Plasmid Isolation Scheme

Modified from Ahn S.C., Baek B.S., Oh T., Song C.S., & Chatterjee B.(2000) Rapid mini-scale plasmid isolation technique for DNA sequencing and restriction mapping. *Biotechniques*. **29,** 466-468.

1. Inoculate 2mL LB medium (antibiotic) with a bacterial clone, culture overnight with vigorous shaking at 37°C and harvest bacteria by centrifugation at 11000x **g** for 1 min.

2. Aspirate culture supernatant and resuspend the pellet completely in 100 μL **resuspension buffer**.

3. Add 100 μL **lysis buffer** and mix gently at room temperature.

4. Add 120 μL **neutralization buffer** and mix gently for 3 min at room temperature.

5. Remove bacterial debris by centrifugation at 11000x **g** for 1 min and transfer supernatant to a fresh 1.5 mL microcentrifuge tube.

6. Add 200 μL isopropanol to precipitate plasmid DNA and mix thoroughly for 1 min at room temperature.

7. Collect DNA pellet by centrifugation at 11000x g for 30 s and aspirate supernatant.

8. Wash DNA pellet with 500 μL 70% ethanol.

9. Collect the DNA pellet by centrifugation at 11000x **g** for 30 s and aspirate supernatant.

10. Add 100 μL sterile water to dissolve air-dried DNA.

Solutions:

resuspension buffer:
- 50mM Tris-HCl, pH 8.0
- 10mM EDTA, pH 8.0
- 20 μg RNaseA

Lysis buffer
- 200 mM NaOH
- 0, 1% SDS

neutralization buffer
- 3 M potassium acetate, pH 5.5