Bench Protocol: QIAprep Spin Miniprep Kit Using a Microcentrifuge

This protocol is designed for the purification of up to 20 µg high-copy plasmid DNA from 1–5 ml overnight *E. coli* culture in LB medium. New users and users wanting to purify low-copy plasmids and cosmids, large plasmids (>10 kb), and DNA prepared using other methods should refer to the detailed protocols provided in the *QIAprep Miniprep Handbook*, 2nd ed.

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Things to do before starting

- Add RNase A solution to Buffer P1.
- Optional: Add LyseBlue reagent to Buffer P1.
- Add ethanol (96–100%) to Buffer PE.
- Check Buffers P2 and N3 for salt precipitation and redissolve at 37°C if necessary.

Procedure

1. **Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube.**
2. **Add 250 µl Buffer P2 and mix thoroughly by inverting the tube 4–6 times.**

   If using LyseBlue reagent, solution turns blue.
3. **Add 350 µl Buffer N3 and mix immediately and thoroughly by inverting the tube 4–6 times.**

   If using LyseBlue reagent, solution turns colorless.
4. **Centrifuge for 10 min at 13,000 rpm (~17,900 x *g*) in a table-top microcentrifuge.**
5. **Apply the supernatant (from step 4) to the QIAprep spin column by decanting or pipetting.**
6. **Centrifuge for 30–60 s. Discard the flow-through.**
7. **Recommended: Wash the QIAprep spin column by adding 0.5 ml Buffer PB and centrifuging for 30–60 s. Discard the flow-through.**

   This step is only required when using *endA*$^+$ or other bacteria strains with high nuclease activity or carbohydrate content (see *QIAprep Miniprep Handbook* for more details)
8. **Wash QIAprep spin column by adding 0.75 ml Buffer PE and centrifuging for 30–60 s.**
9. **Discard the flow-through, and centrifuge for an additional 1 min to remove residual wash buffer.**
10. **To elute DNA, place the QIAprep column in a clean 1.5 ml microcentrifuge tube. Add 50 µl Buffer EB or water to the center of each QIAprep spin column, let stand for 1 min, and centrifuge for 1 min.**

Introduction

The QIAprep Miniprep system provides a fast, simple, and cost-effective plasmid miniprep method for routine molecular biology laboratory applications. QIAprep Miniprep Kits use silica membrane technology to eliminate the cumbersome steps associated with loose resins or slurries. Plasmid DNA purified with QIAprep Miniprep Kits is immediately ready for use. Phenol extraction and ethanol precipitation are not required, and high-quality plasmid DNA is eluted in a small volume of Tris buffer (included in each kit) or water. The QIAprep system consists of four products with different handling options to suit every throughput need.

Low throughput

The **QIAprep Spin Miniprep Kit** is designed for quick and convenient processing of 1–24 samples simultaneously in less than 30 minutes. QIAprep spin columns can be used in a microcentrifuge or on any vacuum manifold with luer connectors (e.g., QIAvac 24 Plus, or QIAvac 6S with QIAvac Luer Adapters).

The **QIAprep Spin Miniprep Kit** can be fully automated on the **QIAcube**. The innovative QIAcube uses advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. Sample preparation using the QIAcube follows the same steps as the manual procedure (i.e., lyse, bind, wash, and elute) enabling you to continue using the QIAprep Spin Miniprep Kit for purification of high-quality plasmid DNA.

The QIAcube is preinstalled with protocols for purification of plasmid DNA, genomic DNA, RNA, viral nucleic acids, and proteins, plus DNA and RNA cleanup. The range of protocols available is continually expanding, and additional QIAGEN protocols can be downloaded free of charge at www.qiagen.com/MyQIAcube .

Medium throughput

For medium throughput requirements the **QIAprep 8 Miniprep Kit and QIAprep 8 Turbo Miniprep Kit** utilize 8-well strips on QIAvac 6S allowing up to 48 minipreps to be performed simultaneously in approximately 40 and 30 minutes respectively. In addition, the **QIAprep 8 Turbo BioRobot® Kit** enables automated purification of up to 48 minipreps in 50 minutes on BioRobot systems.

