MINI-PREP DNA

“Mini-prep” DNA is a term often used to describe the purity of the DNA more than the quantity.

A more precise name would be “alkaline lysis” miniprep DNA.
(higher quality DNA is “CsCl banded” or “Column purified”)

Types of MP DNA

<table>
<thead>
<tr>
<th>DNA Type</th>
<th>Method Description</th>
<th>Purity</th>
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<tbody>
<tr>
<td>-MP DNA:</td>
<td>Alkaline Lysis prep followed by EtOH ppt.</td>
<td>&gt; moderate</td>
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<tr>
<td>-Clean MP:</td>
<td>Alkaline Lysis followed by Phenol Choloroform and EtOH ppt.</td>
<td>&gt; good</td>
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<tr>
<td>-Qiagen MP:</td>
<td>Alkaline Lysis followed by various types of Qiagen columns.</td>
<td>&gt; sequencing</td>
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<pre><code>                                                                                 | grade        |
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NOTES: Always start the growth of DNA from a single colony of a freshly streaked plate.
Grow a 1-5 ml Overnight culture to start, (overnight is a relative term: 8-16 hrs)
If you are making more DNA, seed a larger flask with this overnight stock.
Don’t try growing 200+ mls from a single colony.

Alkaline Lysis:
A modification of the Ish-Horowitz Protocol, but the volumes of Solns I, II and III are identical.

Solutions are the same as for the Qiagen protocols:

<table>
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<th>Solution</th>
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<tr>
<td>P1</td>
<td>(Solution I) Stock 6.06g Tris base</td>
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<tr>
<td></td>
<td>3.72 g Na2 EDTA.2H2O in 800 mls H2O, then adjust pH to 8.0 with HCl, qS to 1000 w/ H2O</td>
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<tr>
<td>P2</td>
<td>(Solution II) dissolve 4.0 g NaOH pellets in 475 mls, H2O</td>
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<tr>
<td>P3</td>
<td>(Solution III) dissolve 294.5g Potassium Acetate in 500 mls H2O (not more!!!)</td>
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<td></td>
<td>-then adjust pH to 5.5 with Glacial Acetic Acid (approx... 110 mls)</td>
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<td>-Adjust volume to 1 liter with H2O</td>
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METHOD: -For 1-5 ml cultures use 250 ul of P1/P2/P3
-For larger cultures use 4 mls of each per 100-200 mls of LB used

1. Spin down bacteria and discard the media
2. **Add 250 ul P1**
3. Mix until all bacteria are resuspended
4. **Add 250 ul P2**
5. Mix gently and let stand 2-4 min (NOT longer than 5 min)
6. **Add 250 ul P3** (or 350ul N3 if QIAprrep)
7. Mix gently
8. Place in micro fuge and spin 12000 rpm for 5 min.

https://labs.feinberg.northwestern.edu/arisp/protocols-reagents/index.html
9. **Transfer supernatant** to a fresh tube
10. **Add 700 ul Propanol**, Mix gently
11. Centrifuge 15 min at 12000 rpm
12. Remove supernatant and dry slightly
13. **Add 750 ul 70% EtOH**, Spin again
14. Remove supernatant and dry DNA pellet thoroughly
15. Resuspend in **50-100 ul TE** (use 5 ul for test digests)

**“Clean” MP DNA**

9b. To the transferred supernatant, Add 700 ul Phenol/CH$_2$Cl$_2$ (1:0.8)
9c. Mix vigorously, and Centrifuge 12000 rpm for 5 min
9d. Transfer supernatant to fresh tube and continue with step 10 above.