

Second Edition

September 2007

## **Gentra® Puregene® Handbook**

For purification of archive-quality DNA from  
human whole blood

bone marrow

buffy coat

buccal cells

body fluids

cultured cells

tissue

mouse tail

yeast

bacteria



**Sample & Assay Technologies**

Trademarks: QIAGEN®, Gentra®, Puregene®, Clotspin® (QIAGEN Group); Ficoll® (Amersham Biosciences); Scope® (Procter & Gamble Co.); Listerine® (McNEIL-PPC, Inc).

© 2007 QIAGEN, all rights reserved.

# Contents

<b>Kit Contents</b>	<b>5</b>
<b>Storage</b>	<b>7</b>
<b>Product Use Limitations</b>	<b>7</b>
<b>Product Warranty and Satisfaction Guarantee</b>	<b>8</b>
<b>Technical Assistance</b>	<b>8</b>
<b>Quality Control</b>	<b>8</b>
<b>Safety Information</b>	<b>9</b>
<b>Introduction</b>	<b>10</b>
Principle and procedure	10
<b>Equipment and Reagents to Be Supplied by User</b>	<b>12</b>
<b>Important Notes</b>	<b>13</b>
Storage of blood samples	13
Preparation of buffy coat	13
Yield and quality of purified DNA	13
Optimized handling for higher throughputs	14
Processing large-volume samples	15
Enhanced productivity protocols	17
<b>Protocols</b>	
■ <b>DNA Purification from Whole Blood or Bone Marrow Using the Gentra Puregene Blood Kit</b>	<b>19</b>
■ <b>Enhanced Productivity DNA Purification from Whole Blood Using the Gentra Puregene Blood Kit</b>	<b>22</b>
■ <b>DNA Purification from Compromised Blood Samples Using the Gentra Puregene Blood Kit</b>	<b>24</b>
■ <b>DNA Purification from Buffy Coat Using the Gentra Puregene Blood Kit</b>	<b>26</b>
■ <b>DNA Purification from a Buccal Brush Using the Gentra Puregene Buccal Cell Kit</b>	<b>28</b>
■ <b>DNA Purification from Buccal Cells in Mouthwash Using the Gentra Puregene Buccal Cell Kit</b>	<b>30</b>
■ <b>DNA Purification from Body Fluid Using the Gentra Puregene Blood Kit</b>	<b>32</b>
■ <b>DNA Purification from Cultured Cells Using the Gentra Puregene Cell Kit</b>	<b>34</b>
■ <b>DNA Purification from 1–2 x 10<sup>6</sup> Fixed Cells Using the Gentra Puregene Tissue Kit</b>	<b>37</b>
■ <b>DNA Purification from Tissue Using the Gentra Puregene Tissue Kit</b>	<b>39</b>

■	DNA Purification from Fixed Tissue Using the Gentra Puregene Tissue Kit	41
■	DNA Purification from FFPE Tissue Using the Gentra Puregene Tissue Kit	43
■	DNA Purification from Mouse Tail Tissue Using the Gentra Puregene Mouse Tail Kit	45
■	DNA Purification from Yeast Using the Gentra Puregene Yeast/Bact. Kit	47
■	DNA Purification from Gram-Negative Bacteria Using the Gentra Puregene Yeast/Bact. Kit	49
■	DNA Purification from Gram-Positive Bacteria Using the Gentra Puregene Yeast/Bact. Kit	51
	<b>Troubleshooting Guide</b>	53
	<b>Appendix A: Protocol for Purifying Viral DNA</b>	60
	<b>Appendix B: Determination of Concentration, Yield, Purity, and Length of DNA</b>	61
	<b>Appendix C: Repurifying DNA Samples</b>	62
	<b>Appendix D: Removal of RNA from Purified DNA</b>	64
	<b>Appendix E: Concentrating DNA</b>	65
	<b>Ordering Information</b>	66

