

SOP-104

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# Plasmid DNA

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Procedures for amplification and purification.

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Agar plates with required antibiotics

## Bacterial Stocks

Glycerol stocks stored at -80 C

Lyophilized stocks that have to be re-suspended in media

Liquid cultures

10 ml glass tubes

Qiagen Plasmid DNA Purification Kit

Table Top Centrifuges

Eppendorf Tubes

Pipettes

Spectrometer

**Principle and procedure**

QIAGEN plasmid purification protocols are based on a modified alkaline lysis procedure, followed by binding of plasmid DNA to QIAGEN Anion-Exchange Resin under appropriate low-salt and pH conditions. RNA, proteins, dyes, and low-molecular-weight impurities are removed by a medium-salt wash. Plasmid DNA is eluted in a high-salt buffer and then concentrated and desalted by isopropanol precipitation. No expensive equipment such as ultracentrifuges and HPLC or toxic reagents such as phenol and ethidium bromide are required.

**Plasmid size**

Plasmids up to approximately 150 kb can be purified using QIAGEN plasmid purification protocols. Constructs larger than 45–50 kb, however, may exhibit somewhat reduced elution efficiencies. Pre-warming the elution buffer to 50°C may help to increase the yield of large plasmids. (Qiagen Plasmid Purification Handbook, 3<sup>rd</sup> Ed, 2005)

**Plasmid/cosmid copy number**

High- and low-copy plasmids and cosmids should be purified using the standard protocols. Very low-copy plasmids and very low-copy cosmids (<10 copies per cell) should be purified using the alternative protocol, which uses extremely large culture volumes to obtain good yields of very low-copy constructs. (Qiagen Plasmid Purification Handbook, 3<sup>rd</sup> Ed, 2005)

**Host strains**

The strain used to propagate a plasmid can have a substantial influence on quality of the purified DNA. Host strains such as DH1, DH5a®, and C600 yield high-quality DNA with QIAGEN protocols. The slower growing strain XL1-Blue also yields DNA of very high quality. Strain HB101 and its derivatives, such as TG1 and the JM100 series, contain large amounts of carbohydrates that are released during lysis and can inhibit enzyme activities if not completely removed. In addition, some strains, such as JM101, JM110, and HB101, have high levels of endonuclease activity and yield DNA of lower quality. If the quality of purified DNA is not as expected, a change of host strain should be considered. If difficulty is encountered with strains such as TG1 and Top10F, we recommend either reducing the amount of culture volume or doubling the volumes of Buffers P1, P2, and P3 in order to improve the ratio of biomass to lysis buffers for optimized lysis conditions. (Qiagen Plasmid Purification Handbook, 3<sup>rd</sup> Ed, 2005)

**Procedure**

**Evening before. Set up overnight bacterial cultures in 5 ml tubes. Use appropriate media and antibiotics. Flame loop and pick a bacterial colony or scrap frozen stock. Inoculate media and incubate culture at 37 C with shaking over night (~18 hr).**

**1. Re-suspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube.**

Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet. If LyseBlue reagent has been added to Buffer P1, vigorously shake the buffer bottle to ensure LyseBlue particles are completely dissolved. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

Note: For gram positive cells like *B. subtilis*, add 1 mg / ml lysozyme to P1. Allow cells to incubate at 37 C for 10 minutes. Then continue with rest of protocol.

**2. Add 250 µl Buffer P2 and mix thoroughly by inverting the tube 4–6 times.**

Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min. If LyseBlue has been added to Buffer P1 the cell suspension will turn blue after addition of Buffer P2. Mixing should result in a homogeneously colored suspension. If the suspension contains localized colorless regions or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.

**3. Add 350 µl Buffer N3 and mix immediately and thoroughly by inverting the tube 4–6 times.**

To avoid localized precipitation, mix the solution thoroughly, immediately after addition of Buffer N3. Large culture volumes (e.g.  $\geq 5$  ml) may require inverting up to 10 times. The solution should become cloudy. If LyseBlue reagent has been used, the suspension should be mixed until all trace of blue has gone and the suspension is colorless. A homogeneous colorless suspension indicates that the SDS has been effectively precipitated.

**4. Centrifuge for 10 min at 13,000 rpm (~17,900 x g) in a table-top microcentrifuge.**  
A compact white pellet will form.

**5. Apply the supernatants 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 necessary to remove trace nuclease activity when using endA<sup>+</sup> strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5 $\alpha$ <sup>TM</sup> do not require this additional wash step.

**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.**

**Important:** Residual wash buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.

**10. Place the QIAprep column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of each QIAprep spin column, let stand for 1 min, and centrifuge for 1 min.**

### **Spectrophotometry**

DNA concentration can be determined by measuring the absorbance at 260 nm ( $A_{260}$ ) in a spectrophotometer using a quartz cuvette. For greatest accuracy, readings should be between 0.1 and 1.0. An absorbance of 1 unit at 260 nm corresponds to 50 µg genomic DNA per ml ( $A_{260} = 1 = 50 \text{ µg/ml}$ ). This relation is valid only for measurements made at neutral pH, therefore, samples should be diluted in a low-salt buffer with neutral pH (e.g., Tris·Cl, pH 7.0).

If you will use more than one cuvette to measure multiple samples, the cuvettes must be matched. Spectrophotometric measurements do not differentiate between DNA and RNA, so RNA contamination can lead to overestimation of DNA concentration. Phenol has an absorbance maximum of 270–275 nm, which is close to that of DNA. Phenol contamination mimics both higher yields and higher purity, because of an upward shift in the  $A_{260}$  value.

### **Purity of DNA**

The ratio of the readings at 260 nm and 280 nm ( $A_{260}/A_{280}$ ) provides an estimate of DNA purity with respect to contaminants that absorb UV light, such as protein. The  $A_{260}/A_{280}$  ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting  $A_{260}/A_{280}$  ratio can vary greatly. Lower pH results in a lower  $A_{260}/A_{280}$  ratio and reduced sensitivity to protein contamination. For accurate  $A_{260}/A_{280}$  values, we recommend measuring absorbance in a slightly alkaline buffer (e.g., 10 mM Tris·Cl, pH 7.5). Make sure to zero the spectrophotometer with the appropriate buffer. Pure DNA has an  $A_{260}/A_{280}$  ratio of 1.7–1.9. Scanning the absorbance from 220–320 nm will show whether there are contaminants affecting absorbance at 260 nm. Absorbance scans should show a peak at 260 nm and an overall smooth shape.

Section  
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## References

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Short Protocols in Molecular Biology. 4<sup>th</sup> Edition. Editors Frederick M. Ausubel, Roger Brent, Robert E. Kingston, David D. Moore, J.G. Seidman, John A Smith and Kevin Struhl. John Wiley & Sons, Inc. 1999

Qiagen Plasmid Purification Handbook, 3<sup>rd</sup> Ed, 2005

<http://www.qiagen.com/>