QIAGEN Plasmid Mini Handbook

For Plasmid Mini Kit

March 1999

QIAGEN

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Kit Contents

Plasmid Kits Catalog No.	Mini (25) 12123	Mini (100) 12125
QIAGEN-tip 20	25	100
Buffer P1	20 ml	40 ml
Buffer P2	20 ml	40 ml
Buffer P3	20 ml	40 ml
Buffer QBT	40 ml	110 ml
Buffer QC	120 ml	480 ml
Buffer QF	30 ml	110 ml
RNase A*	2 mg	4 mg
Handbook	1	1

**Provided as a 100 mg/ml solution*

Storage Conditions

QIAGEN® Plasmid Kits should be stored dry and at room temperature (15–25°C). After addition of RNase A, Buffer P1 should be stored at 2–8°C and is stable for six months. All other buffers and components and the RNase A stock solution can be stored for two years at room temperature without showing any reduction in performance, capacity, or quality of separation.

Technical Assistance

Click Here for Contact Addresses

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. They are always available to discuss any general or specific questions you may have. If you have any questions or experience any problems regarding QIAGEN Plasmid Mini Kits, or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are also a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors listed on page 35.

Introduction

QIAGEN Plasmid Kits will dramatically change the way you isolate nucleic acids. Time-consuming and tedious methods, which often involve the use of toxic substances, are replaced by the QIAGEN procedure. This rapid purification protocol, based on the remarkable selectivity of patented QIAGEN Resin, allows the isolation of ultrapure supercoiled plasmid DNA with high yields in just 70 minutes. It requires no expensive equipment such as ultracentrifuges and HPLC, involves only a few steps, and completely avoids the use of toxic reagents such as phenol and ethidium bromide — making the isolation of pure nucleic acids almost as simple as filtration.

Plasmid DNA purified with QIAGEN-tips is free of all contaminants, and ideally suited for use in such demanding procedures as transfection, automated or manual sequencing, and enzymatic modification. DNA prepared with QIAGEN-tips performs equally well or better than DNA prepared with time-consuming methods such as cesium chloride gradient centrifugation, and is typically recovered with yields greater than 90%.

The QIAGEN Principle

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.

Each disposable QIAGEN-tip is designed to operate by gravity flow, reducing the amount of hands-on time required for the purification procedure. QIAGEN-tips are ideally suited for rapid and simple preparation of 1–12 samples.

QIAGEN continually strives to streamline nucleic acid purification. For users processing >12 samples, we recommend the QIAwell® Plasmid Purification System, a state-of-the-art product for rapid, high-throughput isolation of up to 20 µg of ultrapure double-stranded DNA. The QIAwell System allows parallel processing of 8–96 samples in under 2 hours by using a vacuum to draw samples through multiwell modules.

The standard protocol on pages 9–11 of this handbook is for purification of up to 20 µg high-copy plasmid from cultures of *E. coli* using QIAGEN-tip 20. For purification of low-copy plasmids, cosmids, BACs, PACs, P1s, and M13 replicative form on QIAGEN-tip 20 refer to the recommendations on pages 12 and 13.

Please take a few minutes to read this handbook carefully before beginning the DNA preparation. If QIAGEN Plasmid Kits are new to you, please pay particular attention to Appendix A (page 20) and be sure to follow the detailed instructions beginning on page 7.

New Features

Users familiar with the *QIAGEN Plasmid Mini Handbook* should note the following new features which have been introduced since the last edition of this handbook:

- RNase A is now supplied in solution — simply add the RNase A solution to Buffer P1 before use. Buffer P1 containing RNase A is stable for 6 months when stored at 4°C.

- Protocol for BACs, PACs, and P1s — recommendations for purification of large DNA constructs are now included on page 13.

QIAGEN Plasmid Purification Procedure

Plasmid Sizes

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. Prewarming the elution buffer (Buffer QF) to 50°C may help to increase yield.

Culture Media and Culture Volume

QIAGEN plasmid purification protocols are optimized for use with cultures grown in standard Luria-Bertani (LB-Miller) medium (see Appendix B, pages 27–28 for composition) to a cell density of approximately 1 x 10^9 cells/ml (A_{600} = 1–1.5). This is generally attained at 14–16 h after inoculation. Table 1 indicates the culture volumes recommended for use with each QIAGEN-tip. Overloading the system with larger culture volumes will affect the separation characteristics of QIAGEN Resin, leading to reduced yield and purity of the preparation. Rich media are not recommended for plasmid preparation with QIAGEN-tips. If rich media are used, growth time must be optimized, and culture volumes reduced.

Table 1. Recommended culture volumes for QIAGEN-tips

High-copy plasmids (3–5 µg DNA/ml LB)*
(pBluescript®, pUC, pTZ, pGEM®, chloramphenicol-amplified low-copy plasmids, etc.)

QIAGEN-tip	DNA capacity	LB culture volume	Expected DNA yield‡
QIAGEN-tip 20	20 µg	3 ml	9–20 µg
QIAGEN-tip 100	100 µg	25 ml	75–100 µg
QIAGEN-tip 500	500 µg	100 ml	300–500 µg
QIAGEN-tip 2500	2.5 mg	500 ml	1.5–2.5 mg
QIAGEN-tip 10000	10 mg	2.5 liters	7.5–10 mg

Low-copy plasmids (0.2–1 µg DNA/ml LB)*
(pBR322, cosmids, etc.)

QIAGEN-tip	DNA capacity	LB culture volume	Expected DNA yield‡
QIAGEN-tip 20	20 µg	10 ml†	5–15 µg
QIAGEN-tip 100	100 µg	100 ml	20–100 µg
QIAGEN-tip 500	500 µg	500 ml	100–500 µg
QIAGEN-tip 2500	2.5 mg	2.5 liters	0.5–2.5 mg
QIAGEN-tip 10000	10 mg	5 liters†	1–5 mg

** In common host strains, such as XL1-Blue, DH5α, HB101, and JM109.*
† Requires increased amounts of alkaline lysis buffers.
‡ Actual yields depend on culture volume, media, copy number, insert size, and host strain.

Set-up of QIAGEN-tips

QIAGEN-tips may be placed in tubes using tip holders provided with the kits (Figure 1A). Alternatively, QIAGEN-tip 20 may be placed in QIArack 1 (Figure 1B; Cat. No. 19011) which has a removable collection tray that can be used for wash steps.

