June 2015

QIAGEN® Genomic DNA Handbook

For

Blood

Cultured cells

Tissue

Mouse tails

Yeast

Bacteria (Gram-negative and some Gram-positive)

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

Blood & Cell Culture DNA Kit	Mini	Midi	Maxi
Catalog no.	**13323**	**13343**	**13362**
QIAGEN Genomic-tip 20/G	25		
QIAGEN Genomic-tip 100/G		25	
QIAGEN Genomic-tip 500/G			10
Tip Holders	6	5	5
Buffer C1	40 ml	170 ml	250 ml
Buffer G2*	70 ml	260 ml	260 ml
Buffer QBT	60 ml	2 x 60 ml	2 x 60 ml
Buffer QC	2 x 60 ml	2 x 205 ml	6 x 60 ml
Buffer QF	2 x 30 ml	140 ml	200 ml
QIAGEN Protease	1 vial†	2 vials†	2 vials†
Quick-Start Protocol	1	1	1

* Not compatible with disinfection reagents containing bleach. Contains guanidine HCl. Take appropriate safety measures and wear gloves when handling. See page 5 for safety information.

† Resuspension volume 1.4 ml

Note: QIAGEN Protease, QIAGEN Proteinase K, or other required enzymes must be purchased separately as specified.

Genomic DNA Buffer Set (for 75 mini, 25 midi, or 10 maxi preparations)	
Catalog no.	**19060**
Buffer C1	250 ml
Buffer Y1	160 ml
Buffer B1	120 ml
Buffer B2*	50 ml
Buffer G2*	260 ml
Buffer QBT	2 x 60 ml
Buffer QC	1 x 205 ml 1 x 240 ml
Buffer QF	200 ml
Handbook	1

QIAGEN Genomic-tip	20/G	100/G	500/G
Catalog no.	**10223**	**10243**	**10262**
Genomic-tip	25	25	10
Tip Holders	6	5	5
Handbook	1	1	1

* Not compatible with disinfection reagents containing bleach. Contains guanidine HCl. Take appropriate safety measures and wear gloves when handling. See page 5 for safety information.

Storage

QIAGEN Genomic-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 performance, capacity, or quality of separation.

Buffer C1 and Buffer Y1 should always be stored at 2–8°C. All other buffers may be stored at either 2–8°C or at room temperature. Long-term storage at 2–8°C is recommended.

Lyophilized QIAGEN Protease can be stored at room temperature for up to 6 months without any decrease in performance. For storage longer than 6 months or if ambient temperatures constantly exceed 25°C, QIAGEN Protease should be stored dry at 2–8°C.

Reconstituted QIAGEN Protease is stable for 2 months when stored at 2–8°C. Incubating the QIAGEN Protease stock solution at room temperature for prolonged periods of time should be avoided. Storage at –30 to –15°C will prolong its life, but repeated freezing and thawing should be avoided. Dividing the solution into aliquots and storage at –30 to –15°C is recommended.

QIAGEN Proteinase K is stable at room temperature for at least one year. For longer-term storage, we recommend storing at 2–8°C.

Dissolved lysozyme, lysostaphin, lyticase, and zymolase must be stored at –30 to –15°C, but repeated freezing and thawing should be avoided. When storage at –30 to –15°C is necessary, dividing the enzyme solutions into aliquots is recommended.

Intended Use

Blood & Cell Culture DNA Kits, Genomic DNA Buffer Set, and QIAGEN Genomic-tips are intended for molecular biology applications. These products are 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 safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

CAUTION: DO NOT add bleach or acidic solutions directly to waste containing Buffer B2 or Buffer G2.

Buffer B2 and Buffer G2 contain guanidine hydrochloride, which can form highly reactive compounds when combined with bleach.

If liquid containing Buffer B2 or Buffer G2 is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of Blood & Cell Culture DNA Kit, Genomic DNA Buffer Set, and QIAGEN Genomic-tip is tested against predetermined specifications to ensure consistent product quality.

Introduction

Blood & Cell Culture DNA Kits and QIAGEN Genomic-tips, together with the Genomic DNA Buffer Set, provide an easy, safe, and reliable method for the isolation of pure high-molecular–weight genomic DNA direct from whole blood, buffy coat, lymphocytes, cultured cells, tissues, mouse tails, yeast, Gram-negative, and some Gram-positive bacteria.

The simple purification procedure, based on the remarkable selectivity of QIAGEN Anion-Exchange Resin results in negligible DNA shearing and allows isolation of high yields of pure genomic DNA in less than 3 hours.

The exceptionally large size of the obtained DNA makes it especially suitable for the preparation of high-quality libraries for next generation sequencing (NGS) on different platforms and is recommended by several core facilities.

It requires no expensive equipment, involves only a few steps, and completely avoids the use of toxic and hazardous organic solvents such as phenol and chloroform. The gravity-flow operation of QIAGEN Genomic-tips reduces hands-on time to a minimum, making the procedure ideal for rapid parallel processing of multiple samples. With QIAGEN Genomic-tips, isolation of genomic DNA is as easy as filtration.

Genomic DNA purified with QIAGEN Genomic-tips ranges in size from 20–150 kb (Figure 1, page 8), with an average length of 50–100 kb, and is free of all contaminants. DNA purified using QIAGEN resin is ideally suited for use in demanding procedures such as:

- Next generation sequencing
- Sanger sequencing
- RFLP techniques for genetic linkage studies (Figure 2, page 8)
- Screening of embryonic stem-cell clones for gene targeting
- Screening of transgenic animals
- Southern-blotting techniques
- PCR

Note: For rapid and simple preparation of up to 50 µg, 225 µg, or 750 µg of genomic DNA for subsequent PCR or Southern-blotting applications, QIAamp® Kits are recommended. DNA can be purified from most human and animal tissues, such as muscle, liver, heart, brain, or tumors, as well as from blood and body fluids, without mechanical homogenization. Protocols for tissue, rodent tails, insects, yeast, other fungi, and some bacterial species are provided. QIAamp DNA Blood Kits (cat. nos. 51104, 51106, 51183, 51185, 51192, and 51194) permit fast and efficient DNA purification of up to 50 µg of DNA in under 20 minutes from fresh or frozen whole blood, as well as plasma, serum, buffy coat, bone marrow, or cell suspensions. Midi and Maxi Kits can be

used to isolate up to 225 µg or 750 µg of DNA, respectively, in under an hour. For high-throughput applications, the QIAamp 96 DNA Blood Kit (cat. nos. 51161 and 51162) allows purification of genomic DNA from as many as 192 samples in 2–3 hours. For isolation of DNA from paraffin-embedded tissue sections, the QIAamp DNA FFPE Tissue Kits (cat. no. 56404) provide special lysis conditions to release DNA from tissue sections and to overcome inhibitory effects caused by formalin crosslinking of nucleic acids. For high-throughput DNA purification from mouse tails, the DNeasy® 96 Blood & Tissue Kit (cat. nos. 69581 and 69582) is also available. Viral RNA and DNA can also be efficiently purified from plasma, serum, urine, and cell-free body fluids using QIAamp RNA Kits (cat. nos. 52904 and 52906). For more information, contact QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

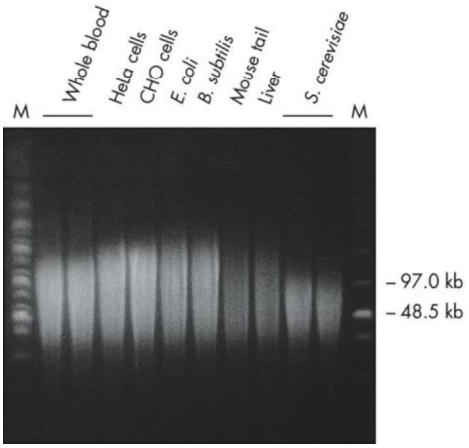


Figure 1. Pulsed-field gel electrophoresis of DNA (2 µg per lane) purified with QIAGEN Genomic-tips. M: markers.

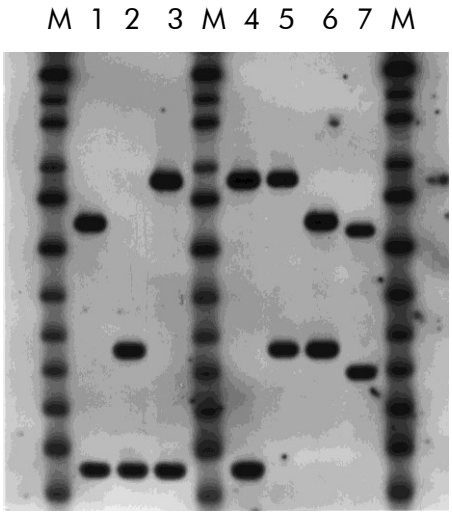


Figure 2. RFLP analysis of genomic DNA purified with the Blood & Cell Culture DNA Kit. Three µg of DNA were loaded per lane. (Data kindly provided by Dr. M. Prinz, Forensic Institute, University of Cologne, Germany.) **1**: mother; **2, 3**: children; **4**: child; **5**: father; **6**: child; **7**: K562 cells; **M**: 0.5–22.6 kb markers.

The QIAGEN Principle

The QIAGEN genomic DNA purification procedure is designed for direct isolation of chromosomal DNA 20–150 kb in size (Figure 1, page 8), directly from whole blood, buffy coat, lymphocytes, cultured cells, tissues, mouse tails, yeast, Gram-negative bacteria, and some Gram-positive bacteria. The procedure is based on optimized buffer systems for careful lysis of cells and/or nuclei, followed by binding of genomic DNA to QIAGEN resin under appropriate low-salt and pH conditions. RNA, proteins, dyes, and low-molecular-weight impurities are removed by a medium-salt wash. Genomic DNA is eluted in a high-salt buffer and then concentrated and desalted by isopropanol precipitation.

Each disposable QIAGEN Genomic-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 Genomic-tips are ideally suited for rapid and simple preparation of multiple samples.

The flowchart on page 12 provides a general overview of the QIAGEN genomic DNA purification procedure.

The QIAGEN Genomic DNA Purification Procedure

Sample volumes and capacities of QIAGEN genomic-tips

QIAGEN protocols are optimized for use with fixed sample volumes corresponding to the capacity of the QIAGEN Genomic-tip used (see Table 1, page 10). Overloading tips with an excessive amount of sample will lead to reduced flow rates, extend the time required for loading, washing, and elution, and may affect the purity and yield of the eluted DNA.

QIAGEN Genomic-tips are available in a variety of sizes for preparation of as little as 20 µg or as much as 500 µg of DNA. The maximum DNA-binding capacities of QIAGEN Genomic-tips 20/G, 100/G, and 500/G are 20 µg, 100 µg, and 500 µg, respectively. Actual yields will depend on the tissue, body fluid, or cell type used.

Table 1. QIAGEN Genomic-tip capacities

	QIAGEN Genomic-tip		
	20/G	**100/G**	**500/G**
DNA	1–20 µg	10–100 µg	80–500 µg
Blood	0.1–1 ml	1–5 ml	5–20 ml
Cells	5×10^6	2×10^7	1×10^8
Tissue	20 mg	100 mg	400 mg
Yeast	1.5×10^9	7.0×10^9	3.5×10^{10}
Bacteria	4.5×10^9	2.2×10^{10}	1.0×10^{11}

To avoid clogging of the QIAGEN Genomic-tip and to ensure high yields of pure genomic DNA, the appropriate amount of sample should be used. For more information on specific samples, refer to the specific recommendations preceding each protocol (pages 18, 25, 31, 37, or 43).

Preparation of the lysate

To ensure good flow rates, a brief vortexing of the lysate before loading on the QIAGEN Genomic-tip is recommended. Vortexing the lysate has a minimal effect on the size of the DNA, and it accelerates the QIAGEN procedure by eliminating poor flow rates associated with clogging. Up to 20 seconds of vortexing only slightly reduces the average size of genomic DNA from 20–150 kb to 20–130 kb as shown in Figure 3.

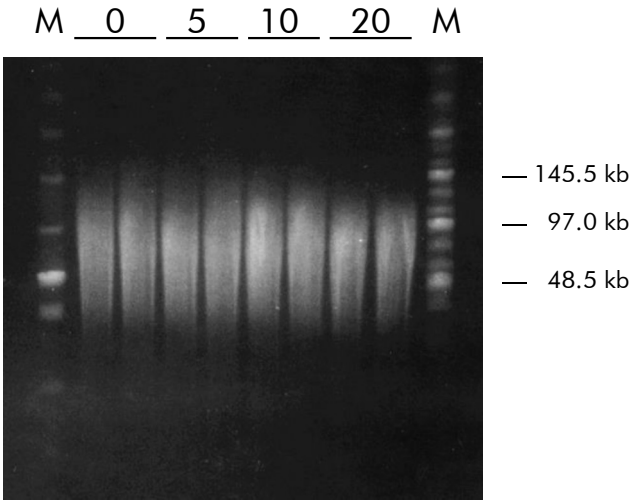


Figure 3. Vortexing has a minimal effect on DNA size. Genomic DNA (2 µg per lane) purified with Blood & Cell Culture Kit was vortexed for 0, 5, 10, or 20 s, as indicated, then analyzed by pulsed-field gel electrophoresis. **M**: markers.

