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Simple and rapid preparation of plasmid template by a filtration method using microtiter filter plates

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ABSTRACT

We developed a new simple high-throughput plasmid DNA extraction procedure, based on a modified alkaline lysis method, using only one 96-well microtiter glassfilter plate. In this method, cell harvesting, lysis by alkaline and plasmid purification are performed on only one microtiter glassfilter plate. After washing out RNAs or other contaminants, plasmid DNA is eluted by low-ion strength solution, although precipitated chromosomal DNA is not eluted. The plasmid prepared by this method can be applied to sequencing reactions or restriction enzyme cleavage.

As the gene mapping and sequencing programs expand, the need for a high-throughput template preparation method is rapidly increasing. Although a number of procedures for the preparation of plasmid DNA have been reported, they are not completely satisfactory in terms of cost-performance, time, labour, yields, quality for sequencing and application to complete automation. In this report, we describe a new simple high-throughput plasmid DNA extraction procedure, based on a modified alkaline lysis method (1,2).

Escherichia coli SOLR transformed with a recombinant pBluescript SK(+) containing a 5.6 kb mouse cDNA fragment was cultivated overnight in 1 ml LB medium containing 100 µg/ml ampicillin. An aliquot of 600 µl of culture was transferred into 96-well microtiter glassfilter plate (Multiscreen glassfilter FB, 1.0 µm pore, Millipore, USA) using a 96-channel autopipetter, Quadra 96 model 320 (Tomtec, USA), and filtrated by vacuum using vacuum manifold (Millipore, USA). An aliquot of 25 µl of solution I (50 mM glucose, 25 mM Tris–HCl, pH 8.0, 10 mM EDTA, 10 mg/ml lysozyme) was added into each well and was incubated for 5 min. Then, 50 µl of solution II (0.2 N NaOH, 1% SDS) was added and was kept at room temperature for 5 min, followed by addition of 160 µl of solution III–guanidine–HCl (GuHCl) (0.7 M potassium acetate, pH 4.8, 5.3 M guanidine–HCl). After 5 min incubation, the mixtures were then filtrated by vacuum sucker and washed three times with 300 µl of 80% ethanol. Then, each well was washed with 150 µl of 80% ethanol–20% glycerol and was dryed by subsequent 20 min vacuum. Finally, the plasmid DNA was eluted with 50 µl TE buffer.

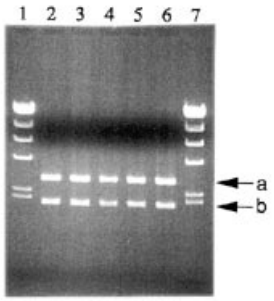

Figure 1. Agarose gel electrophoretic pattern of plasmids prepared by the method described in this report. Plasmid DNAs [pBluescript SK(+)::5.6 kb mouse cDNA] were digested with *Xho*I which cleaved the site in insert DNA and cloning site, and electrophoresed in 0.8% agarose gel, TAE buffer. Lanes 1 and 7, λ-*Hin*dIII digest as molecular size marker; lanes 2–6, representative plasmid DNAs prepared by this method, picked randomly from 96-well. (**a**) Vector and partial insert DNA; (**b**) partial insert DNA.

The concentrations of the resultant plasmid DNAs were 0.1–0.15 µg/µl. This concentration is suitable for fluorescent dideoxy sequencing using a DNA sequencer. The total yields were 4–6 µg per well which is enough for sequencing more than three times. The plasmid DNA was of very high quality and no contamination of RNA, chromosomal DNA or proteins were observed. The ratio of optical density at 260/280 nm was 1.9–2.4 whose average was 2.25 (Fig. 1).

To test whether the quality of the plasmid DNA prepared in this method is appropriate for sequencing, the DNA sample was subjected to dye-terminator sequencing using an ABI 377. The resultant electropherogram is shown in Figure 2. The pattern shows low background and high resolution with very sharp peaks. The extracted DNA has enough quality to be sequenced >500 bp.

