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OPTIMAL HIGH-QUALITY PLASMID EXTRACTION FROM ESCHERICHIA COLI BACTERIA USING TB MEDIUM

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ABSTRACT

Plasmid DNA extraction is a fundamental technique in molecular biology and biomedicine. While traditional methods based on Luria–Bertani (LB) medium are sufficient for routine laboratory applications, many advanced downstream uses—such as transfection, gene therapy, and DNA vaccination—require large quantities of plasmid DNA with high purity and structural integrity. In this study, we systematically evaluated Terrific Broth (TB) as an alternative culture medium to enhance plasmid yield and quality in Escherichia coli. Cultures grown in TB achieved significantly higher cell densities and produced markedly increased plasmid yields compared with LB-grown cultures. Agarose gel electrophoresis confirmed that plasmid DNA extracted from TB cultures was predominantly in the supercoiled form with minimal degradation. Furthermore, several practical protocol modifications—including direct inoculation from frozen stocks, controlled freezing–thawing of bacterial pellets, and optimized elution strategies—reduced processing time and overall cost while improving yield. Collectively, these results demonstrate that TB medium is a robust and efficient platform for producing high-quality plasmid DNA suitable for demanding molecular and biomedical applications.

KEYWORDS

Plasmid Extraction, Escherichia coli, Terrific Broth; DNA yield; supercoiled plasmid.

1. INTRODUCTION

The isolation of plasmid DNA from bacteria is a crucial technique in molecular biology and is an essential step in many procedures such as cloning, DNA sequencing, transfection, and gene therapy. These manipulations require the isolation of high purity plasmid DNA. The purified plasmid DNA can be used for immediate use in all molecular biology procedures such as digestion with restriction enzymes, cloning, PCR, transfection, in vitro translation, blotting and sequencing (Figueroa-Bossi N et al., 2022; Voss C.,2007; Prather KJ, et al., 2003). Lysis formulas may vary depending on whether you want to extract DNA/RNA/Plasmid. All methods of lysing bacteria will yield plasmid solutions contaminated with chromosomal DNA and RNA. Centrifugation removes the vast majority of chromosomal DNA (it will form a pellet, while plasmid DNA remains soluble), and treatment with RNase will eliminate contaminating RNA (Xiang N et al., 2023). Alkaline lysis is a method used in molecular biology, to isolate plasmid DNA or other cell components such as proteins by breaking the cells open. Bacteria containing the plasmid of interest is first grown and then allowed to lyse with an alkaline lysis buffer consisting of a detergent sodium dodecyl sulfate (SDS) and a strong base sodium hydroxide. The detergent cleaves the phospholipid bilayer of membrane, and the alkali denatures the proteins which are involved in maintaining the structure of the cell membrane. During this step disruption of most cells is done, chromosomal as well as plasmid DNA are denatured, and the resulting