QIAGEN Protease and QIAGEN Proteinase K

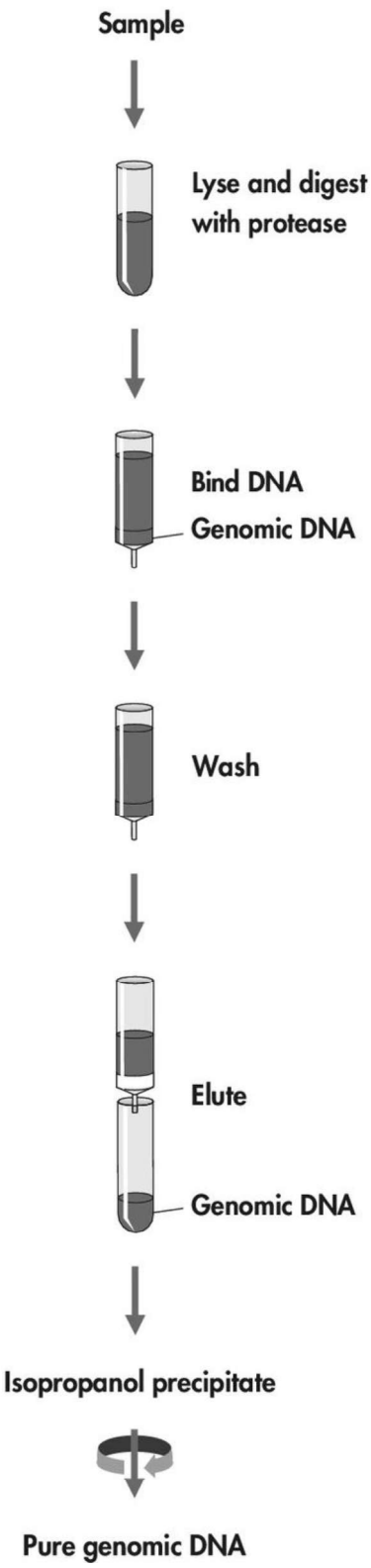
Both QIAGEN Protease and QIAGEN Proteinase K offer broad substrate specificity and high activity for a wide range of salt, denaturant, detergent, pH, and temperature conditions. Both proteases provide high activity in buffers commonly used in most DNA and RNA isolation procedures and are quality-guaranteed by QIAGEN. However, subtle differences between the two, as described below, should be considered when planning protease digestions.

QIAGEN Protease is a serine protease isolated from a recombinant *Bacillus* strain and is an economical alternative to proteinase K for isolation of DNA from a variety of sources. QIAGEN Protease is completely free of DNase and RNase activities. In the presence of >0.5% SDS, >1% sarkosyl, or high concentrations of other strong detergents, however, the EDTA concentration must be <8 mM for full activity over extended incubation times. If you are using QIAGEN Protease for a sample which requires a modified protocol, contact QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com) for advice about whether your lysis conditions are compatible with QIAGEN Protease. Kits contain enough QIAGEN Protease for blood samples. For other sample types additional QIAGEN Protease must be purchased (see ordering information, page 69).

QIAGEN Proteinase K is a subtilisin-type protease isolated from the saprophytic fungus *Tritirachium album* and is particularly suitable for short digestion times. It possesses a high specific activity which remains stable over a wide range of temperatures and pH values with substantially increased activity at higher temperatures. Although proteinase K activity requires 2 bound calcium ions, soluble calcium is not essential for enzymatic activity. This means that EDTA, which may be used to inhibit magnesium-dependent enzymes such as nucleases, will not inhibit proteinase K activity. Proteinase K is not supplied with kits (see ordering information, page 69).

RNase A, lysozyme, lysostaphin, lyticase, and zymolase are not supplied with kits and must be purchased separately. Ensure that the RNase used is completely free of DNase activity.

QIAGEN genomic DNA purification procedure



Setup of QIAGEN Genomic-tips

QIAGEN Genomic-tips may be placed in tubes using tip holders provided with the kits (Figure 4A). Alternatively, QIAGEN Genomic-tips 100/G and 500/G may be placed in QIArack (Figure 4B; cat. no. 19015), which has a removable collection tray that can be used for wash steps.

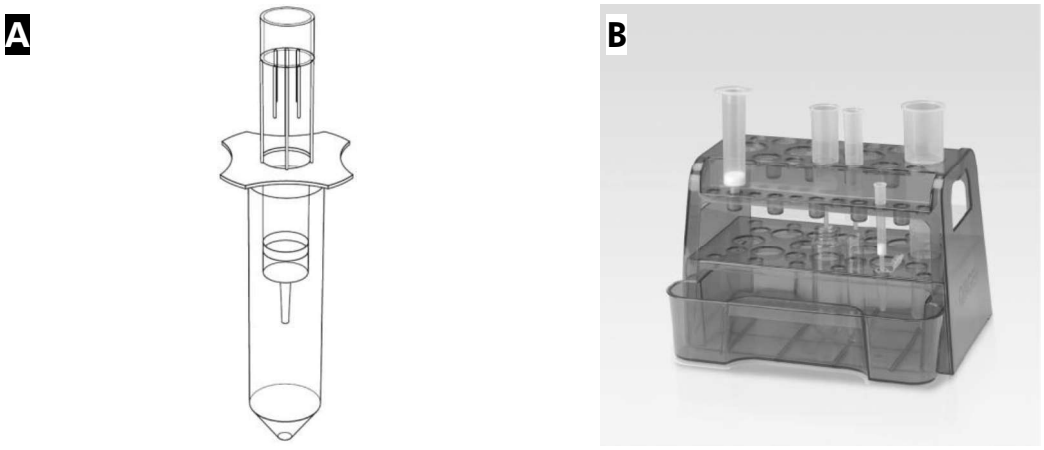


Figure 4. Setup of QIAGEN Genomic-tips A with tip holder or B with QIArack.

Capacity of QIAGEN Genomic-tips

QIAGEN Genomic-tips are available in a variety of sizes for preparation of as little as 20 µg or as much as 500 µg of DNA. The maximum DNA-binding capacities of QIAGEN Genomic-tips 20/G, 100/G, and 500/G are 20 µg, 100 µg, and 500 µg, respectively. Actual yields will depend on the tissue, body fluid, or cell type used.

DNA binding and washing on QIAGEN Genomic-tips

The cleared lysate is loaded onto a pre-equilibrated QIAGEN Genomic-tip by gravity flow. The salt and pH conditions of the lysate 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 Genomic-tip is then washed with a medium-salt buffer (Buffer QC). This buffer completely removes any remaining contaminants, such as traces of RNA and protein (e.g., RNase A), without affecting the binding of the DNA. Buffer QC also disrupts nonspecific interactions and allows removal of nucleic acid-binding proteins without the use of phenol. The low concentration of ethanol in the wash buffer eliminates nonspecific hydrophobic interactions, further enhancing the purity of the bound DNA. The DNA is then efficiently eluted from the QIAGEN Genomic-tip with a high-salt buffer

(Buffer QF). For further information about QIAGEN resin, see Appendix B, starting on page 66.

Desalting and concentration

The eluted 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, pH 8.0, or Tris·Cl, pH 8.5, and is ready for use in Southern blotting, cloning, or any other experimental procedure. Table 2 (page 15) lists average yield and purity of purified DNA.

Analytical gel analysis

The purification procedure can be monitored on an analytical gel (see Figure 5; page 53). Samples for analysis should be removed at steps indicated in the protocols.

Table 2. Average yield and purity of genomic DNA prepared with QIAGEN Genomic-tips

QIAGEN Genomic-tip		Yield	Average A_{260}/A_{280} ratio
Whole blood			
1 ml	20/G	15–20 µg	1.86
5 ml	100/G	80–100 µg	1.78
20 ml	500/G	350–400 µg	1.90
Cultured cells (HeLa)			
5.0×10^6	20/G	15–20 µg	1.79
2.0×10^7	100/G	80–100 µg	1.81
1.0×10^8	500/G	350–450 µg	1.85
Tissue (liver)			
15 mg	20/G	18–20 µg	1.77
80 mg	100/G	85–95 µg	1.77
350 mg	500/G	350–450 µg	1.80
Yeast (*S. cerevisiae*)			
1.5×10^9	20/G	18–20 µg	1.87
7.0×10^9	100/G	85–95 µg	1.76
3.5×10^{10}	500/G	350–450 µg	1.90
Bacteria (*E. coli*, Gram-negative)			
4.5×10^9	20/G	16–20 µg	1.86
2.2×10^{10}	100/G	85–95 µg	1.71
1.0×10^{11}	500/G	300–400 µg	1.74
(*B. subtilis*, Gram-positive)			
4.5×10^9	20/G	16–20 µg	1.87
2.2×10^{10}	100/G	85–95 µg	1.79
1.0×10^{11}	500/G	300–400 µg	1.92

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 safety data sheets (SDSs), available from the product supplier.

For all sample preparation

- Centrifuge and centrifuge tubes
- Isopropanol
- Ethanol, 70%
- QIAGEN Protease (cat. nos. 19155 and 19157) or QIAGEN Proteinase K (cat. nos. 19131 and 19133)*

For blood preparation

- Screw-cap tubes
- Phosphate-buffered saline (PBS)
- Buffers C1, G2, QBT, QC, and QF†
- Ficoll® gradient; lymphocytes only
- 6% Dextran (MW: 250,000); lymphocytes only)

For cell culture preparation

- Screw-cap tubes
- PBS
- Trypsin (optional)
- Buffers C1, G2, QBT, QC, and QF*
- Homogenizer or liquid nitrogen, mortar and pestle

For tissue preparation

- Screw-cap tubes
- Buffers G2, QBT, QC, and QF*
- RNase A

* QIAGEN Protease is supplied in Blood & Cell Culture DNA Kits (cat. nos. 13323, 13343, and 13362). QIAGEN Protease, QIAGEN Proteinase K, or enzyme from another supplier must be purchased separately when using QIAGEN Genomic-tips (cat. nos. 10223, 10243, and 10262) and Genomic DNA Buffer Set (19060).

† Buffers C1, G2, QBT, QC, and QF are provided in the Blood & Cell Culture DNA Kit (cat. nos. 13323, 13343, and 13362) and Buffers C1, Y1, B1, B2, G2, QBT, and QC are provided in the Genomic DNA Buffer Set (19060). Instructions for preparing all necessary buffers are provided in Appendix A (page 64).

For yeast preparation

- Buffers TE, Y1, G2, WBT, QC, and QF*
- RNase A
- Lyticase (zymolase)

For bacteria preparation

- Buffers B1, B2, QC, and QF*
- RNase
- Lysozyme

For isolation of genomic DNA

- Buffer QBT, QC, and QF*
- Vortexer
- Collection tubes
- Elution buffer

* Buffers C1, G2, QBT, QC, and QF are provided in the Blood & Cell Culture DNA Kit (cat. nos. 13323, 13343, and 13362) and Buffers C1, Y1, B1, B2, G2, QBT, and QC are provided in the Genomic DNA Buffer Set (19060). Instructions for preparing all necessary buffers are provided in Appendix A (page 64).

Protocol: Preparation of Blood Samples

This protocol is designed for the rapid, easy, and nontoxic preparation of up to 20 µg (Genomic-tip 20/G), 90 µg (Genomic-tip 100/G), or 400 µg (Genomic-tip 500/G) of genomic DNA from up to 1 ml, 5 ml, or 20 ml of whole blood or 5 x 10^6, 2 x 10^7, or 1 x 10^8 lymphocytes or buffy-coat cells (leukocyte-rich blood concentrate), respectively. The purified genomic DNA ranges from 20 to 150 kb in size. Text marked with a ■ denotes values for mini-preps using the Genomic-tip 20/G; text marked with a ▲ denotes values for midi-preps using the Genomic-tip 100/G, and text marked with a ● denotes values for maxi-preps using the Genomic-tip 500/G.

Storage of blood samples

Storage of blood samples without previous treatment leads to reduced yields of DNA. For best results, use one of the following procedures.

- For short-term storage (up to 3 days), collect blood in tubes containing EDTA as an anticoagulant, and store the tubes at 4°C.

Two alternatives are available for long-term storage:

- Perform steps 1–5 of the protocol beginning on page 21, and then freeze the nuclear pellet at –20°C. When ready to complete the purification, continue with step 6.
- Collect blood in tubes containing a standard anticoagulant and store tubes at –70°C.

Frozen blood should be thawed in a 37°C water bath with mild agitation before beginning the procedure.

Recommended cell densities

Blood is a complex mixture of cells, proteins, metabolites, and many other substances. About 56% of human blood volume is comprised of cells, more than 99% of which are erythrocytes. Human erythrocytes and thrombocytes (platelets, 0.5% of blood components) do not contain nuclei and are therefore unsuitable for preparation of genomic DNA. The only blood cells that contain nuclei are leukocytes (0.3% of cellular blood components).

Blood samples may vary widely in the number of leukocytes they contain, depending on the health of the donor. Healthy blood, for example, contains fewer than 10^7 leukocytes per ml, while blood from an infected donor may have a tenfold higher leukocyte concentration. Although a QIAGEN Genomic-tip 100/G (capacity = 100 µg genomic DNA) can handle up to 5 ml of healthy whole blood, it would be overloaded if more than 0.5 ml of blood from an unhealthy donor were used.

A. Isolation of genomic DNA from whole blood of "healthy" donors (leukocyte concentrations from 5 x 10^6/ml to 1 x 10^7/ml)

Ensure that there are no more than 1 x 10^7 leukocytes per ml of blood, and use no more than ■ 5 x 10^6, ▲ 2 x 10^7, or ● 1 x 10^8 total leukocytes in your sample. Place sample in ■ 1 x 10 ml or ▲ 1 x 50 ml or ● 2 x 50 ml screw-cap tubes. Proceed with protocol, beginning on page 21.

B. Isolation of genomic DNA from whole blood of "unhealthy" donors

1. Leukocyte concentrations higher than 1 x 10^7/ml

Ensure that there are no more than ■ 5 x 10^6, ▲ 2 x 10^7, or ● 1 x 10^8 leukocytes in your sample. Using PBS, adjust the volume to ■ 1 ml, ▲ 5 ml, or ● 2 x 10 ml in ■ 1 x 10 ml, ▲ 1 x 50 ml, or ● 2 x 50 ml screw-cap tubes. Proceed with protocol, beginning on page 21.

2. Leukocyte concentrations lower than 5 x 10^6/ml

Ensure that the blood sample volume contains at least ■ 1 x 10^6, ▲ 5 x 10^6, or ● 2.5 x 10^7 leukocytes in total. Pellet the cells by centrifugation (15 min, 1000 x g). Resuspend the sample in ■ 0.1 ml, ▲ 1 ml, or ● 5 ml of PBS in a ■ 10 ml, ▲ 10 ml, or ● 50 ml screw-cap tube. Proceed with protocol, beginning on page 21.

C. Isolation of genomic DNA from buffy-coat preparations (do not use more than ■ 5 x 10^6, ▲ 2 x 10^7, or ● 1 x 10^8 buffy-coat cells)

Buffy coat is a leukocyte-enriched fraction of whole blood. Preparing a buffy-coat fraction from whole blood is simple and will yield approximately 5–10 times more DNA than an equivalent volume of blood.