Our data demonstrated that plasmid DNA was extracted by filtration through a glass-filter based on the principle that DNA is adsorbed on to the glassfilter surface in 3.5 M GuHCl after alkaline lysis (5). In this condition, RNA is eluted out because DNA is adsorbed by the glassfilter stronger than RNA that is

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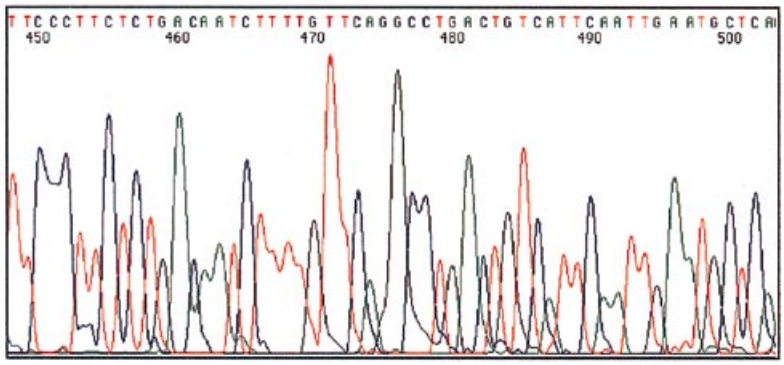

Figure 2. Dideoxy sequencing electropherogram of plasmid DNA [pBluescript SK(+)::5.6 kb mouse cDNA] prepared by the method described in this report. ABI PRISM™ dye terminator cycle sequencing kit with AmpliTaq® DNA polymerase FS was used for the sequencing reaction and the sequence was analyzed with an ABI 377 DNA analyzer.

carrying more hydroxyl groups. Chromosomal DNA denatured in alkaline forms complexes with SDS and cell debris, resulting in its being precipitated through the neutralization. This complex is stable and not solubilized in the presence of GuHCl. In the GuHCl mixture, plasmid DNA is also adsorbed by the glassfilter even in the presence of the chromosomal DNA–SDS–cell debris complex. Most protein components are soluble in this condition and they are easily washed out with 80% ethanol. In the last step for DNA elution, chromosomal DNA is not contaminated, because chromosomal DNA–SDS–cell debris complexes were trapped on the glassfilter without eluting out. Thus, this protocol requires only one microtiter glassfilter plate without transferring each sample to a new glassfilter plate. Any proteinase and RNase treatments can be skipped because RNA and protein do not attach to the glassfilter in the presence of 3.5 M guanidine–HCl. These components are washed out extensively so that it hampers neither the sequencing reaction nor the restriction enzyme cleavage.

This plasmid preparation system is very rapid, simple to prepare a large amounts of samples for large scale sequencing. The system has characteristic features that; (i) it employs a one-way-flow system with 96-well microtiter filter plates without transferring each sample to a new filter plate, enabling us to robotize this system very easy, whereas a previously reported method required at least two microtiter filter plates which should be changed after neutralization of bacterial lysate (4); (ii) this system requires neither prior treatment with RNase nor organic solvents; (iii) the centrifugation step was replaced by vacuum filtration throughout the whole procedure; (iv) this system achieves high speed preparation (<1 h for 96 samples), the highest yield with sufficient quality and concentration, and the lowest cost.

Anderson *et al.* reported a similar method for purification of M13 single stranded DNA, in which the 96-well glass-fiber filtration method was adopted (6). Although M13 single stranded DNA is reported to produce a good quality of sequencing data, the quality of the sequence data with double stranded DNA template has been improved by the development of thermal cycle sequencing. In the case of preparation of M13 single stranded DNA, the yield is low and it requires the tedious steps of pre-cultivation of host strain for infection, centrifugation to remove supernatant and polyethyleneglycol precipitation. Furthermore, the anti-strand of M13 DNA must be prepared to be sequenced from both cloning sites. On the other hand, double stranded DNA is more suitable to sequence from both ends of insert DNA only by changing sequence primer with the same template DNA clone. Also for the full length sequencing of large insert DNA, the nested deletion double strand clone set can be easily prepared, compared with single stranded DNA. Furthermore, their method requires centrifugation steps, transferring each sample to a new 96-well plate or filter plate, precipitating DNA with polyethyleneglycol, and it takes ~2 h for a series of their procedure. On the other hand, in our method reported in this paper, the glassfilter plate does not need to be changed and only five sorts of reagents are serially added in one-way-flow. The final DNA samples can be recovered in final aliquots of 50 µl TE (~0.15 µg/µl).

In conclusion, this method enables the high-throughput plasmid preparation based on the one-way-flow system using only one glassfilter 96-well titer plate without transferring each sample to a new filter plate. The procedure of this method is designed for robotizing plasmid preparation.

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