Modified 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 isopropanol precipitation and, RNA is removed by ammpmium acetate precipitation. The plasmid DNA is then concentrated by isopropanol precipitation. The plasmid DNA purified by this method can be used in in vitro transcription and translation.

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
- Solution I (glucose-TE)
- Solution II (NaOH/SDS solution)
- Solution III (Potassium acetate solution)
- 100% ethanol
- 70% ethanol
- Isopropanol
- 7.5M ammonium acetate

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 4 C or at room temperature
3. Resuspend pellet in 100 µl glucose TE buffer (Solution I).(Let sit 5 min at room temperature;optional) Be sure cells are completely resuspended
4. Add 200 µl NaOH/SDS solution (Solution II), mix by tapping tube with finger,or inverting the tube gently twice,and place on ice.
5. Add 150 µl potassium acetate solution (Solution III) and vortex at highest speed for 2 sec to mix. Be sure mixing is complete
6. Spin for 3 min in microcentrifuge to pellet cell debris and chromosomal DNA. This spin can be at 4 C or at room temperature
7. Carefully transfer the supernatant without any precipitate to another eppendorf and add 450uL 7.5M ammonium acetate. Mix by inverting tube and keep on ice 15 min.

Centrifuge for 15 min. This step precipitates more than 90% of the high molecular weight RNA and a certain quantity of proteins, replacing organic extractions and RNase digestion.

8. Transfer supernatant to another eppendorf and fill with isopropanol. Keep at -20 degrees C for 10 min. and centrifuge for 10 min. Wash pellet with 70% EtOH, dry, and resuspend in 20uL TE. The plasmid DNA should be >90% supercoiled with no visible chromosomal DNA. Yield is approx. 10ug for Bluescript and pUC derivatives.

   This DNA can be used for transcription and translation in cell free assays, but since it contains no Rnase, when you map it with restriction endonucleases you will see a large smear of RNA. If your dropouts are larger than 500 bp, you will still be able to see them. However, if your dropouts are smaller it is a good idea to add some TE+RNase A to either your digests, or your loading buffer.

   Reference: A modification of Birnboim and Doly (1979) by Michael Bender via Yves (Shapiro lab)