Prepare buffy coat by centrifuging whole blood for 10 min at 3300 x *g* at room temperature (15–25°C). After centrifugation, three different fractions are obtained: the upper clear layer is plasma; the intermediate layer is buffy coat, containing concentrated leukocytes; and the bottom layer contains concentrated erythrocytes.

Do not use more than ■ 5 x 10^6, ▲ 2 x 10^7, or ● 1 x 10^8 buffy-coat cells.

Using PBS, adjust the volume to ■ 1 ml, ▲ 5 ml, or ● 2 x 10 ml in ■ 1 x 10 ml, ▲ 1 x 50 ml, ● 2 x 50 ml screw-cap tubes. Mix. Proceed with protocol, beginning on page 21.

D. Isolation of genomic DNA from lymphocyte preparations (do not use more than ■ 5 x 10^6, ▲ 2 x 10^7, or ● 1 x 10^8 lymphocytes)

1. Ficoll gradient preparation of lymphocytes

Use a common Ficoll gradient product (lymphocyte-separation medium). Warm the lymphocyte-separation medium to room temperature (15–25°C) before use.

For details of the procedure, consult the package insert provided with the lymphocyte separation medium.

Use no more than ■ 5 x 10^6, ▲ 2 x 10^7, or ● 1 x 10^8 total lymphocytes in your sample. Resuspend the sample in ■ 1 ml, ▲ 5 ml, or ● 2 x 10 ml of PBS in ■ 1 x 10 ml, ▲ 1 x 50 ml, or ● 2 x 50 ml screw-cap tubes. Proceed with protocol, beginning on page 21.

2. Dextran sedimentation

To ■ 1 ml, ▲ 5 ml, or ● 20 ml of whole blood add ■ 0.35 ml, ▲ 1.7 ml, or ● 6.6 ml of an aqueous solution of 6% Dextran (MW: 250,000; not included in the kit).

Mix well.

Put the tube in a rack and allow sedimentation to take place for 45 min. (Keeping the tube tilted will accelerate the sedimentation). Carefully remove the lymphocyte-rich supernatant to avoid contaminating it with the erythrocyte-rich top layer or the sediment. Use an aliquot with no more than ■ 5 x 10^6, ▲ 2 x 10^7, or ● 1 x 10^8 total lymphocytes. Using PBS, adjust the volume to ■ 1 ml, ▲ 5 ml, or ● 2 x 10 ml in ■ 1 x 10 ml, ▲ 1 x 50 ml, or ● 2 x 50 ml screw-cap tubes.

Proceed with protocol, beginning on page 21.

Sample preparation and lysis of blood

This is the standard sample preparation and lysis protocol for whole blood, buffy-coat cells, and lymphocytes, prior to genomic DNA isolation. The Genomic-tip protocol for nucleic acid isolation begins on page 49. Text marked with a ■ denotes values for mini-preps using the Genomic-tip 20/G; text marked with a ▲ denotes values for midi-preps using the Genomic-tip 100/G; and text marked with a ● denotes values for maxi-preps using the Genomic-tip 500/G.

Important notes before starting

- Read subsection A, B, C, or D of this procedure (pages 19–20) to determine how to prepare the sample and the appropriate amount to use.
- Required reagent volumes are provided in Table 3 (page 22).
- To obtain maximum purity and optimal flow rates, it is very important not to overload the QIAGEN Genomic-tip. Refer to the section on cell densities (pages 18–20).
- Equilibrate Buffer C1 and distilled water to 4°C. Buffer C1 should always be stored at 2–8°C.
- Equilibrate all other buffers to room temperature (15–25°C). These buffers may be stored at either 2–8°C or at room temperature. Long-term storage at 2–8°C is recommended.
- Before using this protocol for the first time, a solution of QIAGEN Protease should be carefully prepared. See step 2 for details of preparation. Alternatively, QIAGEN Proteinase K solution may be used directly.
- Steps 3–7 of the protocol are carried out in standard ■ 10 ml, ▲ 50 ml, or ● 2 x 50 ml screw-cap tubes.
- If using frozen blood or blood which has been stored at 2–8°C, refer to the section on storage of blood samples (page 18).
- Optional: remove aliquots at the indicated steps to monitor the procedure on an analytical gel (page 14).

Table 3. Reagent volumes required per blood prep

Reagent	Mini (tip 20/G)	Midi (tip 100/G)	Maxi (tip 500/G)
C1 (2–8°C)	1.25 ml	6 ml	22 ml
G2	1 ml	5 ml	10 ml
G2	1 ml	5 ml	10 ml
QBT	1 ml	4 ml	10 ml
QC	3 ml	15 ml	30 ml
QF	2 ml	5 ml	15 ml
Distilled water (2–8°C)	3.75 ml	18 ml	66 ml
Isopropanol	1.4 ml	3.5 ml	10.5 ml
70% ethanol	1 ml	2 ml	4 ml
QIAGEN Protease or QIAGEN Proteinase K stock solution	25 µl	95 µl	200 µl

1. **Prepare Buffers C1, G2, QBT, QC, and QF according to the instructions in Appendix A (page 62). Alternatively, the Genomic DNA Buffer Set or the Blood & Cell Culture DNA Kit can be used (for ordering information see page 69).**
2. **Prepare QIAGEN Protease stock solution in distilled water, or use QIAGEN Proteinase K stock solution.**

   If using a kit, dissolve each vial of lyophilized QIAGEN Protease provided in 1.4 ml of distilled water, as indicated on the label. Alternatively, QIAGEN Proteinase K solution may be used directly. See "Preparation of enzyme stock solutions" (page 64) for further details and page 69 for ordering information.

   Storage conditions for QIAGEN Protease and QIAGEN Proteinase K are described on page 5.

3. **Use ■ 0.1–1 ml, ▲ 1–5 ml, or ● 5–20 ml whole blood or ■ 1 ml, ▲ 5 ml, or ● 20 ml of buffy-coat or lymphocyte suspension (pages 19–20). Add 1 volume (■ 0.1–1 ml, ▲ 1–5 ml, or ● 5–20 ml) of ice-cold Buffer C1 and 3 volumes of ice-cold distilled water (■ 0.3–3 ml, ▲ 3–15 ml, or ● 15–60 ml). Mix by inverting the tube several times until the suspension becomes translucent. Incubate for 10 min on ice.**

   Buffer C1 and distilled water must be cold. Keep on ice during use.

   Buffer C1 lyses the cells but stabilizes and preserves the nuclei. Erythrocytes lyse first, releasing the hemoglobin and making the suspension translucent. Frozen blood samples do not visibly change upon lysis.

4. **Centrifuge the lysed blood at 4°C for 15 min at 1300 x *g*. Discard the supernatant.**

   After centrifugation, the small, nuclear pellet is still slightly red due to residual hemoglobin, which is removed in the next step. Centrifugation in a swing-out rotor will make the pellet easier to see.

5. **Add ■ 0.25 ml, ▲ 1 ml, or ● 2 ml of ice-cold Buffer C1 and ■ 0.75 ml, ▲ 3 ml, or ● 6 ml of ice-cold distilled water. Resuspend the pelleted nuclei by vortexing. Centrifuge again at 4°C for 15 min at 1300 x *g*. Discard the supernatant.**

   This wash step removes all residual cell debris and hemoglobin from the nuclear pellet. If the pellet is not white, repeat the wash.

   ● Pellets can be combined into one 50 ml tube for further processing.

   At this point the pellet may be frozen and stored at –20°C if desired. When ready to complete the purification procedure, continue with step 6 of the protocol. The yield of DNA will be the same as for fresh blood samples.

6. **Add ■ 1 ml, ▲ 5 ml, or ● 10 ml of Buffer G2, and completely resuspend the nuclei by vortexing for 10–30 s at maximum speed.**

   Resuspend the nuclei as thoroughly as possible by vortexing. This step is critical for a good flow rate on the QIAGEN Genomic-tip.

7. **Add ■ 25 µl, ▲ 95 µl, or ● 200 µl of QIAGEN Protease or QIAGEN Proteinase K stock solution, and incubate at 50°C for 30–60 min.**

   See step 2 for preparation of stock solutions.

   The length of incubation depends on how well the nuclei were resuspended in step 6. If the suspension is not homogeneous after vortexing, a full 60 min incubation is recommended to avoid clogging the QIAGEN Genomic-tip.

   Buffer G2 lyses the nuclei and denatures proteins such as nucleases, histones, and viral particles. The excess QIAGEN Protease digests the denatured proteins into smaller fragments. Buffer G2 and QIAGEN Protease, in combination, strip the genomic DNA of all bound proteins, facilitating efficient removal during purification. It is important that the lysate becomes clear at this stage. If necessary, extend the incubation time, or pellet the particulate matter by centrifugation for 10 min at 5000 x *g*, 4°C.

   Optional: Take a ■ 300 µl, ▲ 300 µl, or ● 150 µl aliquot and save it for an analytical gel (aliquot 1).

8. **Proceed with the Genomic-tip protocol on page 49.**

   The sample should be loaded onto the QIAGEN Genomic-tip promptly. If left too long, it may become cloudy due to further precipitation of protein. The sample must then be centrifuged or filtered before loading to prevent clogging of the QIAGEN Genomic-tip.

Protocol: Preparation of Cell Culture Samples

This protocol is designed for the rapid, easy, and nontoxic preparation of up to 20 µg (Genomic-tip 20/G), 90 µg (Genomic-tip 100/G), or 400 µg (Genomic-tip 500/G) of genomic DNA from up to 5 x 10^6, 2 x 10^7, or 1 x 10^8 cultured cells, respectively. The purified genomic DNA ranges in size from 20 to 150 kb. Text marked with a ■ denotes values for mini-preps using the Genomic-tip 20/G, text marked with a ▲ denotes values for midi-preps using the Genomic-tip 100/G, and text marked with a ● denotes values for maxi-preps using the Genomic-tip 500/G.

Storage of cell samples

Frozen cell samples should be thawed before beginning the procedure. Spin down the cells, wash them once with PBS, and resuspend them in PBS to a concentration of 10^7 cells/ml. Proceed with step 1 of the protocol, beginning on page 27. If cells are frozen whole, avoid repeated freeze/thaw cycles, as this will lead to considerable degradation of the DNA.

For long-term storage of the cells before isolation of genomic DNA, perform steps 1–5 of the protocol beginning on page 27, and then freeze the nuclear pellet at –20°C.

When ready to complete the extraction, continue with step 6. The yield of DNA will be the same as for fresh samples.

Recommended cell densities

Isolation of genomic DNA from cell culture (do not use more than ■ 5 x 10^6, ▲ 2 x 10^7, or ● 1 x 10^8 cells)

A. Cells grown in suspension

1. **Centrifuge the appropriate number of cells for 10 min at 1500 x *g* in a centrifuge tube.**
2. **Discard supernatant, ensuring all media is completely removed.**
3. **Wash the cells twice in PBS, and resuspend in cold PBS (4°C) to a final concentration of 10^7 cells/ml.**

   Note: Do not use more than ■ 0.5 ml, ▲ 2 ml, or ● 10 ml of this suspension for each QIAGEN Genomic-tip (■ 5 x 10^6, ▲ 2 x 10^7, or ● 1 x 10^8 cells).
4. **Proceed with the protocol, beginning on page 27.**

B. Cells grown in a monolayer

Cells grown in a monolayer can either be harvested by scraping or by using trypsin treatment. Cells are then collected as a cell pellet prior to lysis.

To harvest by scraping:

1. **Wash the monolayer twice with cold PBS (4°C).**
2. **Scrape the cells into 2 ml of cold PBS using a rubber policeman, and transfer to a centrifuge tube on ice.**
3. **Wash the culture vessel with another 2 ml of cold PBS, and add to the centrifuge tube.**
4. **Recover the cells by centrifuging at 1500 x *g* for 10 min at 4°C.**
5. **Discard the supernatant, and resuspend the cells in cold PBS to a final concentration of 10^7 cells/ml.**

   Note: Do not use more than ■ 0.5 ml, ▲ 2 ml, or ● 10 ml of this suspension for each QIAGEN Genomic-tip (■ 5 x 10^6, ▲ 2 x 10^7, or ● 1 x 10^8 cells).
6. **Proceed with the protocol, beginning on page 27.**

To harvest using trypsin treatment:

1. **Add 1–2 ml of 0.25% trypsin in PBS to the cell monolayer.**
2. **Wait until the cells detach from the culture vessel, then transfer the suspension to a centrifuge tube on ice.**
3. **Wash the culture vessel with 2 ml of cold PBS, and add to the centrifuge tube.**
4. **Recover the cells by centrifuging at 1500 x *g* for 10 min at 4°C.**
5. **Discard the supernatant, resuspend in 4 ml of cold PBS, and re-centrifuge at 1500 x *g* for 10 min at 4°C.**
6. **Repeat step 5.**
7. **Discard the supernatant, and resuspend the cells in PBS to a final concentration of 10^7 cells/ml.**

   Note: Do not use more than ■ 0.5 ml, ▲ 2 ml, or ● 10 ml of this suspension for each QIAGEN Genomic-tip (■ 5 x 10^6, ▲ 2 x 10^7, or ● 1 x 10^8 cells).
8. **Proceed with the protocol, beginning on page 27.**

Sample preparation and lysis of cell cultures

This is the standard sample preparation and lysis protocol for cultured cells, prior to genomic DNA isolation. The Genomic-tip protocol for nucleic acid purification begins on page 49. Text marked with a ■ denotes values for mini-preps using the Genomic-tip 20/G; text marked with a ▲ denotes values for midi-preps using the Genomic-tip 100/G; and text marked with a ● denotes values for maxi-preps using the Genomic-tip 500/G.

Important notes before starting

- Read subsection A or B of this procedure on pages 25–26 to determine how to prepare the sample and the appropriate amount to use.
- Required reagent volumes are provided in Table 4 (page 28).
- To obtain maximum purity and optimal flow rates, it is very important not to overload the QIAGEN Genomic-tip. Refer to the section on cell densities (pages 25–26).
- Equilibrate Buffer C1 and distilled water to 4°C. Buffer C1 should always be stored at 2–8°C.
- Equilibrate all other buffers to room temperature (15–25°C). These buffers may be stored at either 2–8°C or at room temperature. Long-term storage at 2–8°C is recommended.
- Before using this protocol for the first time, a solution of QIAGEN Protease should be carefully prepared. See step 2 for details of preparation. Alternatively, QIAGEN Proteinase K solution may be used directly.
- Steps 3–7 of the protocol are carried out in standard ■ 10 ml, ▲ 10 ml, or ● 50 ml screw-cap tubes.
- If using frozen cells, refer to the section on storage of cell samples (page 31).
- **Optional**: remove aliquots at the indicated steps to monitor the procedure on an analytical gel (page 53).

