

Rapid Preparation of Total Nucleic Acids from *E. coli* for Multi-purpose Applications

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Separate protocols are commonly used to prepare plasmid DNA, chromosomal DNA, or total RNA from *E. coli* cells. Various methods for the rapid preparation of plasmid DNA have been developed previously, but the preparation of the chromosomal DNA and total RNA are usually laborious. We report here a simple, fast, reliable, and cost-effective method to extract total nucleic acids from *E. coli* by direct lysis of the cells with phenol. Five distinct and sharp bands, which correspond to chromosomal DNA, plasmid DNA, 23S rRNA, 16S rRNA, and a mixture of small RNA, were observed when analyzing the prepared total nucleic acids on a regular 1-2% agarose gel. The simple and high-quality preparation of the total nucleic acids in a single tube allowed us to rapidly screen the recombinant plasmid, as well as to simultaneously monitor the change of the plasmid copy number and rRNA levels during the growth of *E. coli* in the liquid medium.

Keywords: *E. coli*, Plasmid copy number, rRNA levels, STE/phenol, Total nucleic acids

Introduction

As the most popular host for DNA manipulations and a model prokaryote, *E. coli* plays an important role in current biological science. Preparation of the nucleic acids (including chromosome DNA, plasmid DNA and RNA from *E. coli* cells) is essential for various analysis purposes.

The rapid preparation of plasmid DNA from *E. coli* is a basic technique of DNA manipulation. Existing methods for the rapid preparation of plasmid DNA usually include a lysis

step and an isolation/purification step (Birnboim and Doly, 1979; Kristensen *et al.*, 1991; Tarczynski *et al.*, 1994; Voo and Jacobsen, 1998; Song *et al.*, 1999; Sambrook and Russell, 2001). The collected cells are first broken by alkaline, boiling or phenol/chloroform to allow nucleic acids to be released from the cells, then the plasmid DNA is isolated from the mixed nucleic acids by digestion of the RNA mixture, ethanol precipitation, and/or passing through a resin, etc. The preparation of a high quality of chromosomal DNA and RNA are usually much more laborious and difficult (Sambrook and Russell, 2001).

We developed a simple method to quickly extract a high quality of total nucleic acids, including plasmid DNA, chromosomal DNA, and RNA from *E. coli* cells. Briefly, the collected cells were resuspended in a STE buffer containing 100 mM NaCl, 10 mM Tris buffer, pH 7.0, 1 mM EDTA, followed by the addition of an equal volume of phenol or phenol/chloroform to release the nucleic acids from the cells. This is called the STE/phenol method. Isolation of the plasmid DNA from the mixture of the total nucleic acids was omitted, making the STE/phenol method simpler, faster, and cheaper than any existing method for the preparation of plasmid DNA. Moreover, the chromosomal DNA and total RNA that were prepared using this simple method were qualified for a variety of applications in molecular biology study.

Materials and Methods

Growth of bacteria *E. coli* strains DH5 α and JM109 that were used as the hosts were grown in a LB medium (Sambrook and Russell, 2001) at 37°C. Antibiotics for the *E. coli* selection were added to a final concentration of 50 μ g/ml for ampicillin or 25 μ g/ml for chloramphenicol.

Rapid screening of recombinant DNA by STE/phenol method

The plasmid pLXC113 with a 2 kb DNA fragment being inserted in pBC SK (+) (Stratagene, La Jolla, USA) was deleted with a ExoIII/S1 Deletion Kit (LKB Pharmacia, Uppsala, Sweden). The resulting DNA mixtures were re-ligated and transformed to DH5 α

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competent cells. The transformed clones were re-grown at 37°C for 8-10 h after patching on LB agar plates. The cells were collected from each patch by a sterile toothpick and put into 1.5 ml tubes. The collected cells were suspended in 50 µl of a STE buffer (100 mM NaCl, 10 mM Tris buffer, pH 7.0, 1 mM EDTA), followed by the addition of 50 µl phenol. After 30 sec of vigorous vortex using a QL-901 vortexer (QilinYQ, Jiangsu, China), the mixture was centrifuged for 5 min at 13,000 g. A volume of 10 µl supernatant was loaded on agarose gel to analyze the size of the deleted plasmid.

