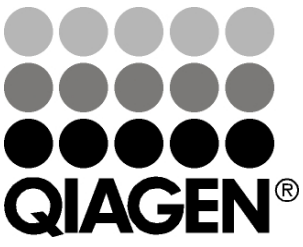
April 2012

QIAGEN® Large-Construct Handbook

For isolation of genomic DNA-free BAC, PAC, P1, and cosmid DNA



Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

Contents

Kit Contents

QIAGEN Large-Construct Kit	**(10)**
Catalog no.	**12462**
QIAGEN-tip 500	10
Buffer P1	250 ml
Buffer P2	250 ml
Buffer P3	250 ml
Buffer QBT	2 x 60 ml
Buffer QC	3 x 240 ml
Buffer QF	200 ml
ATP-Dependent Exonuclease	10 x 80 µg
Buffer EX	110 ml
Buffer QS	110 ml
Exonuclease Solvent	2 x 1.25 ml
RNase A (100 mg/ml)	25 mg
Folded filters	10
QIAGEN-tip 500 holders	10
Quick-Start Protocol	1

Storage

QIAGEN-tips should be stored dry and at room temperature (15–25°C). They can be stored for at least 2 years without showing any reduction in capacity or performance.

After addition of RNase A, Buffer P1 should be stored at 2–8°C and is stable for 6 months. ATP-Dependent Exonuclease should be stored at 15–25°C and is stable for at least 2 years. Freshly prepared ATP solution should be stored in aliquots at –20°C and is stable for up to 12 months. Other buffers and RNase A stock solution can be stored for 2 years at room temperature.

Intended Use

The QIAGEN Large-Construct Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/Support/MSDS.aspx where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAGEN Large-Construct Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The QIAGEN Large-Construct Kit is designed for the purification of large constructs such as BACs, PACs, P1s, and cosmids (up to 250 kb), which are typically present at very low-copy numbers. The kit uses a rapid purification procedure based on the remarkable selectivity of patented QIAGEN Anion-Exchange Resin. An integrated digestion step with ATP-Dependent Exonuclease, provided in the kit, enables efficient removal of genomic DNA contamination to yield ultrapure, genomic DNA-free BAC/PAC/P1/cosmid DNA.

Principle and procedure

The QIAGEN Large-Construct Kit protocol starts with modified alkaline lysis and integrated ATP-Dependent Exonuclease digestion. The ATP-Dependent Exonuclease digestion step ensures selective removal of contaminating genomic DNA, as well as nicked or damaged large-construct DNA. Following the digestion step, large-construct DNA is bound to the QIAGEN resin under appropriate low-salt and pH conditions. RNA, proteins, dyes, and low–molecular-weight impurities are removed by a medium-salt wash. Large-construct DNA is eluted in high-salt buffer and then concentrated and desalted by isopropanol precipitation. No expensive equipment, such as ultracentrifuges and HPLC apparatus, or toxic reagents, such as phenol and ethidium bromide, are required.

Each disposable QIAGEN-tip packed with QIAGEN resin is designed to operate by gravity flow, reducing the amount of hands-on time required for the purification procedure. QIAGEN-tips are highly suited for rapid and simple preparation of multiple samples.

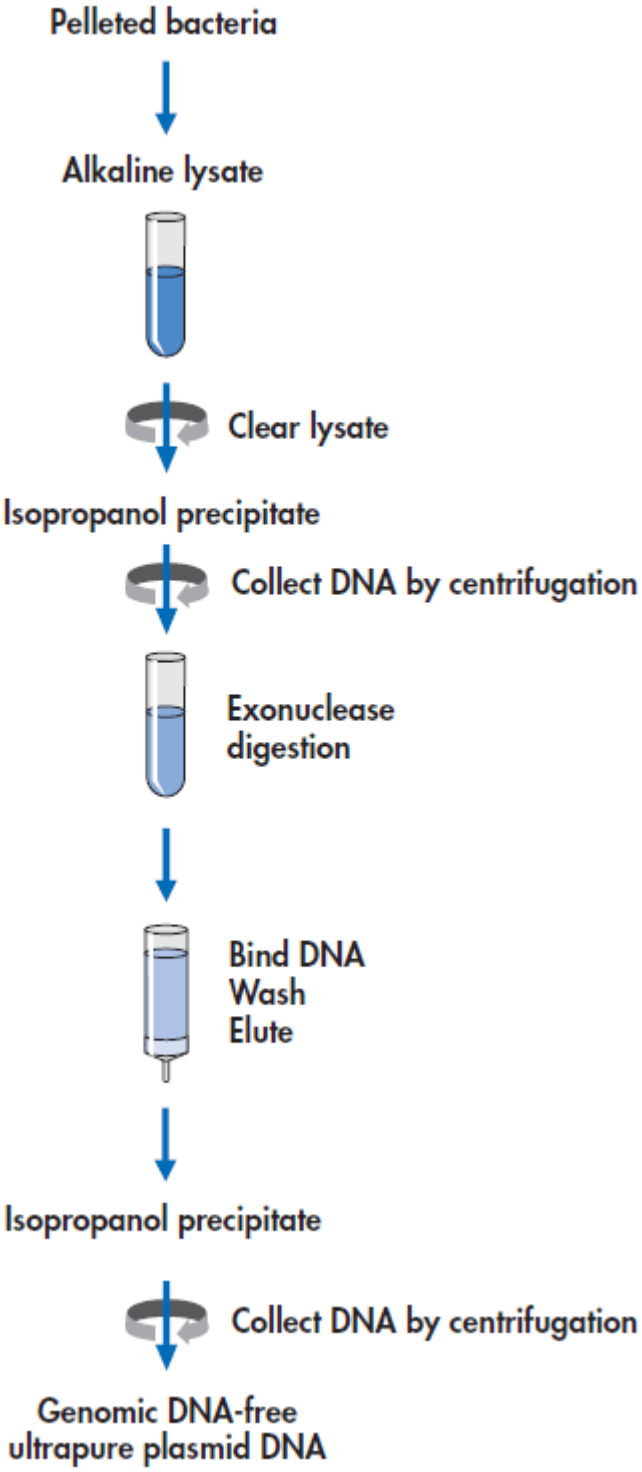
Downstream applications

Purified large-construct DNA is suitable for various critical applications, including subcloning or transfection experiments, or any other sensitive downstream application requiring highest DNA purity. Depending on the construct and the host strain used, yields of up to 50 µg ultrapure, genomic DNA-free BAC/PAC/P1 DNA or up to 200 µg ultrapure, genomic DNA-free cosmid DNA can be obtained.