## Kit Contents

					Plus
Gentra Puregene Blood Kit	(3 ml)	(30 ml)	(120 ml)	(1000 ml)	(1000 ml)
Catalog no.	158422	158445	158467	158389	158489
Volume of blood processed per kit	3 ml	30 ml	120 ml	1000 ml	1000 ml
RBC Lysis Solution	9 ml	100 ml	450 ml	3000 ml	3000 ml
Cell Lysis Solution	3 ml	35 ml	125 ml	1000 ml	1000 ml
Protein Precipitation Solution	1 ml	12 ml	50 ml	350 ml	350 ml
DNA Hydration Solution	1 ml	10 ml	100 ml	500 ml	500 ml
RNase A Solution	15 µl	250 µl	650 µl	—	5 ml
Handbook	1	1	1	1	1

Gentra Buccal Cell Kit	(10)	(100)	(400)
Catalog no.	158822	158845	158867
Number of preps	10	100	400
Cell Lysis Solution	3 ml	35 ml	125 ml
Protein Precipitation Solution	1 ml	12 ml	50 ml
DNA Hydration Solution	1 ml	10 ml	100 ml
Buccal Collection Brushes	10	100	400
RNase A Solution	15 µl	250 µl	650 µl
Glycogen Solution	10 µl	50 µl	200 µl
Handbook	1	1	1

		Plus				
Gentra Puregene Cell Kit		(2 x 10 <sup>7</sup> )	(2 x 10 <sup>8</sup> )	(8 x 10 <sup>8</sup> )	(6.7 x 10 <sup>9</sup> )	(6.7 x 10 <sup>9</sup> )
Catalog no.		158722	158745	158767	158388	158788
Number of cells processed per kit	2 x 10 <sup>7</sup>	2 x 10 <sup>8</sup>	8 x 10 <sup>8</sup>	6.7 x 10 <sup>9</sup>	6.7 x 10 <sup>9</sup>	
Cell Lysis Solution	3 ml	35 ml	125 ml	1000 ml	1000 ml	
Protein Precipitation Solution	1 ml	12 ml	50 ml	350 ml	350 ml	
DNA Hydration Solution	1 ml	10 ml	100 ml	500 ml	500 ml	
RNase A Solution	15 µl	250 µl	650 µl	—	5 ml	
Handbook	1	1	1	1	1	

Gentra Puregene Tissue Kit		(100 mg)	(4 g)	(33 g)
Catalog no.		158622	158667	158689
Amount of tissue processed per kit	100 mg	4 g	33 g	
Cell Lysis Solution	3 ml	125 ml	1000 ml	
Protein Precipitation Solution	1 ml	50 ml	350 ml	
DNA Hydration Solution	1 ml	100 ml	500 ml	
RNase A Solution	15 µl	650 µl	5 ml	
Puregene Proteinase K	15 µl	650 µl	5 ml	
Handbook	1	1	1	

Gentra Puregene Mouse Tail Kit		(100 mg)	(4 g)	
Catalog no.		158222	158267	
Amount of mouse tail processed per kit	100 mg	4 g		
Cell Lysis Solution	3 ml	125 ml		
Protein Precipitation Solution	1 ml	50 ml		
DNA Hydration Solution	1 ml	100 ml		
Puregene Proteinase K	15 µl	650 µl		
Handbook	1	1		

<b>Gentra Puregene Yeast/Bact. Kit</b>	<b>(5 ml)</b>	<b>(200 ml)</b>
<b>Catalog no.</b>	<b>158522</b>	<b>158567</b>
<b>Volume of culture processed per kit</b>	<b>5 ml</b>	<b>200 ml</b>
Cell Lysis Solution	3 ml	125 ml
Protein Precipitation Solution	1 ml	50 ml
DNA Hydration Solution	1 ml	25 ml
Cell Suspension Solution	3 ml	125 ml
Lytic Enzyme Solution	15 $\mu$ l	650 $\mu$ l
RNase A Solution	15 $\mu$ l	650 $\mu$ l
Handbook	1	1

## Storage

Gentra Puregene Kit buffers and reagents must be stored dry at the temperature indicated on the kit label. RNase A Solution, Puregene Proteinase K, Lytic Enzyme Solution, and Glycogen Solution must be refrigerated 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, Gentra Puregene Kits are stable until the expiration date printed on the label and on the kit box.

## Product Use Limitations

Gentra Puregene Kits are intended for general laboratory use. No claim or representation is intended to provide information 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.

## **Product Warranty and Satisfaction Guarantee**

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

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit [www.qiagen.com](http://www.qiagen.com) ).