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lysate is cleared by centrifugation, filtration or magnetic clearing. Subsequent neutralization with potassium acetate allows only the covalently closed plasmid DNA to reanneal and to stay solubilized. Most of the chromosomal DNA and proteins precipitate in a complex formed with potassium and SDS, which is removed by centrifugation. (Birnboim HC, et al, 1979. Tashiro Y, et al. 2012). The bacteria are resuspended in a resuspension buffer (50mM Tris-Cl, 10 mM EDTA, 100 µg/ ml RNase A, pH 8.0) and then treated by 1% SDS (w/v) / alkaline lysis buffer (200mM NaOH) to liberate the plasmid DNA from the E. coli host cells. Neutralization buffer (3.0 M potassium acetate, pH 5.0) neutralizes the resulting lysate and creates appropriate conditions for binding of plasmid DNA to the silica membrane column. After the addition of neutralization buffer, a fluffy white material forms and the lysate becomes less viscous. The precipitated material contains genomic DNA, proteins, cell debris, and SDS. The lysate should be mixed thoroughly to ensure even potassium dodecyl sulphate precipitation. If the mixture still appears viscous, more mixing is required to completely neutralize the solution. A homogeneous colorless suspension indicates that the SDS has been effectively precipitated. Precipitated protein, genomic DNA, and cell debris are then pelleted by a centrifugation step and the supernatant is loaded onto a column. Contamination like salts, metabolites, and soluble macromolecular cellular components are removed by simple washing with ethanolic wash buffer (1.0 M NaCl, 50mM MOPS, pH 7.0, isopropanol (v/v) 15 %). Pure plasmid DNA is finally eluted under low ionic strength conditions with slightly alkaline buffer (5 mM Tris / HCl, pH 8.5) (Shamlou, P. A. 2003; Silva, F., et al. ; Sambrook, J., & Russell, D. W. 2001). Plasmid–host cell interactions have been investigated experimentally using Escherichia coli HB101, these recombinant strains contained different plasmid genes were investigated in Luria broth (LB) Maximum specific growth rates in LB and minimal media were reduced for increasing plasmid content or types per cell. Plasmid copy number increased when specific growth rate was reduced by changing medium composition. For decades, LB medium has served as the standard for cultivating Escherichia coli in plasmid production. However, certain experimental applications, such as in vivo delivery or large-scale transfection, require higher yields and better-quality plasmid DNA. Terrific Broth (TB), a nutrient-rich medium, has been reported to enhance bacterial growth and may improve plasmid yield. In this study, we compared the performance of LB and TB media in terms of bacterial density, plasmid yield, and DNA quality (Wunderlich M. et al.; Reinikainen P. Virkajarvi I. et al.; Greene, R. J., & Rogers, P. L. et al.).

Plasmid DNA extraction from *Escherichia coli* is a foundational technique in molecular biology, underpinning applications such as molecular cloning, DNA sequencing, gene expression studies, transfection, and gene therapy. In recent years, the demand for **large quantities of high-quality plasmid DNA** has increased substantially, driven by advances in DNA vaccines, cell-based therapies, and in vivo gene delivery systems. While Luria–Bertani (LB) medium remains the most commonly used culture medium, it frequently produces insufficient biomass and inconsistent plasmid quality for demanding downstream applications. Key challenges include limited plasmid yield, reduced proportions of supercoiled DNA, long multi-day workflows, and high reagent costs. Accordingly, this study aims to systematically compare Terrific Broth (TB) and LB media for bacterial growth, plasmid yield, and DNA integrity, while introducing practical protocol optimizations. This work demonstrates that TB medium not only enhances bacterial growth but also yields plasmid DNA with superior structural integrity and reduced processing time.

Previous studies have established alkaline lysis as the standard plasmid extraction method, while later research demonstrated that growth conditions strongly influence plasmid copy number and yield. Rich media formulations have been shown to improve plasmid productivity, yet TB remains underutilized in standard plasmid workflows recommended by commercial kits.

2. MATERIALS AND METHODS

2.1. Cell Culture and Harvest

For the LB medium, we choose a single colony from a freshly streaked selective plate and inoculate a starter culture of 2–5 ml LB medium containing the appropriate selective antibiotic. Incubate for approximately 8 h (usually overnight, which is easier for people schedule) at 37°C with vigorous shaking at200- 250 rpm. Then, we dilute the starter culture 1/500 to 1/1000 into 3 ml selective LB medium. Grow at 37°C for 12–16 h with vigorous shaking at 250 rpm. For the TB medium, we used glycerol stocks of plasmid-containing E. coli, which were directly inoculated (10–100 μL) into 250 mL of either LB or TB medium. Cultures were incubated at 37°C and shaken at 250 rpm for 21 hours. Optical density (OD600) was measured using a DU730 UV/Vis spectrophotometer (Beckman Coulter). Harvest the bacterial cells by centrifugation at 5000 speed (Beckman Coulter Avanti J 26) x g for 15 min and remove as much of the supernatant as possible. Then we can keep the pellet at -20°C at least overnight can increase the yield.