Note: BAC/PAC/P1/cosmid DNA prepared by traditional methods typically contains genomic DNA contamination of up to 30%, leading to significant overestimation of the actual DNA yield when this is measured spectrophotometrically. With the QIAGEN Large-Construct Kit, contaminating genomic DNA is removed, so correspondingly lower spectrophotometric readings should be expected.

Note: For less sensitive downstream applications that do not require genomic DNA-free preparations, higher yields can be obtained with the special protocol provided in Appendix A (page 28). This protocol does not include an ATP-Dependent Exonuclease digestion step and provides yields of up to 150 µg BAC/PAC/P1 DNA or up to 400 µg cosmid DNA.

QIAGEN Large-Construct Kit Procedure



Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- A 100 mM ATP solution, prepared according to the instructions given at the beginning of the protocol (page 17)
- ATP disodium salt; e.g., from AppliChem (cat. no. A1348) or Sigma (cat. no. A3377)*

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Important Notes

Complete solutions for BACs

In addition to the QIAGEN Large-Construct Kit for isolation of up to 50 µg ultrapure BAC, PAC, or P1 DNA, or up to 200 µg ultrapure cosmid DNA, QIAGEN also offers a solution for high-throughput minipreps of large constructs. An optimized protocol provided in the R.E.A.L.® Prep 96 Plasmid Kit yields up to 0.8 µg BAC, PAC, or P1 DNA for sequencing and screening applications. Call QIAGEN Technical Services or your local distributor for more information (for contact information, see back cover or visit www.qiagen.com).

General considerations for optimal results

The QIAGEN large-construct purification protocol is an optimized procedure based on the alkaline lysis method of Birnboim and Doly (1). The procedure incorporates a unique treatment with ATP-Dependent Exonuclease, which allows selective preparation of ultrapure genomic DNA-free BAC, PAC, P1, or cosmid DNA without the use of phenol, chloroform, ethidium bromide, or cesium chloride.

Growth of bacterial cultures

Large constructs 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 DNA prepared may depend on a number of factors, including construct copy number, size of insert, host strain, culture volume, and culture medium.

Copy number

Large constructs such as BACs, PACs, P1s, and cosmids are generally present in low or very low copy numbers in cells. The copy number of large constructs can be substantially influenced by the cloned insert, the vector, and the host strain used, resulting in lower than expected yields of DNA.

Host strains

Many large-construct preparations are performed using the host strain DH10B™, which allows successful isolation of high-quality DNA. Cosmids are commonly prepared from a number of different host strains. The host strain used to propagate a construct can have a substantial influence on the quality of the purified DNA. Host strains such as DH1, DH5®α, and C600 yield high-quality DNA with QIAGEN protocols. The slower growing strain XL1-Blue also yields DNA of very high quality that works extremely well in sequencing applications. 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 (3). In addition, some strains such as JM101, JM110, and HB101 have high levels of endonuclease activity, and yield DNA of lower quality than that prepared from strains such as XL1-Blue, DH1, DH5α, and C600. The methylation and growth characteristics of the host strain can also affect DNA isolation. If after performing a QIAGEN large-construct preparation, 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 Top10, we recommend either reducing the amount of culture volume used for cleared lysate preparation, or using the same amount of culture volume but doubling the volumes of Buffers P1, P2, and P3 to improve the ratio of biomass to lysis buffers for optimized lysis conditions.

Inoculation

Bacterial cultures for BAC, PAC, P1, and cosmid preparations should always be grown from a single colony isolated from a freshly streaked selective plate. Subculturing directly from glycerol stocks, agar stabs, and liquid cultures is poor microbiological practice and may lead to loss of the construct. Inoculation from plates that have been stored for a long time may also lead to loss or mutation of the construct.

The desired clone should be streaked from a glycerol stock onto a freshly prepared agar plate containing the appropriate selective agent so that single colonies can be isolated. This procedure should then be repeated to ensure that a single colony of an antibiotic-resistant clone can be isolated. A single colony should be inoculated into 2–5 ml LB medium (see page 15) containing the appropriate selective agent and grown to late logarithmic phase (~8 h). Using a vessel with a volume at least four times greater than the volume of media, the starter culture should then be diluted 1/500 to 1/1000 into a larger volume of selective medium, and grown with vigorous shaking (~300 rpm) to late logarithmic/stationary (12–16 hours). It is often convenient to grow the starter culture during the day and the larger culture overnight for harvesting the following morning.

Antibiotics

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

Culture medium

The QIAGEN Large-Construct Kit protocol is optimized for use with cultures grown in standard LB medium (see page 15), grown to a cell density of approximately 3–4 x 10^9 cells per ml. We advise harvesting cultures after approximately 12–16 hours of growth, which is typically the transition from logarithmic into stationary growth phase. At this time, the ratio of large-construct DNA to RNA is higher than during the logarithmic phase. Also the DNA will not be degraded due to overaging of the culture, as can occur in the late stationary phase. Please note the maximum recommended culture volumes given at the beginning of the protocol.

Several bacterial strains can grow to very high cell densities. It is best to assess the cell density by measuring the OD_{600} of the culture and to reduce the culture volumes accordingly or increase the volumes of lysis buffers P1, P2, and P3, if necessary. A high ratio of biomass to lysis buffers will result in poor lysis conditions and subsequently low DNA yield and purity.

It is not recommended to use super rich growth media such as TB (terrific broth) or 2x YT. Although TB or 2x YT have the obvious advantage of producing more bacteria (2–5 times), this does not necessarily lead to greater yields or higher quality DNA.

If rich media must be used, the culture volumes must be reduced according to the cell density such that they match the capacity of the QIAGEN-tip used. If the culture volume used is too high, alkaline lysis will be inefficient, resulting in lower yield than expected. Furthermore, the excessive viscosity of the lysate will require that it is mixed vigorously, resulting in shearing of bacterial genomic DNA and the large-construct DNA.

Measuring cell density

Photometric measurements of cell density can vary between different spectrophotometers. The optical density reading of a bacterial culture is a measure of the light scattering, which varies depending on the distance between the sample and the detector.

Calibration of each individual spectrophotometer is required to facilitate accurate conversion of OD_{600} measurements into the number of cells per milliliter. This can be achieved by plating serial dilutions of a culture onto LB agar plates in the absence of antibiotics. The counted colonies are used to calculate the cells per milliliter, which is then set in relation to the measured OD_{600} values.