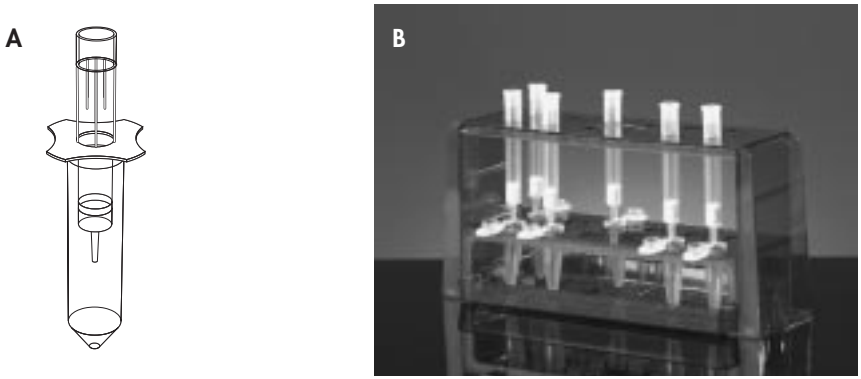

Figure 1. *Set-up of QIAGEN-tips* ***(A)*** *with tip holder or* ***(B)*** *with QIArack 1.*

Plasmid Mini Purification Protocol

This protocol is for Mini (up to 20 µg) preparations of high-copy plasmid DNA from cultures of *E. coli*. For cosmid and low-copy-number plasmid purification see recommendations on page 12. For purification of BACs, PACs, P1s, and purification of double-stranded M13 replicative form see recommendations on page 13.

Important Notes Before Starting

- New users are strongly recommended to read Appendix A (page 20) at the end of this handbook before starting the procedure.
- To ensure high yields of pure DNA, use no more than 3 ml LB culture for high-copy-number plasmids (e.g. pUC, pBluescript®). For low-copy-number plasmids (e.g. pBR322), use no more than 10 ml LB culture and refer to the recommendations on page 12. We do not recommend the use of rich media such as TB or 2x YT for culture. When low-copy-number plasmids containing the ColE1 replication origin are prepared, the yield can be improved by amplification in the presence of chloramphenicol (180 mg/l). They should then be treated as high-copy-number plasmids.
- Add the provided RNase A solution to Buffer P1 before use (spin down RNase A briefly before use). Buffer P1 should then be stored at 2–8°C and is stable for 6 months.
- Check Buffer P2 for SDS precipitation due to low storage temperatures. If necessary, dissolve the SDS by warming to 37°C.
- Pre-chill Buffer P3 at 4°C.
- Optional: To confirm proper purification or to identify a problem, samples may be taken at specific steps for analysis on an agarose gel. Appropriate samples and volumes are indicated in the protocol below.

Procedure

1. **Resuspend the bacterial pellet in 0.3 ml of Buffer P1.**

   Ensure that RNase A has been added to Buffer P1. The bacteria should be resuspended completely, leaving no cell clumps.

2. **Add 0.3 ml of Buffer P2, mix gently, and incubate at room temperature for 5 min.**

   After addition of Buffer P2, the solution should be mixed gently, but thoroughly, by inverting the tube 4–6 times. Do not vortex, as this will result in shearing of genomic DNA. The lysate should appear viscous. Do not allow the lysis reaction to proceed for more than 5 min. After use, the bottle containing Buffer P2 should be closed immediately to avoid any reaction between the NaOH and CO_2 in the air. If the buffer is left open for any length of time, it should be prepared fresh from stock solutions.

3. **Add 0.3 ml of chilled Buffer P3, mix immediately but gently, and incubate on ice for 5 min.**

   Precipitation is enhanced by using chilled Buffer P3 and incubating on ice. After addition of Buffer P3, the solution becomes cloudy and very viscous. To avoid localized potassium dodecyl sulfate precipitation, mix the solution gently, but thoroughly, immediately after addition of Buffer P3. Mix by inverting the tube 4–6 times.

4. **Centrifuge at maximum speed in a microfuge for 10 min. Remove supernatant promptly.**

   Before loading the centrifuge, the sample should be mixed again. Centrifugation should be performed at maximum speed in 1.5-ml or 2-ml microfuge tubes (e.g. 10,000–13,000 rpm in a microfuge). Maximum speed corresponds to 14,000–18,000 x *g* for most microfuges. After centrifugation, the supernatant should be clear. If the supernatant is not clear, a second, shorter centrifugation should be carried out to avoid applying any suspended or particulate material to the column. Suspended material (which causes the sample to appear turbid) will clog the column and reduce or eliminate flow.

   • Remove a 50-µl sample from the cleared lysate and save it for an analytical gel (sample 1).

5. **Equilibrate a QIAGEN-tip 20 by applying 1 ml Buffer QBT, and allow the column to empty by gravity flow.**

   Place QIAGEN-tips into a QIArack 1 over the waste tray or use the tip holders provided with each kit (see "Set-up of QIAGEN-tips" page 8). Flow of buffer will begin automatically by reduction in surface tension due to the presence of detergent in the equilibration buffer. Allow the QIAGEN-tip to drain completely. The resin bed will retain some buffer and will not readily dry out. QIAGEN-tips can therefore be left unattended. Do not force out the remaining buffer.

6. **Apply the supernatant from step 4 to the QIAGEN-tip 20 and allow it to enter the resin by gravity flow.**

   The supernatant should be loaded onto the QIAGEN-tip promptly. If it is left too long and becomes cloudy due to further precipitation of protein, it must be recentrifuged before loading to prevent clogging of the QIAGEN-tip.

   • Remove a 50-µl sample of the flowthrough and save for an analytical gel (sample 2).

7. **Wash the QIAGEN-tip 20 with 4 x 1 ml Buffer QC.**

   Allow Buffer QC to move through the QIAGEN-tip by gravity flow. The first 2 ml are sufficient to remove all contaminants in the majority of plasmid DNA preparations. The second 2 ml ensure complete removal of contaminants in all situations, and will ensure consistent results in sequencing. The second 2 ml are particularly necessary when large culture volumes or bacterial strains producing large amounts of carbohydrate are used. It is particularly important not to force out residual wash buffers. Traces of wash buffer will not affect the elution step.

- Remove a 50-µl sample of the combined wash fractions and save for an analytical gel (sample 3).

8. **Elute DNA with 0.8 ml Buffer QF.**

   Place the upper part of a QIArack 1 over the lower rack fitted with clean 1.5-ml or 2-ml microfuge tubes and collect the samples into the tubes. Alternatively, use the tip holders provided. Flow begins when Buffer QF is added. Drain the QIAGEN-tip by allowing it to empty by gravity flow.

   - Remove a 50-µl sample of the eluate and save for an analytical gel (sample 4).

9. **Precipitate DNA with 0.7 volumes (0.56 ml per 0.8 ml of elution volume) of room-temperature isopropanol. Centrifuge immediately at ≥10,000 rpm for 30 min in a microfuge, and carefully decant the supernatant.**

   Precipitation of DNA with isopropanol should be carried out with all solutions equilibrated to room temperature in order to minimize salt precipitation. 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.

