January 2022

Puregene® DNA Handbook

For purification of archive-quality DNA from
- human whole blood
- bone marrow
- buffy coat
- body fluids
- cultured cells
- tissue

Sample to Insight

QIAGEN

Contents

Kit Contents

Puregene Blood Kit	**(120 ml)**	**(1000 ml)**
Catalog no.	**158023**	**158026**
Volume of blood processed per kit	**120 ml**	**1000 ml**
RBC Lysis Solution	450 ml	3000 ml
Cell Lysis Solution	125 ml	1000 ml
Protein Precipitation Solution	50 ml	350 ml
DNA Hydration Solution	100 ml	500 ml
RNase A Solution	650 µl	5 ml

Puregene Cell Kit	**(8 x 10^8)**	**(6.7 x 10^9)**
Catalog no.	**158043**	**158046**
Number of cells processed per kit	**8 x 10^8**	**6.7 x 10^9**
Cell Lysis Solution	125 ml	1000 ml
Protein Precipitation Solution	50 ml	350 ml
DNA Hydration Solution	100 ml	500 ml
RNase A Solution	650 µl	5 ml

Puregene Tissue Kit	**(4 g)**	**(33 g)**
Catalog no.	**158063**	**158066**
Amount of tissue processed per kit	**4 g**	**33 g**
Cell Lysis Solution	125 ml	1000 ml
Protein Precipitation Solution	50 ml	350 ml
DNA Hydration Solution	100 ml	500 ml
RNase A Solution	650 µl	5 ml
Proteinase K	650 µl	5 ml

Storage

The Puregene kit buffers and reagents must be stored dry at the temperature indicated on the kit label. RNase A Solution and Glycogen Solution must be refrigerated or frozen at the temperatures marked on the labels. All other reagents can be stored at room temperature (15–25°C). When stored at the indicated temperatures, the Puregene kits are stable until the expiration date printed on the label and on the kit box.

Intended Use

The Puregene kits 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.

Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding Puregene kits or QIAGEN products, in general, please do not hesitate to contact us.

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

For technical assistance and more information, please see our Technical Support center at **www.qiagen.com/Support** or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit **www.qiagen.com**).

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.

Quality Control

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

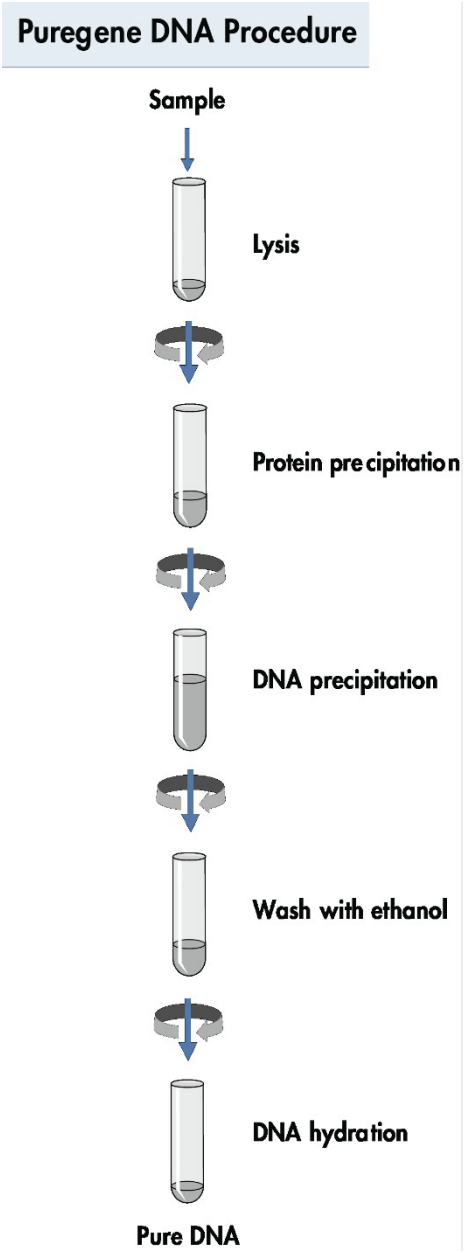
Introduction

The Puregene kits are designed for purification of high-molecular-weight genomic, mitochondrial, or viral DNA from a variety of sample sources. High-quality DNA can be purified from sample types including whole blood, buffy coat, bone marrow, body fluids, cultured cells, animal tissues, Gram-negative bacteria, Gram-positive bacteria, and yeast in as little as 25 minutes. The convenient, scalable purification procedure (see the flowchart on page 9) removes contaminants and enzyme inhibitors such as proteins and divalent cations, and purified DNA is ready for immediate use in sensitive downstream applications or for archiving.

Principle and procedure

Cells are lysed with an anionic detergent in the presence of a DNA stabilizer. The DNA stabilizer limits the activity of intracellular DNases and DNases found elsewhere in the environment. RNA is then removed by treatment with an RNA digesting enzyme. Other contaminants, such as proteins, are removed by salt precipitation. Finally, the genomic DNA is recovered by precipitation with alcohol and dissolved in hydration solution (1 mM EDTA, 10 mM Tris·Cl pH 7.5). Purified DNA typically has an A_{260}/A_{280} ratio between 1.7 and 1.9 and is up to 200 kb in size. The DNA can be safely stored at 2–8, –20, or –80°C.

Supplementary protocols for processing additional sample types or amounts are available at **www.qiagen.com/puregene-kits-SuppProt** or from QIAGEN Technical Services.


Puregene DNA Procedure
Sample
Lysis
Protein precipitation
DNA precipitation
Wash with ethanol
DNA hydration
Pure DNA

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 protocols

- Isopropanol, 100%
- Ethanol, 70%*
- Microcentrifuge tubes (1.5 ml), 15 ml centrifuge tubes, or 50 ml centrifuge tubes
- Pipets and pipet tips
- Vortexer
- Standard laboratory centrifuge or microcentrifuge
- Water baths
- Crushed ice

For compromised blood and samples with low expected yields

- Glycogen Solution, cat. no. 158930 or 949002 and 158183 or 158186

For buccal swabs

- Buccal swabs, mouth wash, scissors, or razor blade

For body fluid and buccal cell samples

- **Optional**: Proteinase K, cat. no. 158918 or 158920 and 158143 or 158146

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

For tissue samples

- Liquid nitrogen
- Mortar and pestle

For tissue in fixative or FFPE tissues

- Xylene
- Microcentrifuge tube pestle

For yeast and gram-positive bacterial cells

- Cell resuspension buffer, for example, Tris /EDTA buffer, pH 7.7
- Lytic enzyme solution, for example, approx.4–5 kU /ml in 30% glycerol.

Important Notes

Storage of blood samples

Whole blood samples treated with EDTA, citrate, or heparin can be used and may be either fresh or frozen. Yield and quality of the purified DNA depend on the storage conditions of the blood. Fresher blood samples yield better results.

For short-term storage, collect blood in tubes containing EDTA, as an anticoagulant, and store the tubes at room temperature (15–25°C) for 1 day or at 4°C for up to 5 days. For applications requiring maximum fragment size, such as Southern blotting, we recommend storage at 2–8°C for up to 3 days only, as low levels of DNA degradation will occur after this time.

For long-term storage, collect blood in tubes containing a standard anticoagulant (preferably EDTA when high-molecular-weight DNA is required), and store at –80°C.

Preparation of buffy coat

Buffy coat is a leukocyte-enriched fraction of whole blood. The efficiency of leukocyte enrichment depends on the procedure used to prepare buffy coat and on the accuracy with which the buffy coat layer is extracted. Prepare buffy coat by centrifuging whole blood samples containing a standard anticoagulant (EDTA, citrate, or heparin) at 2500 x *g* for 10 minutes at room temperature (15–25°C). After centrifugation,

Three different fractions are distinguishable: the upper clear layer is plasma; the intermediate layer is buffy coat, containing concentrated leukocytes; and the bottom layer contains concentrated erythrocytes. Approximately 1 ml leukocyte-containing fraction should be harvested from 10 ml centrifuged whole blood, which gives 10x enrichment. To avoid overloading the DNA purification procedure, do not prepare buffy coat samples of >10x enrichment. If buffy coat samples are of >10x enrichment, use less starting material in the DNA purification procedure.

Yield and quality of purified DNA

The Puregene DNA procedure yields pure DNA, indicated by A_{260}/A_{280} ratios greater than 1.7. The purified DNA is greater than 50 kb in size, typically in the range of 100–200 kb. DNA of this length and purity is suitable for archiving as well as for immediate use in all downstream applications.

The Puregene kits are designed to purify high yields of high-quality DNA. Table 1 lists typical DNA yields from a variety of sample sources. The actual yield obtained will depend on the sample type, genome size of the source organism, and number of cells in the sample. Yield will also depend on the quality of the starting material.

Table 1. Typical DNA yields from a variety of sample types

Sample type and size	Range of expected yields (µg)	Average yield (µg)
Whole blood, 1 ml (7 x 10^6 white cells)	16–50	35
Buccal swabs, 1 swab	0.2–2	1
Body fluids, 1 ml	2–50	25
Cultured cells, 1–2 x 10^6 cells	5–10	7
Solid animal tissue, 10 mg	5–100	50

Optimized handling for higher throughputs

The following points may help with high-throughput sample processing using the Puregene kits.

- Up to 24 fresh whole blood samples can be processed at a time using 24-tube racks. In a typical working day, up to 5 racks (120 samples) can be processed.
- Use dedicated centrifuges with 24-tube rotors.
- Pre-aliquot RBC Lysis Solution and isopropanol on a weekly basis.
- To save time labeling tubes, use removable labels (preferably computer generated) and transfer the label from the lysis tube to the tube used for DNA precipitation. Or prepare two labels: one for each tube.
- Vortex for 10 s at high speed after adding Cell Lysis Solution instead of pipetting up and down to lyse the cells.
- Omit the RNase A Solution treatment step. Alternatively, add RNase A Solution to Cell Lysis Solution before beginning the purification procedure. RNase A Solution is stable in Cell Lysis Solution for at least 8 weeks at room temperature (15–25°C).
- Use a multi-tube vortexer or a vortexer equipped with a platform head.
- Place samples in a rack after adding RBC Lysis Solution, isopropanol, or DNA Hydration Solution and invert the rack rather than inverting each tube individually.
- After the DNA is dissolved in DNA Hydration Solution, transfer DNA to storage tubes labeled with small, computer-generated labels.

Processing large-volume samples

Table 2, Table 3, Table 4, and Table 5 provide information about scaling the Puregene purification procedure for use with larger amounts of starting material.

Table 2. Reagent volumes for scaling whole blood and buffy coat protocols

	Number of white blood cells ($x10^6$)*				
	1.4	**3.5**	**7**	**35**	**70**
Blood volume for preparing buffy coat (µl)	200	500	1000	5000	10,000
Tube size (ml)	1.5	1.5	15	50	50
RBC Lysis Solution (µl)†	3 volumes				
Cell Lysis Solution (µl)	200	500	1000	5000	10,000
RNase A Solution (µl)	1	2.5	5	25	50
Protein Precipitation Solution (µl)	67	167	333	1670	3330
Isopropanol (µl)‡	200	500	1000	5000	10,000
70 % ethanol (µl)	200	500	1000	5000	10,000
DNA Hydration Solution (µl)§	100	100	100	500	1000
Typical DNA yield (µg)¶	7	17.5	35	175	350

* Cell number estimates assume an average of 7 x 10^6 white blood cells per milliliter of whole blood.