Pellet wet weight

If spectrophotometric measurement of the cell density or calibration of the photometer is not possible, another way of estimating the amount of cell

harvest is the assessment of the pellet wet weight. Typically a 1 liter, overnight shaker-culture of *E. coli* with a cell density of 3–4 x 10^9/ml corresponds to a pellet wet weight of approximately 3 g/liter.

Key steps in the QIAGEN Large-Construct protocol

After lysis of bacteria under alkaline conditions and exonuclease digestion, the lysate is applied under defined salt conditions to the QIAGEN-tip. Large-construct 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 large-construct 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, 4). SDS solubilizes the phospholipid and protein components of the cell membrane, leading to lysis and release of the cell contents. NaOH denatures the chromosomal and large-construct DNA, as well as proteins. The optimized lysis time allows maximum release of large-construct DNA from the cell without release of chromosomal DNA, while minimizing the exposure of the large construct to denaturing conditions. Long exposure to alkaline conditions may cause the construct to become irreversibly denatured.

The lysate is neutralized by the addition of acidic potassium acetate (Buffer P3). The high salt concentration causes KDS* to precipitate, and the denatured proteins, the majority of the chromosomal DNA, and cellular debris become trapped in salt-detergent complexes. Since any SDS remaining in the lysate will inhibit binding of DNA to the QIAGEN resin, the solution must be thoroughly but gently mixed to ensure complete precipitation of the detergent.

Separation of the large-construct DNA from chromosomal DNA is based on exonuclease digestion of nicked DNA. Chromosomal DNA is nicked during the lysis process and subsequently digested, while the majority of the large-construct DNA in the solution remains intact. Since chromosomal fragments have a similar negative charge density to large-construct DNA, the two species would not be separated on QIAGEN Anion-Exchange Resin and would elute under the same salt conditions if chromosomal DNA was not removed by the exonuclease digestion step.

* KDS: Potassium dodecyl sulfate

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 the QIAGEN resin under the salt and pH conditions present in the lysate.

The precipitated debris is removed by high-speed centrifugation and subsequent filtration, producing a cleared lysate for exonuclease digestion and subsequent loading onto the QIAGEN-tip. It is important that the lysate is clear at this stage to ensure optimal exonuclease digestion.

Exonuclease digestion of chromosomal DNA

DNA in the cleared lysate is precipitated with isopropanol and redissolved in exonuclease reaction buffer. ATP solution and ATP-Dependent Exonuclease are then added to the sample. Chromosomal DNA and nicked large-construct DNA are digested by the exonuclease and only supercoiled, intact large-construct DNA remains. Subsequent addition of Buffer QS allows adjustment of conditions for DNA binding to the QIAGEN resin.

DNA binding and washing on the QIAGEN-tip

The digested preparation is loaded onto a pre-equilibrated QIAGEN-tip and the sample enters the column by gravity flow. The salt and pH conditions of the digested preparation and the superior selectivity of the QIAGEN resin ensure that only DNA binds, while degraded RNA, cellular proteins, and metabolites are not retained and appear in the flow-through fraction.

The QIAGEN-tip is then washed with medium-salt buffer (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 large-construct DNA. 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 large-construct DNA is then efficiently eluted from the QIAGEN-tip with high-salt buffer (Buffer QF). Use of prewarmed Buffer QF (elution buffer) provides more efficient elution of the large DNA molecules. For further information about QIAGEN Anion-Exchange Resin, see Appendix C, page 33.

Desalting and concentration

The eluted large-construct DNA is desalted and concentrated by isopropanol precipitation. Precipitation is carried out at room temperature (15–25°C) to minimize coprecipitation of salt. After centrifugation, the DNA pellet is washed with 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 redissolved in a small volume of TE buffer, pH 8.0, or Tris·Cl,

pH 8.5, and is ready for use in cloning, sequencing, transfection, labeling, or any other experimental procedure.

Brief considerations for the large-construct purification procedure

Please take a few moments to read this handbook carefully before beginning the DNA preparation.

If you have not previously used QIAGEN purification kits, please pay particular attention to the detailed information provided in "General Considerations for Optimal Results" starting on page 10.

Copy number

Large constructs such as BACs, PACs, P1, and cosmids are generally present in low or very low copy numbers in cells. Although the QIAGEN Large-Construct Kit is specifically developed and adapted for the purification of BACs, PACs, P1s, or cosmids, the actual yield obtained depends on the construct insert, the vector, and the host strain used. Propagation of large constructs, which is a critical factor in the success of the isolation procedure, can vary significantly depending on the insert. Yields may be lower than those obtained for conventional plasmids.

Culture medium

The QIAGEN Large-Construct procedure is optimized for use with cultures grown in standard Luria Bertani (LB) medium to a cell density of approximately 3–4 x 10^9 cells/ml, which typically corresponds to a pellet wet weight of approximately 3 g/liter (see page 12). Please note that a number of slightly different LB culture broths, containing different concentrations of NaCl, are commonly used. We recommend growing cultures in LB medium containing 10 g NaCl per liter (Table 1) to obtain the highest yields of large-construct DNA. Rich media are not recommended for large-construct preparation using QIAGEN-tips.

Table 1. Composition of Luria Bertani medium*

Contents	Amount per liter
Tryptone	10 g
Yeast extract	5 g
NaCl	10 g

* Please refer to Appendix B on page 32 for preparation of LB medium.

Culture volume

Do not exceed the maximum recommended culture volume given at the beginning of the protocol (i.e., 500 ml). Use of larger culture volumes can affect the efficiency of alkaline lysis, leading to reduced yield and purity of the preparation.

Setup of QIAGEN-tips

QIAGEN-tips may be held upright in a suitable collection vessel (e.g., tube or flask) using tip holders provided with the kits. Alternatively, QIAGEN-tips may be placed in a QIArack which has a removable collection tray.

Convenient stopping points in the protocol

The purification procedure can be stopped and continued later by freezing the cell pellets obtained by centrifugation. Frozen cell pellets may be stored at –20°C for several weeks. In addition, the DNA eluted from QIAGEN-tips may be stored overnight at 2–8°C*, after which the protocol can be continued. These stopping points are indicated by the symbol ⊗.

* Longer storage is not recommended.

Protocol: Isolation of genomic DNA-free DNA using the QIAGEN Large-Construct Kit

This protocol is for preparation of up to 50 µg ultrapure, genomic DNA-free BAC, PAC, or P1 DNA, or up to 200 µg ultrapure, genomic DNA-free cosmid DNA using a QIAGEN-tip 500 (Maxi).