High throughput

The **QIAprep 96 Turbo Miniprep Kit** enables up to 96 minipreps to be performed simultaneously in less than 45 minutes on the QIAvac 96. For automated high-throughput plasmid purification the **QIAprep 96 Turbo BioRobot Kit** enables up to 96 minipreps to be processed in 70 minutes.

Applications using QIAprep purified DNA

Plasmid DNA prepared using the QIAprep system is suitable for a variety of routine applications including:

- Restriction enzyme digestion
- Library screening
- In vitro translation
- Sequencing
- Ligation and transformation
- Transfection of robust cells

Principle

The QIAprep miniprep procedure is based on alkaline lysis of bacterial cells followed by adsorption of DNA onto silica in the presence of high salt (1). The unique silica membrane used in QIAprep Miniprep Kits completely replaces glass or silica slurries for plasmid minipreps.

The procedure consists of three basic steps:

- Preparation and clearing of a bacterial lysate
- Adsorption of DNA onto the QIAprep membrane
- Washing and elution of plasmid DNA

All steps are performed without the use of phenol, chloroform, CsCl, ethidium bromide, and without alcohol precipitation.

Preparation and clearing of bacterial lysate

The QIAprep miniprep procedure uses the modified alkaline lysis method of Birnboim and Doly (2). Bacteria are lysed under alkaline conditions, and the lysate is subsequently neutralized and adjusted to high-salt binding conditions in one step. After lysate clearing, the sample is ready for purification on the QIAprep silica membrane. For more details on growth of bacterial cultures and alkaline lysis, please refer to Appendix A on pages 39–42. In the QIAprep Spin and QIAprep 8 miniprep procedures, lysates are cleared by centrifugation, while the QIAprep 8 and 96 Turbo Miniprep kits provide TurboFilter strips or plates for lysate clearing by filtration.

LyseBlue reagent*

Use of LyseBlue is optional and is not required to successfully perform plasmid preparations. See “Using LyseBlue reagent” on page 14 for more information.

* LyseBlue reagent is only supplied with QIAprep Spin Miniprep Kits since multiwell or automated formats do not allow visual control of individual samples.

LyseBlue is a color indicator which provides visual identification of optimum buffer mixing. This prevents common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, genomic DNA, and cell debris. This makes LyseBlue ideal for use by researchers who have not had much experience with plasmid preparations as well as experienced scientists who want to be assured of maximum product yield.

DNA adsorption to the QIAprep membrane

QIAprep columns, strips, and plates use a silica membrane for selective adsorption of plasmid DNA in high-salt buffer and elution in low-salt buffer. The optimized buffers in the lysis procedure, combined with the unique silica membrane, ensure that only DNA will be adsorbed, while RNA, cellular proteins, and metabolites are not retained on the membrane but are found in the flow-through.

Washing and elution of plasmid DNA

Endonucleases are efficiently removed by a brief wash step with Buffer PB. This step is essential when working with *endA*$^+$ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, to ensure that plasmid DNA is not degraded. The Buffer PB wash step is also necessary when purifying low-copy plasmids, where large culture volumes are used.

Salts are efficiently removed by a brief wash step with Buffer PE. High-quality plasmid DNA is then eluted from the QIAprep column with 50–100 µl of Buffer EB or water. The purified DNA is ready for immediate use in a range of applications — no need to precipitate, concentrate, or desalt.

Note: Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water for elution, make sure that the pH value is within this range. Store DNA at –20°C when eluted with water since DNA may degrade in the absence of a buffering agent.

DNA yield

Plasmid yield with the QIAprep miniprep system varies depending on plasmid copy number per cell (see page 39), the individual insert in a plasmid, factors that affect growth of the bacterial culture (see pages 39–42), the elution volume (Figure 1), and the elution incubation time (Figure 2). A 1.5 ml overnight culture can yield from 5 to 15 µg of plasmid DNA (Table 1, page 14). To obtain the optimum combination of DNA quality, yield, and concentration, we recommend using Luria-Bertani (LB) medium for growth of cultures (for composition see page 41), eluting plasmid DNA in a volume of 50 µl, and performing a short incubation after addition of the elution buffer.