† To lyse residual red blood cells in the buffy coat sample, use 3 volumes of RBC Lysis Solution for every 1 volume of buffy coat. For example, 3 ml of RBC Lysis Solution should be used to lyse red blood cells in 1 ml of buffy coat.

‡ If the DNA yield is expected to be low (e.g., if the number of cells in the sample is low) add a DNA carrier, such as glycogen, to the isopropanol during DNA precipitation. Add 1 µl Glycogen Solution (cat. no. 158930 or 949002 and 158183 or 158186) per 600 µl isopropanol.

§ The volume of DNA Hydration Solution used can be increased or decreased for lower or higher DNA concentrations, respectively.

¶ DNA yield assumes 6 pg DNA per human diploid cell and 80% recovery.

Table 3. Reagent volumes for scaling cultured-cell protocols

	Number of cells*		
	0.5–1 x 10^6	**3–5 x 10^6**	**3–5 x 10^7**
Tube size (ml)	1.5	1.5	15
Cell Lysis Solution (µl)	150	600	6000
RNase A Solution (µl)	0.75	3	30
Protein Precipitation Solution (µl)	50	200	2000
Isopropanol (µl)†	150	600	6000
Ethanol, 70% (µl)	150	600	6000
DNA Hydration Solution (µl)‡	50	200	500
Typical DNA yield (µg)§	2–6	15–30	80–300

* The number of cells may be determined by counting with a hemacytometer or other cell counter.

† If the DNA yield is expected to be low (e.g., if the number of cells in the sample is low) add a DNA carrier, such as glycogen, to the isopropanol during DNA precipitation. Add 1 µl Glycogen Solution (cat. no. 158930 or 949002 and 158183 or 158186) per 600 µl isopropanol.

‡ The volume of DNA Hydration Solution used can be increased or decreased for lower or higher DNA concentrations, respectively.

§ DNA yield assumes 6 pg DNA per human diploid cell and 80% recovery.

Table 4. Reagent volumes for scaling body fluid protocols

	Volume of body fluid (ml)			
	0.1	**0.2**	**0.5**	**3**
Tube size (ml)	1.5	15	15	50
Cell Lysis Solution (ml)	0.5	1	2.5	15
RNase A Solution (µl)	3	6	15	90
Protein Precipitation Solution (µl)	200	400	1000	6000
Isopropanol (ml)*	0.6	1.2	3	18
70% ethanol (ml)	0.6	1.2	3	18
DNA Hydration Solution (µl)	100	100	100	200

* If the DNA yield is expected to be low (e.g., if the number of cells in the sample is low) add a DNA carrier, such as glycogen, to the isopropanol during DNA precipitation. Add 1 µl Glycogen Solution (cat. no. 158930 or 949002 and 158183 or 158186) per 600 µl isopropanol.

Table 5. Reagent volumes for scaling tissue protocols

	Weight of tissue (mg)			
	0.5–2.0	**10–20**	**25**	**100–200**
Tube size (ml)	1.5	1.5	2	15
Cell Lysis Solution (µl)	100	600	750	6000
RNase A Solution (µl)	0.5	3	3.75	30
Protein Precipitation Solution (µl)	33	200	250	2000
Isopropanol (µl)*	100	600	750	6000
70% ethanol (µl)	100	600	750	6000
DNA Hydration Solution (µl)†	100	100	200	500
Typical range of DNA yields (µg)‡	0.3–8	5–80	12–100	50–800

* If the DNA yield is expected to be low (e.g., if the number of cells in the sample is low) add a DNA carrier, such as glycogen, to the isopropanol during DNA precipitation. Add 1 µl Glycogen Solution (cat. no. 158930 or 949002 and 158183 or 158186) per 600 µl isopropanol.

† The volume of DNA Hydration Solution used can be increased or decreased for lower or higher DNA concentrations, respectively.

‡ The expected DNA yield range is based on average yields obtained from a variety of tissues. The yield of DNA may vary considerably depending on the tissue type.

Enhanced productivity protocols

Two protocols, the standard protocol and the enhanced productivity protocol, are provided for purification of DNA from 10 ml whole blood. The enhanced productivity protocols can be scaled for other sample sizes. Contact QIAGEN Technical Services for more information (see back cover or visit **www.qiagen.com**).

The criteria below allow you to determine the best protocol for optimal results from your samples and for your workflow needs.

The standard protocol is optimized for:

- Workflows where it is convenient to stop after the addition of Cell Lysis Solution. Samples are stable in Cell Lysis Solution for at least 2 years at room temperature (15–25°C).
- Samples that have been subjected to unknown storage conditions.

The enhanced productivity protocol is optimized for:

- Blood samples that have been stored under optimal conditions. For example, no more than 1 day at room temperature (15–25°C) or 5 days at 4°C or frozen at –80°C for less than 2 years.
- Workflows that require a large number of samples to be processed.
- Workflows where it is not necessary to stop after addition of Cell Lysis Solution.
- Rapid purification of DNA from large blood samples; DNA can be purified from 16 samples in less than 1 hour with the enhanced productivity protocol and large-volume processing (see "Optimized handling for higher throughputs" on page 14).

Protocol: DNA Purification from Whole Blood or Bone Marrow Using the Puregene Blood Kit

This protocol is for purification of genomic DNA from fresh or frozen samples of 300 µl, 3 ml, or 10 ml whole blood using the Puregene Blood Kit. The protocol can also be used for DNA purification from packed cells, buffy coat, or bone marrow.

Important points before starting

- In some steps of the procedure, one of three choices can be made. Choose ■ if processing 300 µl blood samples; choose ▲ if processing 3 ml blood samples; choose ● if processing 10 ml blood samples.
- If processing packed cells or buffy coat samples, directly scale the volume of reagents used in proportion to the volume of the original blood sample (e.g., if a 1 ml buffy coat sample was prepared from 10 ml whole blood, use the volumes of reagents given for 10 ml blood.
- Bone marrow samples may contain more white blood cells than a whole blood sample. After addition of Cell Lysis Solution, make sure that the solution is homogeneous. If the solution is not homogenous, add additional Cell Lysis Solution and scale up the volumes of the other reagents used in the protocol accordingly.
- Frozen blood and bone marrow samples should be thawed quickly in a 37°C water bath with mild agitation and stored on ice before beginning the procedure.

Things to do before starting

- Preheat water bath to 65°C for use step 19 of the procedure.
- **Optional**: Preheat water bath to 37°C for use in step 8 of the procedure.

Procedure

1. Dispense ■ 900 µl, ▲ 9 ml, or ● 30 ml RBC Lysis Solution into a ■ 1.5 ml microcentrifuge tube, ▲ 15 ml centrifuge tube, or ● 50 ml centrifuge tube.
2. Add ■ 300 µl, ▲ 3 ml, or ● 10 ml whole blood or bone marrow, and mix by inverting 10 times.
3. Incubate for ■ 1 min, ▲ 5 min, or ● 5 min at room temperature (15–25°C). Invert at least once during the incubation.

   ■ For fresh blood (collected within 1 h before starting the protocol), increase incubation time to 3 min to ensure complete red blood cell lysis.
4. Centrifuge for ■ 20 s at 13,000–16,000 x *g*, ▲ 2 min at 2000 x *g*, or ● 2 min at 2000 x *g* to pellet the white blood cells.
5. Carefully discard the supernatant by pipetting or pouring, leaving approximately ■ 10 µl, ▲ 200 µl, or ● 200 µl of the residual liquid and the white blood cell pellet.
6. Vortex the tube vigorously to resuspend the pellet in the residual liquid.

   Vortexing greatly facilitates cell lysis in the next step.

   The pellet should be completely dispersed after vortexing.
7. Add ■ 300 µl, ▲ 3 ml, or ● 10 ml Cell Lysis Solution, and pipet up and down to lyse the cells or vortex vigorously for 10 s.

   Usually no incubation is required; however, if cell clumps are visible, incubate at 37°C until the solution is homogeneous.

   Samples are stable in Cell Lysis Solution for at least 2 years at room temperature.
8. **Optional**: If RNA-free DNA is required, add ■ 1.5 µl, ▲ 15 µl, or ● 50 µl RNase A Solution, and mix by inverting 25 times. Incubate for 15 min at 37°C. Then incubate for ■ 1 min, ▲ 3 min, or ● 3 min on ice to quickly cool the sample.
9. Add ■ 100 µl, ▲ 1 ml, or ● 3.33 ml Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.
10. Centrifuge for ■ 1 min at 13,000–16,000 x *g*, ▲ 5 min at 2000 x *g*, or ● 5 min at 2000 x *g*.

The precipitated proteins should form a tight, dark brown pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation.

11. Pipet ■ 300 µl isopropanol into a clean 1.5 ml tube, ▲ 3 ml isopropanol into a clean 15 ml tube, or ● 10 ml isopropanol into a clean 50 ml tube and add the supernatant from the previous step by pouring carefully.

    Be sure that the protein pellet is not dislodged during pouring.

12. Mix by inverting gently 50 times until the DNA is visible as threads or a clump.

13. Centrifuge for ■ 1 min at 13,000–16,000 x *g*, ▲ 3 min at 2000 x *g*, or ● 3 min at 2000 x *g*.

    The DNA may be visible as a small white pellet.

14. Carefully discard the supernatant, and drain the tube by inverting on a clean piece of absorbent paper, taking care that the pellet remains in the tube.

15. Add ■ 300 µl, ▲ 3 ml, or ● 10 ml of 70% ethanol and invert several times to wash the DNA pellet.

16. Centrifuge for ■ 1 min at 13,000–16,000 x *g*, ▲ 1 min at 2000 x *g*, or ● 1 min at 2000 x *g*.

17. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube. Air dry the pellet for ■ 5 min, ▲ 5–10 min, or ● 5–10 min.

    The pellet might be loose and easily dislodged.

    Avoid over-drying the DNA pellet, as the DNA will be difficult to dissolve.

18. Add ■ 100 µl, ▲ 300 µl, or ● 1 ml DNA Hydration Solution and vortex for 5 s at medium speed to mix.

19. Incubate at 65°C for ■ 5 min, ▲ 1 h, or ● 1 h to dissolve the DNA.

20. Incubate at room temperature overnight with gentle shaking. Ensure that the tube cap is tightly closed to avoid leakage. Samples can then be centrifuged briefly and transferred to a storage tube.

Protocol: Enhanced Productivity DNA Purification from Whole Blood Using the Puregene Blood Kit

This protocol is for enhanced productivity purification of genomic DNA from 10 ml whole blood using the Puregene Blood Kit.

The enhanced productivity protocol can be scaled for other sample sizes. Contact QIAGEN Technical Services for more information (see back cover or visit **www.qiagen.com**).

Important points before starting

- See notes for enhanced productivity (page 17) and high-throughput processing (page 14).

Things to do before starting

- Preheat water bath to 65°C for use in step 16 of the procedure.
- Frozen blood samples should be thawed quickly in a 37°C water bath with mild agitation and stored on ice before beginning the procedure.