10. **Wash DNA with 1 ml of 70% ethanol, air-dry for 5 min, and redissolve in a suitable volume of buffer.**

    The DNA pellet should be washed briefly in 70% ethanol, and then recentrifuged. The 70% ethanol serves to remove precipitated salt, as well as to replace isopropanol with the more volatile ethanol, making the DNA easier to redissolve. A second wash with room-temperature 70% ethanol may improve results in more sensitive applications, such as transfection and sequencing. After careful and complete removal of ethanol, the pellet should be air-dried briefly (approximately 5 min) before redissolving in an appropriate volume of TE buffer. Overdrying the pellet will make the DNA difficult to redissolve. Resuspend the DNA pellet by rinsing the walls to recover all the DNA. Pipetting the DNA up and down to promote resuspension may cause shearing, and should be avoided. 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.

Analytical Gel (optional): to analyze the purification procedure as shown in Figure 2 (Appendix A, page 26), precipitate samples 1–4 (from steps 4–8) with 35 µl of isopropanol. Rinse the pellets with 70% ethanol, drain well, air-dry, and resuspend in 10 µl of TE, pH 8.0. Use 2 µl of each for analysis on a 1% agarose gel (1).

1. Sambrook, J. et al., eds. (1989) Molecular cloning: a laboratory manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, p. 1.32.

Special Applications

Purification of Cosmids and Low-Copy-Number Plasmids*

Cosmids and low-copy-number plasmids often require large culture volumes to yield significant amounts of DNA. A few modifications to the protocol will avoid overloading the system. The following procedure is recommended for optimal results.

Note: Many cosmids yield less than 1 µg per ml of culture. Quantitate the amount of DNA obtained in a pilot experiment by comparison to standards on an agarose gel. Adjust LB culture volumes accordingly (maximum 10 ml). If culture volumes greater than 10 ml are required, use the appropriate QIAGEN Plasmid Midi, Maxi, Mega, or Giga Kit.

1. Resuspend the bacterial cells in an appropriate volume of Buffer P1. Use 0.3 ml of P1 for every 3 ml of LB culture.
2. Increase the volumes of Buffers P2 and P3 to match the volume of P1 used in the previous step.
3. Centrifuge at maximum speed (10,000–13,000 rpm) for 10 min at room temperature. Transfer the supernatant promptly to a fresh tube and recentrifuge for 10 min at maximum speed. Transfer the supernatant to a fresh tube. After centrifugation the supernatant should be clear. Suspended or particulate material (causing the sample to be turbid) will clog the column and reduce or eliminate flow. Alternatively, the centrifugation process may be replaced by use of a QIAfilter Midi Cartridge (see ordering information, pages 32–33).
4. Precipitate the DNA in the supernatant with 0.7 volumes room-temperature isopropanol. Centrifuge at maximum speed for 30 min at room temperature and carefully decant the supernatant.
5. Redissolve the DNA pellet in a small volume of TE, pH 7.0, and add Buffer QBT to obtain a final volume of at least 1 ml. Apply the sample to a previously equilibrated QIAGEN-tip 20.

   Note: To compare the amount of plasmid in the culture with the amount obtained after purification, remove an aliquot (10%) of the DNA suspended in TE before adding Buffer QBT. Remove a comparable aliquot (11%) of the final purified sample and quantitate the amount of plasmid each contains by comparison to standards on an agarose gel. A recovery of 80% is typical.
6. Continue with the standard purification protocol at step 7 (page 10).

   Note: An extra 5–10% increase in recovery of cosmid DNA may be obtained by heating Buffer QF to 65°C before elution (step 8).

Purification of BACs, PACs, P1s, and Other Large Plasmids*

BACs, PACs, P1s, and other large plasmid constructs can be purified on QIAGEN-tip 20 with a few modifications to the standard protocol. Recommendations are given below. Cultures should be grown overnight in 5 ml LB medium.

1. Resuspend bacterial cells in 0.6 ml Buffer P1, and also increase volumes of lysis buffers P2 and P3 to 0.6 ml.
2. Elute DNA using 2 x 0.4 ml pre-heated (65°C) Buffer QF.

   Note: Do not heat more Buffer QF than is needed for immediate use.
3. After isopropanol precipitation, redissolve DNA in 20 µl 10 mM Tris·Cl, pH 8.5, incubating overnight at 4°C to redissolve completely.

Purification of Plasmid DNA Prepared by Other Methods*

The DNA must be free of SDS and other anionic detergents. RNA must be digested first with RNase A. Note that QIAGEN columns cannot separate plasmid DNA from chromosomal DNA.

1. Use a QIAGEN-tip 20 for purification of up to 20 µg of DNA.
2. Adjust DNA sample to 750 mM NaCl, 50 mM MOPS, pH 7.0 or dilute sample with 5 volumes of Buffer QBT. The final sample volume should be at least 1 ml.
3. Apply the sample to a QIAGEN-tip 20 previously equilibrated with Buffer QBT.
4. Continue with the standard purification protocol at step 7 (page 10).

Purification of M13 Replicative Form*

M13 replicative form (RF) DNA behaves like a a low-copy plasmid. M13 RF DNA is purified using the standard procedure with one modification: an extra wash step is performed before cell lysis in order to remove all traces of phage supernatant.

1. Resuspend bacterial pellet in 5 ml STE (see page 25 for formulation). Pellet cells again and carefully remove all the supernatant.
2. Continue with the standard purification protocol at step 1 (page 9).

**For further information, contact our Technical Service Group or your local distributor.*

Troubleshooting Guide

The following troubleshooting guide, as well as the information provided in the appendices of this handbook, may be helpful in solving any problems which may arise. Should more assistance be required, please contact our Technical Service Group or your local distributor. For questions related to plasmid templates for DNA sequencing, *The QIAGEN Guide to Template Purification and DNA Sequencing* is available free on request.

		Comments and Suggestions
Low or no yield		
a)	General	If fractions have been saved during the procedure, analysis by agarose gel electrophoresis is the best way to determine at what stage the problem occurred. Should the DNA be located in a particular fraction, it can generally be recovered by isopropanol precipitation.
b)	Sequence related	Problems related to quality of DNA in fluorescent sequencing may need specialized troubleshooting with respect to the specific sequencing system used. *The QIAGEN Guide to Template Purification and DNA Sequencing* contains useful information on sequencing optimization and troubleshooting, and is available free on request.
c)	Insufficient alkaline lysis or overloaded column	Most problems with inconsistent or low yields are attributable to overloading the system. Check the culture volume and yield for use with QIAGEN-tip 20 (Table 1, page 7), and reduce the culture volume accordingly. If larger culture volumes must be used (e.g. for a low-copy-number plasmid), volumes of Buffers P1, P2, and P3 may not be sufficient for efficient lysis. Increasing the volumes of the lysis buffers can help to increase the efficiency of lysis (see "Special Applications" page 12).
d)	Inappropriate salt or pH conditions in buffers	Ensure that any buffers prepared in the laboratory were prepared according to the instructions provided in Appendix B (page 27).
No DNA in the lysate before loading		
a)	Plasmid did not propagate	Please read "Growth of Bacterial Cultures" (Appendix A, page 20), and check that the conditions for optimum growth were met.

