A QUICK AND ECONOMICAL METHOD TO ISOLATE PLASMID DNA FOR LARGE SCALE SEQUENCING

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ABSTRACT: Isolating plasmid DNA for large scale sequencing demands automated liquid handling facility which is expensive and labs with limited resources cannot afford them. In view of the limitations of resources and time we have designed and standardized a simple method based on lysing the bacterial cells by boiling to isolate the plasmid DNA, which can be subsequently sequenced on automated DNA sequencer without the need for further purification. The sequencing quality and the number of base read through are comparable to the standard procedure followed for isolating plasmid DNA.
Key words: plasmid isolation, large scale sequencing.

Experimental procedures:

Plasmid DNA is routinely isolated using either alkaline lysis (1) or boiling lysis (2) Method. Although, the above methods yield quality DNA which can be used for downstream processing, these methods cannot be readily adapted for large scale DNA isolation without the help of robotic instruments. One can isolate plasmid DNA from 96 clones in less than an hour using robotic machine like liquid handling system. But, the cost involved in buying and maintaining such facilities is beyond the scope of small lab.
In order to find an appropriate method requiring less time and manipulation for isolation of plasmid DNA from large number of individual clones we tried using 96 dip well culture plates(used routinely for growing bacterial culture in robotic liquid handling system for isolating plasmid DNA) for plasmid DNA isolation. Bacterial cells were collected by centrifugation in the same dip well plate. Cell pellet suspended in TE buffer and the whole plate was kept in boiling water bath for five minutes and immediately shifted on ice for five minutes. The plate was centrifuged and the supernatant containing the plasmid DNA was transferred to a fresh 96 well costar cell culture plate. The plasmid DNA can subsequently be used for automated sequencing work. The procedure takes ~30 minutes.
We used a c-DNA library constructed from root tips of Arachis hypogia plant cloned in PTZ57R/T vector (Fermentas Life Sciences) with XL-1 blue E.coli as host. The insert size ranges from 200bases to 2.5Kb. 96 independent bacterial clones were inoculated from their glycerol stocks into 1ml LB medium per each dip well with appropriate antibiotic and grown at 37º c for 22 hours in (Innova 4330 refrigerated gyratory incubator).The grown cultures were centrifuged in eppendorf refrigerated centrifuge 5810R at 4000rpmfor 15 min at 4ºc.The supernatant was decanted carefully and any left over medium was removed by inverting the plate and tapping onto filter paper towel. The cell pellet was re-suspended in 50µl of TE buffer with 20µg/ml Rnase free of Dnase. (Novagen) The 96 well plates containing re-suspended bacterial pellet was incubated in a boiling water bath for five minutes. The plate was covered with its lid during boiling to avoid loss of water. The whole plate was immediately transferred onto ice and allowed to remain for 5min.The lysed bacterial culture was centrifuged at 4000rpm for 10min.at 4ºc.

The supernatant was carefully removed and transferred to 96 well costar cell culture plate. The supernatant having plasmid DNA can be directly used to set up the sequencing PCR after DNA estimation by nano drop method. The plasmid DNA is loaded on 1% agarose gel electrophoresis, we observed pure plasmid DNA compared to normal plasmid isolation procedures (Figure 1).

We routinely got a plasmid DNA yield of ~900ng/ml of bacterial culture. We used 125ng to 150ng plasmid DNA for setting up of sequencing reactions. The inserts were sequenced using M13 forward primer (-20), 17mer, (Fermentas.) The sequencing platform used was ABI PRISM 377TM DNA sequencer. The sequence read length was from 800-1200bases(Figure 2).This read length was comparable to the read length obtained using plasmid DNA purified by automated liquid handling system (Data not shown).

Results and discussion:

We have designed and standardized a simple method for isolating plasmid DNA for direct sequencing without the need for further DNA purification. The yield of plasmid DNA and the sequence read length obtained by this method were very well comparable with the standard methods used. The drawback of this method are that the plasmid DNA needs to be used within a month when stored at -20ºc and restriction enzyme digestion analysis cannot be carried out as the E.coli genomic DNA present as contamination will interfere.