Table 2. Recommended culture volumes

Construct	QIAGEN-tip 500
BAC	500 ml
PAC	500 ml
P1	500 ml
Cosmid	500 ml

Important points before starting

- Add RNase A solution to Buffer P1 before use. Use 1 vial of RNase A (centrifuge briefly before use) per bottle of Buffer P1 for a final concentration of 100 µg/ml.
- Use 1 vial of ATP-Dependent Exonuclease per preparation. Resuspend the exonuclease contained in each vial in 225 µl Exonuclease Solvent before use. Mix by tapping the vial, and leave standing for 15 minutes to allow complete solution of the freeze-dried enzyme. Do not vortex.
- Check Buffer P2 for SDS precipitation due to low storage temperatures. If necessary, dissolve the SDS by warming to 37°C.
- Prechill Buffer P3 to 4°C.
- Prewarm elution Buffer QF to 65°C for use in step 16 (e.g., start the prewarming during the exonuclease digestion, step 12).
- Do not exceed the culture volumes recommended above as this will alter the ratio of biomass to lysis reagent and lead to inefficient cell lysis.

Things to do before starting

- Prepare a 100 mM ATP solution for the exonuclease digestion step: Dissolve 2.75 g ATP (dehydrated disodium salt)* in 40 ml distilled water. Adjust the pH to 7.5 with 10 M NaOH (~1 ml). Adjust the volume to 50 ml with distilled water. The ATP solution should be stored at –20°C in 300 µl aliquots and is stable for up to 12 months.

Procedure

1. **Isolate a single colony from a freshly streaked selective plate and inoculate a starter culture of 2–5 ml LB medium containing the appropriate selective antibiotic. Incubate for ~8 h at 37°C with vigorous shaking (~300 rpm).**

   Use a flask with a volume at least 4 times the volume of the culture.

2. **Dilute 0.5–1.0 ml of the starter culture into 500 ml selective LB medium (1/500 to 1/1000 dilution). Grow at 37°C for 12–16 h with vigorous shaking (~300 rpm).**

   Use a flask or vessel with a volume of at least 4 times the volume of the culture. The culture should reach a cell density of approximately 3–4 x 10^9 cells per ml.

3. **Harvest the bacterial cells by centrifugation at 6000 x *g* for 15 min at 4°C.**

   Note that 6000 x *g* corresponds to 6000 rpm in Sorvall® GSA or GS3 or Beckman® JA-10 rotors.

   Remove all traces of supernatant by inverting the open centrifuge tube until all medium has been drained.

   ⊗ If you wish to stop the protocol and continue later, freeze the cell pellet at –20°C.

* Since ATP is often supplied hydrated, it may be necessary to adjust the mass of ATP. Refer to supplier's information (e.g., prepare solution with 3.03 g if using disodium salt trihydrate).

4. **Resuspend the bacterial pellet in 20 ml Buffer P1.**

   For efficient lysis it is important to use a vessel that is large enough to allow complete mixing of the lysis buffers. Ensure that RNase A has been added to Buffer P1. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

   Note: If no centrifugation tubes suitable for 60 ml volumes are available, the resuspended bacteria can be split into 2 x 10 ml. The two batches should be processed separately through steps 5 and 6 and then combined after step 7, before the filtration step.

5. **Add 20 ml Buffer P2, mix gently but thoroughly by inverting 4–6 times, and incubate at room temperature (15–25°C) for 5 min.**

   Do not vortex, as this will result in shearing and subsequent exonuclease digestion of the large BAC/PAC/P1/cosmid 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 acidification from CO_2 in the air.

6. **Add 20 ml chilled Buffer P3, mix immediately but gently by inverting 4–6 times, and incubate on ice for 10 min.**

   Precipitation is enhanced by using chilled Buffer P3 and incubating on ice. After addition of Buffer P3, a fluffy white material forms and the lysate becomes less viscous. The precipitated material contains genomic DNA, proteins, cell debris, and KDS*. Thorough mixing avoids localized KDS precipitation. Do not vortex, as this will result in shearing and subsequent exonuclease digestion of the large BAC/PAC/P1/cosmid DNA.

7. **Centrifuge at ≥20,000 x *g* for 30 min at 4°C. Remove supernatant containing BAC/PAC/P1/cosmid DNA promptly.**

   Before loading into the centrifuge, the sample should be mixed again. Centrifugation should be performed in non-glass tubes (e.g., polypropylene). A centrifugal force of 20,000 x *g* corresponds to 12,000 rpm in a Beckman JA-17 rotor or 13,000 rpm in a Sorvall SS-34 rotor. After centrifugation, the supernatant should be clear.

   Note: If the sample has been processed in 2 batches from step 4, they should be combined now for step 8.

8. **Filter the lysate through a folded filter premoistened with distilled water.**

   This filtration step should be carried out to avoid precipitation of suspended or particulate material in step 9, which could lead to incomplete exonuclease digestion in step 12.

* KDS: Potassium dodecyl sulfate.

9. **Precipitate DNA by adding 0.6 volumes (approximately 36 ml) room-temperature isopropanol to the cleared lysate. Mix and centrifuge immediately at ≥15,000 x *g* for 30 min at 4°C. Carefully decant the supernatant.**

   Isopropanol should be at room temperature to minimize salt precipitation. 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. Besides the large-construct DNA, this isopropanol pellet also contains proteins and genomic DNA and is therefore typically easy to see.

   Note: If no centrifugation tubes suitable for approximately 95 ml are available, the cleared lysate can be split into 2 x 50 ml tubes and centrifuged at 5000 x *g* for 60 min in a centrifuge such as a Heraeus® Minifuge. Continue to process both samples in parallel through step 10 and combine the samples after step 11, before exonuclease digestion.

10. **Wash DNA pellet with 5 ml room-temperature 70% ethanol, and centrifuge at ≥15,000 x *g* for 15 min. Carefully decant the supernatant without disturbing the pellet.**

    The 70% ethanol removes precipitated salt and replaces isopropanol with the more volatile ethanol, making the DNA easier to redissolve.

11. **Place the tube containing the DNA pellet upside down on a paper towel and allow the DNA to air-dry for 2–3 min. Carefully remove any additional liquid visible on the tube opening and carefully redissolve the DNA in 9.5 ml Buffer EX, until the DNA is completely dissolved.**

    DNA should be redissolved by very gentle shaking. Avoid pipetting the DNA as this will cause shearing of large BAC/PAC/P1/cosmid DNA.