2.2. Resuspended Cells

In our study, plasmid DNA was extracted using the IBI Scientific Maxi Fast-Ion Plasmid Kit (IB47122). So, the solution buffers are similar, in our kit, it is called buffer PM I. Resuspend the bacterial per gram of pellet in 10 ml of resuspension buffer (50mM Tris-Cl, 10 mM EDTA, 100 µg/ ml RNase A, pH 8.0). The bacteria should be resuspended completely by vertexing or pipetting up and down until no cell clumps remain.

2.3. Cell Lysis

Per 3g bacteria pellet, add 10 ml of lysis buffer (in our kit called buffer PM II), mix thoroughly by inverting the sealed tube 4–6 times, and incubate at room temperature (15–25°C) for 5 min. Do not vortex, as this will result in the sharing of genomic DNA. The lysate should appear viscous. Do not allow the lysis reaction to proceed for more than 5 min.

2.4. Neutralization

Per 3g bacteria pellet, add 10 ml of neutralization buffer, mix immediately and thoroughly by vigorously inverting 4–6 times, and incubate on ice for 5 min. Precipitation is enhanced by using chilled neutralization buffer and incubating on ice. The buffer aids in complete precipitation of SDS, protein, and genomic DNA. Incomplete neutralization leads to reduced yield. However, released plasmid DNA is very vulnerable at this point and shaking too much or too strongly will damage the DNA. Lysis buffers contain a high concentration of chaotropic salts, which have two important roles in nucleic acid extraction. Firstly, they destabilize hydrogen bonds, van der Waals forces and hydrophobic interactions, leading to destabilization of proteins, including nucleases. Secondly, they disrupt the association of nucleic acids with water, thereby providing optimal conditions for their transfer to silica.

2.5. Binding and Washing

Transfer the supernatant to the equilibrated Plasmid Maxi Column, and the supernatant containing plasmid DNA. Allow the column to empty completely by gravity flow. The lysate through silica membrane, the desired nucleic acids should be bound to the column and impurities such as protein and polysaccharides should be in the flow-through. Columns contain a silica resin that selectively binds to DNA/RNA. The DNA of interest can be isolated by virtue of its ability to bind silica in the presence of high concentrations of chaotropic salts. These salts are then removed with an alcohol-based wash and the DNA is eluted using a low-ionic-strength solution such as TE buffer or water. The binding of DNA to silica seems to be driven by dehydration and hydrogen bond formation, which competes against weak electrostatic repulsion. Hence, a high concentration of salt will help drive DNA adsorption onto silica, and a low concentration will release the DNA. 30ml of wash buffer to the column and allow the column to empty by gravity flow. Flow of buffer will begin automatically by reduction in surface tension due to the presence of detergent in the equilibration buffer. The wash steps will remove such impurities. There are typically two wash steps, although it varies depending on sample type. The first wash will often include a low concentration of chaotropic salts to remove residual proteins and pigments. This is always followed with an ethanol wash to remove the salt.

2.6. Plasmid Elution

According to the kits' manual, elute DNA with 12 ml elution buffer. Collect the elute liquid in a 30 ml microcentrifuge tube. The elution buffer volume and method can be adapted to the subsequent downstream application to achieve higher yield or concentration that the standard method. For maximal DNA elution, allow the buffer to stand in the membrane for a few minutes before centrifugation. Elution Buffer AE (5 mM Tris/HCl, pH 8.5) can be replaced by TE buffer or water as well. At first the elusion buffer is used to wash change the PH or solution situation, so sometime at first, you couldn't get ideal amount DNA, this case, we can increase the volume of elution buffer to 1. 5 times or double it. You can measure the DNA concentration after column and decide whether use more Elution buffer or not. It is important that the elution buffer works quickly without changing the function or activity of the desired protein. Precipitate DNA by adding 0.7 volumes (9 ml per 12 ml of elution volume) isopropanol to the eluted DNA. Mix and centrifuge immediately at 12,000 rpm for 30 min in a microcentrifuge. Carefully decant the supernatant. Wash DNA pellet with 5 ml of 75% ethanol and centrifuge at 12,000 rpm for 10 min and removes precipitated salt and replaces isopropanol with the more volatile ethanol, making DNA easier to redissolve. Carefully aspirate the supernatant by vacuum without disturbing the pellet. Air-dry the pellet for 5–10 min and redissolve the DNA in a suitable volume of buffer (e.g., TE buffer, pH 8.0, or 10mM Tris-Cl, pH 8.5). Redissolve the DNA pellet by rinsing the walls to recover all the DNA.