Testing the effect of vortex time on the yield and quality of the total nucleic acid The *E. coli* strain JM109 was transformed by pBC SK(+). A plasmid-containing colony was grown overnight at 37°C with shaking to the stationary phase (OD_{600} of 3.2) in a 35 ml culture tube containing 10 ml of the liquid LB medium. Then the bacteria cells that were equivalent to OD_{600} of 4 were collected in a 5 ml tube by centrifugation. The pellet was then resuspended in 2 ml of the sterile STE buffer. An equal volume of the phenol/chloroform mixture (1 : 1 volume ratio) was added, followed by a vigorous vortex of 10 to 600 s; an aliquot of 200 µl mixture was taken out at the indicated time followed by centrifugation. A volume of 20 µl supernatant of each sample was loaded for agarose gel electrophoresis. The gels were stained by ethidium bromide, then pictured by a Kodak 290 digital camera and analyzed by the 1D 3.5 image analysis software (Kodak, Rochester, USA).

Monitoring the change of the plasmid copy and rRNA levels during the growth course of the *E. coli* JM109 strain containing pBC SK(+) were grown to OD_{600} of about 2, as described previously. The culture was diluted to 50 ml of the fresh liquid LB medium in a 250 ml flask to a final concentration of OD_{600} of 0.01 then continued to culture at 37°C. The first samples were taken after 3 hrs of growth, and the following samples were taken every hour to determine the cell density (OD_{600}) and to extract total nucleic acid from bacteria cells that were equivalent to OD_{600} of 0.5 by the STE/phenol method with 2 min of vortex. Note that a sterile STE buffer and phenol/chloroform (1 : 1) was used. Total nucleic acid preparations were analyzed by agarose gel electrophoresis as described previously.

Results and Discussion

Rapid screening of recombinant plasmids using STE/phenol method Our initial goal was to develop a simple, fast, and cost-effective method to screen the recombinant plasmids. Using the STE/phenol method (Materials and Methods), we extracted nucleic acids from the DH5α cells that were transformed by recombinant plasmids of different sizes (Fig. 1). The difference in these plasmids, varying from 3.4 kb (lane 13) to 5.4 kb (lanes 1 and 14), was readily distinguished on an agarose gel. The deletion clones that were identified from this analysis were selected for further analysis.

Phenol-chloroform was previously used to prepare plasmid DNA (Tarczynski *et al.*, 1994; Song *et al.*, 1999). However, a prior isolation/purification of the plasmid DNA slows the

preparation process, and expensive gel matrix is required in some cases. Although the plasmid purification step is also omitted from the alkaline lysis-based toothpick method (Sambrook and Russell, 2001), the lysis solution that is used for alkaline lysis is required to be freshly prepared for each use and the lysed cells are required to be treated with the neutralization buffer. In contrast, the STE buffer that was used in the STE/phenol method is stable at room temperature for up to a year and no further reagent is required after the addition of the phenol. Therefore, the STE/phenol method is simpler and faster than all of the existing methods in the mini-preparation of plasmid for rapid screening. It should be more effective when a large number of samples need to be analyzed at the same time, such as high throughput screening of recombinant plasmids. Please note that the prepared plasmid is also qualified for PCR (data not show).

In addition to the plasmid bands, a sharp band migrating near the wells of the gel was clearly demonstrated for each sample, indicating that the chromosomal DNA is also released (Fig. 1). In order to minimize the effect of ribosomal RNA bands on the visualization of various sizes of plasmids, the STE buffer that was used in this experiment was not sterilized to keep the contaminated RNase. Therefore, the 16S and 23S rRNA bands were not observed, but a small RNA band corresponding to the digested RNA was visualized (Fig. 1). RNase A or RNase T1 could also be added to the STE buffer to remove the RNA.