Procedure

1. Dispense 30 ml RBC Lysis Solution into a 50 ml centrifuge tube. Add 10 ml whole blood, and mix by inverting.
2. Incubate for 5 min at room temperature (15–25°C). Invert gently 3 times during the incubation.
3. Centrifuge for 2 min at 2000 x *g* to pellet the white blood cells.
4. Carefully discard the supernatant, leaving less than 200 µl of the residual liquid and the pellet.
5. Vortex the tube vigorously for 10 s to resuspend the pellet in the residual liquid.

Vortexing greatly facilitates cell lysis in the next step.

The pellet should be completely dispersed after vortexing.

6. Add 3.33 ml Protein Precipitation Solution to the center of the sample. Add 10 ml Cell Lysis Solution, and vortex vigorously for 20 s to lyse the cells and precipitate the proteins.
7. Centrifuge for 6 min at 2000 x *g*.

   The precipitated proteins should form a tight, dark brown pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation.
8. Pipet 10 ml isopropanol into a clean 50 ml centrifuge tube and add the supernatant from the previous step by pouring carefully.

   Be sure the protein pellet is not dislodged during pouring.
9. Mix by inverting gently 50 times.
10. Centrifuge for 3 min at 2000 x *g*.

    The DNA will be visible as a small white pellet.
11. Carefully discard the supernatant, and drain the tube by inverting on a clean piece of absorbent paper for 1 min, taking care that the pellet remains in the tube.
12. Add 10 ml of 70% ethanol.
13. Centrifuge for 1 min at 2000 x *g*.
14. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper for 5 min, taking care that the pellet remains in the tube.

    The pellet might be loose and easily dislodged. Avoid over-drying the DNA pellet, as the DNA will be difficult to dissolve.
15. Add 1 ml DNA Hydration Solution and vortex for 5 s at medium speed to mix.
16. Incubate at 65°C for 1 h to dissolve the DNA.
17. Incubate at room temperature overnight with gentle shaking. Ensure that the tube cap is tightly closed to avoid leakage. Samples can then be centrifuged briefly and transferred to a storage tube.

Protocol: DNA Purification from Compromised Blood Samples Using the Puregene Blood Kit

This protocol is for purification of genomic DNA from 10 ml compromised whole blood using the Puregene Blood Kit. Blood samples stored at –20°C, or at room temperature (15–25°C) for more than 24 hours, or at 2–8°C for more than 5 days are considered compromised.

Things to do before starting

- Preheat water baths to 37 and 65°C for use in steps 7 and 20, respectively, of the procedure.
- Frozen blood samples should be thawed quickly in a 37°C water bath with mild agitation and stored on ice before beginning the procedure.

Procedure

1. Dispense 30 ml RBC Lysis Solution into a 50 ml centrifuge tube. Add 10 ml whole blood or bone marrow, and mix by inverting.
2. Incubate for 5 min at room temperature (15–25°C). Invert gently at least once during the incubation.
3. Centrifuge for 5 min at 2000 x *g*.
4. Carefully discard the supernatant by pipetting and leave approximately 3.5 ml of the supernatant and the brownish pellet in the tube.
5. Vortex the tube vigorously to resuspend the pellet in the residual liquid.

   Vortexing greatly facilitates cell lysis in the next step.

   The pellet should be completely dispersed after vortexing.
6. Add 10 ml Cell Lysis Solution, and vortex vigorously for 10 s to lyse the cells.

7. Incubate at 37°C for at least 2 h.

   Samples can additionally be incubated at room temperature overnight to ensure that the solution is homogenous.

   Note: Do not incubate overnight at 37°C.

8. **Optional**: If RNA-free DNA is required, add 50 µl RNase A Solution, and mix by inverting 25 times. Incubate for 15 min at 37°C. Then incubate for 5 min on ice to quickly cool the sample.
9. Add 4.5 ml Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.
10. Centrifuge for 10 min at 2000 x *g*.

    The precipitated proteins should form a tight, dark brown pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation.

11. Pipet 13.5 ml isopropanol into a clean 50 ml centrifuge tube. Add 135 µl Glycogen Solution (cat. no. 158930 or 949002 and 158183 or 158186). Add the supernatant from the previous step by pouring carefully.

    Be sure the protein pellet is not dislodged during pouring.

12. Mix by inverting gently 50 times.
13. Centrifuge for 3 min at 2000 x *g*.
14. The DNA will be visible as a small white pellet. The pellet might be loose and easily dislodged.
15. Carefully discard the supernatant, and drain the tube by inverting on a clean piece of absorbent paper, taking care that the pellet remains in the tube.
16. Add 10 ml of 70% ethanol and invert several times to wash the DNA pellet.
17. Centrifuge for 1 min at 2000 x *g*.
18. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube. Allow to air dry for 5–10 min.

    The pellet might be loose and easily dislodged. Avoid over-drying the DNA pellet, as the DNA will be difficult to dissolve.

19. Add 500 µl DNA Hydration Solution and vortex for 5 s at medium speed to mix.
20. Incubate at 65°C for 1 h to dissolve the DNA.
21. Incubate at room temperature overnight with gentle shaking. Ensure that the tube cap is tightly closed to avoid leakage. Samples can then be centrifuged briefly and transferred to a storage tube.

Protocol: DNA Purification from Body Fluid Using the Puregene Blood Kit

This protocol is for purification of genomic DNA from 50 µl or 1 ml body fluid (e.g., CSF, plasma, saliva, serum, sputum, synovial fluid, urine, whole blood, or milk) using the Puregene Blood Kit.

Important point before starting

- In some steps of the procedure, one of two choices can be made. Choose ■ if processing 50 µl body fluid samples with low protein content or 25 µl body fluid samples with high protein content; choose ▲ if processing 1 ml body fluid samples with low protein content or 0.5 ml body fluid samples with high protein content.

Things to do before starting

- Preheat water baths to 55°C for use in step 2b and 65°C for use in steps 2a and 17 of the procedure.
- Body fluids with low cell numbers might require concentration by centrifuging the sample. Pellet cells from 3–40 ml body fluid by centrifuging at 2000 x *g* for 10 min. Remove the supernatant, leaving behind the desired volume of residual fluid. Thoroughly suspend the pellet in the residual fluid by pipetting up and down 10 times. Place the sample on ice for immediate use or store frozen at –80°C.
- Frozen body fluid samples should be thawed quickly in a 37°C water bath with mild agitation and stored on ice before beginning the procedure.
- Optional: Preheat water baths to 37°C for use in step 3 of the procedure.

Procedure

1. For samples with a normal protein content, follow step 1a. For samples with a high protein content, follow step 1b.

   1a. Dispense ■ 250 µl Cell Lysis Solution into a sterile 1.5 ml microcentrifuge tube or ▲ 5 ml Cell Lysis Solution into a sterile 15 ml centrifuge tube. Add ■ 50 µl or ▲ 1 ml body fluid, and mix by pipetting up and down.

   1b. Dispense ■ 275 µl Cell Lysis Solution into a sterile 1.5 ml microcentrifuge tube or ▲ 5.5 ml Cell Lysis Solution into a sterile 15 ml centrifuge tube. Add ■ 25 µl or ▲ 0.5 ml body fluid, and mix by pipetting up and down.

2. Complete cell lysis by following step 2a or 2b below:

   2a. Heat at 65°C for 15 min.

   2b. If maximum yield is required, add ■ 1.5 µl or ▲ 30 µl Proteinase K (cat. no. 158918 or 158920 and 158143 or 158146), mix by inverting 25 times, and incubate at 55°C for 1 h to overnight.

3. **Optional**: If RNA-free DNA is required, add ■ 1.5 µl or ▲ 30 µl RNase A Solution, and mix by inverting 25 times. Incubate for 15 min at 37°C. Incubate for ■ 1 min or ▲ 3 min on ice to quickly cool the sample.

   Samples can be incubated at 37°C for up to 1 h.

4. Add ■ 100 µl or ▲ 2 ml Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.

5. Incubate for 5 min on ice.

6. Centrifuge ■ 3 min at 13,000–16,000 x *g* or ▲ 10 min at 2000 x *g*.

   The precipitated proteins should form a tight pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation.

7. Pipet ■ 300 µl isopropanol into a clean 1.5 microcentrifuge tube or ▲ 6 ml isopropanol into a clean 15 ml centrifuge tube, and add the supernatant from the previous step by pouring carefully.

   Be sure the protein pellet is not dislodged during pouring.

8. If the DNA yield is expected to be low (■ <1 µg or ▲ <10 µg), add ■ 0.5 µl or ▲ 10 µl Glycogen Solution (cat. no. 158930 or 949002 and 158183 or 158186).
9. Mix by inverting gently 50 times.
10. Incubate for 5 min at room temperature (15–25°C).
11. Centrifuge for ■ 5 min at 13,000–16,000 x *g* or ▲ 10 min at 2000 x *g*.
12. Carefully discard the supernatant, and drain the tube by inverting on a clean piece of absorbent paper, taking care that the pellet remains in the tube.
13. Add ■ 300 µl or ▲ 6 ml of 70% ethanol and invert several times to wash the DNA pellet.
14. Centrifuge for ■ 1 min at 13,000–16,000 x *g* or ▲ 1 min at 2000 x *g*.
15. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube. Allow to air dry for ■ 5 min or ▲ 5–10 min.

    The pellet might be loose and easily dislodged. Avoid over-drying the DNA pellet, as the DNA will be difficult to dissolve.
16. Add ■ 100 µl or ▲ 200 µl DNA Hydration Solution and vortex 5 s at medium speed to mix.
17. Incubate at 65°C for 1 h to dissolve the DNA.
18. Incubate at room temperature overnight with gentle shaking. Ensure that the tube cap is tightly closed to avoid leakage. Samples can then be centrifuged briefly and transferred to a storage tube.

Protocol: DNA Purification from Cultured Cells Using the Puregene Cell Kit

This protocol is for purification of genomic DNA from 1–2 x 10^6 or 1–2 x 10^7 cultured cells using the Puregene Cell Kit.

Important point before starting

In some steps of the procedure, one of two choices can be made. Choose ■ if processing 1–2 x 10^6 cells; choose ▲ if processing 1–2 x 10^7 cells.

Things to do before starting

- Preheat water bath to 65°C for use in step 18 of the procedure.
- Cultured cells can be used fresh or frozen. Collect suspended cultured cells and place on ice until use. Determine the number of cells using a hemacytometer or other cell counter. A 200 µl suspension containing up to 2 x 10^7 cultured cells may be used for the protocol.
- Cell cultures with low cell numbers might require concentration by centrifuging the sample. Pellet cells by centrifuging at 13,000–16,000 x *g* in a 1.5 ml microcentrifuge tube for 5 s. Remove the supernatant, leaving 200 µl residual fluid. Thoroughly suspend the pellet in the residual fluid by pipetting up and down 10 times. Place the sample on ice for immediate use or store frozen at –80°C.
- Frozen cells should be thawed quickly in a 37°C water bath with mild agitation and stored on ice before beginning the procedure.
- **Optional**: Preheat water bath to 37°C for use in step 7 of the procedure.