2.7. Determination of Yield

Plasmid DNA was extracted using the IBI Scientific Maxi Fast-Ion Plasmid Kit (IB47122), and DNA concentration was measured with NanoDrop. To determine the yield, DNA concentration should be determined by both UV spectrophotometry at 260 nm and quantitative analysis on an agarose gel. To quantitate the nucleic acid concentration, dilute the plasmid DNA 1: 100 or 1: 50 (depending on the plasmid copy number) in TE buffer and measure the absorbance (optical density) at 260 nm (A260) and 280 nm (A280). Use TE buffer as the blank. This measurement permits the direct calculation of the nucleic acid concentration using the formula [DNA] (μg/mL) = A260 × Dilution factor × 50 where 50 is the extinction coefficient of DNA. The ratio A260/A280 provides a reasonable estimate of the purity of the preparation (Brown, L. R. 2006).

2.8. Analytical Gel Analysis

DNA integrity was assessed via agarose gel electrophoresis. Removing and saving aliquots during the purification procedure is recommended. If the plasmid DNA is of low yield or low in quality, the samples can be analyzed by agarose gel electrophoresis to determine at what stage of the purification procedure the problem occurred (Wood, W. N., (2017). The supercoiled (SC) topological isomers ratio in each purified fraction was calculated from Gel Image data by ImageJ according to the following equation:

$$\%\,\text{SC pDNA} = 100 \times \frac{\text{SC pDNA}}{\text{OC pDNA} + \text{SC pDNA} + \text{LIN pDNA}}$$

3. RESULTS

After 20 hours of incubation, TB medium supported significantly higher bacterial growth (OD600 = 2.358) compared to LB (OD600 = 1.815). Correspondingly, plasmid DNA yield from TB was 12.5 mg per 250 mL culture (7000 ng/µL), compared to only 3 mg from LB (3000 ng/µL). Agarose gel electrophoresis revealed that plasmids extracted from TB cultures were primarily in circular (supercoiled) form with sharp, intact bands. In contrast, LB-derived plasmid DNA showed slightly increased linear forms and reduced intensity, suggesting lower yield and partial degradation.