    Note: If the sample has been processed in two batches from step 9, they should be combined now for step 12.

12. **Add 200 µl ATP-Dependent Exonuclease and 300 µl ATP solution to the dissolved DNA, mix gently but thoroughly, and incubate in a water bath or heating block at 37°C for 60 min.**

    Ensure that the ATP-Dependent Exonuclease has been completely dissolved in Exonuclease Solvent, as described in the "Important points before starting" on page 17.

    During this step genomic DNA and nicked BAC/PAC/P1/cosmid DNA will be digested by the exonuclease. Only supercoiled DNA will remain for further purification.

    Note: If the solution appears turbid after exonuclease digestion, an optional centrifugation step at ≥15,000 x *g* for 5 min should be performed. Remove supernatant containing BAC/PAC/P1/cosmid DNA promptly after centrifugation.

13. **Equilibrate a QIAGEN-tip 500 by applying 10 ml Buffer QBT, and allow the column to empty by gravity flow.**

    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. QIAGEN-tips can be left unattended, since the flow of buffer will stop when the meniscus reaches the upper frit in the column.

14. **Add 10 ml Buffer QS to the DNA sample from step 12, apply the whole sample to the QIAGEN-tip, and allow it to enter the resin by gravity flow.**
15. **Wash the QIAGEN-tip with 2 x 30 ml Buffer QC.**
16. **Elute DNA with 15 ml Buffer QF, prewarmed to 65°C.**

    Use of prewarmed Buffer QF will make elution of the large DNA molecules more efficient. Collect the eluate in a 30 ml tube. Use of polycarbonate centrifuge tubes is not recommended as polycarbonate is not resistant to the alcohol used in subsequent steps.

    ⊗ If you wish to stop the protocol and continue later, store the eluate at 4°C. Storage periods longer than overnight are not recommended.

17. **Precipitate DNA by adding 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 to minimize salt precipitation. 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. 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.

    Note: Samples can be centrifuged in 50 ml tubes, as described in step 9.

18. **Wash DNA pellet with 5 ml room-temperature 70% ethanol, and centrifuge at ≥15,000 x *g* for 15 min. Carefully decant the supernatant without disturbing the pellet.**

    The 70% ethanol removes precipitated salt and replaces isopropanol with the more volatile ethanol, making the DNA easier to redissolve.

19. **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 the DNA, especially if glass tubes have been used. Alternatively, dissolve DNA overnight at room temperature or at 55°C for 1–2 h with gentle agitation. 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. The DNA may also be difficult to dissolve if the buffer used is too acidic. DNA dissolves best under slightly alkaline conditions.

Determination of yield

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

Reliability of DNA quantitation by spectrophotometry

The concentration of nucleic acids in solution can be readily calculated from the absorbance at 260 nm. For reliable spectrophotometric DNA quantitation, A_{260} readings should lie between 0.1 and 1.0. A_{260} readings below 0.1 and above 1.0 are considerably less reproducible. Furthermore, readings above 3.0 are incorrect, which can potentially leading to underestimation of the DNA quantity. When working with small amounts of DNA, quantitation by agarose gel electrophoresis may be more effective.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
Low or no DNA yield; no DNA in the lysate	
a) Large construct did not propagate	Please read "Growth of bacterial cultures" (starting on page 10), and check that the conditions for optimal growth are met.
b) Alkaline lysis was inefficient	If the cell densities are too high or a larger than recommended amount of culture was used, poor lysis may result, since the volumes of Buffers P1, P2, and P3 may not be sufficient for efficiently releasing the large-construct DNA. Reduce the culture volume or proportionally increase volumes of Buffers P1, P2, and P3. Please read "Growth of bacterial cultures" (starting on page 10) and check that the conditions for optimal growth are met.
c) Lysate incorrectly prepared	Check Buffer P2 for SDS precipitation resulting from low storage temperatures, and if necessary dissolve the SDS by warming. The bottle containing P2 should always be closed immediately after use. Ensure that any lysis buffers prepared in the laboratory are prepared correctly (see page 32). If necessary, use fresh Buffers P1, P2, and P3. Please read "Growth of bacterial cultures" (starting on page 10) and check that the conditions for optimal growth are met.
DNA in the flow-through fraction	
a) Inappropriate salt or pH conditions in buffers	Check the culture volume and yield against the capacity of the QIAGEN-tip, as detailed at the beginning of the protocol. Reduce the culture volume accordingly.

	Comments and suggestions
b) Column flow was uneven	Store QIAGEN-tips at room temperature (15–25°C). If 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.
c) Column was overloaded	Do not exceed the recommended culture volume given at the beginning of the protocol (i.e., 500 ml).

DNA in the Buffer QC wash fraction

Buffer QC composition was incorrect	Check pH and salt concentration of Buffer QC. Recover DNA by precipitation, and purify on a new QIAGEN-tip.

No DNA in eluate

a) No DNA in the lysate	See above.
b) Buffer QF composition was incorrect	Check pH and salt concentration of Buffer QF (elution buffer). Recover DNA by eluting with fresh buffer. Preheat the elution buffer to 65°C.
c) DNA passed through in the flow-through or wash fraction	See previous two sections.

DNA digested in exonuclease step

a) DNA was sheared during lysis	Avoid any vortexing or pipetting up and down during lysis steps 5 and 6. Use a vessel that is large enough to allow complete mixing of the lysis buffers without vigorous agitation. The culture volume and biomass should not exceed the recommended amount, as this will lead to very viscous lysis conditions requiring excess mechanical force to mix the reagents.
b) DNA was sheared during redissolving	Avoid any vortexing or pipetting up and down when redissolving the DNA in step 11.

Little or no DNA after precipitation

a) No DNA in eluate	See above.