Procedure

1. Harvest cells according to step 1a (for cells grown in suspension) or 1b (for cells grown in a monolayer).

   1a. Cells grown in suspension (do not use more than 2 x 10^7 cells): Determine the number of cells. Centrifuge the appropriate number of cells for 5 min at 300 x *g* in a 1.5 ml microcentrifuge tube. Remove the supernatant leaving behind 200 µl residual fluid. Thoroughly suspend the pellet in the residual fluid by pipetting up and down until the cells are resuspended. Continue with step 2.

   1b. Cells grown in a monolayer (do not use more than 2 x 10^7 cells): Cells grown in a monolayer can be detached from the culture flask by either trypsinization or using a cell scraper.

   To trypsinize cells:

   Aspirate the medium and wash cells with balanced salt solution. Aspirate the balanced salt solution and add 0.10–0.25% trypsin.* After cells have detached from the dish or flask, collect them in medium,* and determine the number of cells. Transfer the appropriate number of cells (maximum 2 x 10^7 cells) to a 1.5 ml microcentrifuge tube. Centrifuge for 5 min at 300 x *g*. Remove the supernatant leaving behind 200 µl residual fluid. Thoroughly suspend the pellet in the residual fluid by pipetting up and down until the cells are resuspended. Continue with step 2.

   Using a cell scraper:

   Detach cells from the dish or flask. Transfer the appropriate number of cells (maximum 2 x 10^7 cells) to a 1.5 ml microcentrifuge tube and centrifuge for 5 min at 300 x *g*. Remove the supernatant leaving behind 200 µl residual fluid. Thoroughly suspend the pellet in the residual fluid by pipetting up and down until the cells are resuspended. Continue with step 2.

* 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.

2. Add ■ 1–2 x 10^6 or ▲ 1–2 x 10^7 cells in balanced salt solution or culture medium to a ■ 1.5 ml microcentrifuge tube or ▲ 15 ml centrifuge tube.
3. Centrifuge for ■ 5 s at 13,000–16,000 x *g* or 3 min at ▲ 500 x *g* to pellet cells.

   Carefully discard the supernatant by pipetting or pouring, leaving approximately ■ 20 µl or ▲ 200 µl residual liquid.
4. Vortex the tube vigorously to resuspend the cells in the residual supernatant.

   Vortexing greatly facilitates cell lysis in the next step.
5. Add ■ 300 µl or ▲ 3 ml Cell Lysis Solution to the resuspended cells and pipet up and down or vortex on high speed for 10 s to lyse the cells.

   Usually no incubation is required; however, if cell clumps are visible after mixing, incubate at 37°C until the solution is homogeneous. Samples are stable in Cell Lysis Solution for at least 2 years at room temperature (15–25°C).
6. **Optional**: If RNA-free DNA is required, add ■ 1.5 µl or ▲ 15 µl RNase A Solution, and mix by inverting 25 times. Incubate for 5 min at 37°C. Incubate for ■ 1 min or ▲ 3 min on ice to quickly cool the sample.

   Sample can be incubated at 37°C for up to 1 h.
7. Add ■ 100 µl or ▲ 1 ml Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.
8. Centrifuge for ■ 1 min at 13,000–16,000 x *g* or ▲ 10 min at 2000 x *g*.

   The precipitated proteins should form a tight pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation.
9. Pipet ■ 300 µl isopropanol into a clean 1.5 ml microcentrifuge tube or ▲ 3 ml isopropanol into a clean 15 ml centrifuge tube and add the supernatant from the previous step by pouring carefully.

   Be sure the protein pellet is not dislodged during pouring.
10. Mix by inverting gently 50 times.
11. Centrifuge for ■ 1 min at 13,000–16,000 x *g* or ▲ 3 min at 2000 x *g*.
12. The DNA will be visible as a small white pellet.

13. Carefully discard the supernatant, and drain the tube by inverting on a clean piece of absorbent paper, taking care that the pellet remains in the tube.
14. Add ■ 300 µl or ▲ 3 ml of 70% ethanol and invert several times to wash the DNA pellet.
15. Centrifuge for ■ 1 min at 13,000–16,000 x *g* or ▲ 1 min at 2000 x *g*.
16. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper for 5 s, taking care that the pellet remains in the tube. Allow to air dry for ■ 5 min or ▲ 5–10 min.

    The pellet might be loose and easily dislodged. Avoid over-drying the DNA pellet, as the DNA will be difficult to dissolve.
17. Add ■ 100 µl or ▲ 400 µl DNA Hydration Solution and vortex for 5 s at medium speed to mix.
18. Incubate at 65°C for 1 h to dissolve the DNA.
19. Incubate at room temperature overnight with gentle shaking. Ensure that the tube cap is tightly closed to avoid leakage. Samples can then be centrifuged briefly and transferred to a storage tube.

Protocol: DNA Purification from 1–2 x 10^6 Fixed Cells Using the Puregene Tissue Kit

This protocol is for purification of genomic DNA from 1–2 x 10^6 cells fixed in methanol–acetic acid, 70% ethanol, or 95% ethanol using the Puregene Tissue Kit.

Things to do before starting

- Preheat water baths to 37°C for use in step 7, 55°C for use in step 5b, and 65°C for use in steps 5a and 20 of the procedure.

Procedure

1. Transfer 1–2 x 10^6 cells fixed in methanol–acetic acid (3:1), 70% ethanol, or 95% ethanol to a 1.5 ml microcentrifuge tube.
2. Centrifuge at 13,000–16,000 x *g* for 5 s to pellet cells.
3. Remove as much supernatant as possible.

   Note: Do not wash cells with PBS as this may result in a significantly reduced DNA yield due to cell lysis.
4. Add 300 µl Cell Lysis Solution to the cell pellet and pipet up and down or vortex at high speed for 10 s to lyse the cells.
5. Complete cell lysis by following step 5a or 5b below:

   5a. Heat at 65°C for 15–60 min to complete cell lysis. If cell clumps are visible after the incubation, homogenize the cells with a microcentrifuge tube pestle.

   5b. If maximum yield is required, add 1.5 µl Proteinase K (cat. no. 158918 or 158920 and 158143 or 158146) to the cell lysate, mix by inverting 25 times, and incubate at 55°C for 1 h or until cell clumps have dispersed.
   Sample can be incubated at 55°C overnight.

   If possible, invert tube periodically during the incubation.

6. Add 1.5 µl RNase A Solution, and mix the sample by inverting the tube 25 times.
7. Incubate at 37°C for 15 min.

   Sample can be incubated at 37°C for up to1 h.
8. Incubate for 1 min on ice to quickly cool the sample.
9. Add 100 µl Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.
10. Centrifuge for 3 min at 13,000–16,000 x *g*.

    The precipitated proteins should form a tight pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation.
11. Pipet 300 µl isopropanol into a clean 1.5 ml microcentrifuge tube, and add the supernatant from the previous step by pouring carefully.

    Be sure the protein pellet is not dislodged during pouring.
12. If the DNA yield is expected to be low (<2 µg), add 0.5 µl Glycogen Solution (cat. no. 158930 or 949002 and 158183 or 158186).
13. Mix by inverting gently 50 times.
14. Centrifuge for 1 min at 13,000–16,000 x *g*.

    The DNA will be visible as a small white pellet.
15. Carefully discard the supernatant, and drain the tube by inverting on a clean piece of absorbent paper, taking care that the pellet remains in the tube.
16. Add 300 µl of 70% ethanol and invert several times to wash the DNA pellet.
17. Centrifuge for 1 min at 13,000–16,000 x *g*.
18. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube. Allow to air dry for 5 min.

    The pellet might be loose and easily dislodged. Avoid over-drying the DNA pellet, as the DNA will be difficult to dissolve.

19. Add 100 µl DNA Hydration Solution and vortex 5 s at medium speed to mix.
20. Incubate at 65°C for 1 h to dissolve the DNA.
21. Incubate at room temperature (15–25°C) overnight with gentle shaking. Ensure that the tube cap is tightly closed to avoid leakage. Samples can then be centrifuged briefly and transferred to a storage tube.

Protocol: DNA Purification from Tissue Using the Puregene Tissue Kit

This protocol is for purification of genomic DNA from 5–10 mg or 50–100 mg fresh or frozen solid tissue using the Puregene Tissue Kit.

Important point before starting

- In some steps of the procedure, one of two choices can be made. Choose ■ if processing 5–10 mg tissue; choose ▲if processing 50–100 mg tissue.

Things to do before starting

- Preheat water baths to 37°C for use in step 3, 55°C for use in step 2b, and 65°C for use in steps 2a and 15 of the procedure.

Procedure

1. Dissect tissue sample quickly and freeze in liquid nitrogen.

   Grind ■ 5–10 mg or ▲ 50–100 mg frozen or fresh tissue in liquid nitrogen with a mortar and pestle. Work quickly and keep tissue on ice at all times, including when tissue is being weighed.

2. Dispense ■ 300 µl or ▲ 3 ml Cell Lysis Solution into a ■ 1.5 ml or ▲ 15 ml grinder tube on ice, and add the ground tissue from the previous step. Complete cell lysis by following step 2a or 2b below:

   2a. Heat at 65°C for 15 min to 1 h.

   2b. If maximum yield is required, add ■ 1.5 µl or ■ 15 µl Proteinase K, mix by inverting 25 times, and incubate at 55°C for 3 h or until tissue has completely lysed. Invert tube periodically during the incubation.
   The sample can be incubated at 55°C overnight for maximum yields.

3. Add ■ 1.5 µl or ▲ 15 µl RNase A Solution, and mix the sample by inverting 25 times. Incubate at 37°C for 15–60 min.
4. Incubate for ■ 1 min or ▲ 3 min on ice to quickly cool the sample.
5. Add ■ 100 µl or ▲ 1 ml Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.
6. Centrifuge for ■ 3 min at 13,000–16,000 x *g* or ▲ 10 min at 2000 x *g*.

   The precipitated proteins should form a tight pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation.
7. Pipet ■ 300 µl or ▲ 3 ml isopropanol into a clean ■ 1.5 ml microcentrifuge tube or ▲ 15 ml centrifuge tube and add the supernatant from the previous step by pouring carefully.

   Be sure the protein pellet is not dislodged during pouring.

   Note: If the DNA yield is expected to be low (<1 µg), add ■ 0.5 µl Glycogen Solution (cat. no. 158930 or 949002 and 158183 or 158186).
8. Mix by inverting gently 50 times.
9. Centrifuge for ■ 1 min at 13,000–16,000 x *g* or ▲ 3 min at 2000 x *g*.
10. Carefully discard the supernatant, and drain the tube by inverting on a clean piece of absorbent paper, taking care that the pellet remains in the tube.
11. Add ■ 300 µl or ▲ 3 ml of 70% ethanol and invert several times to wash the DNA pellet.
12. Centrifuge for ■ 1 min at 13,000–16,000 x *g* or ▲ 1 min at 2000 x *g*.
13. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube. Allow to air dry for ■ 5 min or ▲ 5–10 min.

    The pellet might be loose and easily dislodged. Avoid over-drying the DNA pellet, as the DNA will be difficult to dissolve.
14. Add ■ 100 µl or ▲ 400 µl DNA Hydration Solution and vortex for 5 s at medium speed to mix.

15. Incubate at 65°C for 1 h to dissolve the DNA.
16. Incubate at room temperature (15–25°C) overnight with gentle shaking. Ensure that the tube cap is tightly closed to avoid leakage. Samples can then be centrifuged briefly and transferred to a storage tube.

