molecular biology

GenElute™ Five-Minute Plasmid Miniprep Kit: The Fastest Method for Plasmid Preparation

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Application Notes

- **Sophisticated** – no more pelleting cells or clearing lysates
- **Flexible** – binding column works with any standard laboratory vacuum manifold
- **Plentiful** – up to 5 μg of plasmid DNA or enough for 15 sequencing reactions
- **Capable** – DNA suitable for immediate capillary DNA sequencing

Introduction

Plasmid preparation is often an essential step in molecular biology. This step can be time consuming and labor intensive, especially for researchers who need to screen hundreds or even thousands of clones in order to identify a few desired ones. Even though mini preparations of plasmid DNA are sufficient in most basic experiments, each preparation can take about 20-30 minutes and include a lot of hands-on steps.

The GenElute™ Five-Minute Plasmid Miniprep Kit circumvents many of the challenges and obstacles associated with current methods for small-scale plasmid DNA isolation. The conventional steps of cell harvesting, resuspension, alkaline lysis, neutralization, and lysate clearing have all been eliminated, resulting in the fastest method for isolating plasmid DNA. An overnight *E. coli* culture in LB broth is treated briefly with a lysis reagent to effect rapid cell lysis and RNA degradation in the presence of culture medium. Moreover, in contrast to alkaline lysis, overexposure to the lysis reagent does not compromise plasmid integrity. Therefore, the procedure is not only rapid, but also flexible and can be easily scaled to current demands. Following lysis, released plasmid DNA is captured and purified on a silica-based binding column. Purified plasmid DNA is suitable for capillary DNA sequencing, restriction digestion, and PCR. Typical yields of high copy plasmid are 2-6 μg from 400 μl of overnight culture.

Common procedures for plasmid preparation rely on the alkaline lysis method described by Birnboim and Doly over two decades ago. Typically, *E. coli* cells are harvested from overnight culture, resuspended in buffer, and lysed with NaOH and SDS. The lysate is then neutralized with an acidic salt solution, and the resultant precipitate of cellular debris and SDS-salt complex is removed by centrifugation or filtration to produce a cleared lysate, from which plasmid DNA can be isolated by various methods, such as cesium chloride density gradient ultra-centrifugation, phenol/chloroform extraction, anion exchange chromatography, or silica-based chromatography.

While the combination of alkaline lysis with silica-based chromatography has greatly increased the speed of plasmid preparation, harvesting of bacteria and resuspension of the bacterial pellet prior to alkaline lysis remains a laborious task for manual preparation and an obstacle for automation. Moreover, alkaline lysis is time sensitive and needs to be controlled attentively, as overexposure to alkaline lysis solution can cause irreversible damage to plasmid DNA and render it unsuitable for downstream application. Lysate clearing is also a time-consuming step. All these become more burdensome when a large number of small samples are to be prepared. Alternative methods, such as boiling and microwave radiation for cell lysis, and glass bead binding to circumvent the lysate clearing step, have attempted to simplify the process.

Materials and Methods

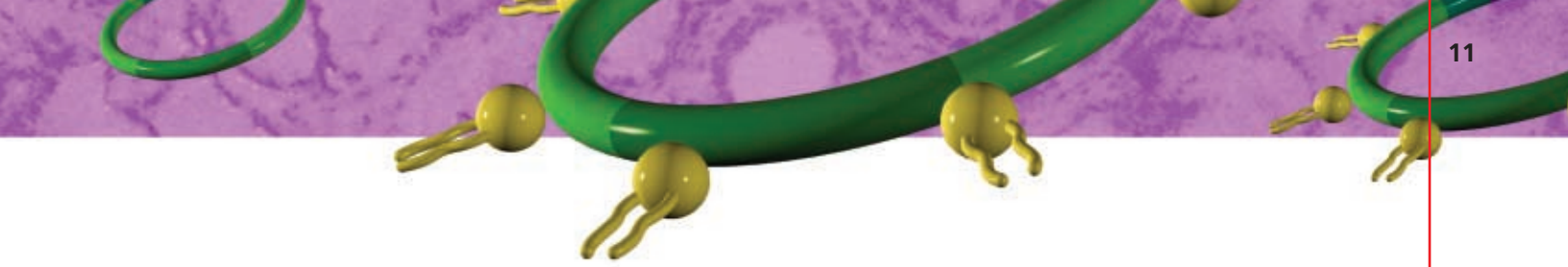
All materials were supplied by Sigma-Aldrich Corporation (St. Louis, MO) unless otherwise stated.