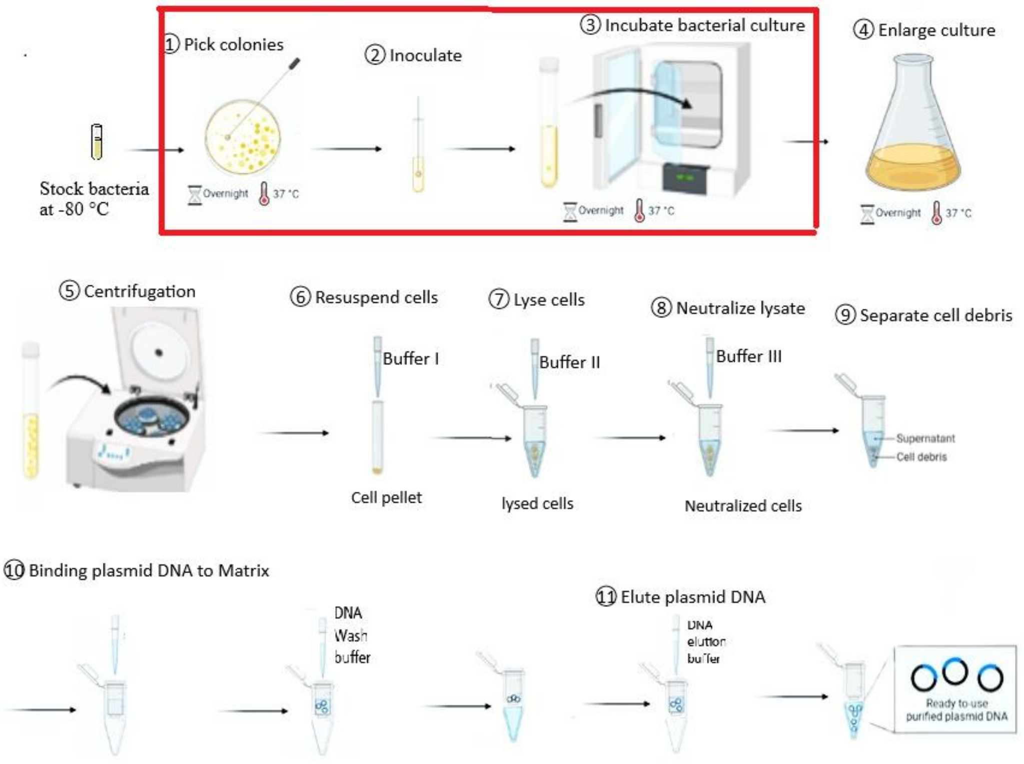

Figure 1. Flowchart of E. coli plasmid purification.

The steps within red tangle are removed in our experiments. The experiment is designed by us to use two days less than normal Plasmid extraction for a laboratory experiment. Our experiment used the plasmid M43-EH in E. coli host. We inoculated 10 µl bacteria from stocked 80°C into 250ml flask. And they are incubated at 37°C and 250 rpm speed.

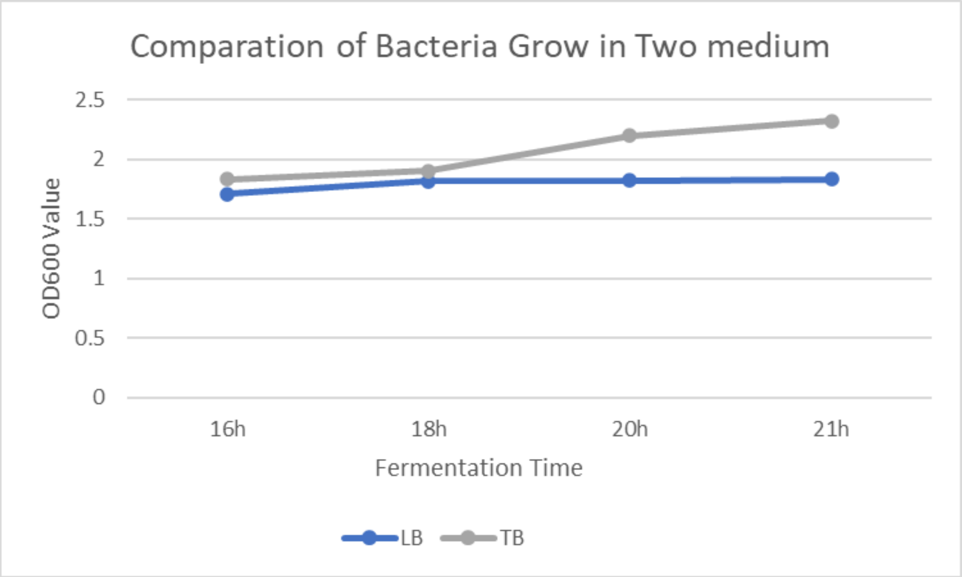

Figure 2. Cell concentration by spectrometer

Plasmid DNA was produced in cell culture (E. coli -EH) using a growth medium TB that has proven to result in higher amounts of plasmid DNA, in agreement with its ability to produce higher cell titters.