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/or protocols in this handbook or sample and assay technologies. For contact information, visit **www.qiagen.com**.

		Comments and suggestions
All protocols		
Cells are incompletely lysed		
a)	Too many cells were used	The amount of Cell Lysis Solution used was insufficient for the number of cells. If too many cells are used, cell lysis will be incomplete; the Cell Lysis Solution will become very viscous and cells will clump. Add more Cell Lysis Solution to completely lyse the cells. To prevent incomplete cell lysis, either count cells with a hemacytometer or other cell counter or weigh tissue samples prior to adding Cell Lysis Solution.
b)	Cell clumps were present after adding Cell Lysis Solution	Cells may clump if cells are not completely resuspended prior to addition of Cell Lysis Solution. To lyse the cells in the clumps, incubate sample at either 37°C or room temperature (15–25°C) with periodic mixing until the solution is homogeneous. Cell clumps may be dispersed more quickly by adding Proteinase K (cat. no. 158918 or 158920 and 158143 or 158146) and incubating at 55°C until cells are completely lysed (1 h to overnight).
c)	Incomplete lysis of cells purified from a Ficoll® gradient	Ficoll was not adequately removed. Wash cells once in PBS to remove Ficoll prior to adding Cell Lysis Solution. After addition of Cell Lysis Solution, incubate the lysate at 65°C to complete lysis, if necessary.

		Comments and suggestions
Protein pellet soft, loose, or absent		
a)	Sample was not cooled sufficiently before adding Protein Precipitation Solution	To obtain a tight protein pellet, be sure that the sample is cooled to room temperature or below (≤20–22°C) prior to adding Protein Precipitation Solution. To obtain a tight protein pellet: • Vortex the sample again for 20 s to mix the Protein Precipitation Solution uniformly with the cell lysate. • Incubate sample on ice for 5–15 min to facilitate formation of a tight pellet. • Centrifuge according to the protocol to pellet the precipitated proteins.
b)	Protein Precipitation Solution was not mixed uniformly with the cell lysate	Be sure to vortex vigorously for the full 20 s as specified in the protocol.
c)	Centrifuge speed set incorrectly	Set centrifuge speed to the g-force specified in the protocol. For microcentrifuge tube preps, set the centrifuge speed to maximum. For tabletop and other centrifuges, the speed should usually be set to 2000 x *g*. If a *g*-force of 2000 x *g* cannot be attained by your centrifuge, increase centrifugation time to achieve the same total *g*-force. For example, 2000 x *g* for 10 min is equivalent to a total *g*-force of 20,000 x *g* x time (min). If your centrifuge only achieves 1600 x *g*, centrifuge at 1600 x *g* for 12.5 min [(1600 x *g*) (12.5 min) = 20,000 x *g* x min)]. **Note**: 2000 x *g* and 2000 rpm are not equivalent. Use this equation to check if your rpm is set correctly *g*(rcf)=1.12*r*(rpm/1000)2 where r is the radius of the rotor in mm.
Samples are slow to rehydrate		
a)	Samples were not mixed during the hydration step	Incubate with gentle shaking to facilitate hydration of the DNA.
b)	The DNA pellet was dried too long prior to adding DNA Hydration Solution	DNA pellets that are too dry will require a longer time to rehydrate completely. To rehydrate, incubate at 65°C for 1 h and at room temperature overnight. DNA in DNA Hydration Solution can be stored at room temperature for up to 1 year. Using heat or vacuum to dry DNA pellets is not recommended. **Note**: Incubation at 65°C overnight is not recommended as it will reduce the DNA size.
c)	Protein contamination in the rehydrated DNA sample	Protein contamination usually results from exceeding the recommended amount of sample material. Repurify the DNA sample according to “Appendix C: Repurifying DNA Samples” on page 68.

		Comments and suggestions
A_{260}/A_{280} too high		
	RNA contamination	Ratios above 2.0 may indicate the presence of RNA. RNA can be moved with one of the following: • Increase RNase incubation time in cell lysate from 15 min to 30–60 min. • Remove contaminating RNA using the protocol in "Appendix D: Removal of RNA from Purified DNA" on page 70.
Purified DNA is less than 50 kb in size		
a)	DNA is degraded	Improper sample collection or storage of starting material can cause DNA to degrade. Samples rehydrated in water are not stable and the DNA may degrade over time. Collect and store samples using methods that preserve DNA integrity. For long-term sample storage (>5 days), store samples frozen at –80°C, or, alternatively, in Cell Lysis Solution at room temperature. For short-term sample storage (<5 days), store samples at 4°C or, alternatively, in Cell Lysis Solution at room temperature.
b)	DNA is sheared	Over-handling (e.g., homogenizing tissue samples for too long) in Cell Lysis Solution or DNA Hydration Solution can cause the DNA to shear. When processing tissue samples, place fresh tissue directly into Cell Lysis Solution and homogenize immediately or freeze tissue immediately upon collection. This minimizes DNase activity and results in increased DNA size. **Note**: The Puregene method is a very gentle method that produces a minimum amount of shearing compared to organic or other methods of DNA extraction. Vortexing for 20 s at the protein precipitation step of the procedure will not affect the size or quality of the purified DNA.
Low DNA yield		
a)	Insufficient number of cells in the starting sample material	Count cells or weigh tissue prior to beginning the cell lysis step. Make sure to use the specified amount of starting material for a given protocol. Too few cells will result in a lower DNA concentration during the DNA precipitation step and will reduce DNA precipitation efficiency. The result is a low DNA yield. Adding a carrier, such as glycogen, will help to maximize DNA yield. We recommend adding 0.5 µl Glycogen Solution (cat. no. 158930 or 949002 and 158183 or 158186) per 300 µl isopropanol.
b)	Cells not completely lysed	Cells were not completely lysed due to adding too many cells or too much tissue to the Cell Lysis Solution. Count cells or weigh tissue prior to beginning cell lysis step. Too many cells may overload the chemistry inhibiting complete cell lysis, which results in a low DNA yield.

		Comments and suggestions
c)	Cell clumps present in the sample after adding Cell Lysis Solution	Cells may clump if cells are not completely resuspended prior to addition of Cell Lysis Solution. To lyse the cells in the clumps, incubate sample at either 37°C or room temperature with periodic mixing until the solution is homogeneous. Cell clumps may be dispersed more quickly by adding Proteinase K (cat. no. 158918 or 158920 and 158143 or 158146) and incubating at 55°C until cells are completely lysed (1 h to overnight).
d)	Insufficient number of cells	If fewer than 200,000 cells per 300 µl Cell Lysis Solution are used for DNA purification or if the DNA yield is expected to be low (<1 µg), add a DNA carrier such as glycogen to the isopropanol. We recommend adding 0.5 µl Glycogen Solution (cat. no. 158930 or 949002 and 158183 or 158186) per 300 µl isopropanol.
e)	Sample not completely hydrated	Sample can be hydrated by incubating with gentle shaking either overnight at room temperature or by for 1 h at 65°C (samples may be incubated at 65°C for a total of 2 h without affecting DNA quality).
DNA concentration low		
	Low DNA yield	Precipitate the DNA solution and rehydrate in a smaller volume of DNA Hydration Solution as described in “Appendix E: Concentrating DNA” on page 72.
Blood protocols		
Red blood cells in the sample were not completely lysed		
	Higher than average number of red blood cells in the sample	Repeat the incubation with RBC Lysis Solution to lyse the remaining red blood cells. Add 3 volumes RBC Lysis Solution for each volume of sample, incubate for 10 min at room temperature, and then centrifuge according to the original protocol followed.

	Comments and suggestions
Blood clots are present in the whole blood sample	
The sample was not mixed or stored properly during blood collection	The sample may be handled as follows: For best results use the Clotspin® protocol. Call QIAGEN Technical Services for more information. Purify DNA from the unclotted portion of the sample only; that is, when blood is removed from the collection tube, leave clots behind and purify DNA from unclotted portion. Remove large clots from white blood cell pellet. To facilitate clot removal, resuspend cells in PBS and remove the clots with either a forceps or pipet tip. After removing the clot, centrifuge to pellet the white cells, carefully discard the supernatant and proceed with the purification protocol. Remove small clots by digestion with Proteinase K (cat. no. 158918 or 158920 and 158143 or 158146). Add Proteinase K to the Cell Lysis Solution and incubate at 55°C with periodic mixing until the clots are lysed completely. **Note**: Make sure to lyse blood clots completely before proceeding to the protein precipitation step of the procedure to ensure maximum DNA yield with minimum protein contamination.
White blood cell pellet is loose after centrifugation	
Centrifuge settings need to be optimized	If following a large volume protocol (15 or 50 ml tube) increase centrifugation time from 2 to 5 min. Set centrifuge speed to the *g*-force specified in the protocol. For microcentrifuge tube preps, set the centrifuge speed to maximum. For tabletop and other centrifuges, the speed should usually be set to 2000 x *g*. If a *g*-force of 2000 x *g* cannot be attained by your centrifuge, increase centrifugation time to achieve the same total g-force. For example, 2000 x *g* for 10 min is equivalent to a total *g*-force of 20,000 x *g* x time (min). If your centrifuge only achieves 1600 x *g*, centrifuge at 1600 x *g* for 12.5 min [(1600 x *g*) (12.5 min) = 20,000 x *g* x min)]. **Note**: 2000 x *g* and 2000 rpm are not equivalent. Use this equation to check if your rpm is set correctly *g*(rcf)=1.12*r*(rpm/1000)2, where r is the radius of the rotor in mm.

		Comments and suggestions
Fixed cell protocol		
Incomplete cell lysis of cells fixed in methanol, acetic acid or ethanol		
a)	Fixative was not adequately removed from the sample prior to adding Cell Lysis Solution	Remove fixative completely after centrifuging to pellet the cells; do not wash cells with PBS as this will result in a greatly reduced yield.
b)	Cell clumps are present after adding Cell Lysis Solution to the sample	Incubate samples at 65°C until cells are completely lysed (cell clumps are dispersed). It is also possible to homogenize the cells with a pestle if heat treatment is not effective. Cell clumps may be dissolved more quickly by adding Proteinase K (cat. no. 158918 or 158920 and 158143 or 158146) and incubating at 55°C until cells are completely lysed (1 h to overnight).

Appendix A: Additional User-Developed Protocols

Sample type and size	Range of expected yields (µg)	Average yield (µg)
Buccal swabs, 1 swab	0.2–2	1
Animal tissue, 10 mg	5–100	50

Protocol: DNA purification from a buccal brush using the Puregene Cell Kit

This protocol is for purification of genomic DNA from 1 buccal brush using the Puregene Cell Kit.

Additional equipment and reagents

- Buccal swabs for collecting buccal cells
- Glycogen Solution, cat. no. 158930 or 949002 and 158183 or 158186
- Proteinase K, cat. no. 158918 or 158920 and 158143 or 158146

Things to do before starting

- Preheat water baths to 55°C for use in step 3b and 65°C for use in steps 3a and 17 of the procedure.
- **Optional**: Preheat water bath to 37°C for use in step 5 of the procedure.

Procedure

1. To collect buccal cells, scrape the inside of the mouth 10 times with a Buccal Collection Brush.

   For best results, wait at least 1 h after eating or drinking to collect buccal cells.

