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A home made kit for plasmid DNA mini-preparation

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Many methods have been used to isolate plasmid DNA, but some of them are time consuming especially when extracting a large number of samples. Here, we developed a rapid protocol for plasmid DNA extraction based on the alkaline lysis method of plasmid preparation (extraction at pH 8.0). Using this new method, a good plasmid preparation can be made in approximately one hour. The plasmids are suitable for any subsequent molecular applications in the laboratory. By applying the recommendations to avoid contaminations and to maximize the plasmid yield and quality during extraction, this protocol could be a valuable reference especially when analyzing a large number of samples.

Key words: Plasmid extraction, PCR, restriction enzymes, sequencing, contamination.

INTRODUCTION

Over the past few years, plasmid DNA has been recognized as the most powerful tool in several biotechnological methods. Based on its molecular properties (closed circular molecule, easily restrictable, harboring an ideal marker gene for selection and rapidly amplifiable in a host system independently), plasmid DNA has been at the center of the most recent and advanced technologies in many disciplines including medicine, agriculture, molecular biology, industry, and biocontrol. In research, plasmid DNA is used as a vector allowing the study and generation of Genetically Modified Organisms (GMO) i.e. the transfer and the subcloning of transgenes across boundaries of species, and functional characterizations of several genes (genomics) within a species. The use of high quality plasmid DNAs often determines the success in various manipulations of genetic material during routine applications such as polymerase chain reaction (PCR) amplification, DNA sequencing, and subcloning of transgenes. Therefore, protocols for extraction of plasmid DNA with high yield and quality have been give serious attention (Sambrook et al., 1989). Several methods for plasmid DNA preparation usually known as plasmid mini-prep, or plasmid DNA miniprep (Birnboim, 1983; Hansen et al., 1995), and commercial kits have been made available. However, some of these methods give relatively low yield and are time consuming especially when carrying out molecular analysis of a large number of samples.

In practice, problems are often linked to the isolation of pure (high quality) plasmid DNA. These problems often arise due to contamination by phenolic compounds and polysaccharides. Acidic polysaccharides are potential inhibitors of Hind III restriction enzyme (Do and Adams, 1991) and they also inhibit classical primer PCR by inhibiting Taq DNA polymerase activity (Demeke and Adams, 1992; Fang et al., 1992; Pandey et al., 1996). These contaminations distort the results in many analytical applications and therefore lead to wrong interpretations. In our laboratory, we are engaged often in a large number of plasmid preparations for several purposes such as subcloning of transgenes, PCR amplifications, transformation, gene cloning and the screening of positive clones. Therefore, we found it necessary to develop a rapid and efficient protocol for plasmid preparation, enabling us to handle many samples in one experiment, and increasing the number of replicates per day. The protocol described here is relatively simple and rapid. It provides high yield and quality plasmid DNA. It is consistently restrictable, amplifiable by PCR, suitable for cloning and for sequencing. The yield of the plasmid DNA using this protocol is higher than that obtained with commercial kits.

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PROCEDURE

The first step of a plasmid DNA preparation is the amplification of the plasmid in a host organism, generally

a competent *E. coli*. The following procedure works remarkably well with 2 ml culture of *E. coli*.

1. Set up a 2 ml overnight culture of *E. coli* harboring the plasmid in 2.5 ml eppendorf tubes with appropriate antibiotic for the amplification of the plasmid. It is necessary to inoculate this culture with a single colony of transformed *E. coli*.
2. Harvest the cells by centrifugation at 5000 g for 5 min at room temperature. If one needs to stop the protocol and continue later, the cell pellets can be stored at -20 C.
3. Resuspend the bacterial pellet in 200 l of Solution I (see recipe) containing 4 g/ml lysozyme (from a lysozyme stock of 20 mg/ml in 10 mM Tris-HCl, pH 8.0). *Note*: Lysozyme must be freshly added to solution I. Moreover, it will not work efficiently if the pH of the solution is less than 8.0. Incubate the suspension at room temperature for 5 min.
4. Add 400 l of freshly prepared solution II (see recipe) and mix well by inverting gently 4 to 6 times to avoid breaking the plasmid. Do not vortex.
5. Immediately add 200 l of solution III (see recipe) and mix very gently by pipetting up and down and incubate at 4 C or in ice for 5 min without shaking. *Note*: After successfully completing step 5, a white precipitate must form and hang in suspension in the sample, otherwise no plasmid DNA will be recovered at the end of the procedure. Incubating the mixture in ice enhances the precipitation. The precipitated material contains genomic bacteria DNA, proteins, cell debris and SDS. The solution should be mixed thoroughly to avoid localized ammonium dodecyl sulfate precipitation.
6. Centrifuge at 10000 g for 5 min at room temperature (before loading the samples in the centrifuge, they should be mixed again) and carefully transfer the supernatant into a new eppendorf tubes. Avoid transferring the white debris with supernatant otherwise, step 6 must be repeated.
7. Add 0.6 volume of isopropanol i.e. 0.6 ml isopropanol for 1 ml of supernatant from step 6. Mix gently by inverting 4 to 6 times and keep at room temperature for 10 min.
8. Centrifuge as described in step 6 and discard the supernatant. The plasmid DNA will be precipitated in the pellet.
9. Wash the pellet with 400 l of 70 % (v/v) ethanol and centrifuge at 10000 g for 3 min at room temperature. Remove the supernatant and air-dry the pellet to get rid of the residual ethanol. This takes approximately 10-20 min for the residual ethanol to dry off.
10. Resuspend the pellet in 30 to 50 l of 10 mM Tris-HCl pH 8.0 or in sterile distilled MilliQ water. Add 1 l of RNase A (from a RNase A stock of 10 mg/ml stored at - 20 C) and incubate at 37 C for 5 min to digest away all contaminating *E. coli* RNA. The suspension can be kept at -20 C for further use.
11. Check an aliquot of 2 l of the plasmid prep in 1% (w/v) agarose gel (test gel) for the purity and quality. The plasmid DNA can be quantified using spectrophotometer at 260/280 nm.