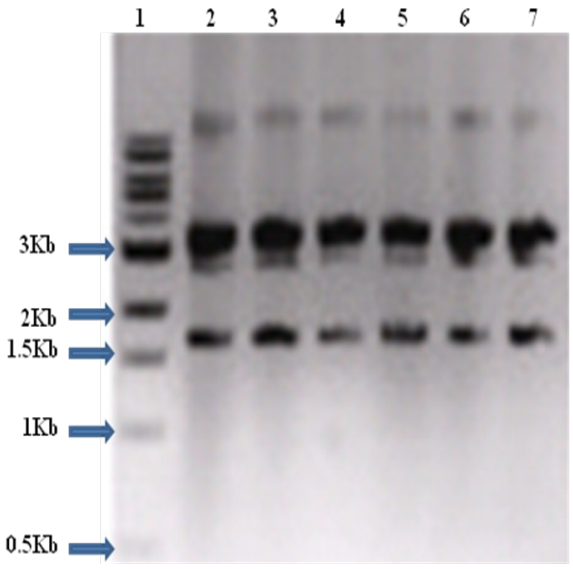

Fig1: 1% Agarose gel electrophoresis of plasmid DNA from different wells of the same clone DNA preparation. Lane 1: 1Kb ladder, Lane 2: AH16A1, Lane 3: AH16A6, Lane4: AH16A12, Lane5: AH16B11, Lane6: AH16C8, Lane7: AH16E12.

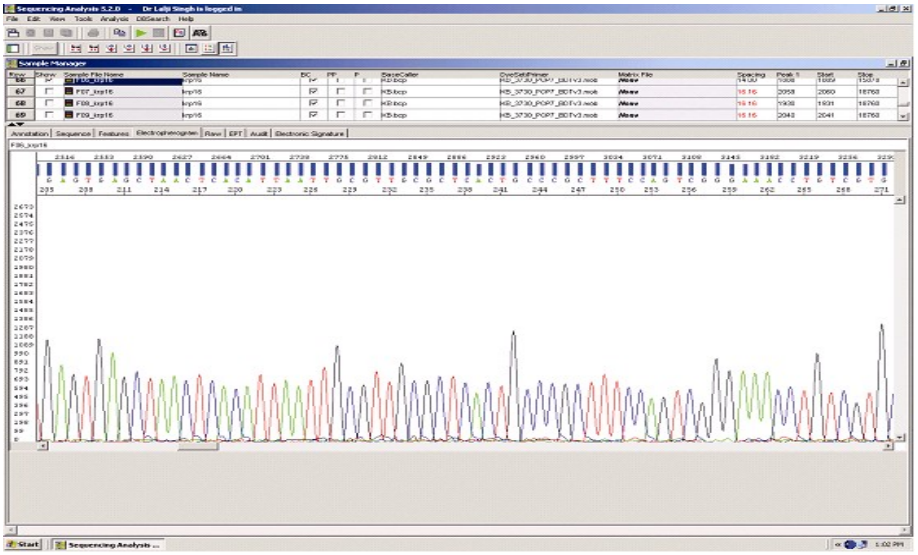

Figure 2: DNA sequence chromatogram for clone AH16A1

Conclusion:

Our method provides quick and economical method to isolate plasmid DNA for large scale sequencing.

REFERENCES

1. Birnboim,H.C.and J.Doly. A rapid alkaline extraction procedure for screening Recombinant plasmid DNA. **Nucleic Acids Res** 1979,7:1513

2. Holmes,D.S. and M.Quigley.A rapid boiling method for the preparation of bacterial Plasmids. **Anal.Biochem** 1981.

3. Vinograd,J.and J.Lebowitz. Physical and topological properties of circular DNA. **J.Gen.Physiol**.49:103, 1966.

4. Sambrook,J.,Fritsch,E.F., and Maniatis.T." A Molecular Laboratory Manual,"2nd Edition.Cold Spring Harbor Laboratory Press, Cold Spring Harbor,NY. 1989.

5. Maxam AM, Gilbert W. A new method for sequencing DNA.Proc Natl Acad Sci U S A. 1977; 74:560-564
6. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc. Natl Acad Sci. U S A 1977;74:5463-5467
7. Schuster SC. Next-generation sequencing transforms today's biology. Nat.Methods. 2008; 5: 16-18