DNA may be purified immediately, or samples may be stored on the collection brush for up to 1 month at room temperature (15–25°C).

2. Dispense 300 µl Cell Lysis Solution into a 1.5 ml microcentrifuge tube. Remove the collection brush from its handle using sterile scissors or a razor blade, and place the detached head in the tube.

   Samples are stable in Cell Lysis Solution for at least 2 years at room temperature.

   If 300 µl Cell Lysis Solution is not sufficient to cover the head, the protocol must be scaled up to use a larger volume. Contact QIAGEN Technical Services for more information (see back cover or visit www.qiagen.com).

3. Complete cell lysis by following step 3a or 3b below:

   3a. Incubate at 65°C for at least 15 min (up to 60 min for maximum yield).

   3b. If maximum yield is required, add 1.5 µl Proteinase K (cat. no. 158918 or 158920 and 158143 or 158146), mix by inverting 25 times, and incubate at 55°C for at least 1 h (up to overnight for maximum yield).

4. Remove the collection brush head from the Cell Lysis Solution, scraping it on the sides of the tube to recover as much liquid as possible.

5. **Optional**: If RNA-free DNA is required, add 1.5 µl RNase A Solution, and mix by inverting 25 times. Incubate for 15 min at 37°C. Incubate for 1 min on ice to quickly cool the sample.

   Samples can be incubated at 37°C for up to 1 h.

6. Add 100 µl Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.

7. Incubate for 5 min on ice.

8. Centrifuge for 3 min at 13,000–16,000 x *g*.

   The precipitated proteins should form a tight pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation.

9. Pipet 300 µl isopropanol and 0.5 µl Glycogen Solution (cat. no. 158930 or 949002 and 158183 or 158186) into a clean 1.5 ml microcentrifuge tube, and add the supernatant from the previous step by pouring carefully.

Be sure the protein pellet is not dislodged during pouring.

10. Mix by inverting gently 50 times.
11. Centrifuge for 5 min at 13,000–16,000 x *g*.
12. Carefully discard the supernatant, and drain the tube by inverting on a clean piece of absorbent paper, taking care that the pellet remains in the tube.
13. Add 300 µl of 70% ethanol and invert several times to wash the DNA pellet.
14. Centrifuge for 1 min at 13,000–16,000 x *g*.
15. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube. Allow to air dry for 5 min.

    The pellet might be loose and easily dislodged. Avoid over-drying the DNA pellet, as the DNA will be difficult to dissolve.
16. Add 100 µl DNA Hydration Solution and vortex for 5 s at medium speed to mix.
17. Incubate at 65°C for 1 h to dissolve the DNA.
18. Incubate at room temperature overnight with gentle shaking. Ensure that the tube cap is tightly closed to avoid leakage. Samples can then be centrifuged briefly and transferred to a storage tube.

Protocol: DNA purification from buccal cells in mouthwash using the Puregene Cell Kit

This protocol is for purification of genomic DNA from buccal cells in 10 ml mouthwash using the Puregene Cell Kit.

Additional equipment and reagents

- Mouthwash for collecting buccal cells
- Glycogen Solution, cat. no. 158930 or 949002 and 158183 or 158186
- Proteinase K, cat. no. 158918 or 158920 and 158143 or 158146

Things to do before starting

- Preheat water bath to 65°C for use in step 22 of the procedure.

Procedure

1. Dispense 10 ml Original Mint Scope® Mouthwash (Procter & Gamble) or Listerine® mouthwash (McNEIL-PPC, Inc) into a 50 ml centrifuge tube.
2. Collect buccal cells by rinsing the mouth with mouthwash and then spit the mouthwash into a 50 ml tube.

   For best results, wait at least 1 h after eating or drinking to collect buccal cells. Samples may be stored at room temperature (15–25°C) for up to 7 days.
3. Centrifuge for 5 min at 2000 x *g* to pellet cells.

   If the cell pellet is too loose, repeat the centrifugation.
4. Carefully discard the supernatant by pipetting or pouring, leaving the pellet undisturbed.
5. Add 1 ml Cell Lysis Solution, and mix by inverting 50 times.
6. Incubate for 15 min at room temperature.
7. Add 10 µl Proteinase K (cat. no. 158918 or 158920 and 158143 or 158146), and mix by inverting 3 times.

8. Vortex vigorously at high speed for 20 s to mix.
9. Incubate for 10 min at room temperature.
10. Add 340 µl Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.
11. Incubate for 10 min on ice.

    Incubation on ice is important to ensure a tight pellet in the next step.
12. Centrifuge for 10 min at 2000 x *g*.

    The precipitated proteins should form a tight, green pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation.
13. Pipet 1 ml isopropanol and 2 µl Glycogen Solution into a clean 15 or 50 ml centrifuge tube.
14. Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into the tube containing isopropanol and Glycogen Solution. Keep samples on ice while transferring supernatant to ensure pellet remains tight.

    Be sure the protein pellet is not dislodged during pouring. Keeping the samples on ice is important to ensure a tight pellet.
15. Mix by inverting gently 50 times.
16. Centrifuge for 5 min at 2000 x *g*.
17. Carefully discard the supernatant, and drain the tube by inverting on a clean piece of absorbent paper, taking care that the pellet remains in the tube.
18. Add 1 ml of 70% ethanol and invert several times to wash the DNA pellet.
19. Centrifuge for 1 min at 2000 x *g*.
20. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube. Allow to air dry for 5–10 min.

    The pellet might be loose and easily dislodged. Avoid over-drying the DNA pellet, as the DNA will be difficult to dissolve.

21. Add 400 µl DNA Hydration Solution and vortex for 5 s at medium speed to mix.
22. Incubate at 65°C for 1 h to dissolve the DNA.
23. Incubate at room temperature overnight with gentle shaking. Ensure that the tube cap is tightly closed to avoid leakage. Samples can then be centrifuged briefly and transferred to a storage tube.

Protocol: DNA purification from fixed tissue using the Puregene Tissue Kit

This protocol is for purification of genomic DNA from 5–10 mg fixed tissue using the Puregene Tissue Kit.

Things to do before starting

- Preheat water baths to 37°C for use in step 5, 55°C for use in steps 3a and 3b, and 65°C for use in steps 1, 4, and 18 of the procedure.

Procedure

1. Briefly blot excess fixative from tissue on clean absorbent paper. Dispense 300 µl Cell Lysis Solution into a 1.5 ml microcentrifuge tube, and add 5–10 mg tissue. Incubate for 15 min at 65°C to soften the tissue.
2. Homogenize using 30–50 strokes with a microcentrifuge tube pestle.
3. If maximum yield is required, perform steps 3a and 3b. Otherwise go directly to step 4.
   - 3a. Add 1.5 µl Proteinase K, mix by inverting 25 times, and incubate at 55°C for 3 h. Continue with step 3b or step 5. Step 4 is not required.
     Samples can be incubated at 55°C overnight for maximum homogenization. Invert tube periodically during the incubation.
   - 3b. If tissue is not completely digested after an overnight incubation, add an additional 1.5 µl Proteinase K and continue incubation at 55°C for 3 h. Continue with step 5.
     Samples can be incubated at 55°C overnight for maximum homogenization. Invert tube periodically during the incubation.
4. Incubate lysate at 65°C for 15–60 min.
5. Add 1.5 µl RNase A Solution, and mix the sample by inverting 25 times. Incubate at 37°C for 15 min.
6. Incubate for 1 min on ice to quickly cool the sample.

7. Add 100 µl Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.
8. Centrifuge for 3 min at 13,000–16,000 x *g*.

   The precipitated proteins should form a tight pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation.
9. Pipet 300 µl isopropanol into a clean 1.5 ml microcentrifuge tube and add the supernatant from the previous step by pouring carefully.

   Be sure that the protein pellet is not dislodged during pouring.
10. If the DNA yield is expected to be low (<10 µg), add 0.5 µl Glycogen Solution (cat. no. 158930 or 949002 and 158183 or 158186).
11. Mix by inverting gently 50 times.
12. Centrifuge for 5 min at 13,000–16,000 x *g*.
13. Carefully discard the supernatant, and drain the tube by inverting on a clean piece of absorbent paper, taking care that the pellet remains in the tube.
14. Add 300 µl ml of 70% ethanol and invert several times to wash the DNA pellet.
15. Centrifuge for 1 min at 13,000–16,000 x *g*.
16. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube. Allow to air dry for 5 min.

    The pellet might be loose and easily dislodged. Avoid over-drying the DNA pellet, as the DNA will be difficult to dissolve.
17. Add 100 µl DNA Hydration Solution and vortex for 5 s at medium speed to mix.
18. Incubate at 65°C for 1 h to dissolve the DNA.
19. Incubate at room temperature (15–25°C) overnight with gentle shaking. Ensure that the tube cap is tightly closed to avoid leakage. Samples can then be centrifuged briefly and transferred to a storage tube.

Protocol: DNA purification from FFPE tissue using the Puregene Tissue Kit

This protocol is for purification of genomic DNA from 5–10 mg formalin-fixed paraffin-embedded tissue using the Puregene Tissue Kit.

Additional equipment and reagents

- Xylene or Deparaffinization Solution, cat. no. 19093 or 939018

Things to do before starting

- Preheat water baths to 37°C for use in step 20, 55°C for use in steps 18 and 19, and 65°C for use in step 33 of the procedure.

Procedure

1. Finely cut the tissue and transfer 5–10 mg into a 1.5 ml microcentrifuge tube.
2. Add 300 µl xylene and incubate for 5 min with gentle shaking at room temperature (15–25°C).

   Wear gloves, safety goggles, and a laboratory coat when handling xylene. Avoid contact with skin, eyes, and clothing and work in a fume hood.
3. Centrifuge for 1–3 min at 13,000–16,000 x *g* to pellet the tissue.
4. Carefully discard the supernatant.
5. Add 300 µl xylene and incubate for 5 min with gentle shaking at room temperature.
6. Centrifuge for 1–3 min at 13,000–16,000 x *g* to pellet the tissue.
7. Carefully discard the supernatant.
8. Add 300 µl xylene and incubate for 5 min with gentle shaking at room temperature.
9. Centrifuge for 1–3 min at 13,000–16,000 x *g* to pellet the tissue.
10. Carefully discard the supernatant.

11. Add 300 µl of 96–100% ethanol, and incubate for 5 min at room temperature with gentle shaking.
12. Centrifuge for 1–3 min at 13,000–16,000 x *g* to pellet the tissue.
13. Carefully discard the supernatant.
14. Add 300 µl of 96–100% ethanol, and incubate 5 min at room temperature with gentle shaking.
15. Centrifuge for 1–3 min at 13,000–16,000 x *g* to pellet the tissue.
16. Carefully discard the supernatant.
17. Add 300 µl Cell Lysis Solution, and homogenize using 30–50 strokes with a microcentrifuge tube pestle.
18. Add 1.5 µl Proteinase K, mix by inverting 25 times, and incubate at 55°C for 3 h.

    Samples can be incubated at 55°C overnight for maximum homogenization. Invert tube periodically during the incubation.
19. If tissue is not completely digested after an overnight incubation, add an additional 1.5 µl Proteinase K and continue incubation at 55°C for 3 h.