		Comments and Suggestions
b)	Lysate was prepared incorrectly	Check age and storage conditions of buffers. If necessary use fresh Buffers P1, P2, and P3.
c)	Buffer P2 precipitated	Check Buffer P2 for SDS precipitation due to low storage temperature and dissolve the SDS by warming to 37°C. The bottle containing Buffer P2 should always be closed immediately after use.
d)	Cell resuspension incomplete	Pelleted cells should be completely resuspended in Buffer P1. Do not add Buffer P2 until an even suspension is obtained.

DNA is found in the flowthrough fraction

a)	Column was overloaded with DNA	Check the culture volume and yield for use with QIAGEN-tip 20 (Table 1, page 7) and reduce the culture volume accordingly.
b)	RNase A digestion was insufficient or omitted	If Buffer P1 is more than 3 months old, add more RNase A. Recover DNA by digesting the flowthrough fraction with RNase A, and purifying on a new QIAGEN-tip.
c)	SDS (or other ionic detergent) was in lysate	Ensure that Buffer P3 is chilled before use, and lysate is incubated on ice before centrifugation. Promptly load cleared lysate onto the QIAGEN-tip after centrifugation. If lysate is too viscous for effective mixing of Buffer P3, reduce culture volume or increase volumes of Buffers P1, P2, and P3.
d)	Lysis buffers incompletely mixed	Ensure complete mixing of all buffers.
e)	Column flow was uneven	Ensure that the QIAGEN-tips are stored at room temperature. If they are stored under cold, damp conditions for prolonged periods of time, the resin may clump. This problem can be overcome by shaking the column before use.

DNA is found in the wash fraction

a)	Column was overloaded with DNA	Check the culture volume and yield for use with QIAGEN-tip 20 (Table 1, page 7) and reduce the culture volume accordingly.
b)	Wash buffer was incorrect	Check pH and salt concentration of Buffer QC. Recover DNA by precipitation, and purify on a new QIAGEN-tip (see "Purification of Plasmid DNA Prepared by Other Methods" page 13).

No DNA in eluate

a)	Elution buffer was incorrect	Check pH and salt concentration of Buffer QF. Recover DNA by eluting with fresh buffer.
b)	No DNA in the lysate before loading	See appropriate section on page 14.
c)	DNA is in the flowthrough or wash fraction	See two previous sections.

Little or no DNA upon precipitation

a)	DNA failed to precipitate	Ensure that the precipitate is centrifuged at maximum speed (≥10,000 rpm) in a microfuge for 30 min. Make sure isopropanol was used for precipitation.
b)	DNA pellet was lost	Isopropanol pellets are glassy and may be difficult to see. Mark the expected location of the pellet before centrifugation. Pipet or pour off supernatant gently.
c)	DNA was poorly resuspended	Check that DNA is completely resuspended. Be sure to wash any DNA off the walls, particularly if a fixed-angle rotor is used. Up to half of the total DNA may be smeared on the walls. Alternatively, a swinging bucket rotor can be used to ensure that the pellet is located at the bottom of the tube.

DNA is difficult to resuspend

a)	Pellet was overdried	Air-dry pellet instead of using vacuum, especially if the DNA is of high molecular weight. Recover DNA by warming the solution slightly, and allowing more time for resuspension.

		Comments and Suggestions
b)	Residual ethanol in pellet	Carefully pipet off the supernatant after the 70% ethanol wash step and dry pellet at room temperature.
c)	Residual isopropanol in pellet	Ensure that pellets are washed with 70% ethanol.
d)	Salt carryover in pellet	Ensure that isopropanol is at room temperature and that supernatant is completely removed. Salt concentration may be decreased by increasing the volume of TE buffer used for resuspension.
e)	Buffer pH was too low	DNA does not dissolve well in acidic solutions. Ensure that the pH of the TE buffer used is ≥8.0.
RNA in the eluate		
a)	RNase A digestion was insufficient	Check culture volume against recommended volumes in Table 1 (page 7), and reduce if necessary. If Buffer P1 is more than 3 months old, add more RNase A. Increase volume of wash buffer (Buffer QC). Recover DNA by precipitating the eluate, digesting with RNase A, and purifying on a new QIAGEN-tip (see "Purification of Plasmid DNA Prepared by Other Methods" page 13).
Genomic DNA in the eluate		
a)	Mixing of the lysate was too vigorous	The lysate must be handled gently after addition of Buffer P2 to prevent shearing of chromosomal DNA. Reduce culture volume if lysate is too viscous for gentle mixing.
b)	Lysis time was too long	Ensure that the lysis step does not exceed 5 min.
c)	Column was overloaded	Check the culture volume and yield for use with QIAGEN-tip 20 (Table 1, page 7) and reduce the culture volume accordingly.
d)	Cell lysis during culture	Do not grow cultures for more than 12–16 hours.

DNA is nicked/sheared/degraded

a)	Endonuclease-containing host	Refer to "Host Strains" (page 21) and consider changing *E. coli* host strain.
b)	Nuclease contamination	Check buffers for nuclease contamination and replace if necessary. Use autoclaved glassware and plasticware, and wear gloves.
c)	DNA was poorly buffered	Resuspend DNA in TE, pH 8.0, to inhibit nuclease activity and maintain stable pH during storage.
d)	Shearing during resuspension	Resuspend DNA gently, without vortexing or vigorous pipetting.

DNA does not perform well

a)	DNA was nicked	Check percentage of nicked DNA in lysate on an agarose gel. If significant nicked DNA is present reduce culture volume or use an alternative host strain.
b)	Salt concentration too high	Ensure that isopropanol is at room temperature for precipitation, and that the pellet is washed twice with room-temperature 70% ethanol.
c)	Residual protein	Check culture volume, media, and host-strain growth. Increase volume of wash buffer (Buffer QC).

DNA contains extra bands

a)	Possible deletion mutants	Some sequences are poorly maintained in plasmids. Check for deletions by restriction analysis. Cosmid clones, in particular, should always be prepared from freshly streaked, isolated colonies, since cosmids are not stable in *E. coli* for long periods of time.
b)	Plasmid multimers are present	Digest DNA with a restriction enzyme with a unique site in the plasmid. Monomers and multimers will form one band on an agarose gel (see "Analysis of Plasmid DNA" page 25).
c)	Plasmid has formed denatured supercoils	This species runs faster than closed circular DNA on a gel and is resistant to cutting. Do not incubate cells for longer than 5 min in Buffer P2 (see Figure 2, page 26).

Blocked QIAGEN-tip		
a)	Lysate was turbid	Ensure that the lysate is clear before it is loaded onto the column. Chill Buffer P3, and incubate the lysate on ice before centrifugation. Check *g*-force and centrifugation time.

Appendix A: Important Background Information

The QIAGEN plasmid purification procedure is based on the optimized alkaline lysis method of Birnboim and Doly (1). The procedure has been condensed to three steps, and in combination with purification on QIAGEN Resin, allows selective preparation of ultrapure plasmid DNA without the use of phenol, chloroform, ethidium bromide, or cesium chloride. It can be used for the preparation of plasmid, cosmid, or double-stranded M13 DNA.