Table 4. Reagent volumes required per cell culture prep

Reagent	Mini (tip 20/G)	Midi (tip 100/G)	Maxi (tip 500/G)
C1 (2–8°C)	0.75 ml	3 ml	12 ml
G2	1 ml	5 ml	10 ml
QBT	1 ml	4 ml	10 ml
QC	3 ml	15 ml	30 ml
QF	2 ml	5 ml	15 ml
Distilled water (4°C)	2.25 ml	9 ml	36 ml
Isopropanol	1.4 ml	3.5 ml	10.5 ml
70% ethanol	1 ml	2 ml	4 ml
QIAGEN Protease or QIAGEN Proteinase K stock solution	25 µl	95 µl	200 µl

1. **Prepare Buffers C1, G2, QBT, QC, and QF according to the instructions in Appendix A (page 62).**

   Alternatively, the Genomic DNA Buffer Set or the Blood & Cell Culture DNA Kits can be used (for ordering information see page 69).

2. **Prepare QIAGEN Protease stock solution in distilled water, or use QIAGEN Proteinase K stock solution.**

   If using a kit, dissolve each vial of lyophilized QIAGEN Protease provided in 1.4 ml of distilled water, as indicated on the label. Alternatively, QIAGEN Proteinase K solution may be used directly. See "Preparation of enzyme stock solutions" (page 64) for further details and page 69 for ordering information.

   Storage conditions for QIAGEN Protease and QIAGEN Proteinase K are described on page 5.

3. **Use ■ 0.5 ml, ▲ 2 ml, or ● 10 ml cell suspension (1 x 10^7 cells/ml, pages 25–26). Add 1 volume (■ 0.5 ml, ▲ 2 ml, or ● 10 ml) of ice-cold Buffer C1 and 3 volumes of ice-cold distilled water (■ 1.5 ml, ▲ 6 ml, or ● 30 ml). Mix by inverting the tube several times. Incubate for 10 min on ice.**

   Buffer C1 and distilled water must be equilibrated to 4°C. Keep on ice during use.

   Buffer C1 lyses the cells but stabilizes and preserves the nuclei.

4. **Centrifuge the lysed cells at 4°C for 15 min at 1300 x *g*. Discard the supernatant.**

   After centrifugation there should be a small nuclear pellet visible. Centrifugation in a swing-out rotor will make the pellet easier to see.

5. **Add ■ 0.25 ml, ▲ 1 ml, or ● 2 ml of ice-cold Buffer C1 and ■ 0.75 ml, ▲ 3 ml, or ● 6 ml of ice-cold distilled water. Resuspend the pelleted nuclei by vortexing. Centrifuge again at 4°C for 15 min at 1300 x *g*. Discard the supernatant.**

   This wash step removes all residual cell debris from the nuclear pellet.

   At this point the pellet may be frozen and stored at –20°C if desired. When ready to complete the purification procedure, continue with step 6 of the protocol. The yield of DNA will be the same as for fresh samples.

6. **Add ■ 1 ml, ▲ 5 ml, or ● 10 ml of Buffer G2, and completely resuspend the nuclei by vortexing for 10–30 s at maximum speed.**

   Resuspend the nuclei as thoroughly as possible by vortexing. This step is critical for a good flow rate on the QIAGEN Genomic-tip.

7. **Add ■ 25 µl, ▲ 95 µl, or ● 200 µl of QIAGEN Protease or QIAGEN Proteinase K stock solution, and incubate at 50°C for 30–60 min.**

   See step 2 for preparation of stock solutions.

   The length of incubation depends on how well the nuclei were resuspended in step 6. If the suspension was not homogeneous after vortexing, a full 60 min incubation is recommended to avoid clogging the QIAGEN Genomic-tip.

   Buffer G2 lyses the nuclei and denatures proteins such as nucleases, histones, and viral particles. The excess QIAGEN Protease digests the denatured proteins into smaller fragments. Buffer G2 and QIAGEN Protease, in combination, strip the genomic DNA of all bound proteins, facilitating efficient removal during purification. It is important that the lysate becomes clear at this stage. If necessary, extend the incubation time, or pellet the particulate matter by centrifugation for 10 min at 5000 x *g*, 4°C.

   Optional: Take a ■ 300 µl, ▲ 300 µl, or ● 150 µl aliquot and save it for an analytical gel (aliquot 1).

8. **Proceed with the Genomic-tip protocol on page 49.**

   The sample should be loaded onto the QIAGEN Genomic-tip promptly. If left too long, it may become cloudy due to further precipitation of protein. The sample must then be centrifuged or filtered before loading to prevent clogging of the QIAGEN Genomic-tip.

Protocol: Preparation of Tissue Samples

This protocol is designed for the rapid, easy, and nontoxic preparation of up to 20 µg (Genomic-tip 20/G), 90 µg (Genomic-tip 100/G), or 400 µg (Genomic-tip 500/G) of genomic DNA from not more than 20 mg, 100 mg, or 400 mg of tissue, respectively. The purified genomic DNA ranges in size from 20 to 150 kb. Text with a ■ denotes values for mini-preps using the Genomic-tip 20/G; text with a ▲ denotes values for midi-preps using the Genomic-tip 100/G; and text with a ● denotes values for maxi-preps using the Genomic-tip 500/G.

Storage of tissue samples

The DNA yield will decrease for tissue samples stored at either 2–8°C or –20°C without previous treatment. To avoid this decrease in yield upon storage, add the tissue sample to 20% glycerol or 20% DMSO, and store samples in liquid nitrogen. Frozen tissue samples do not need to be thawed before beginning the procedure. Pellet the frozen tissue by centrifugation, and discard the supernatant containing glycerol or DMSO before homogenization in liquid nitrogen.

Recommended sample amounts

QIAGEN protocols are optimized for use with fixed cell densities corresponding to the capacity of the QIAGEN Genomic-tip used. Overloading tips with DNA from an excessive number of cells (too much tissue) will lead to reduced performance of the system.

Yield of DNA and flow characteristics of the QIAGEN Genomic-tips depend on the number of cells in the sample and on the size of the genome. Tissue samples may vary in the number of cells they contain depending on the age, the organ, and the organism of origin. There is good correlation between cell number and weight in different organs and between different mammalian species. Weighing the tissue samples before starting the preparation and referring to Table 5 (page 32) is recommended. Liver and spleen are transcriptionally very active organs. Tissue samples derived from these organs have a very high protein and RNA content. Thus, when preparing genomic DNA from liver or spleen, use only 75–85% of the standard amount of starting material.

Table 5. Recommended amounts of tissue

	QIAGEN Genomic-tip		
	20/G	**100/G**	**500/G**
DNA	15–20 µg	70–95 µg	350–450 µg
Heart	≤20 mg	≤100 mg	≤400 mg
Lung	≤20 mg	≤100 mg	≤400 mg
Muscle	≤20 mg	≤100 mg	≤400 mg
Brain	≤20 mg	≤100 mg	≤400 mg
Mouse tail	≤20 mg	≤100 mg	≤400 mg
Liver	≤15 mg	≤80 mg	≤350 mg
Spleen	≤15 mg	≤80 mg	≤350 mg

Sample preparation and lysis of tissue

This is the standard sample preparation and lysis protocol for different animal tissues, such as lung, liver, spleen, tumors, and mouse tails, prior to genomic DNA isolation. The Genomic-tip protocol for nucleic acid purification begins on page 49. Text with a ■ denotes values for mini-preps using the Genomic-tip 20/G; text with a ▲ denotes values for midi-preps using the Genomic-tip 100/G; and text with a ● denotes values for maxi-preps using the Genomic-tip 500/G.

Important notes before starting

- Read the recommended sample amounts section of this procedure on page 31 to determine how to prepare the sample and the appropriate amount to use.
- Required reagent volumes are provided in Table 6 (page 34).
- To obtain maximum purity and optimal flow rates, it is very important not to overload the QIAGEN Genomic-tip. Refer to the section on sample amounts (page 31).
- Equilibrate buffers to room temperature (15–25°C). The required buffers may be stored at either 2–8°C or at room temperature. Long-term storage at 2–8°C is recommended.
- Before using this protocol for the first time, a stock solution of QIAGEN Protease should be carefully prepared. See step 2 for details. RNase A and QIAGEN Proteinase K stock solutions may be used directly in the procedure.
- Note that when using this protocol, additional QIAGEN Protease may need to be ordered. QIAGEN Protease sufficient for ■ 14, ▲ 5, or ● 2 preps is supplied with the kit. To carry out additional preps, order ■ 1, ▲ 1, or ● 2 vials of QIAGEN Protease (cat. no. 19155, see page 69).
- Steps 3–5 of the protocol are carried out in standard ■ 10 ml, ▲ 50 ml, or ● 50 ml screw-cap tubes.
- If using frozen tissue, Refer to the section on storage of tissue samples (page 31).
- **Optional**: remove aliquots at the indicated steps to monitor the procedure on an analytical gel (page 53).

Table 6. Reagent volumes required per tissue prep

Reagent	Mini (tip 20/G)	Midi (tip 100/G)	Maxi (tip 500/G)
G2	2 ml	9.5 ml	19 ml
QBT	1 ml	4 ml	10 ml
QC	3 ml	15 ml	30 ml
QF	2 ml	5 ml	15 ml
Isopropanol	1.4 ml	3.5 ml	10.5 ml
70% ethanol	1 ml	2 ml	4 ml
RNase A stock solution*	4 µl	19 µl	38 µl
QIAGEN Protease or QIAGEN Proteinase K stock solution*	100 µl	500 µl	1000 µl

* To be supplied by the user.

1. **Prepare Buffers G2, QBT, QC, and QF according to the instructions in Appendix A (page 62), or use the Genomic DNA Buffer Set (for ordering information see page 69).**
2. **For each prep, add ■ 4 µl, ▲ 19 µl, or ● 38 µl of RNase A stock solution (100 mg/ml) to a ■ 2 ml, ▲ 9.5 ml, or ● 19 ml aliquot of Buffer G2. Prepare QIAGEN Protease stock solution in distilled water, or use QIAGEN Proteinase K stock solution.**

   If using a kit, dissolve each vial of lyophilized QIAGEN Protease provided in 1.4 ml of distilled water, as indicated on the label. Alternatively, QIAGEN Proteinase K solution may be used directly. See "Preparation of enzyme stock solutions" (page 64) for further details and page 69 for ordering information.

   Storage conditions for QIAGEN Protease and QIAGEN Proteinase K are described on page 5.

   RNase A should be added to Buffer G2 to a final concentration of 200 µg/ml and will be stable for 6 months when stored at 2–8°C.

3. Do not use more than ■ 20 mg, ▲ 100 mg, or ● 400 mg of tissue (■ 15 mg, ▲ 80 mg, or ● 350 mg of liver or spleen). Homogenize tissue as described in step 3A or step 3B.

See page 31 for the appropriate amount of starting material.

Liver and spleen tissue have very high protein and RNA content. Therefore, when preparing genomic DNA from liver or spleen, only 75–85% of the standard amount of starting material should be used.

For frozen tissue samples stored in 20% glycerol or 20% DMSO, pellet the sample by centrifugation, and discard the supernatant before homogenization. See the section on storage of tissue samples (page 31).

3A. Thoroughly homogenize the tissue mechanically in ■ 2 ml, ▲ 9.5 ml, or ● 19 ml of Buffer G2 (with RNase A) using a homogenizer (e.g., Ultra Turrax®, Polytron®). Proceed with step 4A.

Add the RNase A to Buffer G2 according to step 2 before adding the buffer to the tissue sample.

Note: Extensive mechanical homogenization can result in shearing of the DNA. This may be critical if the DNA is intended for next generation sequencing library preparation.

3B. Alternatively, grind the tissue to a fine powder with liquid nitrogen in a precooled mortar and pestle. Proceed with step 4B.

Grind the tissue as thoroughly as possible. This step is critical for lysis and for a good flow rate on the QIAGEN Genomic-tip.

4. Transfer material as described in step 4A or 4B.

4A. Transfer the homogenate from step 3A to a ■ 10 ml, ▲ 50 ml, or ● 50 ml screw-cap tube. Add ■ 0.1 ml, ▲ 0.5 ml, or ● 1.0 ml of the QIAGEN Protease or QIAGEN Proteinase K stock solution prepared in step 2 to the homogenate. Mix well by vortexing. Proceed with step 5.

4B. Transfer the ground tissue from step 3B to a ■ 10 ml, ▲ 50 ml, or ● 50 ml screw-cap tube. Add ■ 2 ml, ▲ 9.5 ml, or ● 19 ml of Buffer G2 (with RNase A) and ■ 0.1 ml, ▲ 0.5 ml, or ● 1.0 ml of the QIAGEN Protease or QIAGEN Proteinase K stock solution prepared in step 2 to the ground tissue. Mix well by vortexing. Proceed with step 5.

Add the RNase A to Buffer G2 according to step 2 before adding the buffer to the ground tissue.

5. **Incubate at 50°C for 2 h.**

   The length of incubation depends on how well the tissue sample has been homogenized in step 3. Lysates should be clear after incubation. If particulate matter is still observed after 2 h, extend the incubation time until the lysate is clear to avoid clogging the QIAGEN Genomic-tip.

   Note: If the sample contains particulate matter, centrifuge at 5000 x *g* for 10 min at 4°C. Discard the pellet and transfer only the supernatant onto the QIAGEN Genomic-tip.

   Buffer G2 lyses the nuclei and denatures proteins such as nucleases, histones, and viral particles. The excess QIAGEN Protease digests the denatured proteins into smaller fragments. Buffer G2 and QIAGEN Protease, in combination, strip the genomic DNA of all bound proteins, facilitating efficient removal during purification.

   Degradation of RNA by RNase A ensures complete removal during column procedure.