    Samples can be incubated at 55°C overnight for maximum homogenization. Invert tube periodically during the incubation.
20. Add 1.5 µl RNase A Solution, and mix the sample by inverting 25 times. Incubate at 37°C for 15 min.
21. Incubate for 1 min on ice to quickly cool the sample.
22. Add 100 µl Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.
23. Centrifuge for 3 min at 13,000–16,000 x *g*.

    The precipitated proteins should form a tight pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation.
24. Pipet 300 µl isopropanol into a clean 1.5 ml microcentrifuge tube and add the supernatant from the previous step by pouring carefully.

    Be sure the protein pellet is not dislodged during pouring.

25. If the DNA yield is expected to be low (<10 µg), add 0.5 µl Glycogen Solution (cat. no. 158930 or 949002 and 158183 or 158186).
26. Mix by inverting gently 50 times.
27. Centrifuge for 5 min at 13,000–16,000 x *g*.
28. Carefully discard the supernatant, and drain the tube by inverting on a clean piece of absorbent paper, taking care that the pellet remains in the tube.
29. Add 300 µl ml of 70% ethanol and invert several times to wash the DNA pellet.
30. Centrifuge for 1 min at 13,000–16,000 x *g*.
31. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube. Allow to air dry for 5 min.

    The pellet might be loose and easily dislodged. Avoid over-drying the DNA pellet, as the DNA will be difficult to dissolve.
32. Add 100 µl DNA Hydration Solution and vortex for 5 s at medium speed to mix.
33. Incubate at 65°C for 1 h to dissolve the DNA.
34. Incubate at room temperature overnight with gentle shaking. Ensure that the tube cap is tightly closed to avoid leakage. Samples can then be centrifuged briefly and transferred to a storage tube.

Protocol: DNA purification from mouse tail tissue using the Puregene Tissue Kit

This protocol is for purification of genomic DNA from 5 mm mouse tail using the Puregene Tissue Kit.

Things to do before starting

- Preheat water baths to 55 and 65°C for use in steps 4 and 16, respectively, of the procedure.
- **Optional**: Preheat water bath to 37°C for use in step 5 of the procedure.

Procedure

1. Cut 5 mm (5–10 mg) fresh or frozen mouse tail tissue into small pieces.
2. Dispense 300 µl Cell Lysis Solution into a 1.5 ml microcentrifuge tube and add the tissue from the previous step.
3. Add 1.5 µl Proteinase K to the lysate, and mix by inverting 25 times.
4. Incubate at 55°C overnight or until the tissue has completely lysed.

   Invert tube periodically during the incubation.

   Note: After the incubation, undigested vertebrae and hair can be removed from the tube.
5. **Optional**: If RNA-free DNA is required, add 1.5 µl RNase A Solution (cat. no. 158153 or 158156), and mix by inverting 25 times. Incubate for 15–60 min at 37°C. Incubate for 1 min on ice to quickly cool the sample.
6. Add 100 µl Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.
7. Centrifuge for 3 min at 13,000–16,000 x *g*.

   The precipitated proteins should form a tight pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation.
8. Pipet 300 µl isopropanol into a clean 1.5 ml microcentrifuge tube and add the supernatant from the previous step by pouring carefully.

Be sure the protein pellet is not dislodged during pouring.

9. Mix by inverting gently 50 times.
10. Centrifuge for 1 min at 13,000–16,000 x *g*.

    The DNA may be visible as a small white pellet.

11. Carefully discard the supernatant, and drain the tube by inverting on a clean piece of absorbent paper, taking care that the pellet remains in the tube.
12. Add 300 µl of 70% ethanol and invert several times to wash the DNA pellet.
13. Centrifuge for 1 min at 13,000–16,000 x *g*.
14. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube. Allow to air dry for 5 min.

    The pellet might be loose and easily dislodged. Avoid over-drying the DNA pellet, as the DNA will be difficult to dissolve.

15. Add 100 µl DNA Hydration Solution and vortex 5 s at medium speed to mix.
16. Incubate at 65°C for 1 h to dissolve the DNA.
17. Incubate at room temperature overnight with gentle shaking. Ensure that the tube cap is tightly closed to avoid leakage. Samples can then be centrifuged briefly and transferred to a storage tube.

Protocol: DNA Purification from Gram-Negative Bacteria Using the Puregene Cell Kit

This protocol is for purification of genomic DNA from fresh or frozen samples of 0.5 ml Gram-negative bacterial cultures using the Puregene Cell Kit.

Things to do before starting

- Preheat water baths to 37°C for use in step 6, 65°C for use in step 18, and 80°C for use in step 5 of the procedure.
- Gram-negative bacterial cultures can be used either fresh or frozen. Typically, an overnight culture contains 1–3 x 10^9 cells/ml. Due to the small genome size of Gram-negative bacteria, up to 3 x 10^9 cells may be used for the protocol. Thus, culture can either be used directly, or, if necessary, concentrated by centrifuging. To concentrate, pellet 1 ml of overnight culture at 13,000–16,000 x *g* for 1 min. Remove the supernatant, leaving 200 µl residual fluid. Thoroughly suspend the pellet in the residual fluid by pipetting up and down 10 times. Place the sample on ice for immediate use or store frozen at –80°C.
- Frozen bacterial samples should be thawed and equilibrated to room temperature (15–25°C) before beginning the procedure.

Procedure

1. Prepare an overnight culture.
2. Transfer 500 µl of the culture (containing approximately 0.5–1.5 x 10^9 cells) to a 1.5 ml microcentrifuge tube on ice.
3. Centrifuge for 5 s at 13,000–16,000 x *g* to pellet cells.
4. Carefully discard the supernatant by pipetting or pouring.
5. Add 300 µl Cell Lysis Solution, and mix by pipetting up and down. Incubate sample at 80°C for 5 min to lyse the cells.

   Samples are stable in Cell Lysis Solution for at least 2 years at room temperature (15–25°C).
6. Add 1.5 µl RNase A Solution, and mix by inverting 25 times. Incubate for 15–60 min at 37°C.
7. Incubate for 1 min on ice to quickly cool the sample.
8. Add 100 µl Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.
9. Centrifuge for 3 min at 13,000–16,000 x *g*.

   The precipitated proteins should form a tight pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation.
10. Pipet 300 µl isopropanol into a clean 1.5 ml microcentrifuge tube and add the supernatant from the previous step by pouring carefully.

    Be sure the protein pellet is not dislodged during pouring.
11. Mix by inverting gently 50 times.
12. Centrifuge for 1 min at 13,000–16,000 x *g*.

    The DNA will be visible as a small white pellet.
13. Carefully discard the supernatant, and drain the tube by inverting on a clean piece of absorbent paper, taking care that the pellet remains in the tube.
14. Add 300 µl of 70% ethanol and invert several times to wash the DNA pellet.
15. Centrifuge for 1 min at 13,000–16,000 x *g*.

16. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube. Allow to air dry for 5 min.

    The pellet might be loose and easily dislodged. Avoid over-drying the DNA pellet, as the DNA will be difficult to dissolve.

17. Add 100 µl DNA Hydration Solution and vortex for 5 s at medium speed to mix.
18. Incubate at 65°C for 1 h to dissolve the DNA.
19. Incubate at room temperature overnight with gentle shaking. Ensure tube cap is tightly closed to avoid leakage. Samples can then be centrifuged briefly and transferred to a storage tube.

Protocol: DNA Purification from Gram-Positive Bacteria Using the Puregene Cell Kit

This protocol is for purification of genomic DNA from fresh or frozen samples of 0.5 ml Gram-positive bacterial cultures using the Puregene Cell Kit.

Things to do before starting

- Preheat water baths to 37°C for use in steps 5 and 9, 65°C for use in step 21, and 80°C for use in step 8 of the procedure.
- Frozen bacterial samples should be thawed and equilibrated to room temperature (15–25°C) before beginning the procedure.

Procedure

1. Prepare an overnight culture.

   Transfer 500 µl of the cell culture (containing approx. 0.5–1.5 x 10^9 cells) to a 1.5 ml microcentrifuge tube on ice.
2. Centrifuge for 5 s at 13,000–16,000 x *g* to pellet cells.

   Longer centrifuge times may be necessary for some species to form a tight pellet.
3. Carefully discard the supernatant by pipetting or pouring.
4. Add 300 µl cell resuspension buffer (not provided), and pipet up and down.
5. Add 1.5 µl lytic enzyme solution (not provided), and mix by inverting 25 times. Incubate for 30 min at 37°C.
6. Centrifuge for 1 min at 13,000–16,000 x *g* to pellet cells.
7. Carefully discard the supernatant with a pipet.
8. Add 300 µl Cell Lysis Solution, and pipet up and down to lyse the cells.

   An incubation for 5 min at 80°C may be necessary to lyse cells of some species.
9. Add 1.5 µl RNase A Solution, and mix by inverting 25 times. Incubate for 15–60 min at 37°C.

10. Incubate for 1 min on ice to quickly cool the sample.
11. Add 100 µl Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.

    Note: For species with high polysaccharide content, incubate the sample on ice for 15–60 min.
12. Centrifuge for 3 min at 13,000–16,000 x *g*.

    The precipitated proteins should form a tight pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation.
13. Pipet 300 µl isopropanol into a clean 1.5 ml microcentrifuge tube and add the supernatant from the previous step by pouring carefully.

    Be sure the protein pellet is not dislodged during pouring.
14. Mix by inverting gently 50 times.
15. Centrifuge for 1 min at 13,000–16,000 x *g*.

    The DNA will be visible as a small white pellet.
16. Carefully discard the supernatant, and drain the tube by inverting on a clean piece of absorbent paper, taking care that the pellet remains in the tube.
17. Add 300 µl of 70% ethanol and invert several times to wash the DNA pellet.
18. Centrifuge for 1 min at 13,000–16,000 x *g*.
19. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube. Allow to air dry for 5 min.

    The pellet might be loose and easily dislodged. Avoid over-drying the DNA pellet, as the DNA will be difficult to dissolve.
20. Add 100 µl DNA Hydration Solution and vortex 5 s at medium speed to mix.
21. Incubate at 65°C for 1 h to dissolve the DNA.
22. Incubate at room temperature (15–25°C) overnight with gentle shaking. Ensure tube cap is tightly closed to avoid leakage. Samples can then be centrifuged briefly and transferred to a storage tube.

Protocol: DNA Purification from Yeast Using the Puregene Cell Kit

This protocol is for purification of genomic DNA from fresh or frozen samples of 1 ml overnight yeast cultures (approximately 1–2 x 108 cells) using the Puregene Cell Kit.

Things to do before starting

- Preheat water baths to 37°C for use in steps 6 and 20 and 65°C for use in step 21 of the procedure.
- Frozen yeast samples should be thawed and equilibrated to room temperature (15–25°C) before beginning the procedure.

Procedure

1. Prepare an overnight culture containing 1–2 x 10^8 cells.
2. Transfer 1 ml of the cell suspension to a 1.5 ml microcentrifuge tube on ice.
3. Centrifuge for 5 s at 13,000–16,000 x *g* to pellet cells.
4. Carefully discard the supernatant by pipetting or pouring.
5. Add 300 µl cell resuspension buffer (not provided), and pipet up and down.
6. Add 1.5 µl lytic enzyme solution (not provided), and mix by inverting 25 times. Incubate for 30 min at 37°C.
7. Centrifuge for 1 min at 13,000–16,000 x *g* to pellet cells.
8. Carefully discard the supernatant by pipetting or pouring.
9. Add 300 µl Cell Lysis Solution, and pipet up and down to lyse the cells.
10. Add 100 µl Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.
11. Centrifuge for 3 min at 13,000–16,000 x *g*.