1. Growth of Bacterial Cultures

Plasmids are generally prepared from bacterial cultures grown in the presence of a selective agent such as an antibiotic (2,3). The yield and quality of the plasmid DNA prepared may depend on a number of factors including plamid copy number, size of insert, host strain, culture volume, and culture medium.

Plasmid Copy Number

Plasmids vary widely in their copy number (Table 2), depending on the replication origin they contain (pMB1 or pSC101 for example) which determines whether they are under relaxed or stringent control; and depending on the size of the plasmid and its associated insert. Some plasmids, such as the pUC series and derivatives have mutations which allow them to reach very high copy numbers within the bacterial cell. Plasmids based on pBR322 are generally present in lower copy numbers, while cosmids and very large plasmids are often maintained at very low copy numbers per cell.

Table 2. Origins of replication and copy numbers of various plasmids and cosmids (2)

DNA construct	Origin of replication	Copy number	Classification
Plasmids			
pUC vectors	ColE1	500–700	high copy
pBluescript® vectors	ColE1	300–500	high copy
pGEM® vectors	pMB1*	300–400	high copy
pTZ vectors	pMB1*	>1000	high copy
pBR322 and derivatives	pMB1*	15–20	low copy
pACYC and derivatives	p15A	10–12	low copy
pSC101 and derivatives	pSC101	~5	very low copy
Cosmids			
SuperCos	ColE1	10–20	low copy
pWE15	ColE1	10–20	low copy

* *The pMB1 origin of replication is closely related to that of ColE1 and falls in the same incompatibility group. The high-copy-number plasmids listed here contain mutated versions of this origin.*

Host Strains

Most *E. coli* strains can be used successfully to isolate plasmid DNA, although the strain used to propagate a plasmid can have an effect on the quality of the purified DNA. Host strains such as DH1, DH5α, and C600 yield high quality DNA with the QIAGEN protocol. The slower growing strain, XL1-Blue, also yields DNA of very high quality and works extremely well for sequencing. Strain HB101 and its derivatives, such as TG1 and the JM100 series, contain large amounts of carbohydrate which is released during lysis and can inhibit enzyme activities if not completely removed (3). In addition, these strains have high levels of endonuclease activity, and yield DNA of lower quality than that prepared from strains such as DH1, DH5α, and C600. The methylation and growth characteristics of the strain should also be taken into account when a plasmid host is chosen. If after performing a QIAGEN plasmid preparation, the quality of purified DNA is not as expected, a change of host strain should be considered.

Inoculation

Bacterial cultures for plasmid preparation should always be grown from a single colony picked from a selective plate. Subculturing directly from glycerol stocks, agar stabs, and liquid cultures is poor microbiological practice and may lead to loss of the plasmid. Inoculation from plates that have been stored for a long time may also lead to loss or mutation of the plasmid. For minipreps, a single colony should be inoculated into 3 ml or less of media containing the appropriate selective agent, and grown to saturation (12–16 hours).

Antibiotics

Antibiotic selection should be applied at all stages of growth. Many plasmids in use today do not contain the *par* locus, which ensures that the plasmids segregate equally during cell division. Daughter cells which do not receive plasmids will replicate much faster than plasmid-containing cells in the absence of selective pressure, and can quickly take over the culture.

The stability of the selective agent should also be taken into account. Resistance to ampicillin, for example, is mediated by ß-lactamase which is encoded by the plasmid-linked *bla* gene and which hydrolyzes ampicillin. Levels of ampicillin in the culture medium are thus continually depleted. This phenomenon is clearly demonstrated on ampicillin plates, where "satellite colonies" appear as the ampicillin is hydrolyzed in the vicinity of a growing colony. It is important to innoculate cultures from freshly prepared plates to ensure that the antibiotic is effective. Ampicillin is also sensitive to temperature, and when in solution should be stored frozen in single-use aliquots.

Culture Media

QIAGEN protocols are optimized for use with cultures grown in standard Luria Bertani (LB) medium, at a cell density of approximately 1 x 10^9 cells per ml (approximately 1–1.5 A_{600} units/ml). Table 1 on page 6 of this manual shows the culture volumes recommended for use with each type of QIAGEN column and several types of plasmids.

It is not necessary to use super rich growth media such as TB (terrific broth) or 2x YT for most commonly used high-copy plasmids. These rich media will lead to extraordinarily high cell densities. Cultures grown in TB may yield 2–5 times the number of cells as those grown in LB.

If these media are used, the culture volumes must be reduced according to the cell density measured to match the capacity of the QIAGEN-tip used, and growth time should be reduced to 8–10 hours. If too much culture volume is used, alkaline lysis will be inefficient, the column will be overloaded, and the performance of the system reduced. Furthermore, the excessive viscosity of the lysate would require vigorous mixing, which may result in shearing of bacterial genomic DNA and thus contamination of the plasmid DNA.

Care must also be taken when using strains which grow unusually fast or to very high cell densities. It is best to calculate the cell density of the culture and adjust the volumes accordingly.

Chloramphenicol Amplification

The copy numbers of the current generation of plasmids are so high that selective amplification in the presence of chloramphenicol is not necessary to achieve high yields. However, when low-copy-number plasmids containing the pMB1 or ColE1 origin of replication are prepared, the yield can be improved by adding chloramphenicol (170 mg/liter) to amplify the copy number (2). Cultures of bacteria containing low-copy-number plasmids amplified in the presence of chloramphenicol should be treated as if they contain high-copy-number plasmids when choosing the appropriate culture volumes for the QIAGEN-tips to be used.

Cosmids

QIAGEN Plasmid Kits are also ideal for purification of cosmid DNA. Due to their relatively large size and slow replication time, cosmids are generally present in low copy numbers in bacterial cells. For this reason cosmids should be treated as low-copy-number plasmids when determining which QIAGEN-tip to use. Yields of cosmids containing the ColE1 replication origin may also be enhanced by addition of chloramphenicol to the culture medium. In order to obtain sufficient cosmid DNA, it is often necessary to use culture volumes much larger than those normally recommended for use on QIAGEN-tips. A few changes in the procedure are necessary to avoid overloading the QIAGEN-tip. See the detailed protocol on page 11. Cosmid DNA prepared with QIAGEN-tips is suitable for all downstream applications, including sequencing (manual or automated).

Purification of M13 Replicative Form

The replicative form of bacteriophage M13 behaves like a low-copy-number plasmid, and can be purified using QIAGEN Plasmid Kits (page 13). The only modification of the procedure is the introduction of a wash step before the cells are lysed. This step removes all traces of the phage-rich culture supernatant from the bacterial pellet and prevents contamination of the double-stranded M13 RF with single-stranded phage DNA.

