Re-Purification of Plasmid DNA Prepared by Methods other than QIAGEN Tips

Before using this protocol, the DNA must be free of SDS and other anionic detergents. If the prep is contaminated with RNA, the RNA must first be digested with RNase A (cat. no. 19101). Note that QIAGEN-tips cannot separate plasmid DNA from chromosomal DNA — this separation is achieved during the alkaline lysis procedure.

1. Choose a QIAGEN-tip appropriate for the amount of DNA to be purified. QIAGEN-tips 100, 500, 2500, and 10000 are appropriate for purifying up to 100 µg, 500 µg, 2.5 mg, and 10 mg DNA, respectively.

2. Either adjust the DNA sample to 750 mM NaCl, 50 mM MOPS, pH 7.0, or resuspend the DNA sample in Buffer QBT. (The volume of Buffer QBT added should be at least 10 times the volume of the original sample solvent.) The final sample volume should be at least 5, 12, 40, or 90 ml for QIAGEN-tips 100, 500, 2500, or 10000, respectively.

3. Apply the sample to a QIAGEN-tip previously equilibrated with Buffer QBT.

4. Proceed with the appropriate protocol below.
(Also described in the QIAGEN Plasmid Purification Handbook from step 11. See page 22 for Midi/Maxi preparations and page 27 for Mega/Giga preparations.)

For ▲QIAGEN-tip 100 or ● QIAGEN-tip 500:

5. Wash the QIAGEN-tip with ▲2 x 10 ml or ● 2 x 30 ml Buffer QC.
Allow Buffer QC to move through the QIAGEN-tip by gravity flow. The first wash is sufficient to remove all contaminants in the majority of plasmid DNA preparations. The second wash is especially necessary when large culture volumes or bacterial strains producing large amounts of carbohydrates are used.
☞ Remove a▲400 µl or ● 240 µl sample from the combined wash fractions and save for an analytical gel (sample 3).

6. Elute DNA with ▲5 ml or ● 15 ml Buffer QF.
Collect the eluate in a 10 ml or 30 ml tube. Use of polycarbonate centrifuge tubes is not recommended as polycarbonate is not resistant to the alcohol used in subsequent steps.
☞ Remove a ▲100 µl or ● 60 µl sample of the eluate and save for an analytical gel (sample 4).
If you wish to stop the protocol and continue later, store the eluate at 4°C. Storage periods longer than overnight are not recommended.

7. Precipitate DNA by adding ▲3.5 ml or ● 10.5 ml (0.7 volumes) room-temperature isopropanol to the eluted DNA. Mix and centrifuge immediately at ≥15,000 x *g* for 30 min at 4°C. Carefully decant the supernatant.
All solutions should be at room temperature in order to minimize salt precipitation, although centrifugation is carried out at 4°C to prevent overheating of the sample. A centrifugal force of 15,000 x *g* corresponds to 9500 rpm in a Beckman JS-13 rotor and 11,000 rpm in a Sorvall SS-34 rotor. Alternatively, disposable conicalbottom centrifuge tubes can be used for centrifugation at 5000 x *g* for 60 min at 4°C. Isopropanol pellets have a glassy appearance and may be more difficult to see than the fluffy, salt-containing pellets that result from ethanol precipitation. Marking the outside of the tube before centrifugation allows the pellet to be more easily located. Isopropanol pellets are also more loosely attached to the side of the tube, and care should be taken when removing the supernatant.

8. Wash DNA pellet with ▲2 ml or ● 5 ml of room-temperature 70% ethanol, and centrifuge at ε15,000 x *g* for 10 min. Carefully decant the supernatant without disturbing the pellet.
Alternatively, disposable conical-bottom centrifuge tubes can be used for centrifugation at 5000 x *g* for 60 min at 4°C. The 70% ethanol removes precipitated salt and replaces isopropanol with the more volatile ethanol, making the DNA easier to redissolve.

