A one-tube plasmid DNA mini-preparation suitable for sequencing

Giannino Del Sal, Guidalberto Manfioletti and Claudio Schneider*

International Center for Genetic Engineering and Biotechnology, Padriciano 99, 34012 Trieste, Italy
Submitted August 8, 1988

The cationic detergent CTAB (cetyl trimethyl ammonium bromide) has been employed in the past for DNA and RNA separations (1,2). We have previously developed two simple methods for obtaining single strand DNA (3) and lambda DNA (4); here we extend the use of this detergent for the mini scale preparation of plasmid DNA . The advantage of this method is that all the manipulations, from the centrifugation of bacterial cells to the sequencing reactions, are performed in the same tube. The method described is a modification of the boiling prep (5). A single colony is inoculated in1.5 ml of L-Broth (50 µg/ml ampicillin) in 15 ml disposable tubes and cultured overnight . Bacterial cells are spun in an Eppendorf microfuge, resuspended in 200 µl of STET buffer (8%w/v sucrose, 0.1%v/v TritonX-100, 50 mM EDTA, 50 mM Tris-HCl pH 8.0) and incubated at RT for 5 minutes after addition of 4 µl of lysozyme (50 mg/ml). Samples are boiled for 45 seconds and centrifuged for 10 minutes. The pellet is removed using a toothpick ; 8 µl of CTAB (5%w/v) (Sigma-cat.#H5882) are added and the precipitate centrifuged for 5 minutes at RT. The pellet is resuspended in 300 µl of 1.2 M NaCl , by vigorous vortexing, and reprecipitated by addition of 750 µl of ethanol and centrifugation for 10 minutes. The final pellet is rinsed in 70% ethanol/water dried under vacuum and resuspended in 20 µl of TE. The average yield, using Bluescript plasmid (Stratagene), is around 5 µg of plasmid DNA. One tenth is used for yield check up and/or restriction analysis. The rest is used for the following sequencing steps without any RNase treatment.
Denaturation takes place by addition of 2 µl of 2 M NaOH in a final volume of 20 µl at 68 °C for 20 minutes. Following denaturation 8 µl of 5 M ammonium acetate pH 5.4 are added, and DNA is precipitated by addition of 100 µl of ethanol and incubation for 5 minutes in dry ice. After centrifugation for 10 minutes at RT, the denatured DNA is washed with 70% ethanol/water dried under vacuum, and used for the dideoxy-sequencing reactions according to the protocols of Sequenase (USB) or T7 Sequencing kit (Pharmacia).

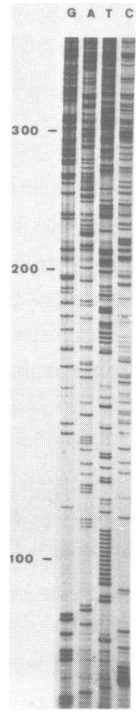

Fig. 1: Dideoxy sequencing using T7 polymerase (Pharmacia).

Acknowledgements
This work has been partially funded by " Progetto Strategico Genoma Umano " CNR, ITALY.
GDS is supported by "Associazione Italiana per la Ricerca sul Cancro" and the Istituto di Anatomia Patologica, Univ.di Trieste. GM is supported by "Area per la Ricerca-Trieste".

*To whom correspondence should be addressed

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