In vitro Transcription

Plasmid DNA preparations are free of any detectable proteins or other contaminants when purified on QIAGEN-tips according to the recommended protocol. Although RNase A is used in large amounts in the QIAGEN procedure, DNA purified on QIAGEN-tips is free of detectable RNase A activity and is suitable for *in vitro* transcription. If, however, any RNase A activity should be found in the purified DNA sample, it can be easily eliminated by phenol extraction or by addition of an RNase inhibitor (e.g. RNasin).

1. *Birnboim, H.C. and Doly, J. (1979) A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucl. Acids Res.* ***7****, 1513-1522.*
2. *Sambrook, J. et al., eds. (1989) Molecular cloning: a laboratory manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor.*
3. *Ausubel, F.M. et al., eds. (1991) Current protocols in molecular biology, Wiley Interscience, New York.*

2. Purification of Plasmid DNA

After lysis of the bacteria under alkaline conditions, the lysate is applied under defined salt conditions to the QIAGEN-tip where the plasmid DNA is selectively bound and purified from RNA, proteins, and other cellular contaminants.

Preparation of the Cell Lysate

DNA yield depends on the quality of the cell lysate used. Preparation of a cleared cell lysate is therefore a critical step in the QIAGEN purification procedure, which has been carefully designed to provide ideal lysis conditions.

After harvesting and resuspension, the bacterial cells are lysed in NaOH/SDS (Buffer P2) in the presence of RNase A (1,2). SDS disrupts the phospholipid and protein components of the cell membrane, leading to lysis and release of the cell contents. NaOH denatures the chromosomal and plasmid DNAs, as well as proteins. The optimized lysis time allows maximum release of plasmid DNA without release of chromosomal DNA, while minimizing the exposure of the plasmid to denaturing conditions. Long exposure to alkaline conditions may cause the plasmid to become irreversibly denatured. This denatured form of the plasmid runs faster on agarose gels and is resistant to restriction enzyme digestion (see Figure 2, page 26).

The lysate is neutralized by the addition of acidic potassium acetate (Buffer P3). The high salt concentration causes SDS to precipitate, and the denatured proteins, chromosomal DNA, and cellular debris become trapped in salt/detergent complexes. Plasmid DNA, being smaller and covalently closed, renatures correctly and remains in solution. Since any SDS remaining in the lysate will inhibit binding of DNA to the QIAGEN Resin, the solution must be gently but thoroughly mixed to ensure complete precipitation of the detergent. Precipitation is enhanced by using pre-chilled Buffer P3, and carrying out the precipitation on ice.

To prevent contamination of plasmid DNA with chromosomal DNA, vigorous stirring and vortexing must be avoided during lysis. Separation of plasmid from chromosomal DNA is based on coprecipitation of the cell wall-bound chromosomal DNA with the insoluble complexes containing salt, detergent, and protein. Plasmid DNA remains in the clear supernatant. Vigorous treatment during the lysis procedure will shear the bacterial chromosome, leaving free chromosomal DNA fragments in the supernatant. Since chromosomal fragments are chemically indistinguishable from plasmid DNA under the conditions used, the two species will not be separated on QIAGEN Resin and will elute under the same salt conditions. Mixing during the lysis procedure should therefore be carried out only by gentle inversion.

1. *Birnboim, H.C. and Doly, J. (1979) A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucl. Acids Res.* ***7****, 1513-1522.*
2. *Birnboim, H.C. (1983) A rapid alkaline extraction method for the isolation of plasmid DNA. Methods Enzymol.* ***100****, 243-255.*

The precipitated debris is removed by high speed centrifugation, producing a cleared lysate for loading onto the QIAGEN-tip. It is important that the lysate is clear at this stage to ensure good flow rates and ultimately, to obtain protein-free plasmid DNA preparations.

RNase A, which is added at the beginning of the procedure, digests the liberated RNA efficiently during the alkaline lysis. The resulting RNA fragments do not bind to QIAGEN Resin under the salt and pH conditions of the lysate.

Purification on the QIAGEN-tip

The cleared lysate is loaded onto a pre-equilibrated QIAGEN-tip by gravity flow. The salt and pH conditions in the lysate and the superior selectivity of the QIAGEN Resin ensure that only plasmid DNA binds, while degraded RNA, cellular proteins, and metabolites are not retained, and appear in the flowthrough fraction.

The QIAGEN-tip is then washed with buffer containing 1 M NaCl (Buffer QC) which completely removes any remaining contaminants, such as traces of RNA and protein (e.g. RNase A), without affecting the binding of the plasmid DNA (see Figure 2, page 26). Buffer QC also disrupts nonspecific interactions, and allows removal of nucleic acid-binding proteins without the use of phenol. The low concentration of alcohol in the wash buffer eliminates nonspecific hydrophobic interactions, further enhancing the purity of the bound DNA. The plasmid DNA is then efficiently eluted from the QIAGEN-tip with Buffer QF containing 1.25 M NaCl at pH 8.5. For further information about QIAGEN Anion-Exchange Resin, see Appendix C (page 29).

Desalting and Concentration

The eluted plasmid DNA is desalted and concentrated by isopropanol precipitation. Precipitation is carried out at room temperature to minimize coprecipitation of salt. After centrifugation, the pellet is washed with room temperature 70% ethanol to remove residual salt and to replace the isopropanol with ethanol, which is more volatile and easily removed. The purified DNA is briefly air-dried and resuspended in a small volume of TE (pH 8.0 or higher to enhance solubility), and is ready for use in transfection, sequencing, labeling, cloning, or any other experimental procedure.

Analysis of Plasmid DNA

A 1% analytical agarose gel comparing the fractions at each stage of the QIAGEN purification procedure is shown in Figure 2. The entire procedure, including the efficiency of plasmid recovery, can be easily monitored by saving equal proportions of each fraction and precipitating the nucleic acids with isopropanol before loading them on the gel.

L: Cleared lysate containing supercoiled and open-circular plasmid DNA and degraded RNA.

F: Flow-through fraction containing only degraded RNA is depleted of plasmid DNA which is bound to the QIAGEN Resin.

W1: First wash fraction, in which the remaining traces of RNA are removed without affecting the binding of the DNA.

W2: Second wash fraction, which ensures that the resin is completely cleared of RNA and other contaminants, leaving only pure plasmid DNA on the column.

E: The eluate containing pure plasmid DNA with no other contaminating nucleic acids.

M: Lambda DNA digested with *Hin*dIII.

Lanes 1–5 demonstrate some atypical results that may be observed in some preparations, depending on plasmid type and host strain.

1: Supercoiled (lower band) and open-circular form (upper band) of the high-copy plasmid, pUC18, with an additional band of denatured supercoiled DNA migrating just below the supercoiled form. This form may result from prolonged alkaline lysis with Buffer P2 and is resistant to restriction digestion.

2: Multimeric forms of supercoiled plasmid DNA (pTZ19) which may be observed with some host strains, and should not be mistaken for genomic DNA. Multimeric plasmid DNA can easily be distinguished from genomic DNA by a simple restriction digestion: linearization of a plasmid sample displaying multimeric bands will yield one defined band with the size of the linearized plasmid monomer (see lane 3).