   Optional: Take a ■ 300 µl, ▲ 300 µl, or ● 150 µl aliquot and save it for an analytical gel (aliquot 1).

6. **Proceed with the Genomic-tip protocol on page 49.**

   The sample should be loaded onto the QIAGEN Genomic-tip promptly. If left too long, it may become cloudy due to further precipitation of protein. The sample must then be centrifuged or filtered before loading to prevent clogging of the QIAGEN Genomic-tip.

Protocol: Preparation of Yeast Samples

This protocol is designed for the rapid, easy, and nontoxic preparation of up to 20 µg (Genomic-tip 20/G), 90 µg (Genomic-tip 100/G), or 450 µg (Genomic-tip 500/G) of genomic DNA from yeast (*Saccharomyces* spp., *Candida* spp.). The purified genomic DNA ranges in size from 20 to 100 kb. Text marked with a ■ denotes values for mini-preps using the Genomic-tip 20/G; text marked with a ▲ denotes values for midi-preps using the Genomic-tip 100/G; and text marked with a ● denotes values for maxi-preps using the Genomic-tip 500/G.

Recommended culture volumes

QIAGEN protocols are optimized for use with fixed cell densities corresponding to the capacity of the QIAGEN Genomic-tip used. Overloading tips with DNA from an excessive number of cells (too much culture volume) will lead to reduced performance of the system.

Yield of DNA and flow characteristics of the QIAGEN Genomic-tips depend on the number of yeast cells in culture and on the size of the genome.

Yeast growth is usually measured using a spectrophotometer. However, it is very difficult to give specific and reliable recommendations for the relations between OD values and cell number in cultures. Cell density is influenced by a variety of factors (e.g., species, media, incubation time, and shaker speed), and OD readings of cultures measure light scattering rather than absorption. Measurements of light scattering are highly dependent on the distance between the sample and the detector. Therefore readings vary between different types of spectrophotometers. In addition, different species may show different OD values at the same wavelength.

We therefore recommend calibrating the spectrophotometer used by comparing OD measurements at appropriate wavelengths with viable cell densities determined by plating experiments (e.g., see Ausubel, F.M. et al., eds. (1991) *Current Protocols in Molecular Biology*. New York: Wiley Interscience). OD readings should be between 0.05 and 0.3 to ensure significance. Samples with readings above 0.3 should be diluted so that the readings fall within this range. The dilution factor should then be used in calculating the number of cells per ml. Table 7 (page 38) provides a range of culture volumes for *S. cerevisiae*, which may be helpful.

QIAGEN protocols are optimized for use with yeast cultures grown in standard YPD media (see Appendix A, page 64) to a cell density of approximately 3×10^8 cells/ml.

Note: Do not use more than ■ 1.5×10^9, ▲ 7×10^9, or ● 3.5×10^{10} cells for each Genomic-tip.

Table 7. Recommended culture volumes for *S. cerevisiae* cultures

	QIAGEN Genomic-tip		
	20/G	**100/G**	**500/G**
DNA	5–20 µg	25–90 µg	200–450 µg
Culture volume	4–5 ml	15–20 ml	80–100 ml
Number of cells	1.5×10^9	7.0×10^9	3.5×10^{10}

Volumes given are for *S. cerevisiae* cultures grown in YPD medium to an OD_{600} value of 2.0 on a Beckman® DU®-40 spectrophotometer or an OD_{600} value of 3.6 on a Beckman DU-7400 spectrophotometer. OD_{600} values depend on the length of the light path and therefore differ between spectrophotometers.

Note: Culture volumes for other species of yeast may differ.

Sample preparation and lysis of yeast

This is the standard sample preparation and lysis protocol for yeast, prior to genomic DNA isolation. The Genomic-tip protocol for nucleic acid purification begins on page 49. Text marked with a ■ denotes values for mini-preps using the Genomic-tip 20/G; text marked with a ▲ denotes values for midi-preps using the Genomic-tip 100/G; and text marked with a ● denotes values for maxi-preps using the Genomic-tip 500/G.

Important notes before starting

- Read the recommended culture volumes section on pages 37–38 to determine how to prepare the sample and the appropriate amount to use. Note that culture volumes may differ for other species of yeast.
- Required reagent volumes are provided in Table 8 (page 40).
- To obtain maximum purity and optimal flow rates, it is very important not to overload the QIAGEN Genomic-tip. Refer to the section on culture volumes (pages 37–38).
- Equilibrate buffers to room temperature (15–25°C). Buffer Y1 should be stored at 2–8°C. The other buffers may be stored either at 2–8°C or at room temperature. Long-term storage at 2–8°C is recommended.
- Before using this protocol for the first time, stock solutions of lyticase (zymolase) and QIAGEN Protease should be carefully prepared. See step 2 for details of preparation. RNase A and QIAGEN Proteinase K stock solutions may be used directly in the procedure. See Appendix A (page 65) for ordering information for lyticase or zymolase.
- Steps 4–10 of the protocol are carried out in standard ■ 10 ml, ▲ 50 ml, or ● 50 ml screw-cap tubes.
- **Optional**: remove aliquots at the indicated steps to monitor the procedure on an analytical gel (page 53).

Table 8. Reagent volumes required per yeast prep

Reagent	Mini (tip 20/G)	Midi (tip 100/G)	Maxi (tip 500/G)
TE	2 ml	4 ml	12 ml
Y1	1 ml	4 ml	12 ml
G2	2 ml	5 ml	15 ml
QBT	1 ml	4 ml	10 ml
QC	3 ml	15 ml	30 ml
QF	2 ml	5 ml	15 ml
QF	2 ml	5 ml	15 ml
Isopropanol	1.4 ml	3.5 ml	10.5 ml
70% ethanol	1 ml	2 ml	4 ml
RNase A stock solution	4 µl	10 µl	30 µl
Lyticase (zymolase) stock solution (1000 U/ml)	100 µl	250 µl	1000 µl
QIAGEN Protease or QIAGEN Proteinase K stock solution	45 µl	100 µl	400 µl

1. **Prepare Buffers TE, Y1, G2, QBT, QC, and QF according to the instructions in Appendix A (page 62), or use the Genomic DNA Buffer Set (for ordering information see page 69). When using the Genomic DNA Buffer Set for the first time, be sure to add 160 µl of β-mercaptoethanol (14.3 M) to the 160 ml bottle of Buffer Y1.**

2. **For each prep, add ■ 4 µl, ▲ 10 µl, or ● 30 µl of RNase A solution (100 mg/ml) to a ■ 2 ml, ▲ 5 ml, or ● 15 ml aliquot of Buffer G2. Dissolve lyticase (zymolase) in distilled water to a final concentration of 1000 U/ml. Prepare QIAGEN Protease stock solution in distilled water, or use QIAGEN Proteinase K stock solution.**

   If using a kit, dissolve each vial of lyophilized QIAGEN Protease provided in 1.4 ml of distilled water, as indicated on the label. Alternatively, QIAGEN Proteinase K solution may be used directly. See "Preparation of enzyme stock solutions" (page 64) for further details and page 69 for ordering information.

   Storage conditions for QIAGEN Protease and QIAGEN Proteinase K are described on page 5.

   Lyticase or zymolase should be dissolved in distilled water to a concentration of 1000 U/ml and should be stored at –20°C. For efficient lysis of some yeast species, zymolase rather than lyticase is recommended. Use the appropriate enzyme for the particular species. See Appendix A (page 65) for ordering information for lyticase or zymolase.

   RNase A should be added to Buffer G2 to a final concentration of 200 µg/ml and will be stable for 6 months when stored at 2–8°C.

3. **Pellet yeast cells from an appropriate volume of culture by centrifuging at 3000–5000 x *g*, 4°C, for 5–10 min. Discard the supernatant, ensuring that all liquid is completely removed.**

   See pages 37–38 for the appropriate starting volume.

   Due to the different growth characteristics of yeast species, performing a preliminary experiment to determine the optimal starting volume is recommended.

4. **Resuspend the cell pellet from Step 3 in ■ 2 ml, ▲ 4 ml, or ● 12 ml of TE buffer by vortexing. This wash eliminates remaining media components.**

5. **Pellet the cells by centrifuging at 3000–5000 x *g*, 4°C, for 5–10 min. Discard the supernatant and resuspend the pellet in ■ 1 ml, ▲ 4 ml, or ● 12 ml of Buffer Y1 by vortexing at top speed.**

   Resuspend the cell pellet as thoroughly as possible by vortexing.

   For efficient preparation of spheroplasts, it is important to have a homogeneous suspension.

6. **Add ■ 100 µl, ▲ 250 µl, or ● 1000 µl of lyticase (zymolase) stock solution (1000 U/ml), and incubate at 30°C for at least 30 min.**

   Use the stock solution from step 2.

   During incubation, the lyticase (zymolase) enzymatically breaks down the cell wall.

7. **Pellet the spheroplasts by centrifuging at 5000 x *g*, 4°C, for 10 min.**
8. **Resuspend the spheroplast pellet in ■ 2 ml, ▲ 5 ml, or ● 15 ml of Buffer G2 (with RNase A), and mix by inverting the tube several times or by vortexing for a few seconds.**

   Take care to add the RNase A to Buffer G2 according to step 2 before adding the buffer to the spheroplasts.

   Resuspend the spheroplast pellet as thoroughly as possible by vortexing. For efficient lysis of spheroplasts it is important to have a homogeneous suspension.
9. **Add ■ 45 µl, ▲ 100 µl, or ● 400 µl of QIAGEN Protease or QIAGEN Proteinase K stock solution, and incubate at 50°C for at least 30 min.**

   Use the stock solution from step 2.

   The length of incubation depends on how well the spheroplasts were resuspended in step 8. If the suspension is not homogeneous after vortexing, a longer incubation is recommended to avoid clogging the QIAGEN Genomic-tip. The lysate will not become clear during incubation. Buffer G2 lyses the nuclei and denatures proteins such as nucleases, histones, and other DNA-binding proteins. The excess QIAGEN Protease digests the denatured proteins into smaller fragments. Buffer G2 and QIAGEN Protease, in combination, strip the genomic DNA of all bound proteins, facilitating efficient removal during purification.

   Degradation of RNA by RNase A ensures complete removal during the column procedure.
10. **Pellet the cellular debris by centrifuging at 5000 x *g*, 4°C, for 10 min. Retain the supernatant, and discard the pellet.**

    After centrifugation there will be a white pellet of cellular debris and a clear, particle-free supernatant.

    Optional: Take a ■ 500 µl, ▲ 300 µl, or ● 250 µl aliquot of the supernatant and save it for an analytical gel (aliquot 1).
11. **Proceed with the Genomic-tip protocol on page 49.**

    The sample should be loaded onto the QIAGEN Genomic-tip promptly. If left too long, it may become cloudy due to further precipitation of protein. The sample must then be centrifuged or filtered before loading to prevent clogging of the QIAGEN Genomic-tip.

Protocol: Preparation Gram-negative and some Gram-Positive Bacterial Samples

This protocol is designed for the rapid, easy, and nontoxic preparation of up to 20 µg (Genomic-tip 20/G), 90 µg (Genomic-tip 100/G), or 450 µg (Genomic-tip 500/G) of genomic DNA from Gram-negative and some Gram-positive (*B. subtilis*, *Staphylococcus* spp., *Lactobacillus* spp.) bacteria. The purified genomic DNA ranges in size from 20 to 160 kb. Text marked with a ■ denotes values for mini-preps using the Genomic-tip 20/G; text marked with a ▲ denotes values for midi-preps using the Genomic-tip 100/G; and text marked with a ● denotes values for maxi-preps using the Genomic-tip 500/G.

Recommended culture volumes

QIAGEN protocols are optimized for use with fixed cell densities corresponding to the capacity of the QIAGEN Genomic-tip used. Overloading tips with DNA from an excessive number of cells (too much culture volume) will lead to reduced performance of the system.

Yield of DNA and flow characteristics of the QIAGEN Genomic-tips depend on the number of bacteria in culture and on the size of the bacterial genome.

Bacterial growth is usually measured using a spectrophotometer. However, it is very difficult to give specific and reliable recommendations for the relations between OD values and cell number in cultures. Cell density is influenced by a variety of factors (e.g., species, media, incubation time, and shaker speed), and OD readings of cultures measure light scattering rather than absorption. Measurements of light scattering are highly dependent on the distance between the sample and the detector. Therefore readings vary between different types of spectrophotometers. In addition, different bacterial species show different OD values at the same wavelength.

We therefore recommend calibrating the spectrophotometer used by comparing OD measurements at appropriate wavelengths with viable cell densities determined by plating experiments (e.g., see Ausubel, F.M. et al., eds. (1991) *Current Protocols in Molecular Biology*. New York: Wiley Interscience). OD readings should be between 0.05 and 0.3 to ensure significance. Samples with readings above 0.3 should be diluted so that the readings fall within this range. The dilution factor should then be used in calculating the number of cells per ml. If using bacteria containing plasmid to prepare total DNA (genomic and plasmid DNA), start with half of the culture volume recommended for bacteria without plasmid. Table 9 (page 44) provides a range of culture volumes for several bacterial species, which may be helpful.

QIAGEN protocols are optimized for use with bacterial cultures grown in standard Luria Bertani (LB) media. Note that a number of slightly different LB

culture broths, containing different concentrations of NaCl, are in common use. We recommend growing cultures in LB medium containing 10 g NaCl per liter (see page 64).

Note: Do not use more than ■ 4.5 x 10^9, ▲ 2.2 x 10^{10}, or ● 1 x 10^{11} cells for each Genomic-tip.

Table 9. Recommended bacterial culture volumes

	QIAGEN Genomic-tip		
	20/G	**100/G**	**500/G**
DNA	5–20 µg	25–90 µg	200–400 µg
Culture volume			
E. coli	0.6–1.2 ml	3.0–6.0 ml	15.0–30.0 ml
E. coli + plasmid	0.4–0.8 ml	2.0–4.0 ml	10.0–20.0 ml
Xanthomonas spp.	0.3–0.6 ml	1.5–3.0 ml	7.5–15.0 ml
Bacillus subtilis	1.2–1.8 ml	6.0–9.0 ml	30.0–45.0 ml
Number of bacteria	4.5 x 10^9	2.2 x 10^{10}	1 x 10^{11}

Volumes given are for bacterial cultures grown in LB medium to an OD_{600} value of 2.0 on a Beckman DU-40 spectrophotometer or an OD_{600} value of 3.6 on a Beckman DU-7400 spectrophotometer. OD_{600} values depend on the length of the light path and therefore differ between spectrophotometers.

