Alkaline Lysis Plasmid Preparation from *E. coli*

This protocol yields plasmid DNA that is suitable for restriction digests and cloning purposes. This preparation method works well on all *E. coli* strains and also exponentially growing *Rhizobium meliloti* cultures.

1. Grow a 2-3 ml culture in rich media with appropriate antibiotic selection. (10 ml culture of *R. meliloti*)

2. Pellet 1.5 ml in a microfuge tube for 30 sec-2 min. (10 ml for *R. meliloti*)

3. Pour off supernatant and vortex pellet until a homogenous cell paste is obtained.

4. Add 0.2 ml freshly made Lysozyme solution, mix gently and incubate on ice for 5 min.

Lysozyme solution: 1 ml GET* + 5 mg lysozyme

5. Add 0.4 ml of freshly made NaOH - SDS solution. Mix gently by rocking tube. The solution should turn translucent as cells lyse. Incubate on ice 5 min.

NaOH - SDS solution (per ml): 0.7 ml water
0.2 ml 1 M NaOH
0.1 ml 10% SDS

6. Add 0.3 ml Potassium Acetate Stock** and vortex gently. A precipitate should form. Can be frozen at this point to increase yield but not required.

7. Centrifuge for 10 min on maximum.

8. Carefully remove 0.75 ml of the supernatant and transfer to a clean and labeled microfuge tube. Be careful not to take any material at the interface. Add 0.45 ml (0.6 volume) of isopropanol and mix well using the vortexer.

9. Centrifuge for 10 min on maximum.

10. pour off supernatant and wash briefly with 0.4 ml cold 70% ethanol (rock tube 5 times). Pour off ethanol wash and spin 30 sec in microfuge. Remove remaining liquid with a micropipet and dry pellet in a vacuum.

11. Dissolve pellet in 20 μl TE buffer. Use one to three microliters for restriction digests. Store plasmid prep at 4° for days-weeks or -20° for months-years.

GET*: 50 mM glucose, 10 mM EDTA, 25 mM Tris-HCl pH 8.0

Potassium Acetate Stock**:
60 ml 5 M potassium acetate
28.5 ml glacial acetic acid
11.5 ml water
(pH 4.8)
store at room temperature