    The precipitated proteins should form a tight pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation.
12. Pipet 300 µl isopropanol into a clean 1.5 ml microcentrifuge tube and add the supernatant from the previous step by pouring carefully.

Be sure the protein pellet is not dislodged during pouring.

13. Mix by inverting gently 50 times.
14. Centrifuge for 1 min at 13,000–16,000 x *g*.

    The DNA may be visible as a small white pellet.
15. Carefully discard the supernatant, and drain the tube by inverting on a clean piece of absorbent paper, taking care that the pellet remains in the tube.
16. Add 300 µl of 70% ethanol and invert several times to wash the DNA pellet.
17. Centrifuge for 1 min at 13,000–16,000 x *g*.
18. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube. Allow to air dry for 5 min.

    The pellet might be loose and easily dislodged. Avoid over-drying the DNA pellet, as the DNA will be difficult to dissolve.
19. Add 100 µl DNA Hydration Solution and vortex for 5 s at medium speed to mix.
20. Add 1.5 µl RNase A Solution, and mix by vortexing by 1 s. Pulse spin to collect liquid, and incubate at 37°C for 15–60 min.
21. Incubate at 65°C for 1 h to dissolve the DNA.
22. Incubate at room temperature (15–25°C) overnight with gentle shaking. Ensure tube cap is tightly closed to avoid leakage. Samples can then be centrifuged briefly and transferred to a storage tube.

Protocol: Protocol for Purifying Viral DNA

The Puregene procedure can be used to purify viral DNA.

The whole blood and buffy coat protocols or the cultured cell protocol should be used to purify viral DNA that is white blood cell-associated or cell-associated.

The body fluid protocol should be used to purify viral nucleic acids that are not cell associated.

Appendix B: Determination of Concentration, Yield, Purity, and Length of DNA

Determination of concentration, yield, and purity

DNA yields are determined from the concentration of DNA in the eluate, measured by absorbance at 260 nm. A simple measure of sample purity can be 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.

For simplified, accurate and fast determination of sample concentration and purity use the QIAxpert® spectrophotometer.

Determination of DNA size

The size of genomic DNA can be determined by gel electrophoresis. For manual agarose gel electrophoresis, the DNA should be concentrated by alcohol* precipitation and reconstituted by gentle agitation in approximately 30 µl Buffer TE, pH 8.0,* for at least 30 minutes at 60°C. Avoid drying the DNA pellet for more than 10 minutes at room temperature (15–25°C) since over-dried genomic DNA is very difficult to redissolve. Load 3–5 µg DNA per well.

For high-sensitive, automated analysis and sizing of DNA samples use the QIAxcel® capillary electrophoresis system.

* 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.

Appendix C: Repurifying DNA Samples

Purified DNA samples containing high-protein contents can be purified with the following protocol.

Use the recommended reagent volumes given in Table 6 below.

Table 6. Recommended reagent volumes for repurifying DNA samples

Reagent	Relative volume	Volume (µl)			
Cell Lysis Solution	5 volumes	500	1000	2500	5000
Proteinase K	N/A	3	6	15	30
Protein Precipitation Solution	2 volumes	200	400	1000	2000
Isopropanol	6 volumes	600	1200	3000	6000
Glycogen Solution	N/A	1	2	5	10
Ethanol, 70%	6 volumes	600	1200	3000	6000
DNA Hydration Solution	1 volume	100	200	500	1000

1. Add 5 volumes Cell Lysis Solution to the DNA purified sample. Pipet up and down to mix.
2. Incubate sample at 65°C until protein particulates have dissolved.

   To obtain maximum yield, it is important that particulates are dissolved completely before proceeding.
3. If it is difficult to dissolve the protein pellet, Proteinase K (cat. no. 158918 or 158920 and 158143 or 158146) may be added. Incubate at 55°C until particulates have dissolved (1 h to overnight).
4. Add 2 volumes Protein Precipitation Solution and vortex vigorously at high speed for 20 s.
5. Centrifuge for 3 min at 13,000–16,000 x *g* (microcentrifuge tube) or for 10 min at 2000 x *g* (15 or 50 ml centrifuge tube) to pellet the proteins.

6. Dispense 6 volumes isopropanol into a clean tube. Carefully add the supernatant from the previous step by pouring.
7. If the DNA yield is expected to be low (<1 µg), add Glycogen Solution (cat. no. 158930 or 949002 and 158183 or 158186).
8. Mix by inverting gently 50 times.
9. Centrifuge for 1 min at 13,000–16,000 x *g* (microcentrifuge tube) or for 5 min at 2000 x *g* (15 ml or 50 ml centrifuge tube) to pellet the DNA.
10. Carefully pour off supernatant and wash DNA with 6 volumes of 70% ethanol.
11. Centrifuge for 1 min at 13,000–16,000 x *g* (microcentrifuge tube) or for 1 min at 2000 x *g* (15 or 50 ml centrifuge tube). Carefully pour off the ethanol.
12. Allow DNA to air dry for up to 15 min.
13. Add 1 volume of DNA Hydration Solution (or appropriate volume) and vortex for 5 s at medium speed to mix.
14. Incubate at 65°C for 1 h to dissolve the DNA.
15. Incubate at room temperature (15–25°C) overnight with gentle shaking. Ensure that the tube cap is tightly closed to avoid leakage.

Appendix D: Removal of RNA from Purified DNA

If purified DNA contains RNA, determination of yield and concentration by absorbance measurements at 260 and 280 nm may be inaccurate.

The following protocol can be used to remove RNA from a purified DNA sample.

Use the recommended reagent volumes given in Table 7 below.

Table 7. Recommended reagent volumes for removing RNA from purified DNA

Reagent	Relative volume	Volume to add for a 100 µl sample (µl)
Protein Precipitation Solution	0.5 volumes	50
Ethanol, 96–100%	2 volumes	200
Glycogen Solution	N/A	0.5
Ethanol, 70%	3 volumes	300

1. Add the volume of RNase A Solution given in the DNA purification protocol. Incubate at 37°C for 15–60 min.
2. Add Protein Precipitation Solution and 96–100% ethanol to the DNA sample.
3. If DNA yield is expected to be low, add Glycogen Solution (cat. no. 158930 or 949002 and 158183 or 158186).
4. Invert gently 50 times to mix, and incubate at room temperature (15–25°C) for 15 min.
5. Centrifuge for 5 min at 13,000–16,000 x *g* (microcentrifuge tube) or for 10 min at 2000 x *g* (15 or 50 ml centrifuge tube).
6. Carefully discard the supernatant by pouring.
7. Add 70% ethanol. Invert gently to wash the DNA.
8. Centrifuge for 1 min at 13,000–16,000 x *g* (microcentrifuge tube) or for 2 min at 2000 x *g* (15 or 50 ml centrifuge tube).

9. Carefully discard the supernatant by pouring. Drain on clean absorbent paper and allow to air dry for up to 15 min.
10. Add 1 volume of DNA Hydration Solution (or appropriate volume) and vortex for 5 s at medium speed to mix.
11. Incubate at 65°C for 1 h to dissolve the DNA.
12. Incubate at room temperature overnight with gentle shaking. Ensure that the tube cap is tightly closed to avoid leakage.

Appendix E: Concentrating DNA

The following protocol is used to concentrate low-concentration DNA samples by precipitation.

Use the recommended reagent volumes given in Table 7, page 70.

1. Add Protein Precipitation Solution and 96–100% ethanol to the DNA sample.
2. If DNA yield is expected to be low, add Glycogen Solution (cat. no. 158930 or 949002 and 158183 or 158186).
3. Invert gently 50 times to mix, and incubate at room temperature (15–25°C) 15 min.
4. Centrifuge for 5 min at 13,000–16,000 x *g* (microcentrifuge tube) or for 10 min at 2000 x *g* (15 ml or 50 ml centrifuge tube).
5. Carefully pour off the supernatant.
6. Add 70% ethanol. Invert gently to wash the DNA.
7. Centrifuge for 1 min at 13,000–16,000 x *g* (microcentrifuge tube) or for 2 min at 2000 x *g* (15 or 50 ml centrifuge tube).
8. Carefully pour off supernatant. Drain on clean absorbent paper and allow DNA to air dry for up to 15 min.
9. Add DNA Hydration Solution and vortex for 5 s at medium speed to mix.

   Note: Adding a smaller volume of DNA Hydration Solution used previously will increase the DNA concentration.
10. Incubate at 65°C for 1 h to dissolve the DNA.
11. Incubate at room temperature overnight with gentle shaking. Ensure that the tube cap is tightly closed to avoid leakage.

Ordering Information

Product	Contents	Cat. no.
DNA from whole blood and bone marrow		
Puregene Blood Kit (120 ml)	For 120 ml blood: RBC Lysis Solution, RNase A Solution, and Reagents	158023
Puregene Blood Kit (1000 ml)	For 1000 ml blood: RBC Lysis Solution, RNase A Solution, and Reagents	158026
Puregene Cell Kit — for purification of archive-quality DNA from cell cultures and cell suspensions		
Puregene Cell Kit (8 x 10^8)	For 8 x 10^8 cells: RNase A Solution and Reagents	158043
Puregene Cell Kit (6.7 x 10^9)	For 6.7 x 10^9 cells: Reagents	158046
Puregene Tissue Kit — for purification of archive-quality DNA from tissue		
Puregene Tissue Kit (4 g)	For 4 g tissue: RNase A Solution, Proteinase K, and Reagents	158063
Puregene Tissue Kit (33 g)	For 33 g tissue: RNase A Solution, Proteinase K, and Reagents	158066
Accessories		
Puregene RBC Lysis Solution (450 ml)	450 ml RBC Lysis Solution	158103
Puregene RBC Lysis Solution (1000 ml)	1000 ml RBC Lysis Solution	158106

Puregene Cell Lysis Solution (125 ml)	125 ml Cell Lysis Solution	158113
Puregene Cell Lysis Solution (1000 ml)	1000 ml Cell Lysis Solution	158116
Puregene Protein Precipitation Solution (50 ml)	50 ml Protein Precipitation Solution	158123
Puregene Protein Precipitation Solution (350 ml)	350 ml Protein Precipitation Solution	158126
Puregene DNA Hydration Solution (100 ml)	100 ml DNA Hydration Solution	158133
Puregene DNA Hydration Solution (500 ml)	500 ml DNA Hydration Solution	158136
Proteinase K (650 µl)	650 µl Puregene Proteinase K	158918/ 158143
Proteinase K (5 ml)	5 ml Puregene Proteinase K	158920/ 158146
RNase A Solution (650 µl)	650 µl RNase A Solution	158922/ 158153
RNase A Solution (5 ml)	5 ml RNase A Solution	158156
Glycogen Solution (500 µl)	500 µl Glycogen Solution	158930/ 158183
Glycogen Solution (5 ml)	5 ml Glycogen Solution	949002/ 158186

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Document Revision History

Document	Changes	Date
HB-0326-004	Updated the product names and catalog numbers.	January 2022

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