Note: Culture volumes for other bacterial species may differ.

Sample preparation and lysis of bacteria

This is the standard sample preparation and lysis protocol for Gram-negative and some Gram-positive bacteria (*B. subtilis*, *Staphylococcus* spp., *Lactobacillus* spp.), prior to genomic DNA isolation. The Genomic-tip protocol for nucleic acid purification begins on page 49. Text marked with a ■ denotes values for mini-preps using the Genomic-tip 20/G, text marked with a ▲ denotes values for midi-preps using the Genomic-tip 100/G, and text marked with a ● denotes values for maxi-preps using the Genomic-tip 500/G.

Important notes before starting

- Read the recommended culture volumes section on pages 43–46 to determine how to prepare the sample and the appropriate amount to use. Note that culture volumes may differ for other species of bacteria.
- Required reagent volumes are provided in Table 10 (page 46).
- To obtain maximum purity and optimal flow rates, it is very important not to overload the QIAGEN Genomic-tip. Refer to the section on culture volumes (pages 43–46).
- Equilibrate buffers to room temperature (15–25°C). The required buffers may be stored at either 2–8°C or at room temperature. Long-term storage at 2–8°C is recommended.
- Before using this protocol for the first time, stock solutions of lysozyme and QIAGEN Protease should be carefully prepared. See step 2 for details of preparation. RNase A and QIAGEN Proteinase K stock solutions may be used directly. See Appendix A (page 65) for ordering information for lysozyme.
- Steps 4–6 of the protocol are carried out in standard 10 ml, 50 ml, or 50 ml screw-cap tubes.
- **Optional**: remove aliquots at the indicated steps to monitor the procedure on an analytical gel (page 53).

Table 10. Reagent volumes required per bacterial prep

Reagent	Mini (tip 20/G)	Midi (tip 100/G)	Maxi (tip 500/G)
B1	1 ml	3.5 ml	11 ml
B2	0.35 ml	1.2 ml	4 ml
QBT	1 ml	4 ml	10 ml
QC	3 ml	15 ml	30 ml
QF	2 ml	5 ml	15 ml
Isopropanol	1.4 ml	3.5 ml	10.5 ml
70% ethanol	1 ml	2 ml	4 ml
RNase A stock solution	2 µl	7 µl	22 µl
Lysozyme stock solution (100 mg/ml)	20 µl	80 µl	300 µl
QIAGEN Protease or QIAGEN Proteinase K stock solution	45 µl	100 µl	500 µl

1. **Prepare Buffers B1, B2, QBT, QC, and QF according to the instruction in Appendix A (page 62), or use the Genomic DNA Buffer Set (for ordering information see page 69).**

2. **For each prep, add ■ 2 µl, ▲ 7 µl, or ● 22 µl of RNase A solution (100 mg/ml) to a ■ 1 ml, ▲ 3.5 ml, or ● 11 ml aliquot of Buffer B1. Dissolve lysozyme in distilled water to a concentration of 100 mg/ml. Prepare QIAGEN Protease stock solution in distilled water, or use QIAGEN Proteinase K stock solution.**

   If using a kit, dissolve each vial of lyophilized QIAGEN Protease provided in 1.4 ml of distilled water, as indicated on the label. Alternatively, QIAGEN Proteinase K solution may be used directly. See "Preparation of enzyme stock solutions" (page 64) for further details and page 69 for ordering information.

   Storage conditions for QIAGEN Protease and QIAGEN Proteinase K are described on page 5.

   Lysozyme should be dissolved in distilled water to a concentration of 100 mg/ml and should be stored at –20°C. For efficient lysis of some bacterial species, enzymes other than lysozyme may be necessary. Lysis of *Staphylococcus* spp., for example, is much more efficient with lysostaphin. Use the appropriate enzyme for the particular species. See Appendix A (page 65) for ordering information for lysozyme.

   RNase A should be added to Buffer B1 to a concentration of 200 µg/ml and will be stable for 6 months when stored at 2–8°C.

3. **Pellet bacteria from an appropriate volume of culture by centrifugation at 3000–5000 x *g* for 5–10 min. Discard the supernatant, ensuring that all liquid is completely removed.**

   See pages 43–44 for the appropriate starting volume.

   Because of the different growth characteristics of bacterial species, performing a preliminary experiment to determine the optimal starting volume is recommended.

4. **Resuspend the bacteria pellet from step 3 in ■ 1 ml, ▲ 3.5 ml, or ● 11 ml of Buffer B1 (with RNase A) by vortexing at top speed.**

   Add the RNase A to Buffer B1 according to step 2 before adding the buffer to the bacteria.

   Resuspend the bacterial pellet as thoroughly as possible by vortexing.

   For efficient lysis of the bacteria, it is important to have a homogeneous suspension.

5. **Add ■ 20 µl, ▲ 80 µl, or ● 300 µl of lysozyme stock solution (100 mg/ml), and ■ 45 µl, ▲ 100 µl, or ● 500 µl of QIAGEN Protease or QIAGEN Proteinase K stock solution. Incubate at 37°C for at least 30 min.**

   Use the stock solutions from step 2.

   The length of incubation depends on how well the bacteria were resuspended in step 4.

   If the suspension is not homogeneous after vortexing, a longer incubation is recommended to avoid clogging the QIAGEN Genomic-tip.

   During incubation, the lysozyme enzymatically breaks down the bacterial cell wall, while the detergents in Buffer B1 ensure complete lysis of the bacteria.

   Degradation of RNA by RNase A ensures complete removal during column procedure.

6. **Add ■ 0.35 ml, ▲ 1.2 ml, or ● 4 ml of Buffer B2, and mix by inverting the tube several times or by vortexing for a few seconds. Incubate at 50°C for 30 min.**

   Mix the lysate and Buffer B2 very thoroughly. This step is important for efficient deproteinization.

   Buffer B2 denatures proteins such as nucleases and DNA-binding proteins. The excess QIAGEN Protease digests the denatured proteins into smaller fragments, facilitating efficient removal during purification.

   Buffer B2 and QIAGEN Protease, in combination, strip the genomic DNA of all bound proteins. It is important that the lysate becomes clear at this stage. If necessary, extend the incubation time, or pellet the particulate matter by centrifugation for 10 min at 5000 x *g*, 4°C.

   Optional: Take a ■ 300 µl, ▲ 300 µl, or ● 150 µl aliquot and save it for an analytical gel (aliquot 1).

7. **Proceed with the Genomic-tip protocol on page 49.**

   The sample should be loaded onto the QIAGEN Genomic-tip promptly. If left too long, it may become cloudy due to further precipitation of protein. The sample must then be centrifuged or filtered before loading to prevent clogging of the QIAGEN Genomic-tip.

Protocol: Isolation of Genomic DNA from Blood, Cultured Cells, Tissue, Yeast, or Bacteria using Genomic-tips

All samples must first be prepared according to the relevant sample preparation and lysis protocol (pages 18–48).

Text with a ■ denotes values for mini-preps using the Genomic-tip 20/G; text with a ▲ denotes values for midi-preps using the Genomic-tip 100/G, and ● denotes values for maxi-preps using the Genomic-tip 500/G.

1. **Equilibrate a QIAGEN Genomic-tip 20/G, Genomic-tip 100/G, or Genomic-tip 500/G with ■ 1 ml, ▲ 4 ml, or ● 10 ml of Buffer QBT, and allow the QIAGEN Genomic-tip to empty by gravity flow.**

   Place a QIAGEN Genomic-tip over a tube using a tip holder or into a QIArack over the waste tray. Equilibrate the QIAGEN Genomic-tip with the volume of buffer described in the protocol. Flow begins automatically by reduction in surface tension due to the presence of detergent (0.15% Triton® X-100) in the equilibration buffer. Allow the QIAGEN Genomic-tip to drain completely. The flow of buffer will stop when the meniscus reaches the upper frit. The frit prevents the QIAGEN Genomic-tip from running dry, allowing it to be left unattended. Do not force out the remaining buffer, as this will necessitate restarting the flow with a syringe and adapter.

2. **Vortex the sample (from the last step of the specific sample preparation and lysis protocol) for 10 s at maximum speed and apply it to the equilibrated QIAGEN Genomic-tip. Allow it to enter the resin by gravity flow.**

   Vortexing the genomic DNA has a minimal effect on the size of the DNA, and it accelerates the QIAGEN procedure by eliminating poor flow rates associated with clogging. The average size of genomic DNA is reduced by only 10 kb when vortexed for up to 20 s (see Figure 3, page 10).

   The sample should be loaded onto the QIAGEN Genomic-tip promptly. If left too long, it may become cloudy due to further precipitation of protein. The sample must then be centrifuged or filtered before loading to prevent clogging of the QIAGEN Genomic-tip.

   Once the QIAGEN Genomic-tip is loaded with the clear and particle-free sample, flow will begin unassisted. Allow gravity to determine the flow rate. The flow rate will depend on the sample source, the number of cells from which the DNA sample was prepared, and on genome size.

   Particularly concentrated genomic DNA lysates may exhibit diminished flow rates due to increased viscosity. Flow can be assisted by the application of gentle positive pressure using a disposable syringe and the appropriate adapter. It may also be helpful to dilute the lysate with an equal volume of Buffer QBT prior to loading.

   When using positive pressure, do not allow the flow rate to exceed ■ 4–10 drops/min, ▲ 10–20 drops/min, or ● 20–40 drops/min.

   Optional: Take a ■ 300 µl, ▲ 300 µl, or ● 150 µl aliquot and save it for an analytical gel (aliquot 2).

3. **Wash the QIAGEN Genomic-tip with ■ 3 x 1 ml, ▲ 2 x 7.5 ml, or ● 2 x 15 ml of Buffer QC.**

   Allow Buffer QC to move through the QIAGEN Genomic-tip by gravity flow. Two washes are sufficient to remove all contaminants in the majority of DNA preparations.

   An additional wash is occasionally necessary if large culture volumes or bacterial strains containing large amounts of carbohydrate are used.

   It is particularly important not to force out residual Buffer QC. Traces of Buffer QC will not affect the elution step.

   Optional: Take a ■ 120 µl, ▲ 600 µl, or ● 300 µl aliquot and save it for an analytical gel (aliquot 3).

4. **Elute the genomic DNA with ■ 2 x 1 ml, ▲ 1 x 5 ml, or ● 1 x 15 ml of Buffer QF.**

   Place the QIAGEN Genomic-tip over a clean ■ 10 ml, ▲ 10 ml, or ● 30 ml collection tube. Use of polycarbonate centrifuge tubes is not recommended as polycarbonate is not resistant to the alcohol used in subsequent steps. Elute with the appropriate volume of Buffer QF, and collect the eluate. Flow begins automatically. Allow the QIAGEN Genomic-tip to drain by gravity flow.

   Use of Buffer QF prewarmed to 50°C will increase yields.

   Optional: Take a ■ 120 µl, ▲ 300 µl, or ● 600 µl aliquot and save it for an analytical gel (aliquot 4).

5. **Precipitate the DNA by adding ■ 1.4 ml, ▲ 3.5 ml, or ● 10.5 ml (0.7 volumes) room-temperature (15–25°C) isopropanol to the eluted DNA. Recover the precipitated DNA as described in step 5A or step 5B.**

5A. Precipitate the DNA by inverting the tube 10 to 20 times, and spool the DNA using a glass rod. Proceed with step 6A.

5B. Alternatively, mix and centrifuge immediately at >5000 x *g* for at least 15 min at 4°C. Carefully remove the supernatant. Proceed with step 6B.

All solutions should be at room temperature to minimize salt precipitation, although centrifugation is carried out at 4°C to prevent overheating of the sample. A centrifugal force of 5000 x *g* is the minimal force required for efficient precipitation. Higher *g*-force is recommended where possible. 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.

6. **Transfer material according to steps 6A and 6B.**

6A. Immediately transfer the spooled DNA to a microcentrifuge tube containing 0.1–2 ml of a suitable buffer (e.g., TE buffer, pH 8.0, or 10 mM Tris·Cl, pH 8.5). Dissolve the DNA overnight on a shaker or at 55°C for 1–2 h.

6B. Wash the centrifuged DNA pellet with ■ 1 ml, ▲ 2 ml, or ● 4 ml of cold 70% ethanol. Vortex briefly and centrifuge at >5000 x *g* for 10 min at 4°C. Carefully remove the supernatant without disturbing the pellet. Air-dry for 5–10 min, and resuspend the DNA in 0.1–2 ml of a suitable buffer (e.g., TE buffer, pH 8.0, or 10 mM Tris·Cl, pH 8.5). Dissolve the DNA overnight on a shaker or at 55°C for 1–2 h.

The 70% ethanol removes precipitated salt and replaces the isopropanol with the more volatile ethanol, making the DNA easier to redissolve. A second wash with cold 70% ethanol may improve results in more sensitive applications.

After careful and complete removal of the ethanol supernatant with a pipet, the pellet should be air-dried briefly before resuspending in a small volume of suitable buffer. Overdrying the pellet will make the DNA difficult to redissolve. Resuspend the DNA pellet by rinsing the walls to recover all the DNA, especially if glass tubes have been used. Pipetting the DNA up and down to promote resuspension may cause shearing and should be avoided. DNA dissolves best under slightly alkaline conditions (pH 8.0–8.5) and does not dissolve easily in acidic buffers.

Determination of yield, purity, and length of the DNA

Yields of genomic DNA will depend on the number of cells and the capacity of the QIAGEN Genomic-tip used. DNA yield is usually determined from the concentration of DNA in the eluate, measured by absorbance at 260 nm. To ensure accuracy, make sure the absorbance readings fall into the linear range of your method (e.g., between 0.1 and 1.0 for spectrophotometric OD). Sample dilution should be adjusted accordingly: for example, an eluate containing 25–50 ng of DNA/µl (A_{260} = 0.5–1) should not be diluted with more than 4 volumes of buffer. Use elution buffer or water (as appropriate) to dilute samples and to calibrate the spectrophotometer. Measure the absorbance at 260 nm and 280 nm, or scan absorbance from 220–320 nm. (A scan will show whether there are other factors affecting absorbance at 260 nm.) Readings on a spectrophotometer are not always accurate, particularly if a single wavelength measurement is taken rather than a scan, and should always be verified by visual examination of the DNA on an agarose gel. Fluorimetric measurements are more accurate and should be used if precise concentrations are needed.

Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. Pure DNA has an A_{260}/A_{280} ratio of 1.7–1.9.

The precise length of genomic DNA is determined by pulsed-field gel electrophoresis (PFGE) through an agarose gel. Standard PFGE conditions are as follows: 1.5% agarose gel in 0.5x TBE electrophoresis buffer; switch intervals, 1–10 s; run time, 16 h; voltage, 170 V.

Analytical gel

To analyze the purification procedure or to find where a problem may have occurred if yields are low, take a proportional aliquot of each of the samples marked in the specific protocol. Precipitate each of the aliquots 1–4 with 0.7 volumes of isopropanol. Rinse the pellets with 70% ethanol, drain well, and resuspend in 20 µl of TE, pH 8.0. Add the appropriate loading buffer, and use 10 µl of the samples for analysis on a 0.5% agarose gel. Run the gel until the bromophenol blue is near the bottom and stain it briefly in an ethidium bromide solution. An example of an analytical gel can be found in Figure 5.

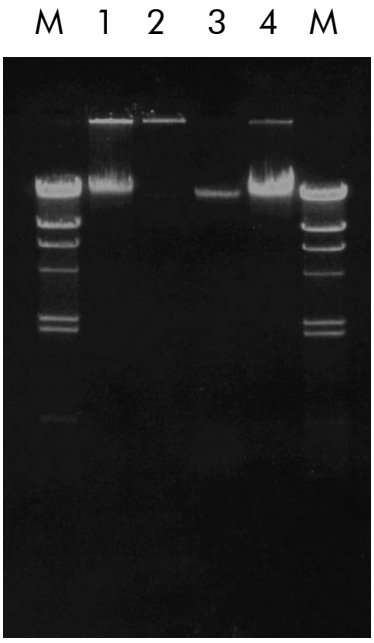


Figure 5. Agarose gel analysis of the genomic DNA purification procedure. 1: nuclear lysate; **2**: flow-through fraction; **3**: wash fraction; **4**: eluate containing pure genomic DNA. **M**: markers.

Special Applications

Purification of genomic DNA prepared by other methods*

Genomic DNA to be loaded on the QIAGEN Genomic-tip must be free of SDS and other anionic detergents and be particle-free to avoid clogging.

Adjust the sample to 750 mM NaCl; 50 mM MOPS, pH 7.0–7.5; and the volume to 1 ml (QIAGEN Genomic-tip 20/G), 5 ml (QIAGEN Genomic-tip 100/G), or 20 ml (QIAGEN Genomic-tip 500/G). If the sample volume is small enough, the conditions may be adjusted by adding 10 or more volumes of Buffer QBT.

Choose a QIAGEN Genomic-tip appropriate for the amount of DNA to be purified (see Table 1 (page 10), and follow the QIAGEN Genomic-tip procedure from step 1 (page 49). Note that alcohol precipitation of DNA is inefficient at concentrations of less than 1 µg/ml, so if low amounts of DNA are being purified, adjust the procedure accordingly.

Preparation of genomic DNA for PCR applications

Genomic DNA purified on QIAGEN Genomic-tips is of high molecular weight and is suitable for use in demanding procedures such as library construction, RFLP analysis, fingerprinting, other Southern blotting based techniques, and PCR.

In many cases, shorter genomic DNA fragments (1–50 kb) are more efficient targets for PCR amplification than longer fragments (>50 kb) (Figure 6, page 55). We therefore recommend shearing the genomic DNA by one of the following methods before adding it to amplification reactions.

- Move the genomic DNA several times through an 18-gauge syringe needle attached to a disposable 5 ml syringe.
- Vortex the genomic DNA with a small amount of ground glass or glass beads for 3–5 min at full speed. Do not carry over any of the glass into the amplification mix.
- Sonicate the genomic DNA for 30 s at level 1 with a Branson B-12 sonicator. **Note**: For rapid and simple preparation of small amounts of genomic DNA for subsequent PCR or Southern-blotting applications, we recommend use of QIAamp Kits.

With QIAamp Kits you can rapidly prepare genomic DNA up to 50 kb in length from blood, buffy coat, plasma, serum, bone marrow, mucus, other body fluids,

* For further information, contact QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

tissue, mouse tails, paraffin-embedded tissue, and cell suspensions using a simple centrifugation protocol. Viral RNA and DNA can also be efficiently purified from plasma and serum using QIAamp Kits.

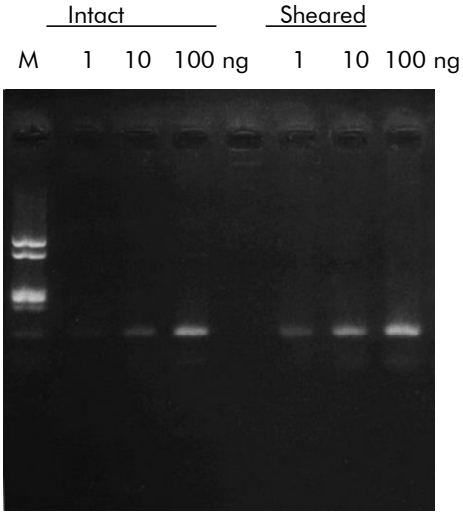


Figure 6. Amplification efficiency of intact or sheared or genomic DNA purified with the Blood & Cell Culture Kit. A 172-bp fragment was amplified from the indicated amounts of template DNA. **M**: marker.

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
Blood sample does not become translucent after addition of Buffer C1	
a) Frozen blood used	This is normal if frozen blood samples are used.
b) Buffer C1 and distilled water added incorrectly	Ensure that both Buffer C1 and distilled water were added to the sample in the correct 1:3 ratio in step 3 (page 23).
Yeast sample does not become clear after lysis in Buffer G2	
Yeast sample prepared	This is normal for yeast samples.
Bacterial sample does not become clear after lysis in Buffer B2	
a) Enzymes not added to sample	Ensure that both lysozyme and QIAGEN Protease (or QIAGEN Proteinase K) were added to the sample.
b) Low enzyme activity	Ensure that the aliquots of lysozyme and QIAGEN Protease (or QIAGEN Proteinase K) used are fresh and have not been repeatedly frozen and thawed. (See page 5 for storage conditions.)
Little or no yield of genomic DNA	
a) QIAGEN Genomic-tip overloaded	Low yields and impure DNA are in most cases attributable to overloading the system (starting with too many cells). Read the recommendations at the beginning of each protocol. Check that the QIAGEN Genomic-tip used is the correct size for the cell number in the sample (Table 1, page 10).

	Comments and suggestions
b) Frozen samples used	The DNA yield will decrease for samples stored at –20°C without previous treatment. If frozen samples from blood, cultured cells, or tissue have been used, read the section on storage of samples preceding each protocol (pages 21, 27, or 33, respectively).
c) Inappropriate salt or pH conditions in buffers	Ensure that any buffers prepared in the laboratory were prepared according to the instructions provided in Appendix A (page 62). If necessary, prepare fresh buffers.
d) Reason unknown	If fractions have been saved during the procedure, analysis by agarose-gel electrophoresis (as described on page 53) is the best way to determine at what stage the problem occurred. If the DNA is located in a particular fraction, it can generally be recovered by isopropanol precipitation. Agarose-gel analysis of an aliquot of the lysate is particularly important since this will show if lysis conditions were optimal.
No DNA in the lysate before loading	
a) Lysate prepared incorrectly	Check age of buffers. Check activity of enzymes. Ensure that any buffers prepared in the laboratory were prepared according to the instructions provided in Appendix A (page 62). If necessary, prepare fresh buffers.
b) Cell count in sample is extremely low	Check cell number in sample. Increase as necessary, but take care not to overload the QIAGEN Genomic-tip.
QIAGEN Genomic-tip blocked	
a) Lysate was not clear	Ensure that the lysate is clear before it is loaded onto the QIAGEN Genomic-tip. If necessary, incubate in lysis buffer and QIAGEN Protease for a longer time. Check *g*-force and centrifugation time, and re-centrifuge the lysate, if necessary. To clear a blocked tip, positive pressure may be applied, using a syringe fitted with an adapter (e.g., a rubber stopper with a hole). Do not exceed recommended flow rates (page 50).

	Comments and suggestions
b) Too much sample processed	Count cells or determine titer before starting the experiment. Thoroughly read the recommendations on sample preparation at the beginning of each protocol. Check that the QIAGEN Genomic-tip used is the correct size for the cell number in the sample (Table 1, page 10). Do not exceed the maximum capacity of the QIAGEN Genomic-tip used.

DNA found in the flow-through fraction

a) QIAGEN Genomic-tip overloaded with DNA	Check the final yield against the maximum capacity of the QIAGEN Genomic-tip. Recover DNA by precipitation, and purify on a new QIAGEN Genomic-tip.
b) SDS (or other ionic detergent) in lysate	If not using the QIAGEN protocol, ensure that anionic detergents are removed by precipitation with potassium acetate before applying the DNA sample to the QIAGEN Genomic-tip. Recover DNA by precipitation, and purify on a new QIAGEN Genomic-tip.
c) pH conditions in buffers	Inappropriate salt or Check the pH of the lysate, which should be around pH 7.0. Ensure that any buffers prepared in the laboratory were prepared according to the instructions provided in Appendix A (page 63). If necessary, prepare fresh buffers.
d) Flow of the QIAGEN Genomic-tip was uneven	Ensure that the QIAGEN Genomic-tips are stored at room temperature (15–25°C). 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 QIAGEN Genomic-tip before use.

DNA found in the wash fraction

a) QIAGEN Genomic-tip was overloaded with DNA	Check the final yield against the maximum capacity of the QIAGEN Genomic-tip. Recover DNA by precipitation, and purify on a new QIAGEN Genomic-tip.

	Comments and suggestions
b) Wash buffer was incorrect	Check the pH and salt concentration of Buffer QC. Recover DNA by precipitation, and purify on a new QIAGEN Genomic-tip.
No DNA in eluate	
a) No DNA in the lysate before loading	Check "No DNA in the lysate before loading" above for possible reasons.
b) DNA passed through in the flow-through or wash fraction	Check "DNA found in the flow-through fraction" and "DNA found in the wash fraction" above for possible reasons.
c) Elution buffer was incorrect	Check the pH and salt concentration of Buffer QF. Recover DNA by eluting with fresh buffer.
d) DNA not eluted efficiently	To increase elution efficiency, prewarm Buffer QF to 50°C.
Little or no DNA upon precipitation	
a) DNA failed to precipitate	Ensure that the precipitate is centrifuged at >5000 x *g* for at least 15 minutes. Recover DNA by centrifuging longer at higher speeds. Try another isopropanol batch, if necessary.
b) DNA too dilute to precipitate efficiently	When DNA concentration in eluate is expected to be less than 1 µg/ml, it is recommended to use a carrier such as glycogen.
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. Decant the supernatant carefully so as not to disturb the pellet.
d) DNA was poorly resuspended	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.

	Comments and suggestions
DNA is difficult to redissolve	
a) DNA pellet was overdried	Air-dry the pellet instead of using a vacuum. High-molecular- weight DNA is very difficult to redissolve when overdried. Redissolve the DNA by warming the solution to 55°C and giving it more time to redissolve. Increase the buffer volume if necessary.
b) Residual isopropanol in pellet	Ensure that pellets are washed with 70% ethanol to remove traces of isopropanol. Redissolve the DNA by warming the solution to 55°C and giving it more time to redissolve. Increase buffer volume if necessary.
c) Too much salt in pellet	Ensure that the isopropanol is at room temperature (15–25°C) for precipitation and that the pellet is washed twice with cold 70% ethanol. Recover the DNA by increasing the volume of buffer used for redissolving.
d) Buffer pH too low	DNA does not dissolve well in acidic solutions. Ensure that the pH of the buffer used for redissolving is ≥8.0.
DNA has a low size distribution	
a) Yeast sample prepared	Yeast genomic DNA has a lower size distribution than genomic DNA of higher eukaryotes. The size of genomic DNA from yeast prepared with QIAGEN Genomic-tips does not exceed 100 kb.
b) Cell pellet stored for excessive time	Process the sample promptly. For blood, cell-culture, or tissue samples, read the section on storage of samples preceding each protocol (pages 21, 27, or 33, respectively).
c) Nuclease contamination	Check buffers for nuclease contamination and replace if necessary. Use sterilized glassware and plasticware, and wear gloves.
d) DNA poorly buffered	Redissolve DNA in TE, pH 8.0, to inhibit nuclease activity and maintain stable pH during storage.
e) DNA has been sheared during preparation	Redissolve DNA gently, without vortexing or vigorous pipetting. Avoid using small pipet tips.

	Comments and suggestions
DNA does not perform well	
a) Too much salt in DNA pellet	Ensure that the isopropanol is at room temperature (15–25°C) for precipitation and that the pellet is washed twice with cold 70% ethanol. Re-precipitate the DNA to remove the salt.
b) DNA is not pure	Count cells or determine titer before starting the experiment. Thoroughly read the recommendations on sample preparation at the beginning of each protocol. Check that the QIAGEN Genomic-tip used is the correct size for the cell number in the sample (Table 1, page 10). Do not exceed the maximum capacity of the QIAGEN Genomic-tip used.

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.