The bioprocess culminates with the downstream processing of pDNA, in which the main goals are the cell lysis and purification of SC pDNA. After pDNA production, which was measured by Nanodop One, the results are as follows (Table 1).

Table 1. The plasmids comparation from the two types of culture medium (For TB-Frozen samples, we frozed bacteria pellet at -20°C over half an hour.)

Sample Name	Nucleic Acid(ng/uL)	A260/A280	A260/A230	A260	A280	Baseline Correction (nm)	Baseline Absorbance
LB medium	2506.429	1.966	2.317	50.129	25.502	340	0.612
LB medium	2996.222	1.968	2.37	59.924	30.442	340	0.506
TB medium	3685.871	1.996	2.365	57.717	38.67	340	0.505
TB medium	4338.345	1.992	2.338	86.242	41.703	340	0.576
TB -Frozen	7193.543	1.97	2.334	143.871	73.045	340	0.787
TB -Frozen	6948.734	1.965	2.325	138.975	70.739	340	0.706

Plasmids are large, covalently closed, double-stranded DNA molecules, which are mostly found as a tightly twisted, supercoiled (SC) topological isomers and could be converted to a less compact form, the open circular (OC), by introducing a single break in one of the DNA strands. Double strand breaks will result in linear pDNA (Prazeres, D. M. F. 2011; Szeberényi, J. 2013).

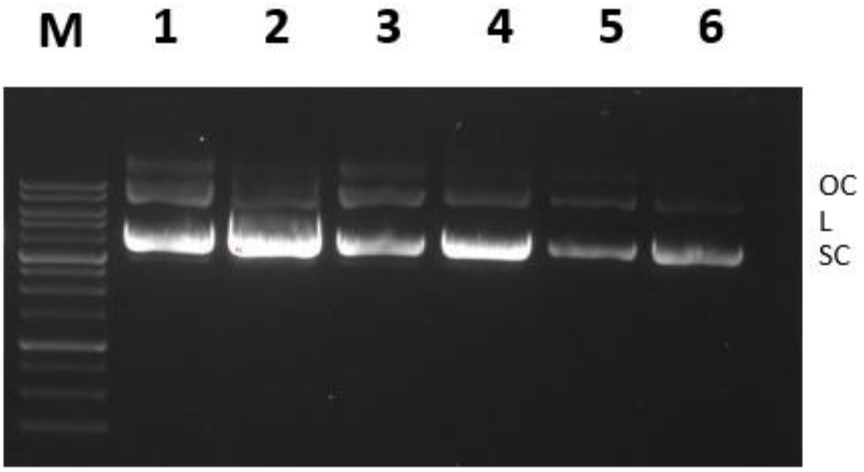

Figure 3. Agarose gel electrophoresis

The clarified E. coli M43-EH lysate samples diluted into 300ng/ µl, normal and after modified alkaline lysis protocol. Lane M—Molecular weight marker (10 kb); Lane 1, 2, 3, 4, 5 and 6—E. coli DH5α lysate samples from different groups of students. (b) Agarose gel electrophoresis showing the identification of the gDNA band; Lane M—Molecular weight marker (10 kb); Lane 1—E. coli M43-EH 900ng plasmids from TB medium, Lane 2—900ng plasmids from LB medium, Lane 3—E. coli M43-EH 600ng plasmids from TB medium, Lane 4—600ng plasmids from LB medium, Lane 5—E. coli M43-EH 300ng plasmids from TB medium, and Lane 6—300ng plasmids from LB medium.

