Molecular Biology

# FeatureArticle

## GenElute™ High Performance (HP) Plasmid Kits: Plasmid DNA from a Midiprep or Maxiprep in 30 Minutes

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### Introduction

Plasmids are an essential tool in molecular biology for manipulating and decoding genetic information. They are small circular molecules of double-stranded DNA that are able to self-replicate and occur naturally in bacterial cells. A typical plasmid is 2-10 thousand base pairs in size. It is their relatively small size that makes them easy to manipulate in the laboratory. Plasmid vectors or cloning vectors are generally modified plasmids that possess a resistance gene (marker) and a multiple cloning site for the insertion of genes of interest. They are the primary vehicle used in the isolation and characterization of genes and their translated proteins. Therefore, it is necessary to have a purification method that yields quality plasmid DNA that is suitable for applications such as cloning, sequencing, PCR, transfection, and protein expression.

Traditional methods for the purification of plasmid DNA include cesium chloride density gradients and phenol/chloroform extractions. Cesium chloride purification separates supercoiled plasmid DNA from chromosomal DNA and nicked plasmid DNA. Supercoiled plasmid intercalates ethidium bromide resulting in an increase in its density. The supercoiled plasmid is separated from the linear and nicked plasmid DNA by ultra-centrifugation in a cesium chloride density gradient. Finally, the supercoiled plasmid will need to be extracted under an ultra-violet light source and undergo dialysis to remove the cesium chloride. A second method is phenol/chloroform extraction, which typically consists of adding phenol to an aqueous DNA sample and a centrifugation step that results in a phase separation. The upper phase containing the DNA is transferred to a new tube and the process is repeated using a 1:1 mixture of phenol/chloroform followed by a final chloroform extraction. It is then necessary to precipitate the DNA with ethanol or isopropanol. These methods can provide quality plasmid DNA but require the use of hazardous materials, are extremely time consuming, and can yield low plasmid recoveries. Therefore, a rapid purification method that is nonhazardous and yields quality plasmid DNA would offer a significant improvement over existing purification technologies.

The GenElute HP Plasmid Kits provide a rapid, non-hazardous method of isolating quality plasmid DNA. The kits do not require the use of any carcinogens such as ethidium bromide or toxic chemicals like phenol and chloroform. Using a simple protocol, the plasmid preparations take only 30 minutes to complete and do not require any alcohol precipitation. These kits feature a filter syringe for rapidly clearing the lysate, and a silica-binding column that can be used with a vacuum or spin purification format. The result is high quality plasmid DNA suitable for most downstream applications. Expected yields are up to 350 µg for the HP Midiprep Kit and 1.2 mg for the HP Maxiprep Kit.

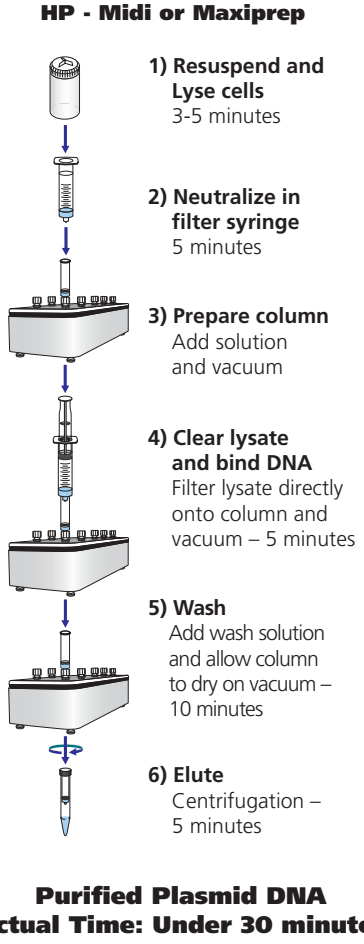


***Figure 1. Overview of the GenElute™ HP Midiprep and Maxiprep Kit procedure using the vacuum format.***

## Materials and Methods

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

### *Culture preparation*

A flask containing LB medium[1] and 100 µg/ml of ampicillin (Product Code A 9518) was inoculated with *E. coli* strain DH5α harboring pCMV-SPORT-βgal (Invitrogen, Carlsbad, CA), which is a 7.9 kb high-copy plasmid. The culture was grown shaking at 300 rpm for 17 hours at 37 °C.

### *Restriction digestion*

Restriction analysis was performed by digesting 250 ng of plasmid DNA with 5 units of *Eco*RI (Product Code R 6265) and 5 units *Hin*d III (Product Code R 1137) at 37 °C for 1.5 hours. Samples were run on a 1 % TBE agarose gel at 100 volts.