STE/phenol method yields a high quality of total nucleic acids We then examined the STE/phenol method to see if it

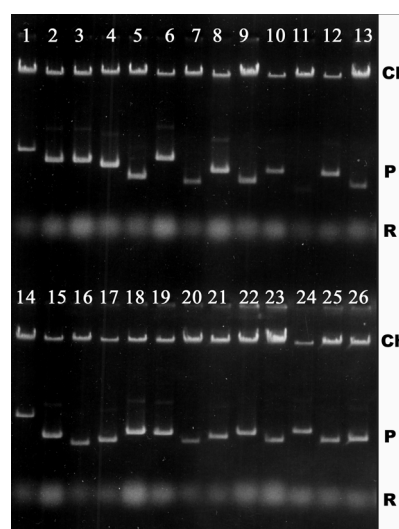


Fig. 1. The different sizes of plasmid prepared by the STE/phenol method were readily detected. The nucleic acids that were prepared (described in “Materials and Methods”) were analyzed on a 1.2% agarose gel. Lane 13, empty vector pBC SK(+); lanes 1 & 14, pBC SK(+) containing a 2 kb insert; other lanes, pBC SK(+) containing the deleted fragments resulting from Exo III/S1 deletion of the 2 kb insert. Plasmid DNA (P), chromosomal DNA (Ch), and the digested RNA (R) are labeled on the right.

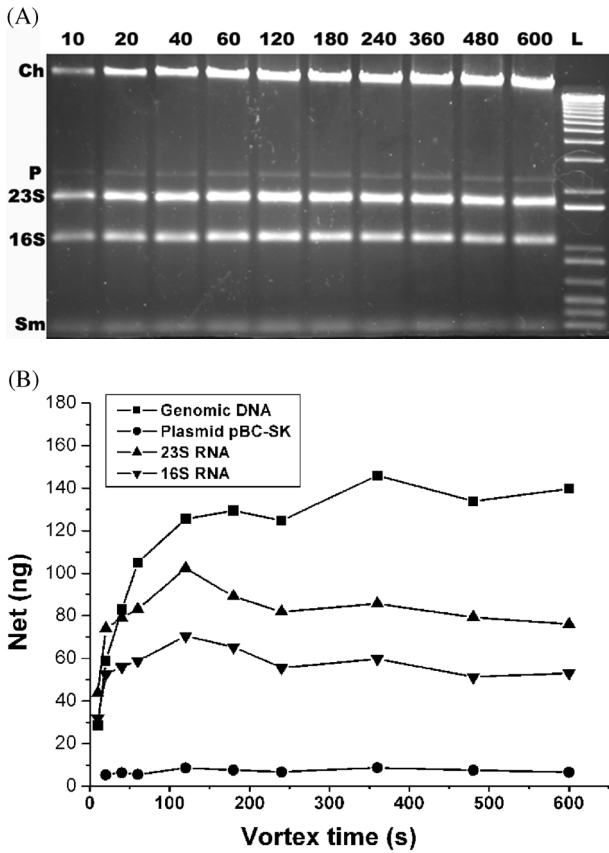


Fig. 2. Effects of the vortex time on the yield and quality of the total nucleic acids. *E. coli* JM109 cells harboring pBC SK(+) were used to extract the total nucleic acids by vortexing for the indicated amount of time (Materials and Methods). (A). The gel showing the total nucleic acids were prepared at each vortex time. Each nucleic acid species is labeled on the left: Ch-chromosomal DNA, P-plasmid DNA, 23S-23S rRNA, 16S-16S rRNA, Sm-a mixture of small RNAs. (B) The result in panel A is analyzed and plotted (Material and Methods).