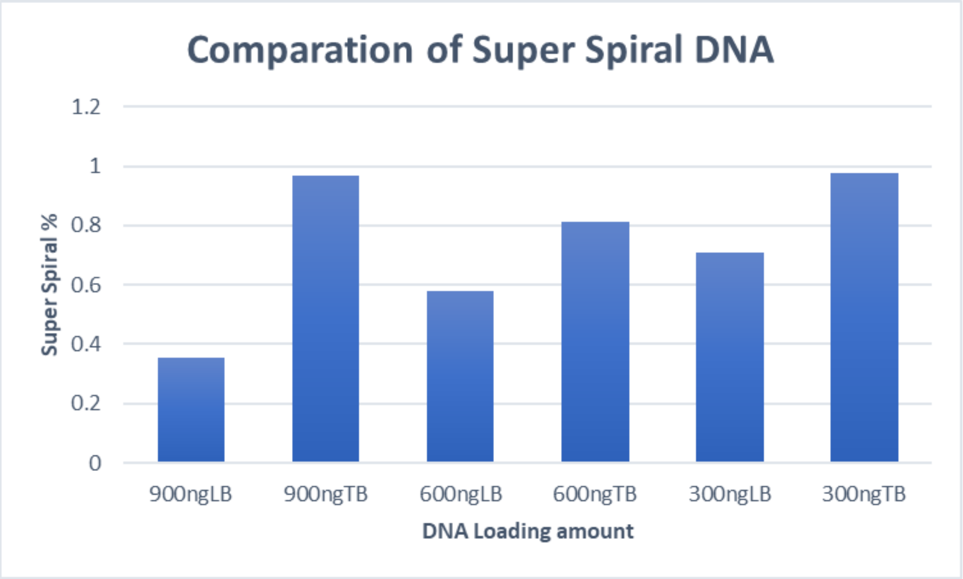

Figure 4. Plasmid DNA (pDNA) mass balance calculations

For supercoiled (SC) pDNA isoforms, according to Agarose gel electrophoresis. For calculate all lines, the supercoiled plasmid DNA (SC pDNA) fraction to relative lane loaded total DNA mass were calculated.

TB samples: Mostly circular plasmid DNA, strong bands, minimal linear or degraded forms.
LB samples: Slightly more linear DNA, indicating a bit more shearing or degradation.

4. DISCUSSION

The papers about isolation of plasmid DNA from bacteria are plenty. It is also easy to find a basic method from many biological companies which supply DNA extraction kits. But still, it is common to meet some different types of problems when we need large amounts high quality plasmids DNA. So, our study will be a solid way to solve this problem.

Yield and quality of plasmid DNA highly depend on the type of culture media used. Usually, plasmids purification is optimized with cultures grown in standard Luria Bertani (LB) medium. For LB medium preparation dissolve 10 g tryptone, 5 g yeast extract, and 10 g NaCl in 800 ml distilled water. Adjust the pH to 7.0 with 1 N NaOH. Adjust the volume to 1 liter by adding distilled water and sterilize by autoclaving.

But for some types of bacteria, which are caused by their species or maybe because they are transfected with some kind of plasmids, they need rich medium TB (Terrific Broth), otherwise it is hard to get bacteria OD600 reach to 2. Our results demonstrate that TB medium significantly enhances E. coli growth and plasmid DNA production. The high yield from TB makes it suitable for applications requiring large-scale or high-quality plasmids, such as transfection or gene therapy studies. The increased proportion of supercoiled DNA in TB samples also suggests improved plasmid integrity, likely due to reduced metabolic stress and enriched nutrients. Previous studies have reported similar benefits of TB over LB in recombinant protein expression and plasmid propagation (Yeung et al., 2008; Sambrook & Russell, 2001).

For the high-quality plasmid's extraction, good bacteria growth is the first important. Terrific Broth medium is modified for most E. coli, which is a very good source of supply. But many manuals about DNA extraction kits suggest that customers should use LB for bacteria growth. But our study indicates that this is not right. Also, care needs to be taken, as overgrowing a culture might lead to a higher percentage of dead or starving cells and the resulting plasmid DNA might be partially degraded or contaminated with chromosomal DNA. To find the optimal culture conditions, the culture medium and incubation times must be optimized for each host strain / plasmid construct combination individually. For the LB medium, 16 to 18 hours, the bacteria growth will reach stationary state, but for the TB medium, even until 21 hours, the bacteria are still growing.