	Comments and suggestions
b) DNA failed to precipitate	Ensure that the precipitate is centrifuged at ≥15,000 x *g* for 30 minutes. Recover DNA by centrifuging longer at higher speeds. Try another isopropanol batch.
c) DNA pellet was lost	Isopropanol pellets are glassy and may be difficult to see. Mark the tube at the expected location of the pellet before centrifugation. Isopropanol pellets may also be loosely attached to the side of the tube, so pour supernatant off gently.
d) DNA was poorly redissolved	Check that DNA is completely redissolved. Be sure to wash any DNA off the walls, particularly if glass tubes and a fixed-angle rotor are 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 difficult to redissolve	
a) Pellet was overdried	Air-dry pellet instead of using a vacuum. Redissolve the high–molecular-weight DNA by warming the solution slightly, and allowing more time for redissolving.
b) Residual isopropanol in pellet	Ensure that pellets are washed with 70%ethanol to remove traces of isopropanol. Redissolve DNA by warming the solution slightly, and allowing more time for redissolving. Increase volume of buffer used for redissolving if necessary.
c) Too much salt in pellet	Ensure that isopropanol is at room temperature for precipitation, and that the pellet is washed twice with room-temperature 70% ethanol. Recover DNA by increasing the volume of buffer used for redissolving.
d) Final buffer pH was too low	Ensure that the pH of the buffer used for redissolving is ≥8.0, since DNA does not dissolve well in acidic solutions.
e) Final resuspension volume too low	Increase resuspension volume if the solution above the pellet is highly viscous.

	Comments and suggestions
Contaminated DNA/poor-quality DNA	
a) Genomic DNA in the eluate	Exonuclease digestion was insufficient. Ensure that the ATP-Dependent Exonuclease was completely dissolved in Exonuclease Solvent. Check ATP solution, reaction time, and volume, as well as storage conditions of ATP solution and exonuclease. Prepare fresh ATP solution if necessary. Do not exceed the recommended culture volume as this may lead to insufficient exonuclease digestion. Ensure that the bacterial lysate is cleared by filtration before proceeding to the exonuclease digestion step to ensure optimal digestion conditions.
b) RNA in the eluate	RNase A digestion was insufficient. Check culture volume against recommended volumes, and reduce if necessary. If Buffer P1 is more than 6 months old, add more RNase A. Recover DNA by precipitating the eluate, digesting with RNase A, and purifying on a new QIAGEN-tip.
c) Nuclease contamination from external sources	Check buffers for nuclease contamination and replace if necessary. Use new glass- and plasticware, and wear gloves.
d) Endonuclease-containing host	See "Host strains" (page 10), and consider changing *E. coli* host strain.
e) Lysis time was too long	Ensure that the lysis step with Buffer P2 (step 5) does not exceed 5 min.
f) Overloaded alkaline lysis	Check the culture volume against the recommended volume and reduce if necessary. Alternatively, increase the volumes of Buffers P1, P2, and P3 proportionally.
g) DNA is nicked/sheared/degraded	DNA was poorly buffered. Redissolve DNA in TE buffer, pH 8.0, to inhibit nuclease activity and maintain a stable pH during storage.
h) Shearing during redissolving	Redissolve DNA gently, without vortexing or vigorous pipetting. Avoid using small pipet tips.

	Comments and suggestions
Poor DNA performance	
a) Too much salt in pellet	Ensure that isopropanol is at room temperature for precipitation, and wash the pellet twice with room-temperature 70% ethanol. Re-precipitate the DNA to remove the salt.
b) Residual protein	Check culture volume against the recommended volume and reduce if necessary. Ensure that the bacterial lysate is cleared properly.
Blocked QIAGEN-tip	
Exonuclease-digested sample was turbid	Ensure that the exonuclease-digested sample is clear before it is loaded onto the column. Centrifuge at >15,000 x *g* for 5 minutes to clarify. To clear a blocked column, positive pressure may be applied, e.g., by using a syringe fitted into a rubber stopper with a hole.

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

1. Birnboim, H. C. and Doly, J. (1979) A rapid alkaline lysis procedure for screening recombinant plasmid DNA. Nucleic Acids Res. ***7***, 1513–1522.
2. Sambrook, J. et al., eds. (1989) Molecular cloning: a laboratory manual, 2nd ed., Cold Spring Harbor Laboratory Press.
3. Ausubel, F. M. et al., eds (1991) Current protocols in molecular biology, New York, Wiley Interscience.
4. Birnboim, H.C. (1983) A rapid alkaline extraction method for the isolation of plasmid DNA. Methods Enzymol. ***100***, 243–255.

Appendix A: Protocol for High Yields of Large-Construct DNA without Removal of Genomic DNA

This streamlined protocol does not include an exonuclease digestion step and provides very high yields of BAC/PAC/P1/cosmid DNA. It is recommended for less sensitive downstream applications that do not require genomic DNA-free preparations, since genomic contamination can comprise up to 30% of the large-construct DNA preparation. Depending on the construct and the host strain used, yields of up to 150 µg BAC/PAC/P1 DNA or up to 400 µg cosmid DNA are obtained with this protocol.

If genomic DNA-free isolate is required, please refer to the standard protocol, which includes an exonuclease digestion step (starting on page 17).

Table 3. Recommended culture volumes

Construct	QIAGEN-tip 500
BAC	500 ml
PAC	500 ml
P1	500 ml
Cosmid	500 ml

Important points before starting

- Add RNase A solution to Buffer P1 before use. Use 1 vial of RNase A (centrifuge briefly before use) per bottle of Buffer P1 for a final concentration of 100 µg/ml.
- Check Buffer P2 for SDS precipitation due to low storage temperatures. If necessary, dissolve the SDS by warming to 37°C.
- Prechill Buffer P3 to 4°C.
- Prewarm elution Buffer QF to 65°C for use in step 12.
- Do not exceed the culture volumes recommended above as this will alter the ratio of biomass to lysis reagent and lead to inefficient cell lysis.

Procedure

A1. Isolate a single colony from a freshly streaked selective plate and inoculate a starter culture of 2–5 ml LB medium containing the appropriate selective antibiotic. Incubate for ~8 h at 37°C with vigorous shaking in a flask with a volume at least 4 times the volume of the culture.

A2. Dilute 0.5–1.0 ml of the starter culture into 500 ml selective LB medium (1/500 to 1/1000 dilution). Grow at 37°C for 12–16 h with vigorous shaking (~300 rpm).

Use a flask or vessel with a volume at least 4 times the volume of the culture. The culture should reach a cell density of approximately 3–4 x 10^9 cells per ml.

A3. Harvest the bacterial cells by centrifugation at 6000 x *g* for 15 min at 4°C.

6000 x *g* corresponds to 6000 rpm in Sorvall GSA or GS3 or Beckman JA-10 rotors. Remove all traces of supernatant by inverting the open centrifuge tube until all medium has been drained.

⊗ If you wish to stop the protocol and continue later, freeze the cell pellet at –20°C.

A4. Resuspend the bacterial pellet in 20 ml Buffer P1.