was a good way to prepare total nucleic acids, including plasmid DNA, chromosomal DNA, and total RNA from *E. coli* cells. We would also like to know if the vortex time affects the release and quality of the total nucleic acids. As shown in Fig. 2, an equal amount of *E. coli* cells that were grown to the stationary phase was used to prepare the total nucleic acids with vortex time varying from 10 s to 10 min. The plasmid DNA, 16S and 23S ribosomal RNA, as well as chromosomal DNA were released from *E. coli* cells with a 10 s vortex, and the levels of all these nucleic acids were increasing as the vortex time prolonged (Fig. 2). The release of plasmid DNA was minimally affected by the vortex time, while the release of chromosomal DNA, as well as the 16S and 23S rRNA, were significantly increased with the vortex time. Almost all species of nucleic acids reached their maximal levels in 2-min vortex, except that the level of the chromosomal DNA slightly increased with further vortex. We noted that the levels of both 16S and 23S rRNA slightly

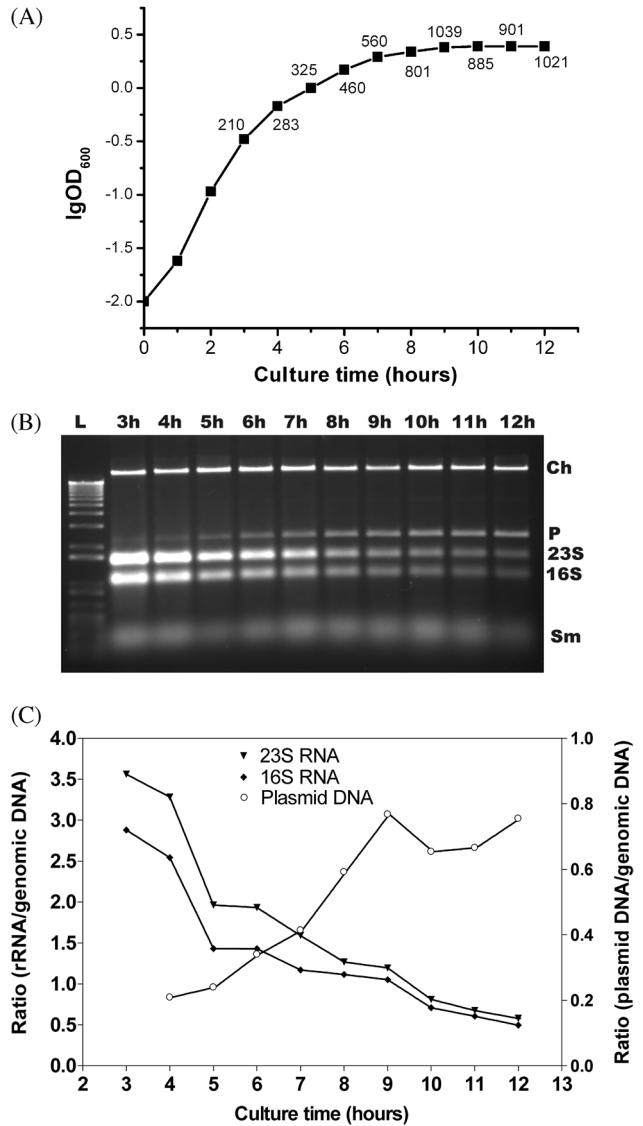


Fig. 3. Monitoring the change of the plasmid copy number and the rRNA levels during bacterial growth. Total nucleic acids were prepared from the bacteria samples that were taken at different growth times. (A) Growth curve is obtained by plotting the cell density (OD₆₀₀) against the growth time. The Y axis indicates the log of the cell density (OD₆₀₀). The plasmid copy number calculated from panels B and C are indicated at the corresponding growth point. (B) An agarose gel showing the total nucleic acids that were prepared. (C) Plot of the levels of 16S rRNA, 23S rRNA and plasmid relative to the genomic DNA in the same loading.

decreased during the prolonged vortex, which might not be explained by the RNA degradation, because the level of the small RNA did not increase.