Culture preparation

Four different types of plasmid, each in a different *E. coli* host strain, were used to prepare plasmid DNA: (1) pCMV-SPORT-β-gal (7.9 kb) in DH5α (Invitrogen, Carlsbad, CA); (2) pCRII-TOPO (5.7 kb, including an insert) in TOP10 (Invitrogen, Carlsbad, CA); (3) pBluescript SK (3.0 kb) in HB101 (Stratagen, La Jolla, CA); (4) pUC18 (2.7 kb) in XL1-Blue (Stratagen, La Jolla, CA). All cultures were grown in 20 ml of LB (Luria-Bertani) broth, supplemented with ampicillin (Product Code A 9518) at 100 μg per ml. Cultures were incubated overnight at 280 rpm, 37 °C.

Purification procedure

For each isolation, a 400-μl aliquot of overnight culture was lysed with 40 μl of reconstituted Lysis Reagent at room temperature for 2 minutes, unless otherwise stated. The lysate was then mixed with 400 μl of Binding Solution and the mixture was transferred to a Binding Column seated in a 2-ml Collection Tube (for spin protocol) or attached to a vacuum manifold (for vacuum protocol). Following the spin protocol, DNA binding was



achieved with 20 seconds of centrifugation at maximum speed (14,000 rpm), and the column was washed first with 700 µl of diluted Wash Solution with 20 seconds of centrifugation, then with 200 µl of diluted Wash Solution with 30 seconds of centrifugation to wash and dry the column. Following the vacuum protocol, DNA binding was achieved with vacuum filtration, and the column was washed with 1 ml of diluted Wash Solution under vacuum, and then dried with 30 seconds of centrifugation. For both protocols, plasmid DNA was eluted in tris buffer (10 mM, pH 8.5) with 30 seconds of centrifugation. Plasmid DNA recovery was determined for each sample with 1 µl of eluate, using a PicoGreen dsDNA Quantitation kit (Molecular Probes, Eugene, OR).

Sequencing

Capillary DNA sequencing was performed by SeqWright DNA Technology Services (Houston, TX). Each sample was provided to SeqWright in 5 µl eluate without adjusting the DNA concentration. Cycle sequencing reactions were performed with a T7 sequencing primer (for pCMV-SPORT-β-gal, pCRII-TOPO, and pBluescript SK) or a M13 Forward sequencing primer (for pUC18) and ABI BigDye™ terminator v3.1. Sequencing reaction products were resolved on an ABI Prism® 3730 DNA Analyzer.

Restriction digestion

Restriction digestion was performed with 3 µl of eluate from each sample, 3 units of *EcoR I* (Product Code R 6252) and 3 units of *Xho I* (Product Code R 6379) in combination (for pCMV-SPORT-β-gal and pCRII-TOPO), or 3 units of *EcoR I* alone (for pBluescript SK and pUC18). All digestions were incubated at 37 °C for 1 hour and analyzed on a 1% agarose gel.

PCR

PCR experiments were performed in a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA), with 1 µl of 100-fold diluted plasmid sample, 200 µM each dNTP, 0.4 µM each of T7 and SP6 primers, and 2.5 units of AccuTaq™ LA DNA Polymerse (Product Code D 8045) in 50 µl reaction volume. PCR products were resolved on a 1% agarose gel.

Results and Discussion

Four *E. coli* host strains (DH5α, TOP10, HB101, and XL1-Blue), each containing a different plasmid, were grown in LB broth and used to prepare plasmid DNA with the GenElute Five-Minute Plasmid Miniprep Kit. LB (Luria-Bertani) broth is recommended for growing cultures for the GenElute Five-Minute Plasmid Miniprep Kit. Cultures grown in rich media, such as TB (Terrific Broth), can produce substantially higher cell densities and will consequently overload the purification system and clog the binding column, resulting in poor plasmid recovery (data not shown).