For efficient lysis it is important to use a vessel that is large enough to allow complete mixing of the lysis buffers. Ensure that RNase A has been added to Buffer P1. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

Note: If no centrifugation tubes suitable for 60 ml volumes are available, the resuspended bacteria can be split into 2 x 10 ml. The two batches should be processed separately through steps 5 and 6 and then combined after step 7, before the filtration step.

A5. Add 20 ml Buffer P2, mix gently but thoroughly by inverting 4–6 times, and incubate at room temperature (15–25°C) for 5 min.

Do not vortex, as this will result in shearing of both genomic and large BAC/PAC/P1/cosmid 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 acidification from CO_2 in the air.

A6. Add 20 ml chilled Buffer P3, mix immediately but gently by inverting 4–6 times, and incubate on ice for 10 min.

Precipitation is enhanced by using chilled Buffer P3 and incubating on ice. After addition of Buffer P3, a fluffy white material forms and the lysate becomes less viscous. The precipitated material contains genomic DNA, proteins, cell debris, and KDS*. Thorough mixing avoids localized KDS precipitation. Do not vortex, as this will result in shearing of the large BAC/PAC/P1/cosmid DNA.

A7. Centrifuge at ≥20,000 x *g* for 30 min at 4°C. Remove supernatant containing BAC/PAC/P1/cosmid DNA promptly.

Before loading into the centrifuge, the sample should be mixed again. Centrifugation should be performed in non-glass tubes (e.g., polypropylene). A centrifugal force of 20,000 x *g* corresponds to 12,000 rpm in a Beckman JA-17 rotor or 13,000 rpm in a Sorvall SS-34 rotor. After centrifugation, the supernatant should be clear.

Note: If the sample has been processed in two batches from step 4, they should be combined now for step 8.

A8. Filter the lysate through a folded filter premoistened with distilled water.

A9. Equilibrate a QIAGEN-tip 500 by applying 10 ml Buffer QBT, and allow the column to empty by gravity flow.

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. QIAGEN-tips can be left unattended, since the flow of buffer will stop when the meniscus reaches the upper frit in the column.

A10. Apply the sample from step 8 to the QIAGEN-tip and allow it to enter the resin by gravity flow. This will require 2 loading steps due to the large sample volume.

A11. Wash the QIAGEN-tip with 2 x 30 ml Buffer QC.

A12. Elute DNA with 15 ml Buffer QF, prewarmed to 65°C.

Use of prewarmed Buffer QF will make elution of the large DNA molecules more efficient. Collect the eluate in a 30 ml tube. Use of polycarbonate centrifuge tubes is not recommended as polycarbonate is not resistant to the alcohol used in subsequent steps.

⊗ If you wish to stop the protocol and continue later, store the eluate at 4°C. Storage periods longer than overnight are not recommended.

* KDS: Potassium dodecyl sulfate

A13. Precipitate DNA by adding 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 to minimize salt precipitation. 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. 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.

A14. Wash DNA pellet with 5 ml room-temperature 70% ethanol, and centrifuge at ≥15,000 x *g* for 15 min. Carefully decant the supernatant without disturbing the pellet.

The 70% ethanol removes precipitated salt and replaces isopropanol with the more volatile ethanol, making the DNA easier to redissolve.

A15. 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 the DNA, especially if glass tubes have been used. Alternatively, dissolve DNA overnight at room temperature or at 55°C for 1–2 h with gentle agitation. 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.

Appendix B: Buffer and Media Composition

Table 4. 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	2–8°C, after addition of RNase A
Buffer P2 (lysis buffer)	200 mM NaOH, 1% SDS	Room temperature (15–25°C)
Buffer P3 (neutralization buffer)	3.0 M potassium acetate, pH 5.5	Room temperature or 2–8°C
Buffer QBT (equilibration buffer)	750 mM NaCl; 50 mM MOPS, pH 7.0; 15% isopropanol; 0.15% Triton®, X-100	Room temperature
Buffer QC (wash buffer)	1.0 M NaCl; 50 mM MOPS, pH 7.0; 15% isopropanol	Room temperature
Buffer QF (elution buffer)	1.25 M NaCl; 50 mM Tris·Cl, pH 8.5; 15% isopropanol	Room temperature
Exonuclease Solvent	20 mM KCl; 20 mM KPO_4, pH 7.5	Room temperature
Buffer TE	10 mM Tris·Cl, pH 8.0; 1 mM EDTA	Room temperature

Preparation of LB medium

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

Appendix C: General Information about QIAGEN Anion-Exchange Resin

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

Large-construct purification using 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 of the buffers used determine whether DNA is bound or eluted from the column. The key advantage of the QIAGEN 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. Large-construct DNA remains tightly bound to the DEAE groups over a wide range of salt concentrations. Impurities such as RNA, protein, carbohydrates, and small metabolites are washed from the QIAGEN resin with medium-salt buffers, while large-construct DNA remains bound until eluted with a high-salt buffer.

The separation range of the QIAGEN resin is extremely broad, extending from 0.1 M to 1.6 M salt, allowing DNA to be efficiently separated from RNA and other impurities. In contrast, conventional anion-exchangers, based on cellulose, dextran, or agarose, have separation ranges of 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 the QIAGEN resin, as well as its ease of use surpass those of conventional anion-exchange resins.

Purity and biological activity

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

Stability

The QIAGEN resin is stable for up to 6 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 6 hours for the same sample by re-equilibrating the resin with Buffer QBT after the first elution. The 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 the QIAGEN resin are strongly influenced by pH. Deviations from the appropriate buffer pH values at a given salt concentration may result in losses 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, pKa 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 the 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.

Ordering Information

Product	Contents	Cat. no.
QIAGEN Large-Construct Kit (10)*	10 QIAGEN-tip 500, Reagents, Buffers, ATP-Dependent Exonuclease*	12462
Related BAC purification products		
R.E.A.L. Prep 96 Plasmid Kit (24) †‡	For 24 x 96 rapid extraction alkaline lysis minipreps: 24 QIAfilter 96 Plates, Square-Well Blocks, Tape Pads, Reagents, Buffers	26173
48-Well Blocks (24)	48-well blocks with 5 ml wells, 24 per case	19577
Related plasmid purification kits		
HiSpeed® Plasmid Kits — for ultrafast purification of transfection-grade plasmid or cosmid DNA		
HiSpeed Plasmid Midi Kit (25)§	25 HiSpeed Midi Tips, 25 QIAfilter Midi Cartridges, 25 QIAprecipitator Midi Modules, plus Syringes, Reagents, Buffers	12643
HiSpeed Plasmid Maxi Kit (10)	10 HiSpeed Maxi Tips, 10 QIAfilter Maxi Cartridges, 10 QIAprecipitator Maxi Modules, plus Syringes, Reagents, Buffers	12662
QIAGEN Plasmid Kits — for purification of transfection-grade plasmid or cosmid DNA		
QIAGEN Plasmid Midi Kit (25)§	25 QIAGEN-tip 100, Reagents, Buffers	12143
QIAGEN Plasmid Maxi Kit (10)	10 QIAGEN-tip 500, Reagents, Buffers	12162
QIAGEN Plasmid Mega Kit (5)	5 QIAGEN-tip 2500, Reagents, Buffers	12181

* ATP solution required for exonuclease digestion is not provided.