Recipe of the solutions

Solution I: 50 mM glucose, 25 mM Tris-HCl pH 8.0, 10 mM EDTA pH 8.0. This solution can be autoclaved or filter sterilized and stored at 4 C. Lysozyme should be freshly added to this solution just before use.

Solution II: 0.2 M NaOH, 1 % (w/v) SDS. Solution II should be freshly prepared from stock solutions of 2 M NaOH and 10 % (w/v) SDS.

Solution III: 8 M ammonium acetate (NH_4-Ac). Approximately 100 ml of solution III can be made, autoclaved and kept at room temperature.

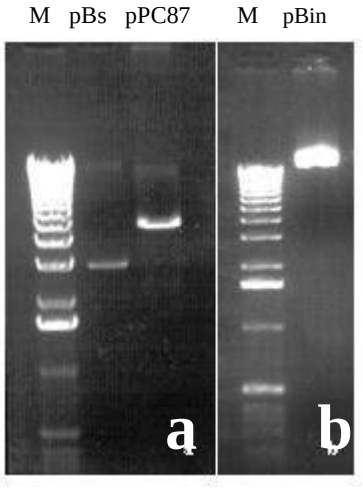

Figure 1. Agarose gel profile of plasmid DNA preparations of different molecular sizes.
a: pBs: pBluescript II KS (2.96 Kb derived from pUC 19; pPC 86: plasmid DNA of 7 Kb carrying the Trp 1 selectable marker, b: Extraction of a large size plasmid DNA pBin 19 (11.7 Kb), M: Molecular weight marker (Kb).

RESULTS AND DISCUSSION

The method described here presents several advantages. It allows preparation of a large number of samples (24 samples) in a maximum of two and half hours. The time required for the plasmid prep using this method depends on the number of samples to be extracted. Approximately 50 min to one hour is the average time required to complete a good plasmid miniprep with this procedure. It is thus possible to carry out several trials in a day. Generally, the number of the extracted samples in an experiment is strictly determined by the capacity of the centrifuge rotor to be used. Using a centrifuge with 24-eppendorf tubes rotor-holder (Sigma-

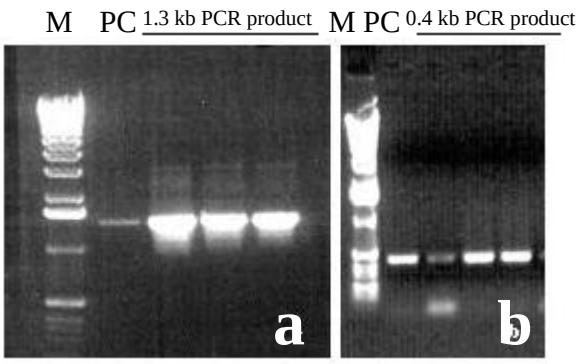

Figure 2. PCR amplifications of different size of DNA fragments from pBin 19 using appropriate primers.
a: PCR product of 1.3 Kb fragment arbitrarily amplified to confirm the quality of the extracted plasmid (results shown in triplicates), **b:** PCR product of NPTII region of pBin 19 (0.46 Kb, results shown in triplicates), **M:** Molecular weight marker (Kb), **PC:** positive control.

202 MK), we were able to obtain rapidly high quality plasmid preps in our laboratory. As recombinant DNA techniques have advanced, it has become unnecessary, for most purposes, to purify large quantities of plasmid DNA. For example, cleavage with restriction endonucleases, ligation, transformation, and even DNA sequencing can be carried out on relatively small quantity but pure plasmid DNA obtained from small-scale (2 ml) cultures. Using our plasmid miniprep protocol, satisfactory quality was obtained. This indicates that the procedure works well for both small and large size plasmid DNA (Figure 1). The plasmid yield was quantified at 260/280 nm and the concentrations obtained ranged between 2 and 2.8 g/ l, which is two to three times more than the concentration obtained using commercial kits. This new method also provides good plasmid for PCR amplification. Arbitrarily, a set of oligonucleotides (primers) was designed for amplification of 460 and 1300 bp DNA fragments using PCR, in order to ascertain the quality of the plasmid prep in subsequence applications. Satisfactory amplification results were obtained (Figure 2). We were also able to sequence our transgenes using the recombinant plasmid preparations (results not shown).

For the precipitation of the plasmid absolute ethanol is often used. In this procedure isopropanol was used (step 7), which also gives a good and high yield of plasmid. It is worthy to note that isopropanol is much cheaper than ethanol. Polysaccharides and other secondary compounds and cell debris released after cell disruption co-precipitate easily with plasmid DNA if the procedure is performed under cold conditions (4 to 8 C), which finally leads to a viscous plasmid preparation. Such plasmid is usually neither restrictable nor suitable for analytical applications. It often remains in the wells during electrophoretic separation. This protocol works perfectly in the summer and less efficient in cold months especially in winter. It is therefore recommended to use 37 C wherever room temperature is below 20 C. The best way to prepare a good plasmid was to carry out the procedure without refrigeration. The use of cold room will lead to a contaminated preparation. We therefore suggest that the protocol would be especially suitable for tropical zones where the temperatures are generally between 23 and 28 C.

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