3: Linearized form of plasmid pTZ19 after restriction digestion with *Eco*RI.

4: Sample contaminated with bacterial chromosomal DNA, which may be observed if the lysate is treated too vigorously, e.g., vortexing during incubation steps with Buffer P2 or P3. Genomic DNA contamination can easily be identified by digestion of the sample with *Eco*RI. A smear is observed, in contrast to the linear band seen after digestion of multimeric plasmid forms.

5: *Eco*RI digestion of a sample contaminated with bacterial genomic DNA which gives a smear above the plasmid DNA.

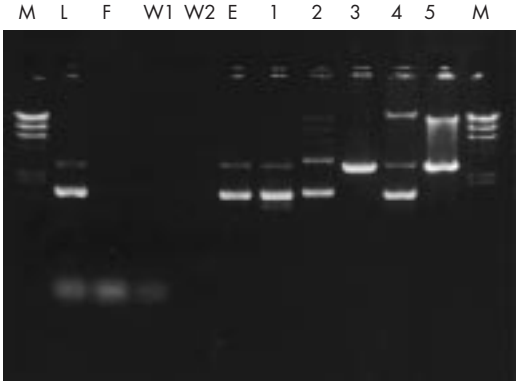

Figure 2: *Agarose gel analysis of plasmid purification procedure.*

Appendix B: Composition and Preparation of Buffers

Composition of Buffers

Buffer	Composition	Storage
Buffer P1 (Resuspension Buffer)	50 mM Tris·Cl, pH 8.0; 10 mM EDTA; 100 µg/ml RNase A	4°C, after addition of RNase A
Buffer P2 (Lysis Buffer)	200 mM NaOH, 1% SDS	room temp.
Buffer P3 (Neutralization Buffer)	3.0 M potassium acetate, pH 5.5	room temp. or 4°C
Buffer QBT (Equilibration Buffer)	750 mM NaCl; 50 mM MOPS, pH 7.0; 15% isopropanol; 0.15% Triton® X-100	room temp.
Buffer QC (Wash Buffer)	1.0 M NaCl; 50 mM MOPS, pH 7.0; 15% isopropanol	room temp.
Buffer QF (Elution Buffer)	1.25 M NaCl; 50 mM Tris·Cl, pH 8.5; 15% isopropanol	room temp.
TE	10 mM Tris·Cl, pH 8.0; 1 mM EDTA	room temp.
STE	100 mM NaCl; 10 mM Tris·Cl, pH 8.0; 1 mM EDTA	room temp.

Preparation of Buffers

Buffer compositions are given per liter of solution. Do not autoclave isopropanol-containing buffers; sterilize by filtration instead.

Buffer calculations ar based on Tris base adjusted to pH with HCl (Tris·Cl). If using Tris-HCl reagent, the qualities used should be recalculated.

P1: Dissolve 6.06 g Tris base, 3.72 g EDTA·2H_2O in 800 ml dH_2O. Adjust the pH to 8.0 with HCl. Adjust the volume to 1 liter with dH_2O. Add 100 mg RNase A per liter of P1.

P2: Dissolve 8.0 g NaOH pellets in 950 ml dH_2O, 50 ml 20% SDS solution. The final volume should be 1 liter.

P3: Dissolve 294.5 g potassium acetate in 500 ml dH_2O. Adjust the pH to 5.5 with glacial acetic acid (~110 ml). Adjust the volume to 1 liter with dH_2O.

QBT: Dissolve 43.83 g NaCl, 10.46 g MOPS (free acid) in 800 ml dH_2O. Adjust the pH to 7.0. Add 150 ml pure isopropanol and 15 ml 10% Triton X-100 solution. Adjust the volume to 1 liter with dH_2O.

QC: Dissolve 58.44 g NaCl and 10.46 g MOPS (free acid) in 800 ml dH_2O. Adjust the pH to 7.0. Add 150 ml pure isopropanol. Adjust the volume to 1 liter with dH_2O.

QF: Dissolve 73.05 g NaCl and 6.06 g Tris base in 800 ml dH_2O and adjust the pH to 8.5 with HCl. Add 150 ml pure isopropanol. Adjust the volume to 1 liter with dH_2O.

STE: Dissolve 5.84 g NaCl, 1.21 g Tris base and 0.37 g EDTA·$2H_2O$ in 800 ml dH_2O. Adjust the pH to 8.0 with HCl. Adjust the volume to 1 liter with dH_2O.

Preparation of LB Medium

Dissolve 10 g tryptone, 5 g yeast extract, and 10 g NaCl in 800 ml dH_2O. Adjust the pH to 7.0 with 1 N NaOH. Adjust the volume to 1 liter with dH_2O. Sterilize by autoclaving.

Appendix C: QIAGEN Anion-Exchange Resin

QIAGEN-tips contain a unique, patented anion-exchange resin which eliminates the need for expensive equipment and reagents such as ultracentrifuges, HPLC/FPLC®, or CsCl. Toxic and mutagenic substances such as phenol, chloroform, and ethidium bromide are not required.

Plasmid purification on QIAGEN Resin is based on the interaction between negatively charged phosphates of the DNA backbone and positively charged DEAE groups on the surface of the resin. The salt concentration and pH conditions of the buffers used determine whether DNA is bound or eluted from the column. The key advantage of QIAGEN Anion-Exchange Resin arises from its exceptionally high charge density. The resin consists of defined silica beads with a particle size of 100 µm, a large pore size, and a hydrophilic surface coating. The large surface area allows dense coupling of the DEAE groups. Plasmid DNA remains tightly bound to the DEAE groups over a wide range of salt concentrations (Figure 3). Impurities such as RNA, protein, carbohydrates, and small metabolites are washed from QIAGEN Resin with medium-salt buffers, while plasmid DNA remains bound until eluted with a high-salt buffer.

The separation range of QIAGEN Resin is extremely broad, extending from 0.1 M to 1.6 M salt (Figure 3), and DNA can be efficiently separated from RNA and other impurities. In contrast, conventional anion-exchangers, based on cellulose, dextran or agarose, have separation ranges only up to 0.4 M salt, so that binding and elution of all substances is limited to a narrow range of salt concentrations. This means that the elution peaks of proteins, RNA, and DNA overlap extensively with one another, and a satisfactory separation cannot be achieved. Thus the separation and purification qualities of QIAGEN Resin as well as its ease of use surpass those of conventional anion-exchange resins.