† Requires use of QIAvac 96; please inquire.

‡ BAC purification using the R.E.A.L. Prep 96 procedure requires the use of 48-well blocks to provide optimal cultivation conditions for the BAC clones. 48-well blocks are not provided in the R.E.A.L. Prep 96 Plasmid Kit and must be purchased separately.

§ Other kit formats/sizes are available; see www.qiagen.com.

Product	Contents	Cat. no.
QIAGEN Plasmid Giga Kit (5)	5 QIAGEN-tip 10000, Reagents, Buffers	12191
QIAGEN Plasmid *Plus* Kits — for the fastest and most convenient purification of transfection-grade plasmid DNA suitable for all applications		
QIAGEN Plasmid *Plus* Maxi Kit (25)	25 QIAGEN Plasmid *Plus* Maxi Columns, Extender Tubes, Reagents, Buffers, 25 QIAfilter Maxi Cartridges	12963
QIAGEN Plasmid *Plus* Midi Kit (25)	25 QIAGEN Plasmid *Plus* Midi Columns, Extender Tubes, Reagents, Buffers, 25 QIAfilter Midi Cartridges	12943
QIAGEN Plasmid *Plus* Giga Kit (5)	5 QIAGEN Plasmid *Plus* Mega Columns, Extender Tubes, Reagents, Buffers, 5 QIAfilter Mega-Giga Cartridges	12991
QIAGEN Plasmid *Plus* Mega Kit (5)	5 QIAGEN Plasmid *Plus* Mega Columns, Extender Tubes, Reagents, Buffers, 5 QIAfilter Mega-Giga Cartridges	12981
QIAGEN Plasmid *Plus* 96 BioRobot Kit (4)	For 4 x 96 plasmid minipreps: TurboFilter 96 Plates and Plasmid *Plus* 96 Plates, Buffers, Reagents, Flat-Bottom Blocks, S-Blocks, and Elution Microtubes; for use with the BioRobot Universal System	960241
QIAfilter Plasmid Kits — for fast purification of transfection-grade plasmid or cosmid DNA		
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
QIAfilter Plasmid Mega Kit (5)	5 QIAGEN-tip 2500, Reagents, Buffers, 5 QIAfilter Mega-Giga Cartridges	12281
QIAfilter Plasmid Giga Kit (5)	5 QIAGEN-tip 10000, Reagents, Buffers, 5 QIAfilter Mega-Giga Cartridges	12291

Product	Contents	Cat. no.
EndoFree® Plasmid Kits — for purification of endotoxin-free advanced transfection-grade plasmid or cosmid DNA		
EndoFree Plasmid Maxi Kit (10)*	10 QIAGEN-tip 500, Reagents, 10 QIAfilter Maxi Cartridges, Endotoxin-free Buffers	12362
EndoFree Plasmid Giga Kit (5)	5 QIAGEN-tip 10000, Reagents, 5 QIAfilter Mega-Giga Cartridges, Endotoxin-free Buffers	12391
Accessories		
QIArack	1 rack for 12 x QIAGEN-tip 20, 8 x QIAGEN-tip 100, 6 x QIAGEN-tip 500 or HiSpeed Midi Tips, 4 x QIAGEN-tip 2500 or HiSpeed Maxi Tips, and 10 QIAfilter Midi or Maxi Cartridges	19015
Plasmid Buffer Set	Buffers P1, P2, P3, QBT, QC, QF, RNase A; for 100 plasmid mini-, 25 midi-, or 10 maxipreps	19046
Buffer P1	500 ml Resuspension Buffer (RNase A not included)	19051
Buffer P2	500 ml Lysis Buffer	19052
Buffer P3	500 ml Neutralization Buffer	19053
RNase A	2.5 ml (100 mg/ml; 7000 units/ml, solution)	19101

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

* Other kit formats/sizes are available; see www.qiagen.com.

Notes

Trademarks: QIAGEN®, EndoFree®, HiSpeed®, R.E.A.L.® (QIAGEN Group); Beckman® (Beckman Instruments, Inc); DH10B™, DH5® (Life Technologies, Inc.); FPLC® (GE Healthcare Bio-Sciences AB); Heraeus® (Heraeus Holding GmbH); Sorvall® (Thermo Fisher Scientific); Triton® (Union Carbide Corp.).

Limited License Agreement for QIAGEN Large-Construct Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
2. Other than expressly stated licenses, QIAGEN makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.
3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

For updated license terms, see www.qiagen.com.

www.qiagen.com

Australia ■ techservice-au@qiagen.com

Austria ■ techservice-at@qiagen.com

Belgium ■ techservice-bnl@qiagen.com

Brazil ■ suportetecnico.brasil@qiagen.com

Canada ■ techservice-ca@qiagen.com

China ■ techservice-cn@qiagen.com

Denmark ■ techservice-nordic@qiagen.com

Finland ■ techservice-nordic@qiagen.com

France ■ techservice-fr@qiagen.com

Germany ■ techservice-de@qiagen.com

Hong Kong ■ techservice-hk@qiagen.com

India ■ techservice-india@qiagen.com

Ireland ■ techservice-uk@qiagen.com

Italy ■ techservice-it@qiagen.com

Japan ■ techservice-jp@qiagen.com

Korea (South) ■ techservice-kr@qiagen.com

Luxembourg ■ techservice-bnl@qiagen.com

Mexico ■ techservice-mx@qiagen.com

The Netherlands ■ techservice-bnl@qiagen.com

Norway ■ techservice-nordic@qiagen.com

Singapore ■ techservice-sg@qiagen.com

Sweden ■ techservice-nordic@qiagen.com

Switzerland ■ techservice-ch@qiagen.com

UK ■ techservice-uk@qiagen.com

USA ■ techservice-us@qiagen.com

1071260 04/2012

Sample & Assay Technologies