### *Sequencing*

Cycle sequencing was performed using 500 ng of plasmid DNA, a T7 sequencing primer, and ABI® BigDye™ terminator (Applied Biosystems, Foster City, CA) chemistry. Sequencing reactions were resolved on an ABI Prism® 377 XL instrument with a 48-cm gel cassette containing 4.5 % AutoPAGE™ Plus acrylamide (Product Code P 8977) at 2.4 kV, 48 °C for 7 hours.

### *Transfection*

Transfections were performed using ESCORT™ IV transfection reagent (Product Code L 3287). CHO-K1 (ATCC # CCL-61; American Type Culture Collection, Manassas, VA) cells were seeded in six-well tissue culture plates and grown to 60-70 % confluency. The cells were transfected with 3 µg of plasmid / 15 µl of transfection reagent. β-galactosidase activity was measured using the β-Galactosidase Reporter Gene Activity Detection Kit (Product Code GAL-A) at 54 hours post-transfection. Absorbance readings were taken at 420 nm and β-gal activity was recorded in units of β-gal/plate.

## Results and Discussion

To evaluate the GenElute HP Midiprep and Maxiprep Kits, plasmid DNA was prepared from a variety of recombinant *E. coli* strains. A representative preparation involved purifying pCMV-SPORT-βgal high-copy plasmid from strain DH5α using the GenElute HP Maxiprep Kit. The culture was grown overnight and reached an absorbance of 2.5 at 600 nm. A 150-ml aliquot of the culture was harvested by centrifugation at 5,000 x g for 10 minutes. The cell pellet was thoroughly resuspended in a Tris-EDTA buffer containing RNase A which degrades RNA and prevents it from contaminating the final plasmid preparation. The suspension was then subjected to a modified alkaline-SDS lysis for 3 minutes (Figure 1).[2] This step breaks apart the bacterial cell wall and releases the internal components. The lysate was then neutralized, resulting in the formation of a white flocculent containing denatured proteins, lipids, SDS, chromosomal DNA, and other cell debris. A binding solution was then added to the lysate and the mixture was transferred to the barrel of a filter syringe and incubated at room temperature for 5 minutes. During this incubation, the binding column was placed on a vacuum manifold and an equilibration solution was added. The vacuum was applied to draw the equilibration solution through the column. Then, the filter syringe barrel was placed over the equilibrated binding column and the plunger was added to expel the clarified lysate solution from the syringe and onto the binding column. The lysate passed through the column while the plasmid DNA bound to the silica membrane.[3] The contaminants were then removed with a simple wash step. Finally, the bound plasmid DNA was eluted in 3 ml of Tris buffer by centrifugation.

The GenElute HP Maxiprep sample was then analyzed for plasmid concentration by determining an absorbance reading at 260 nm. The calculated yield of the preparation was 1.2 mg of plasmid DNA. The purity of the sample was determined by calculating the ratio of absorbance at 260 nm/280 nm. The theoretical absorbance ratio for DNA is between 1.8 and 2.0. Ratios below 1.8 can indicate contamination with proteins, while ratios above 2.0 can indicate contamination with RNA. The sample gave a ratio of 1.9, indicating a plasmid preparation of high purity. The recovery and purity of the sample can also be determined by agarose gel electrophoresis. As seen in Figure 2 (Lane 2), the purified plasmid is predominately in its super-coiled form with no evidence of genomic DNA or RNA contamination.

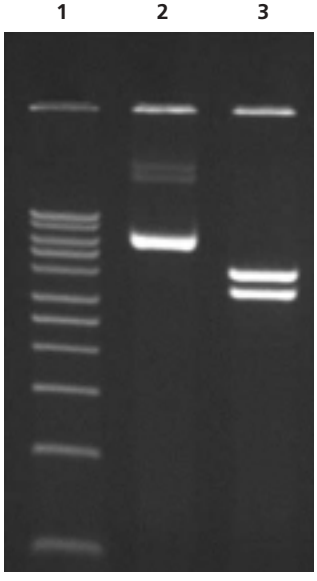


***Figure 2. Restriction digestion of plasmid DNA purified using the GenElute™ HP Maxiprep Kit. Lane 1:*** *1 kb DNA Ladder (Product Code D 0428);* ***Lane 2:*** *250 ng of undigested plasmid DNA;* ***Lane 3:*** *EcoRI and HindIII digestion of 250 ng of plasmid DNA.*

To further assess the quality of the plasmid preparation, the DNA was subjected to restriction digestion. Restriction enzymes are endonucleases that cleave specific DNA sequences. It is these enzymes that make it possible to isolate and characterize specific DNA fragments. However, crude plasmid isolation methods can render the DNA unsuitable for digestion or the preparations can contain impurities that inhibit the endonuclease activity, resulting in inefficient digestions. The plasmid was simultaneously digested with *Eco*RI and *Hin*dIII restriction enzymes. The digest was analyzed on a 1 % agarose gel. As seen in Figure 2 (Lane 3), the digest contained two DNA fragments of the appropriate size and was void of any degraded or undigested DNA.