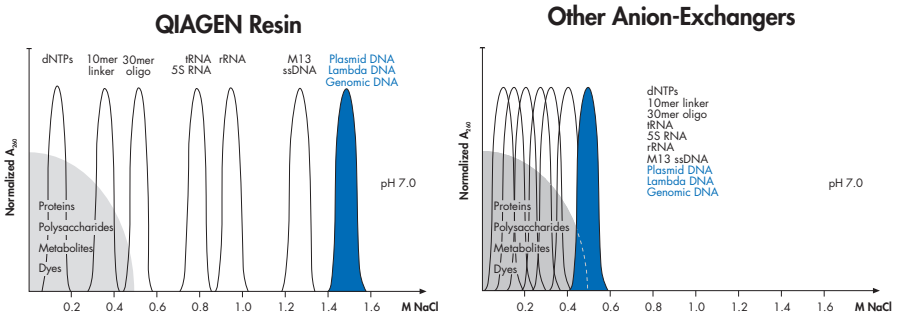

Figure 3. *Separation of nucleic acids at neutral pH on anion-exchange resins.*

DNA Purity and Biological Activity

Nucleic acids prepared on QIAGEN Resin are of comparable (or superior) purity to nucleic acids prepared by two rounds of purification on CsCl gradients. DNA prepared using QIAGEN-tips has been tested with all common restriction endonucleases, polymerases (including *Taq* DNA polymerase), DNA ligases, phosphatases, and kinases. Results were comparable to those achieved using CsCl-prepared DNA. Subsequent procedures such as transfection, transformation, sequencing, cloning, *in vitro* transcription and translation proceed with optimal efficiency.

Capacity and Recovery

The names of the different QIAGEN-tips indicate the binding capacities (in µg) of the columns for double-stranded plasmid DNA, as determined with purified pUC18 DNA. Therefore, QIAGEN-tip 20 has a binding capacity of 20 µg of plasmid DNA.

QIAGEN Resin has different binding capacities for different classes of nucleic acids. The capacity of QIAGEN Resin for RNA, for example, is twice that for plasmid DNA. Conversely, large nucleic acids, such as lambda, cosmids, and genomic DNA, are bound at a slightly lower capacity than plasmid DNA. This relationship between the binding capacity of the QIAGEN Resin and the size of the nucleic acids being prepared must be taken into account when calculating expected yields.

Stability

QIAGEN Resin is stable for up to six hours after equilibration. Beyond this time, the separation characteristics of the resin will begin to change, and it will no longer be effective. QIAGEN-tips may be reused within six hours for the same sample by re-equilibrating the resin with Buffer QBT after the first elution. QIAGEN Resin will not function in the presence of anionic detergents such as SDS, or at a pH less than 4.0.

Buffers

The binding, washing, and elution conditions for QIAGEN Resin are strongly influenced by pH. Figure 4 shows the influence of pH on the salt concentration required for elution of various types of nucleic acids. Deviations from the appropriate pH values of the buffers at a given salt concentration may result in loss of the desired nucleic acid.

Buffers, such as MOPS, sodium phosphate, Tris·Cl, and sodium acetate can be used at the indicated pH. MOPS (3-[N-morpholino]propanesulfonic acid, pK_a 7.2) is frequently the buffer of choice in QIAGEN protocols, since it has a higher buffering capacity at pH 7.0 than sodium phosphate, Tris·Cl, or sodium acetate buffers.

SDS and other anionic detergents interfere with the binding of nucleic acids to QIAGEN Resin by competing for binding to the anion-exchange groups. If SDS is used during sample preparation, it must be removed through steps such as potassium acetate precipitation or alcohol precipitation prior to column application. SDS removal steps are incorporated into the QIAGEN protocols described in this manual.

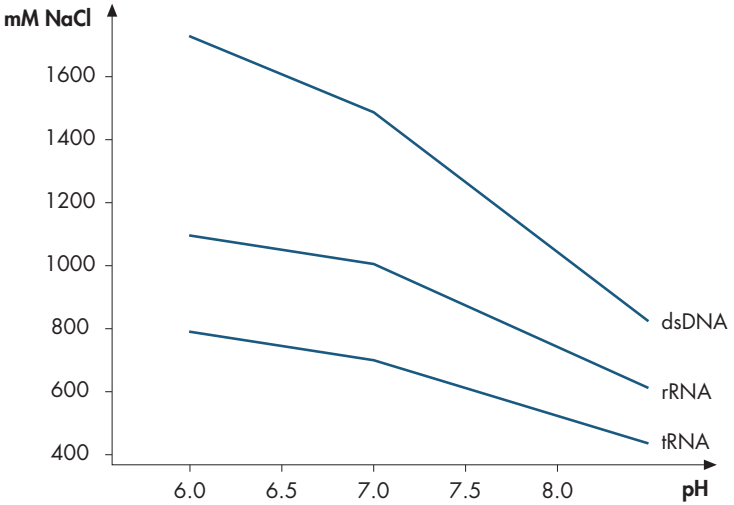

Figure 4. *Elution points of different nucleic acids from QIAGEN Resin as a function of pH.*

Product Use Limitations

QIAGEN Plasmid Mini Kits are developed, designed, and sold for research purposes only. They are not to be used for human diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this text.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department. We will credit your account or exchange the product — as you wish.

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QIAGEN Plasmid Mini Kit (100)	100 QIAGEN-tip 20, Reagents, and Buffers	12125
QIAGEN Plasmid Starter Kits		
QIAGEN Plasmid Starter Kit I	10 QIAGEN-tip 20, 3 QIAGEN-tip 100, 1 QIAGEN-tip 500, Reagents, and Buffers	12129
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QIAwell Plasmid Kits		
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QIAwell 96 Ultra Plasmid Kit (4)†	For 4 x 96 plasmid minipreps, 4 each: QIAfilter 96, QIAwell 96, and QIAprep 96 Plates; Reagents, Buffers, Collection Microtubes (1.2 ml), Caps	16191

** Require use of QIAvac 6S*

† Requires use of QIAvac 96

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QIAGEN Plasmid Giga Kit (5)	5 QIAGEN-tip 10000, Reagents, and Buffers	12191
QIAfilter Plasmid Midi Kit (25)	25 QIAGEN-tip 100, Reagents, Buffers, 25 QIAfilter Midi Cartridges	12243
QIAfilter Plasmid Maxi Kit (10)	10 QIAGEN-tip 500, Reagents, Buffers, 10 QIAfilter Maxi Cartridges	12262
Accessories		
QIArack 1	1 rack for 12 x QIAGEN-tip 20	19011
QIArack 2	1 rack for 8 x QIAGEN-tip 100, and 4 x QIAGEN-tip 500	19014
RNase A	250 mg (70 U/mg; 100 mg/ml))	19101
QIAfilter Midi Cartridges	25 cartridges	19743
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QIAfilter 8 Strips (50)‡	50 QIAfilter 8 Strips for use with QIAwell 8 and QIAwell 8 Plus Plasmid Kits	19624
QIAvac 6S	Vacuum manifold for processing 1–6 QIAGEN 8-well strips	19503
QIAvac 96	Vacuum manifold for processing QIAGEN 96-well plates	19504

‡ *Optional accessories for use with QIAwell 8 Plasmid Kits.*

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