Of the four *E. coli* strains grown in LB broth, HB101 produced the highest cell density with an OD_{600} reading equal to 3.5, while the OD_{600} readings of the other three strains ranged between 2 and 3. Cell lysis occurred rapidly upon the addition of reconstituted Lysis Reagent, even in the presence of culture medium, although there was some variation among the strains. HB101, though having the highest cell density, was the most susceptible to lysis, while XL1-Blue was the least susceptible, for which the lysis time was extended to 3 minutes from the standard protocol of 2 minutes.

It is not necessary, however, to monitor the lysis time, especially when there are a large number of cultures to handle. The lysis step can be extended until it is most convenient to proceed to the next step, since overexposure to the lysis reagent does not adversely affect plasmid DNA integrity. In fact, we have confirmed that plasmid DNA remained stable after exposure to the lysis reagent for 30 minutes, 60 minutes, and even 24 hours (data not shown). This long-term stability allows a large number of cultures to be lysed at the same time. Conversely, for applications that do not require maximum plasmid recovery, cultures can be lysed for 1 minute to reduce the preparation time.

Table 1. Recovery of plasmid DNA from 400 µl overnight culture using the GenElute Five-Minute Plasmid Miniprep Kit

Plasmid	Size (kb)	*E. coli* strain	Protocol	Number of preps	Vol. of elution solution (µl)	Yield (µg)
pCMV-SPORT-β-gal						
	7.9	DH5α	Centrifugation	8	50	7.4 ± 0.5
				8	40	5.7 ± 0.6
			Vacuum	8	40	6.6 ± 0.3
pCRII-TOPO						
	5.7	TOP10	Centrifugation	4	40	4.2 ± 0.5
				4	30	3.9 ± 0.3
			Vacuum	4	40	5.5 ± 0.2
				4	30	4.4 ± 0.3
pBluescript SK						
	3.0	HB101	Centrifugation	4	40	5.8 ± 0.5
				4	30	4.6 ± 0.5
			Vacuum	4	40	5.5 ± 0.2
				4	30	4.7 ± 0.3
pUC18						
	2.7	XL1-Blue	Centrifugation	8	30	2.5 ± 0.6
			Vacuum	8	30	3.3 ± 0.4

Plasmid DNA recovery was quantified by PicoGreen and confirmed by agarose gel electrophoresis. As shown in Table 1, plasmid yields ranged from 2 to 7 µg among the cultures. The highest yields were obtained from the largest plasmid, pCMV-SPORT-β-gal (7.9 kb); and the lowest from the smallest plasmid, pUC18 (2.7 kb). The differences in yield among these different plasmids were expected, as such differences were also observed with other conventional purification kits. In general, the yields of plasmid, as measured by mass (µg), are proportional to the sizes of plasmid if the plasmid copy number and the cell density are similar between cultures. Similar yields were obtained between spin and vacuum protocols within each culture. However, the recovery increased by 10-20% as the volume of elution solution increased from 30 to 40 µl (pCRII-TOPO and pBluescript SK) and from 40 to 50 µl (pCMV-SPORT-β-gal). We recommend 40 µl of elution solution as standard, as this would, in most cases, result in approximately 100 ng/µl plasmid DNA in the eluate, of which 2-3 µl will be sufficient for a restriction digestion or a DNA sequencing reaction, thus making it unnecessary to quantify each sample.

Plasmid preparations from the GenElute Five-Minute Miniprep Kit are especially suitable for capillary DNA sequencing. We have consistently obtained high quality sequencing data across different types of plasmid with 2 µl of eluate without any adjustment in DNA concentration. As shown in Figure 1, the average Read Length at Phred > 20 ranged from 868 to 945 bases among the four different types of plasmid. This level of Phred scores is equivalent to that of other conventional purification kits. A portion of the electropherogram for pCMV-SPORT-β-gal is shown in Figure 2, which reveals strong signal and sharp peaks even after 600 bases, and greater than 900 bases of readable sequence.