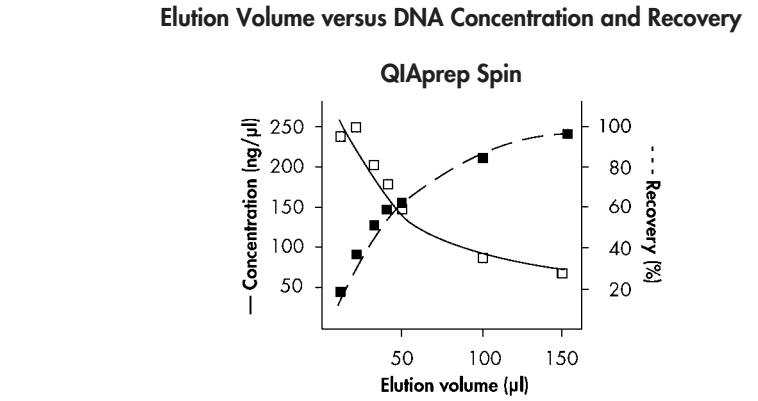

Figure 1 10 µg pUC18 DNA was purified using the QIAprep Spin protocol and eluted with the indicated volumes of Buffer EB. The standard protocol uses 50 µl Buffer EB for elution, since this combines high yield with high concentration. However the yield can be increased by increasing the elution volume.

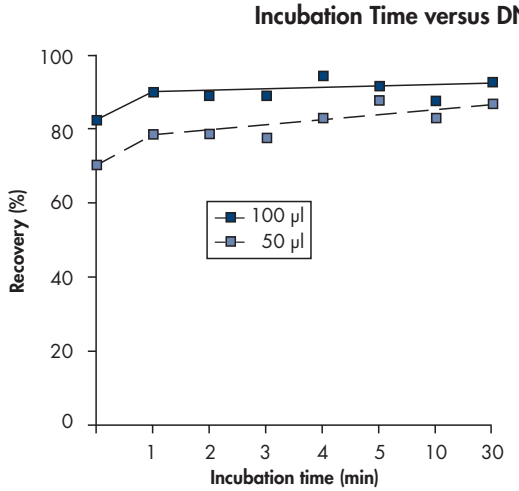

Figure 2 10 µg pBluescript DNA was purified using the QIAprep Spin Miniprep protocol and eluted after the indicated incubation times with either 50 µl or 100 µl Buffer EB. The graph shows that an incubation time of 1 minute and doubling the elution buffer volume increases yield.

Table 1. Effect of Different Compositions of Growth Medium LB on DNA Yield

Culture media	Yield
LB (containing 10 g/liter NaCl)	11.5 µg
LB (containing 5 g/liter NaCl)	9.5 µg

QIAprep Spin Miniprep Kit was used to purify DNA from 1.5 ml LB overnight cultures of XL1-Blue containing pBluescript®. Elution was performed according to the standard protocol (50 µl Buffer EB and 1 min incubation). Use of the recommended LB composition (with 10 g/liter NaCl, also see Appendix A, p. 43) provides optimal plasmid yield.

Using LyseBlue reagent

Using a simple visual identification system, LyseBlue reagent prevents common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, cell debris, and genomic DNA.

LyseBlue can be added to the resuspension buffer (Buffer P1) bottle before use. Alternatively, smaller amounts of LyseBlue can be added to aliquots of Buffer P1, enabling single plasmid preparations incorporating visual lysis control to be performed.

LyseBlue reagent should be added to Buffer P1 at a ratio of 1:1000 to achieve the required working concentration (e.g., 10 µl LyseBlue into 10 ml Buffer P1). Make sufficient LyseBlue/Buffer P1 working solution for the number of plasmid preps being performed.

LyseBlue precipitates after addition into Buffer P1. This precipitate will completely dissolve after addition of Buffer P2. Shake Buffer P1 before use to resuspend LyseBlue particles.

The plasmid preparation procedure is performed as usual. After addition of Buffer P2 to Buffer P1, the color of the suspension changes to blue. Mixing should result in a homogeneously colored suspension. If the suspension contains localized regions of colorless solution or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.

Upon addition of neutralization buffer (Buffer N3), LyseBlue turns colorless. The presence of a homogeneous solution with no traces of blue indicates that SDS from the lysis buffer has been effectively precipitated.