9. 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 10 mM Tris·Cl, pH 8.5).
Redissolve the DNA pellet by rinsing the walls to recover all the DNA, especially if glass tubes have been used. Pipetting the DNA up and down to promote resuspension may cause shearing and should be avoided. Overdrying the pellet will make the DNA difficult to redissolve. DNA dissolves best under slightly alkaline conditions; it does not easily dissolve in acidic buffers.

For ▲QIAGEN-tip 2500 or ● QIAGEN-tip 10000:

5. Wash the QIAGEN-tip with a total of ▲200 ml or a total of ● 600 ml Buffer QC.
Allow Buffer QC to move through the QIAGEN-tip by gravity flow. The first half of the volume of wash buffer is sufficient to remove all contaminants in the majority of plasmid DNA preparations. The second half is particularly necessary when large culture volumes or bacterial strains producing large amounts of carbohydrates are used.
☞ Remove a ▲160 µl or ● 120 µl sample from the combined wash fractions and save for an analytical gel (sample 3).

6. Elute DNA with ▲35 ml or ● 100 ml Buffer QF.
Use of polycarbonate centrifuge tubes for collection is not recommended as polycarbonate is not resistant to the alcohol used in subsequent steps.
☞ Remove a ▲22 µl or 20 µl sample of the eluate and save for an analytical gel (sample 4).
If you wish to stop the protocol and continue later, store the eluate at 4°C. Storage periods longer than overnight are not recommended.

7. Precipitate DNA by adding ▲24.5 ml or ● 70 ml (0.7 volumes) room-temperature isopropanol to the eluted DNA. Mix and centrifuge immediately at ≥15,000 x *g* for 30 min at 4°C. Carefully decant the supernatant.
All solutions should be at room temperature in order to minimize salt precipitation, although centrifugation is carried out at 4°C to prevent overheating of the sample. A centrifugal force of 15,000 x *g* corresponds to 9500 rpm in a Beckman JS-13 rotor and 11,000 rpm in a Sorvall SS-34 rotor. Alternatively, disposable conicalbottom centrifuge tubes can be used for centrifugation at 5000 x *g* for 60 min at 4°C. Isopropanol pellets have a glassy appearance and may be more difficult to see than the fluffy, salt-containing pellets that result from ethanol precipitation. Marking the outside of the tube before centrifugation allows the pellet to be more easily located. Isopropanol pellets are also more loosely attached to the side of the tube, and care should be taken when removing the supernatant.

8. Wash DNA pellet with ▲7 ml or ● 10 ml of room-temperature 70% ethanol, and centrifuge at ε15,000 x *g* for 10 min. Carefully decant the supernatant without disturbing the pellet.
Alternatively, disposable conical-bottom centrifuge tubes can be used for centrifugation at 5000 x *g* for 60 min at 4°C. The 70% ethanol removes precipitated salt and replaces isopropanol with the more volatile ethanol, making the DNA easier to redissolve.

9. Air-dry the pellet for 10–20 min, and redissolve the DNA in a suitable volume of buffer (e.g., TE buffer, pH 8.0, or 10 mM Tris·Cl, pH 8.5).
Redissolve the DNA pellet by rinsing the walls to recover all the DNA, especially if glass tubes have been used. Pipetting the DNA up and down to promote resuspension may cause shearing and should be avoided. Overdrying the pellet will make the DNA difficult to redissolve. DNA dissolves best under slightly alkaline conditions; it does not easily dissolve in acidic buffers.

Determination of yield
To determine the yield, DNA concentration should be determined by both UV spectrophotometry and quantitative analysis on an agarose gel.

Agarose gel analysis
We recommend removing and saving aliquots during the purification procedure (samples 3 and 4). If the plasmid DNA is of low yield or quality, the samples can be analyzed by agarose gel electrophoresis to determine at what stage of the purification procedure the problem occurred (As described in the *QIAGEN Plasmid Purification Handbook* page 41).