Appendix A: Buffers, media and stock solutions

Table 11. Buffer composition and storage

Buffer	Composition	Storage
Buffer B1 (bacterial lysis buffer)	50 mM Tris·Cl, pH 8.0; 50 mM EDTA, pH 8.0; 0.5% Tween®-20; 0.5% Triton X-100	2–8°C or RT
Buffer B2 (bacterial lysis buffer)	3 M guanidine HCl; 20% Tween-20	2–8°C or RT
Buffer C1 (cell lysis buffer)	1.28 M sucrose; 40 mM Tris·Cl, pH 7.5; 20 mM $MgCl_2$; 4% Triton X-100	2–8°C
Buffer G2* (digestion buffer)	800 mM guanidine HCl; 30 mM Tris·Cl, pH 8.0; 30 mM EDTA, pH 8.0; 5% Tween-20; 0.5% Triton X-100	2–8°C or RT
Buffer Y1 (yeast lysis buffer)	1 M sorbitol; 100 mM EDTA; 14 mM β-mercaptoethanol	2–8°C
Buffer QBT (equilibration buffer)	750 mM NaCl; 50 mM MOPS, pH 7.0; 15% isopropanol, 0.15% Triton X-100	2–8°C or RT
Buffer QC (wash buffer)	1.0 M NaCl; 50 mM MOPS, pH 7.0; 15% isopropanol	2–8°C or RT
Buffer QF (elution buffer)	1.25 M NaCl; 50 mM Tris·Cl, pH 8.5; 15% isopropanol	2–8°C or RT
PBS	Standard phosphate-buffered saline, pH 7.4	RT
TE	10 mM Tris·Cl, pH 8.0; 1 mM EDTA, pH 8.0	RT
Culture media		
LB (bacterial culture medium)	10 g/liter tryptone; 5 g/liter yeast extract; 1 g/liter NaCl; pH 7.0	RT
YPD (yeast culture medium)	10 g/liter yeast extract; 20 g/liter peptone; 20 g/liter dextrose	RT

* Not compatible with disinfection reagents containing bleach. Contains guanidine HCl, which is an irritant. Take appropriate safety measures, and wear gloves when handling.

RT: room temperature (15–25°C).

Table 12. Enzyme information

Enzyme	Provider	Storage
QIAGEN Protease	See ordering information on page 69	15°–25°C
QIAGEN Proteinase K	See ordering information on page 69	2–8°C or room temperature (15–25°C)
Lysozyme or lysostaphin	Sigma Chemicals, SERVA/Boehringer Ingelheim Bioproducts*	–20°C
Lyticase or zymolase	Sigma Chemicals*	–20°C

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

Preparation of buffers

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

Buffer C1 and Buffer Y1 should always be stored at 2–8°C. All other buffers may be stored at either 2–8°C or at room temperature (15–25°C). Long-term storage at 2–8°C is recommended. All buffers except Buffer C1 must be equilibrated to room temperature before use.

B1: Dissolve 18.61 g $Na_2EDTA{\cdot}2H_2O$ and 6.06 g Tris base in 800 ml distilled water. Add 50 ml 10% Tween-20 solution and 50 ml 10% Triton X-100 solution. Adjust the pH to 8.0 with HCl. Adjust the volume to 1 liter with distilled water.

B2: Dissolve 286.59 g guanidine HCl in 700 ml distilled water. Add 200 ml of 100% Tween-20. Adjust the volume to 1 liter with distilled water. pH does not need to be adjusted.

C1: Dissolve 438.14 g sucrose, 4.06 g $MgCl_2{\cdot}6H_2O$, and 4.84 g Tris base in 680 ml distilled water. Add 42 g Triton X-100 (100%). Adjust the pH to 7.5 with HCl. Adjust the volume to 1 liter with distilled water.

G2: Dissolve 76.42 g guanidine HCl, 11.17 g $Na_2EDTA{\cdot}2H_2O$, and 3.63 g Tris base in 600 ml distilled water. Add 250 ml 20% Tween-20 solution and 50 ml 10% Triton X-100 solution. Adjust the pH to 8.0 with NaOH. Adjust the volume to 1 liter with distilled water.

Y1: Dissolve 182.2 g sorbitol in 600 ml distilled water. Add 200 ml of a 0.5 M Na_2EDTA (pH 8.0) solution. Add 1 ml of β-mercaptoethanol (14.3 M). Adjust the volume to 1 liter with distilled water. pH does not need to be adjusted.

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

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

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

Preparation of culture media

YPD: Dissolve 10 g yeast extract and 20 g peptone in 800 ml distilled water. Adjust the volume to 900 ml with distilled water. Dissolve 20 g dextrose in 80 ml distilled water, and adjust the volume to 100 ml. Sterilize both solutions promptly by autoclaving. Mix the two solutions before use. (**Note**: Although the dextrose may be added before autoclaving, it is recommended to prepare a separate solution which is added to the other ingredients after autoclaving. This will prevent darkening of the media and will promote optimal growth.)

LB: 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 promptly by autoclaving.

Preparation of enzyme stock solutions

QIAGEN Protease provided in the Blood & Cell Culture DNA Kit should be carefully dissolved in 1.4 ml of distilled water per vial. QIAGEN Protease may also be purchased separately (cat. nos. 19155 and 19157). QIAGEN Protease in solution must be stored at 2–8°C and is stable for 2–3 months. Storage at –20°C will prolong the lifetime of the QIAGEN Protease to one year, but repeated freezing and thawing should be avoided.

QIAGEN Proteinase K may be purchased from QIAGEN in 2 ml or 10 ml solutions (cat. nos. 19131 and 19133). QIAGEN Proteinase K solution is at the proper concentration to be used directly in the QIAGEN Genomic DNA protocols and is stable at room temperature (15–25°C) for at least one year. For longer-term storage, we recommend storing at 2–8°C. If using proteinase K from another supplier, a 20 mg/ml stock solution in distilled water is generally recommended.

Before use, RNase A should be added to Buffer G2 (or Buffer B1 for bacterial DNA isolation) to a concentration of 200 µg/ml and should then be stable for 6 months when stored at 2–8°C.

Lyticase should be dissolved in distilled water to a concentration of 1000 U/ml and stored at –20°C. For efficient lysis of some yeast species, zymolase is the enzyme of choice. Use the appropriate enzyme for the particular species. Dissolved lyticase or zymolase must be stored at –20°C, but repeated freezing and thawing should be avoided. Dividing the enzyme solutions into aliquots is recommended. Lyticase or zymolase can be purchased from Sigma Chemicals, Medac Diagnostika, or Seikagaku Corporation*.

Lysozyme should be dissolved in distilled water to a concentration of 100 mg/ml and stored at –20°C. For efficient lysis of some bacterial species, enzymes other than lysozyme may be necessary. Lysis of *Staphylococcus* spp., for example, is much more efficient with lysostaphin. Use the appropriate enzyme for the particular species.

Dissolved lysozyme or lysostaphin must be stored at –20°C, but repeated freezing and thawing should be avoided. Dividing the enzyme solutions into aliquots is recommended. Lysozyme or lysostaphin can be purchased from Sigma Chemicals, or SERVA/Boehringer Ingelheim Bioproducts.*

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

Appendix B: General information about QIAGEN Anion-Exchange Resin

QIAGEN Genomic-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 also not required.

DNA 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 (Figure 7). 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 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. DNA remains tightly bound to the DEAE groups over a wide range of salt concentrations (Figure 8, page 24). Impurities such as RNA, protein, carbohydrates, and small metabolites are washed from QIAGEN resin with medium-salt buffers, while DNA remains bound until eluted with a high-salt buffer.

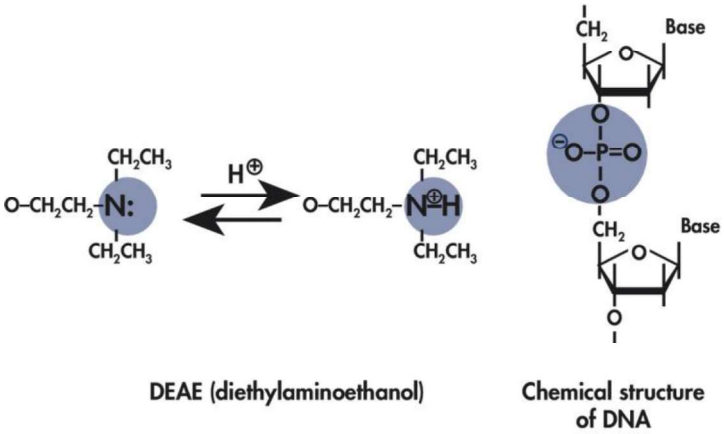


Figure 7. Chemical structure of positively charged DEAE groups of QIAGEN resin, and negatively charged groups of the DNA backbone which interact with the resin.

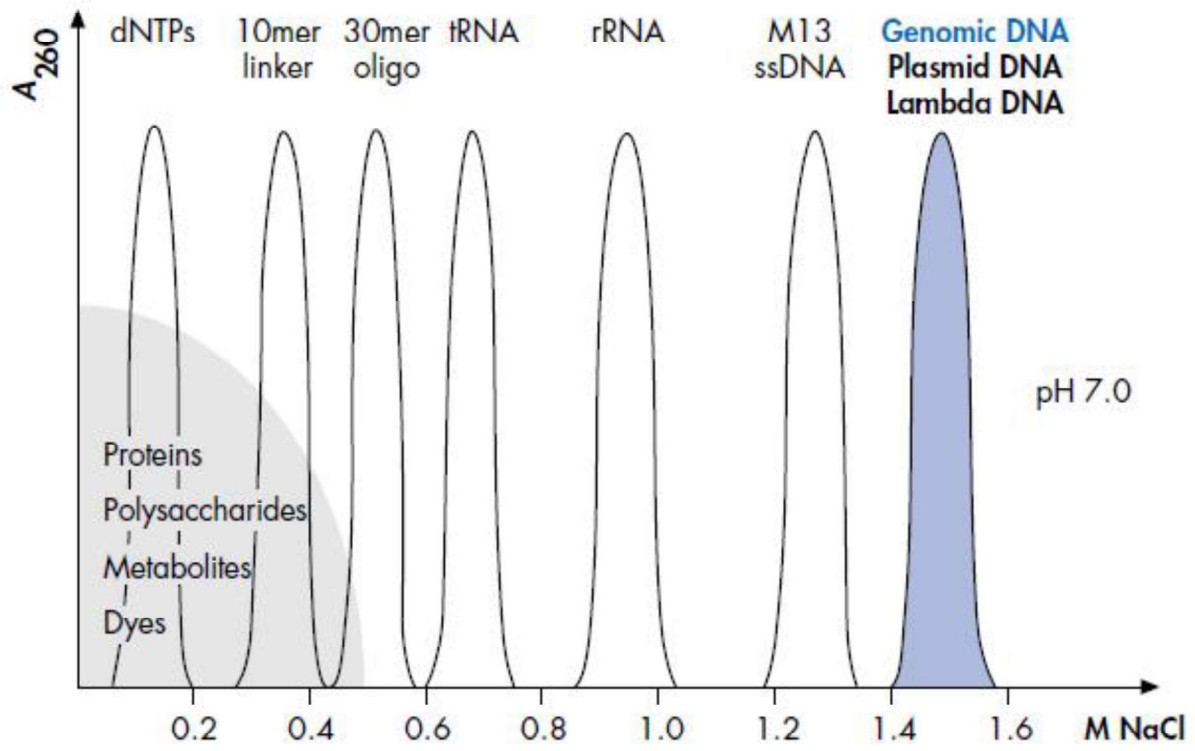


Figure 8. Separation of nucleic acids at neutral pH on QIAGEN resin.

The separation range of QIAGEN resin is extremely broad, extending from 0.1 M to 1.6 M salt (Figure 9, page 68), and DNA can be efficiently separated form 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. The separation and purification qualities of QIAGEN resin, as well as its ease of use, thus exceed those of conventional anion-exchange resins.

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 Genomic-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 2x CsCl-prepared DNA. Subsequent procedures such as transfection, transformation, sequencing, cloning, and in vitro transcription and translation proceed with optimal efficiency.

Capacity and recovery

The names of the different QIAGEN Genomic-tips indicate the binding capacities (in µg) of the columns for double-stranded DNA, as determined with purified genomic DNA. QIAGEN Genomic-tip 100/G, for example, has a binding capacity of 100 µg of DNA.

Stability

QIAGEN resin is stable for up to six hours after equilibration. The separation characteristics of the resin then begin to change, and it is no longer effective.

QIAGEN Genomic-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 9 (page 68) 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 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, 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.

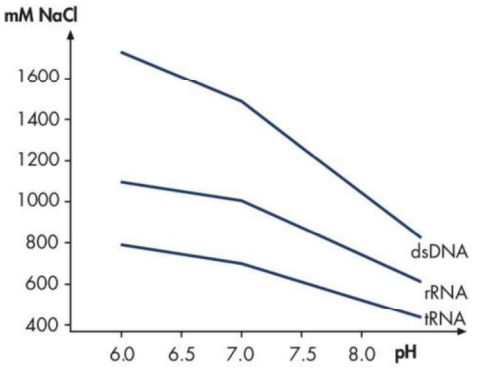


Figure 9. Elution points of different nucleic acids from QIAGEN resin as a function of pH.

Ordering Information

Product	Contents	Cat. no.
Blood & Cell Culture DNA Kits — for preparation of genomic DNA from blood and cultured cells		
Blood & Cell Culture DNA Mini Kit (25)	25 QIAGEN Genomic-tip 20/G, QIAGEN Protease, Buffers	13323
Blood & Cell Culture DNA Midi Kit (25)	25 QIAGEN Genomic-tip 100/G, QIAGEN Protease, Buffers	13343
Blood & Cell Culture DNA Maxi Kit (10)	10 QIAGEN Genomic-tip 500/G, QIAGEN Protease, Buffers	13362
QIAGEN Genomic-tips — for preparation of genomic DNA from blood, cultured cells, tissue, mouse tails, yeast, Gram-negative, and some Gram-positive bacteria		
QIAGEN Genomic-tip 20/G	25 columns	10223
QIAGEN Genomic-tip 100/G	25 columns	10243
QIAGEN Genomic-tip 500/G	10 columns	10262
Accessories		
Genomic DNA Buffer Set	Buffers, including specific lysis buffers for yeast, bacteria, cells, blood, and tissue: Y1, B1, B2, C1, G2, QBT, QC, QF; for 75 mini-, 25 midi-, or 10 maxipreps	19060
QIAGEN Protease (7.5 AU)	7.5 Anson units per vial (lyophilized)	19155
QIAGEN Protease (30 AU)	4 x 7.5 Anson units per vial (lyophilized)	19157
QIAGEN Proteinase K (2 ml)	2 ml (>600 mAU/ml, solution)	19131
QIAGEN Proteinase K (10 ml)	10 ml (>600 mAU/ml, solution)	19133

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.

Limited License Agreement for Blood & Cell Culture DNA Kits, Genomic DNA Buffer Set, and QIAGEN Genomic-tips

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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

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