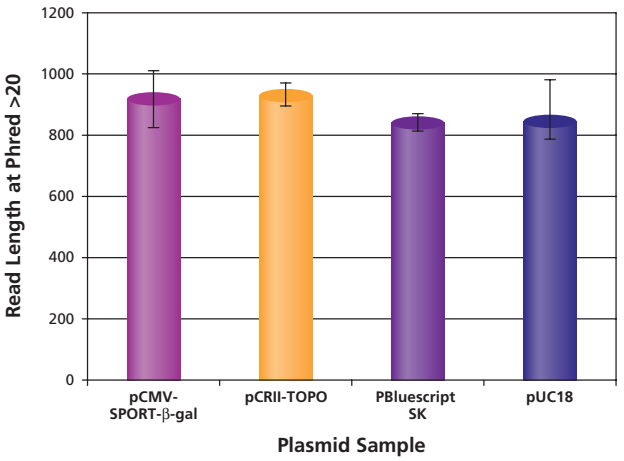


Figure 1. Phred scoring for capillary DNA sequencing of plasmid DNA samples purified with the GenElute Five-Minute Plasmid Miniprep Kit. *Plasmid samples were prepared with the GenElute Five-Minute Plasmid Miniprep Kit and sequenced by SeqWright DNA Technology Service (Houston, TX) with a T7 sequencing primer (pCMV-SPORT-β-gal, pCRII-TOPO, and pBluescript SK) or a M13 Forward sequencing primer (pUC18) and ABI® BigDye™ terminator v3.1 on an ABI Prism® 3730 DNA Analyzer. The Phred score for each plasmid was average of 16 independent minipreps from each type of culture.*

Plasmid preparations from the GenElute Five-Minute Miniprep Kit are also suitable for restriction endonuclease digestion and PCR amplification. Restriction digestion is an established method for identifying and characterizing clones that contain desirable inserts, which can then be amplified in a quantity large enough for use as the substrate in subcloning, probe preparation, and micro-

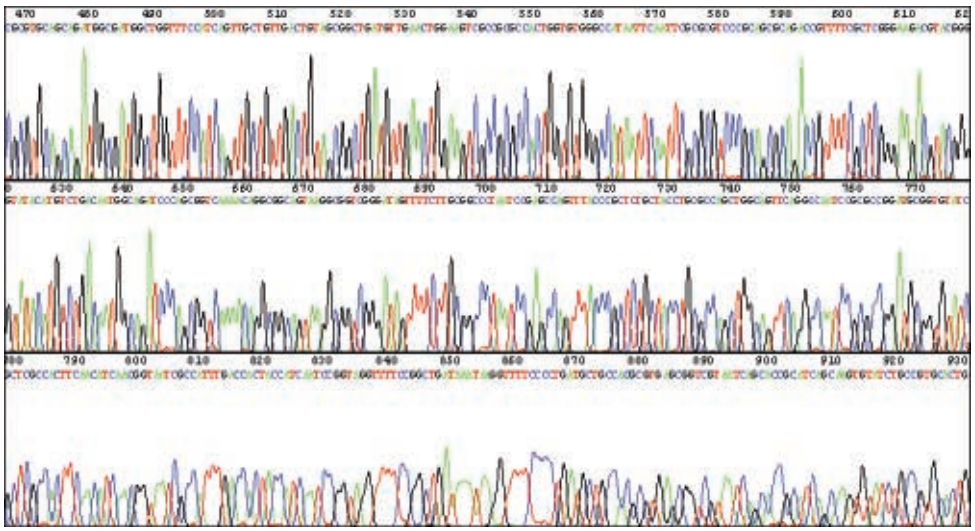


Figure 2. A portion of electropherogram revealing sharp peaks and strong signal between 600 and 900 bases from pCMV-SPORT-β-gal purified with the GenElute Five-Minute Plasmid Miniprep Kit.

array deposition. All plasmid samples isolated from the four cultures were digested to completion after 1 hr at 37 °C, with *Xho I* and *EcoR I* in combination or with *EcoR I* alone. Figure 3 shows the digested products of pCMV-SPORT-β-gal by *EcoR I* and *Xho I,* in agarose gel analysis. Suitability for PCR was confirmed with pCMV-SPORT-β-gal and pCRII-TOPO preparations. Figure 4 shows the PCR products of a 3.6 kb β-gal gene, amplified from pCMV-SPORT-β-gal preparations.