Please note that five visible bands (corresponding to chromosomal DNA, plasmid DNA, 23S rRNA, 16S rRNA, and a mixture of tRNA and 5S rRNA) were distinct and sharp, demonstrating that the high quality, reflected by the high integrity, of the total nucleic acids was produced by this fast

and simple STE/phenol method. Since the largest species of the cellular nucleic acids, chromosomal DNA, was released by this method, we estimate that all of the RNA species are also released by this method. Furthermore, the integrity of all of the visible nucleic acids was not affected by the prolonged vortex for up to 10 min. Such a high quality preparation of the total nucleic acids should be suitable for various analysis purposes.

Total nucleic acids, prepared by STE/phenol method, reveal that the plasmid copy number increases while the rRNA levels decreases during *E. coli* growth

The *E. coli* strain JM109, containing plasmid pBC SK(+), was grown in a LB liquid medium from the cell density of OD₆₀₀ of 0.01 to 2. Culture aliquots were taken at the indicated times. The total nucleic acids were prepared and analyzed by agarose gel electrophoresis, as described. As shown in Fig. 3, the chromosomal DNA level remained relatively stable during bacterial growth, while the level during the late stationary phase decreased slightly, probably due to the slower DNA replication at this phase. In contrast, the plasmid DNA level obviously increased with cell growth, reaching its maximal level at the stationary phase. By comparing the intensity of the plasmid band and the chromosomal band in the same preparation, we calculated that the copy number of pBC SK(+) in JM109 varied from about 200 copies to 1000 copies under the culture conditions that are described. The plasmid copy number linearly increased with growth during the late log phase, and reached the maximal in the stationary phase. Because pBC SK (+) contains the pUC origin and is similar to the pUC plasmids in the aspect of replication, the plasmid number that was obtained for pBC SK (+) in this study is consistent with the copy number of 500-700 that was published for the pUC plasmids (Lin-Chao *et al.*, 1992; Sambrook and Russell, 2001). This validates the plasmid copy number that was obtained with this simple method. Interestingly, the plasmid number was only about 100 copies per cell at the stationary phase under the growth conditions shown in Fig. 2, which was 10-fold lower than that in Fig. 3. These results indicate that the plasmid copy number is dependent on the condition under which the bacteria grow.

The traditional method for measuring the plasmid copy number is very complicated, laborious and/or expensive (Projan *et al.*, 1983; Lin-Chao and Bremer, 1986; Coronado *et al.*, 1994; Schmidt *et al.*, 1996; Fahnert *et al.*, 2000). In most cases, the relative copy number was determined rather than the absolute. Taking advantage of the high quality preparation of both chromosomal DNA and plasmid DNA in one tube by the STE/phenol method, we could rapidly measure not only the relative copy number of the plasmid during the *E. coli* growth, but also the absolute copy number at each growth point. Moreover, the absolute copy number that we obtained with this method was very consistent with data that was previously obtained with a much more complicated method (Lin-Chao *et al.*, 1992). The result obtained by this simple

method also shed new light on the relationship between the plasmid copy number and *E. coli* growth. Therefore, the STE/phenol method provides a simple and effective alternative in measuring the plasmid copy number in *E. coli*.

Meanwhile, using genomic DNA as a powerful internal control, we ambiguously demonstrated that the levels of both 23S and 16S rRNA decreased along with the cell growth. The decrease started with the late log phase, the earliest time point being taken, and steadily progressed to the stationary phase. This finding may reflect the fact that protein synthesis becomes less active in the late log and stationary phases. In the future, it would be interesting to examine the change of the rRNA levels in the early and mid-log phase.

We developed a simple, fast, reliable, and cost-effective method for the preparation of a high quality of total nucleic acids for multiple purposes. In this study, a rapid screening of recombinant DNA with different sizes was performed. This application could be easily extended to high throughput screening of recombinant plasmids. We also monitored the change of the plasmid copy number and rRNA levels by agarose gel electrophoresis. Our research revealed that the rRNA levels decreased significantly while the plasmid copy number increased during the growth of *E. coli* cells in a liquid medium. The total nucleic acids could also be used for Northern and Southern analyses, as well as for other purposes, if the effect of the other nucleic acid species on the interested nucleic acid was properly considered.

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