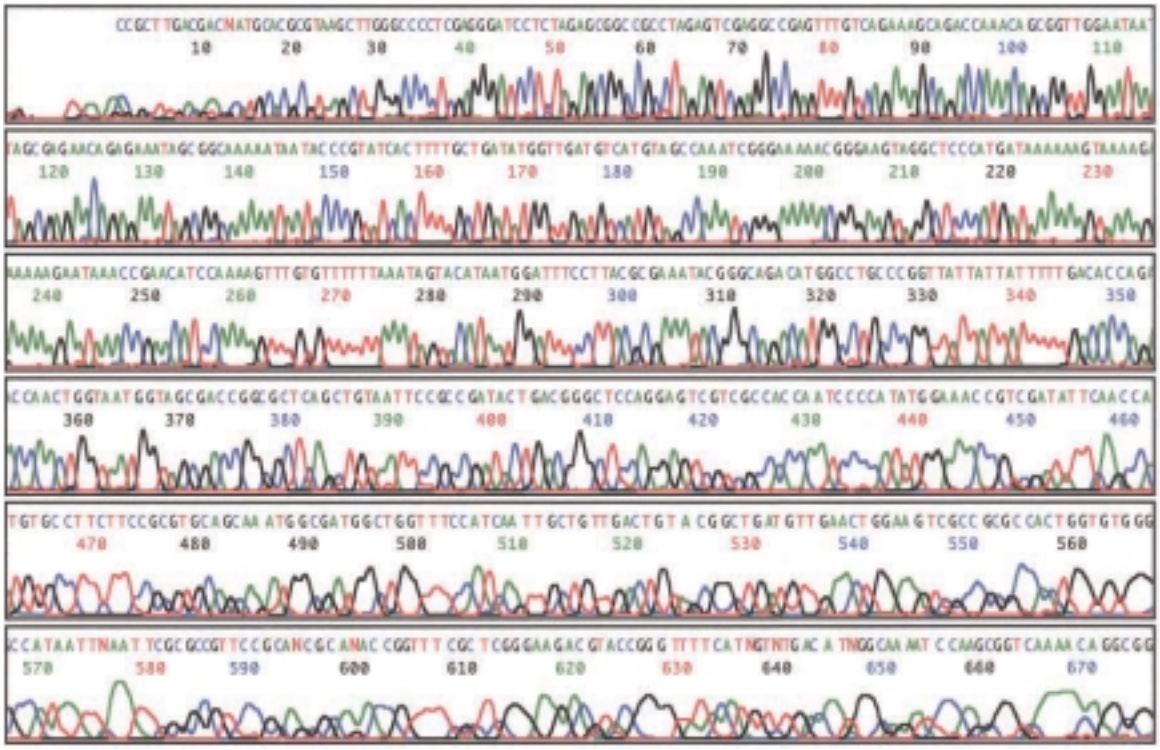

***Figure 3. Electropherogram revealing greater than 600 bases of sequence from pCMV-SPORT β-gal purified from the GenElute™ HP Maxiprep Kit.*** *Details described in Materials and Methods section.*

The quality of the plasmid DNA was also tested by performing automated sequencing. DNA sequencing is the determination of the exact nucleotide sequence in a DNA sample. It is typical for modern sequencing methods to reveal between 500 and 700 bases of nucleotide sequence. However, contaminants in a plasmid preparation can result in smaller read lengths of sequence or none at all. The electropherogram shown in Figure 3 reveals sharp peaks and greater than 600 bases of readable sequence, indicating the plasmid preparation is of high quality.

The performance of the GenElute HP Maxiprep Kit was further evaluated by comparing the product against four other commercially available kits. Plasmid preparations were performed in duplicate using the manufacturers' recommended protocols from a single overnight culture of DH5α harboring pCMV-SPORT-βgal. The average preparation time for each kit is shown in Figure 4. The GenElute HP Maxiprep protocol took only 30 minutes to complete. The closest competitor evaluated took over twice as long, while other kits required almost 2.5 hours to complete. Plasmid recoveries were also compared between the kits (Figure 5). The GenElute HP Maxiprep Kit provided a yield of 1.1 mg of plasmid, which is 25 % greater than the yield of the closest competitor and approximately 50 % greater than the yield from the other kits evaluated.