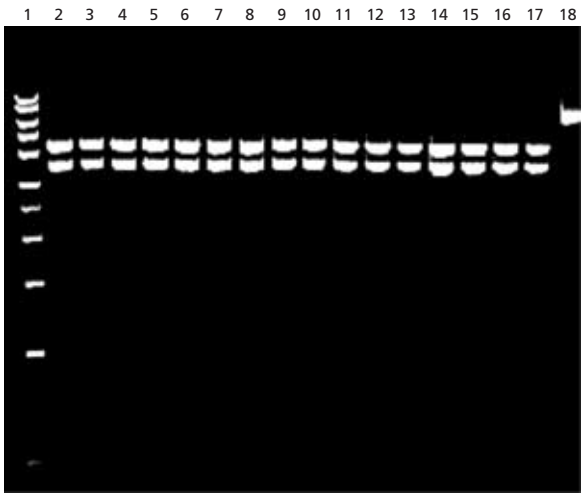


Figure 3. Restriction digestion of pCMV-SPORT-β-gal purified with the GenElute Five-Minute Plasmid Miniprep Kit. *Each digestion was carried out in 15 µl reaction volume at 37 °C for 1 hour, with 3 µl of eluate and 3 units each of EcoR I and Xho I. A quarter of each digestion was fractionated in 1% agarose gel. Lane 1: 1 kb DNA ladder (Product Code D 0428); Lanes 2 to 17: 16 independent plasmid samples prepared with the GenElute Five-Minute Plasmid Miniprep Kit; Lane 18: uncut pCMV-SPORT-β-gal DNA.*

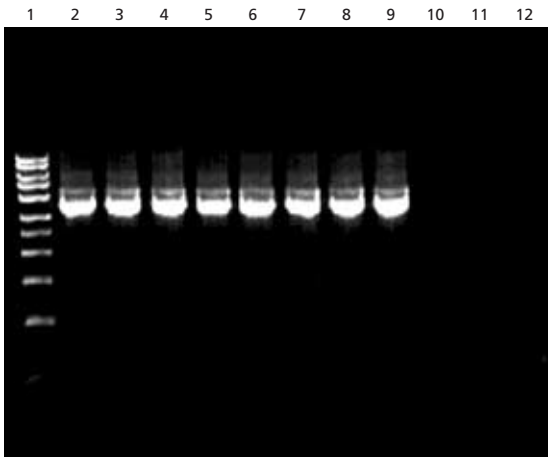


Figure 4. PCR products of a 3.6 kb β-gal gene amplified from pCMV-SPORT-β-gal purified with the GenElute Five Minute Plasmid Miniprep Kit. *Plasmid DNA of pCMV-SPORT-β-gal (7.9 kb) was prepared from 400 µl overnight culture of* E. coli *host strain DH5α with the GenElute Five-Minute Plasmid Miniprep Kit. Purified plasmid DNA sample was diluted 100-fold in TE buffer, and 1 µl of the diluted plasmid DNA was used for PCR amplification. Lane 1: 1 kb DNA Ladder (Product Code D 0428); Lanes 2 to 9: PCR products amplified from 8 independent plasmid minipreps; Lanes 10 to 12: no template controls.*

Conclusions

The GenElute Five-Minute Plasmid Miniprep Kit provides a rapid and simplified procedure for isolating plasmid DNA directly from overnight cultures of recombinant *E. coli.* The streamlined procedure enables preparation of plasmid DNA from overnight culture in 5 minutes. The procedure is also very flexible, and a large number of samples can be processed simultaneously. The small culture volume required for each preparation reduces the cost and time associated with growing and handling overnight cultures. Purified plasmid samples are suitable for capillary DNA sequencing, restriction endonuclease digestion, and PCR amplification.

Acknowledgements

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References

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Ordering Information

Product	Description	Unit
PFM10	GenElute Five-Minute Plasmid Miniprep Kit	10 preps
PFM50	GenElute Five-Minute Plasmid Miniprep Kit	50 preps
PFM250	GenElute Five-Minute Plasmid Miniprep Kit	250 preps