**Plasmid minipreps-Alkaline Lysis**

**Samples:**

1. Grow cells overnight in 2-3ml LB + antibiotic. Note: to make a glycerol stock of the culture: To 0.5ml of overnight culture, add same volume of 80% sterile glycerol and store at -70°C.

2. Pre-chill: GET, 3M K+/5M OAc-, 100% EtOH and 70% EtOH

3. Transfer cultures from overnight incubation to microfuge tubes. Spin in microfuge at room temperature. Aspirate supernatant.

4. Repeat step#3 with remaining culture if a 3ml overnight culture was started

5. Resuspend pellets in 100µl ice-cold Get (or SET). Incubate at room temperature for at least 5 minutes.

6. Add 200µl fresh lysis buffer

<table>
<thead>
<tr>
<th>Lysis buffer</th>
<th>Final concentration</th>
<th>add</th>
<th>Stock</th>
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</thead>
<tbody>
<tr>
<td>0.2N NaOH</td>
<td>0.5ml</td>
<td>2N</td>
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<tr>
<td>1% SDS</td>
<td>250µl</td>
<td>20%</td>
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<tr>
<td>gdw</td>
<td>4.25 ml</td>
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7. Mix by inversion- do not vortex. Incubate on ice for 5 minutes.

8. Add 150µl ice-cold3M K+/5M OAc-. Mix by inversion or gentle vortexing for 10 sec. Incubate on ice for 5 minutes.

9. Microfuge at 4°C for 5 minutes. Transfer supernatant to a fresh tube; avoid white precipitate. Repeat this step if necessary.

10. Add 2.5 volumes ice-cold 100% EtOH-essentially fill the tube by adding about 1 ml. Mix well and allow to precipitate 10-30 minutes at -70°C.

11. Spin in microfuge at 4°C for 5 minutes. Discard supernatant.

12. Rinse pellet with 70% EtOH (pre-chilled), microfuge for 2 minutes at 4°C. Discard supernatant, drain tubes thoroughly on a kimwipe.

13. Dry pellets on bench-top (or in Speed-vac; no heat!). Pellet should be transparent when dry.

14. Resuspend DNA in 10-50µl TE + 10µg/ml RNase A. Store at 4°C until ready to do digests

15. Mini-prepped DNA can be immediately used for restriction analysis (2-5µl per digest). 2µl is typically fine unless you have (or suspect) an unusually low yield. You should get about 1-4µg DNA per 1.5ml culture.