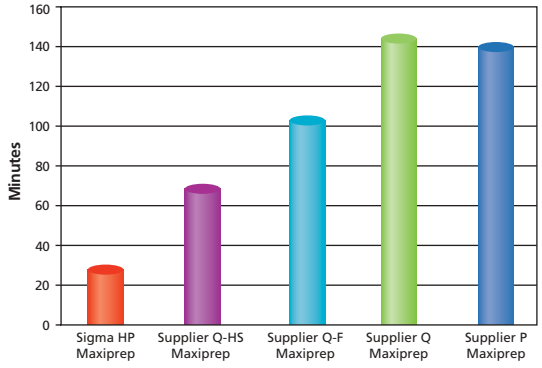


***Figure 4. Comparison of time per isolation using different Maxiprep plasmid purification systems.*** *Samples were prepared in duplicate using the manufacturers' recommended protocols and the average preparation time shown here.*

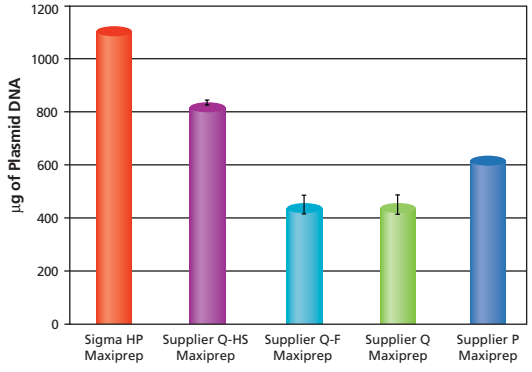


***Figure 5. Comparison of plasmid (pCMV-SPORT-β gal samples) yields using different Maxiprep plasmid purification systems.*** *Samples were prepared in duplicate using the manufacturers' recommended protocols.*

Finally, an important application of plasmid DNA is transfection. This procedure involves the insertion of plasmid DNA into eukaryotic cells to characterize protein expression of DNA cloned into the plasmid. This technique is considered to be highly sensitive, as plasmid preparations of poor quality can result in low transfection efficiencies because impurities can affect cell growth and metabolism. The GenElute HP Maxiprep samples were compared to the three leading suppliers' samples. The data in Figure 6 shows the average of three replicates for each plasmid isolation. The GenElute HP Maxiprep sample shows a high level of efficiency, equivalent to samples prepared from the other competitors, indicating a sample of high quality.

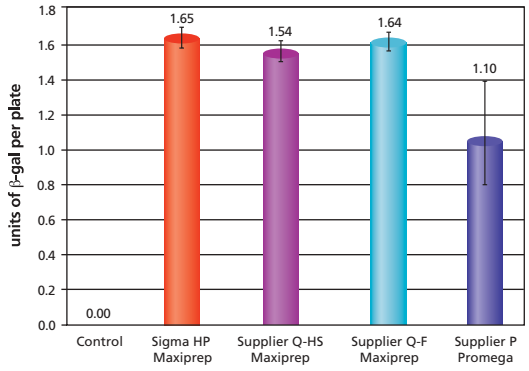


***Figure 6. Comparison of transfection efficiencies into CHO-K1 cells using different Maxiprep plasmid purification systems.*** *The data shows the average of three replicates prepared from each kit. Details described in Materials and Methods section.*

## Conclusions

The GenElute HP Plasmid Midiprep and Maxiprep Kits provide a fast and simple method for the purification of plasmid DNA. Plasmid preparations can be performed in only 30 minutes and involve very little hands-on time. There are no phenol/chloroform extractions or alcohol precipitations required. Yields are up to 350 µg with the Midiprep kit and 1.2 mg with the Maxiprep Kit. These kits have clearly been shown to be faster and deliver higher yields than other commercially available kits. The final plasmid product is of high quality and is suitable for restriction digestion, ligation, sequencing, PCR, transformation, and transfection applications.

## Acknowledgements

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## References

1. Ausubel, F., et al., (eds.) Short Protocols in Molecular Biology, Third Edition, pp. 1.2-1.3 (John Wiley & Sons, Inc., 1997).

2. Birnboim, H.C. and Doly, J., A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7,** 1513-1522 (1979).

3. Vogelstein, B. and Gillespie, D., Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. USA, **76,** 615-619 (1979).

***About the Author***
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## ORDERING INFORMATION

| Product Code | Product Description | Preps |
|---|---|---|
| NA0200S | GenElute™ HP Plasmid Midiprep Kit | 4 |
| NA0200 | GenElute™ HP Plasmid Midiprep Kit | 25 |
| NA0300S | GenElute™ HP Plasmid Maxiprep Kit | 4 |
| NA0300 | GenElute™ HP Plasmid Maxiprep Kit | 10 |
| NA0310 | GenElute™ HP Plasmid Maxiprep Kit | 25 |

## SUGGESTED LITERATURE

| | | |
|---|---|---|
| M 8265 | Molecular Cloning: A Laboratory Manual, 3rd ed., Vols. 1, 2, & 3 (2001) | 1 set |