In our study, we skip the two steps which are suggested by almost all the current protocol commonly used by people, which saves at least two days' time, and reduces some cost. We directly take 10-100 µl from -80°C stock to 250 ml flask, then incubated for 21 hours at 37°C. Our study shows it is not necessary to waste two days' time. But if the bacteria stock at -80°C is kept too long time, or maybe contaminated, we might have to grow them on the Agar plate first. For that the enlarging process is needed only when we grow over litter. Otherwise, we can skip them totally.

We also modified the protocol supplied by commercial plasmids kits for the two steps. First, we collected bacteria pellets and stored them -20°C for over half an hour, which will significantly improve the plasmids yield ($p<0.01$). The frozen and thaw process will help break cells well and release the plasmids. Remember, before putting at -20°C, measure the weight, according to the pellet, choose the right amount Buffer I is always a wise choice, because the bacteria amount varies a lot for each culture, which depends on many factors, like the bacteria amount inoculated, culture instrument brand, etc. Second, for the elution step, we can use more elution buffer, at least half volume suggested by kits' manual more. This way, we may use more elution buffer and some isopropanol, but compared to the expensive kits, we can save lot. Sometimes even double the plasmids yield.

Plasmids present in the bacterium differ in their physical properties such as in size (Kbp), geometry and copy number. But our study is reality and useful for most labs which need more quantitative and quality plasmids. The morphology of pDNA is very sensitive to environment conditions (Meyer, S., et al, 2017). The SC topology has the superior biological activity, because it can reach the perinuclear region of cells more efficiently than open circular and linear isoforms and thus become more easily entrapped in the cell nucleus (Remaut, K., 2006). Based on that, the Food and Drug Administration (FDA) recommends that the SC pDNA content in DNA vaccines should preferably be higher than 80% (Stenler, S., 2014). The plasmids from bacteria in TB medium show better quality and quantities.

The experimental results clearly demonstrate that TB medium substantially enhances both biomass accumulation and plasmid DNA recovery compared with LB medium. The higher OD600 values observed in TB cultures directly translate into increased plasmid yield; however, the quality improvements observed are equally significant. Agarose gel electrophoresis revealed a higher proportion of supercoiled plasmid DNA in TB-derived samples, indicating superior structural integrity and reduced DNA damage.

The improved plasmid quality likely results from the nutrient-rich and well-buffered composition of TB medium, which reduces metabolic stress during prolonged culture and supports sustained plasmid replication. In addition, the freezing–thawing step introduced in this study appears to enhance cell lysis efficiency, thereby increasing plasmid recovery without excessive mechanical disruption. Optimized elution volumes further contribute to improved yield while maintaining acceptable purity ratios.

Together, these findings confirm that TB medium not only increases plasmid quantity but also preserves plasmid topology, making it particularly suitable for applications requiring high biological activity.

While TB medium demonstrated clear advantages, it is not universally applicable to all *E. coli* strains or plasmid constructs. Some strains may require individualized optimization. Additionally, the freezing–thawing modification requires careful handling, particularly for inexperienced users. Future work may focus on strain-specific TB optimization, endotoxin assessment, bioreactor scalability, and integration with automated plasmid purification platforms.

6. CONCLUSION

This study establishes Terrific Broth as an effective medium for high-yield, high-quality plasmid DNA extraction. Compared with LB-based protocols, TB significantly improves bacterial growth, plasmid yield, and the proportion of supercoiled DNA, while reducing processing time by up to two days.

AUTHOR CONTRIBUTIONS

Yuxia Qin designed the study. Ye Xiong performed experimental work. Yuxia Qin drafted the manuscript. Xiaoju Liu analyzed the data. All authors contributed to the critical review and revision of the manuscript. They have seen and approved the final version.

FINANCIAL & COMPETING INTERESTS DISCLOSURE

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