ALKALINE LYSIS MINIPREP

In the following protocol, plasmid DNA is prepared from small amounts of many different cultures of plasmid-containing bacteria. Bacteria are lysed by treatment with a solution containing sodium dodecyl sulfate (SDS) and NaOH (SDS denatures bacterial proteins, and NaOH denatures chromosomal and plasmid DNA). The mixture is neutralized with potassium acetate, causing the plasmid DNA to reanneal rapidly. Most of the chromosomal DNA and bacterial proteins precipitate -- as does the SDS, which forms a complex with potassium -- and are removed by centrifugation. The reannealed plasmid DNA is then concentrated by ethanol precipitation.

Minipreps of Plasmid DNA

Most manipulations are performed in microcentrifuge tubes. This procedure is appropriate for preparation of small amounts of DNA from 1 to 24 cultures of plasmid-containing bacteria.

Materials

- LB medium
- TE buffer
- NaOH/SDS solution (Solution II)
- Potassium acetate solution (Solution III)
- 100% ethanol
- 70% ethanol
- 20ug/ml DNase-free RNase (optional)

1. Inoculate 5 ml LB medium with a single bacterial colony. Grow to saturation (overnight).
2. Spin 1.5 ml of cells 20 sec in a microcentrifuge to pellet. Remove the supernatant with a Pasteur pipet, or aspirate using a pulled Pasteur pipet. This spin can be at 4øC or at room temperature
3. Resuspend pellet in 100ul glucose TE buffer (Solution I).(Let sit 5 min at room temperature;optional) Be sure cells are completely resuspended
4. Add 200ul NaOH/SDS solution (Solution II), mix by tapping tube with finger,or inverting the tube gently twice,and place on ice.
5. Add 150ul potassium acetate solution (Solution III) and vortex at highest speed for 2 sec to mix. Be sure mixing is complete
6. Spin 3 min in microcentrifuge to pellet cell debris and chromosomal DNA. This spin can be at 4øC or at room temperature
7. Transfer supernatant to a fresh tube, phenol extract with 450ul of buffered phenol. Mix well, spin 5 seconds in microfuge to separate phases. Remove organic phase (bottom) carefully with a pulled Pasteur pipet and discard in flammable waste disposal, not down the sink! Add 0.6 vol of 100% isopropanol (300ul), vortex to mix.
8. Spin 5-10 min at room temperature to pellet plasmid DNA and RNA.
9. Remove supernatant, wash the pellet with 200ul of 70% ethanol, and dry pellet under

vacuum.

NOTE: ETHANOL IS HIGHLY FLAMMABLE; USE WITH CAUTION NEAR OPEN FLAME.

10. . Resuspend the pellet in 20ul (TE buffer + 20ug/ml RNase). 1 to 2ul of the resuspended DNA should be used for a restriction digest.

IMPORTANT NOTE: Phenol is an organic solvent and will cause severe burns if it comes into contact with your skin! The smell of it is also very unpleasant and irritating to the nostrils. It should be used with great care, wearing lab coat, gloves, eye protection and in the fume hood. Read the MSDS sheet before using this material.

Troubleshooting

If no plasmid DNA is obtained, try leaving out the RNase, isolating the plasmid DNA from a different strain, precipitating the plasmid DNA with ammonium acetate, or extracting with pheno. If the isolated DNA fails to cut with restriction endonucleases, the most common cause is inadequate washing of the pellets after the ethanol precipitation step. Inadequate washing of the pellets is sometimes, but not always, manifested in the pH of the final DNA solution suspended in TE buffer, which should be >6.5. Placing 5ul of the prep onto a piece of pH paper will establish this point. If the pH of the DNA is normal but it still fails to cut, precipitating the DNA a second time with ethanol, or washing the pellets from the first precipitation with 70% ethanol, will usually clean up the DNA enough to cut it. In general, pH problems can often be avoided by aspirating off the supernatants from the isopropanol precipitation and ethanol washes thoroughly.

Anticipated Results

Approximately 3ug DNA is obtained from 1.5 ml of a culture of cells that contains a pBR322-derived plasmid.

Time Considerations

With practice, this protocol can be used by a skilled practitioner to produce 12 or 24 samples of DNA from 12 saturated bacterial cultures in about 2.5 hr.