MINI-PREP DNA

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Oct 9, 1999

"Mini-prep" DNA is a term often used to describe the purity of the DNA more than the quantity.
A more presise name would be "alkaline lysis" miniprep DNA.
(higher quality DNA is "CsCl banded" or "Column purified")

Types of MP DNA		**Purity**
- MP DNA:	Alkaline Lysis prep followed be EtOH ppt	-› moderate
- Clean MP	Alkaline Lysis followed by Phenol Chloroform and EtOH ppt.	-› good
- Qiagen MP	Alkaline lysis followed by various types of Qiagen columns.	-› Sequencing grade

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: 8-16hrs)
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:

P1 (Solution I) -Stock 6.06 g **Tris base**
3.72 g **Na_2 EDTA.2$H_2$0**
in 800 mls $H_2$0, then adjust the pH to 8.0 with HCl, qs to 1000 w/ $H_2$0
-before use, **add 100 ug/ml RNAse A**

P2 (Solution II) -disolve 4.0 g **NaOH pellets** in 475 mls, $H_2$0
-Add 25 mls 20% **SDS**

P3 (Solution III) -disolve 294.**5g Potassium Acetate** in 500 mls $H_2$0 (not more!!!!)
- then adjust the pH to 5.5 with Glacial Acetic Acid (approx. 110 mls)
- Adjust volumn to 1 liter with $H_2$0

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 QIAprep)
7. Mix gently
8. Place in micro fuge and spin 12000 rpm for 5 min.
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_3Cl_4 (1:0.8)
9c. Mix vigourously, and Centrifuge 12000 rpm for 5 min
9d. Transfer supernatant to fresh tube and continue with step 10 above.