Pre-heat elution solution : 65°C
7. Prepare new tube again , put column on top
 - Column only has 650 μ l , therefore add two times
 - 600 μ l each time
 - Centrifuge 1 minute after each addition , 13000 turns
8. Add washing buffer
 - 600~650 μ l x 2 times
 - Each time centrifuge 1 minute 13000 turns
 - Centrifuge 5 minutes in the end
9. Add elution buffer
 - 40 μ l
 - Add to middle hollow precipitator
 - Centrifuge 13000 turns 1 minute