## **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 Gentra 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/goto/TechSupportCenter](http://www.qiagen.com/goto/TechSupportCenter) or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit [www.qiagen.com](http://www.qiagen.com) ).

## **Quality Control**

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

# **Safety Information**

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

The following risk and safety phrases apply to the components of Gentra Puregene Kits:

## **Puregene Proteinase K Solution**

Contains Proteinase K: sensitizer, irritant. Risk and safety phrases: \* R36/37/38-42/43, S23-24-26-36/37

## **RNase A Solution**

Contains ribonuclease: sensitizer. Risk and safety phrases: R42/43, S23-24-26-36/37

## **Lytic Enzyme Solution**

Contains lyticase: sensitizer, irritant. Risk and safety phrases: R36/37/38-42/43, S23-24-26-36/37

## **24-hour emergency information**

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

Poison Information Center Mainz, Germany

Tel.: +49-6131-19240

\* R36/37/38: Irritating to eyes, respiratory system, and skin; R42/43: May cause sensitization by inhalation and skin contact; S23: Do not breathe spray; S24: Avoid contact with the skin; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36/37: Wear suitable protective clothing and gloves.

# Introduction

Gentra 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 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 also 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 a buffered solution containing a DNA stabilizer. 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°C, -20°C, or -80°C.

Supplementary protocols for processing other sample types or amounts are available at [www.qiagen.com/literature/protocols](http://www.qiagen.com/literature/protocols) 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 material safety data sheets (MSDSs), available from the product supplier.

## For all protocols:

- 100% isopropanol
- 70% ethanol\*
- 1.5 ml microcentrifuge tubes, 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

## For buccal swabs

- Scissors or razor blade

## For mouse tails

- Optional: RNase A Solution, cat. no. 158922

## For body fluid and buccal cell samples

- Optional: Puregene Proteinase K, cat. no. 158918

## For tissue samples

- Liquid nitrogen
- Mortar and pestle

## For tissue in fixative or FFPE tissues

- Xylene
- Microcentrifuge tube pestle

\* Do not use denatured alcohol, which contains other substances such as methanol or methyl ethyl ketone.

## Important Notes

### Storage of blood samples

Fresh or frozen whole blood samples can be used. 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. Tubes containing citrate or heparin may also be used. 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, freeze the blood at –80°C directly after collection. DNA can be isolated from samples stored for less than 2 years 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 800  $\times$  g for 10 minutes at room temperature (15–25°C). After centrifugation, 3 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 Gentra 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.

Gentra Puregene Kits are designed to purify high yields of high-quality DNA. Table 1, page 14 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 ( $7 \times 10^6$ white cells)	16–50	35
Buccal swabs, 1 swab	0.2–2	1
Body fluids, 100 µl	0.2–5	2.5
Cultured cells, 1–2 $\times 10^6$ cells	5–10	7
Solid animal tissue, 1 mg	0.5–10	5
Nonmammalian blood, 1 µl	2.5–7.5	5
Yeast, 1 ml culture	3–6	4.5
Gram-negative bacteria, 1 ml culture	25–75	50
Gram-positive bacteria, 1 ml culture	3–30	17

## Optimized handling for higher throughputs

The following points may help with high-throughput sample processing using Gentra 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 seconds 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

Tables 2–5 provide information about scaling the Gentra 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 cells (x 10 <sup>6</sup> ) <sup>*</sup>					
	0.35	1.4	3.5	7	35	70
<b>Blood volume for preparing buffy coat (μl)</b>	50	200	500	1000	5000	10,000
<b>Tube size (ml)</b>	1.5	1.5	1.5	15	50	50
<b>RBC Lysis Solution (μl)<sup>†</sup></b>				3 volumes <sup>†</sup>		
<b>Cell Lysis Solution (μl)</b>	50	200	500	1000	5000	10,000
<b>RNase A Solution (μl)</b>	0.25	1	2.5	5	25	50
<b>Protein Precipitation Solution (μl)</b>	17	67	167	333	1670	3330
<b>Isopropanol (μl)<sup>‡</sup></b>	50	200	500	1000	5000	10,000
<b>70 % ethanol (μl)</b>	50	200	500	1000	5000	10,000
<b>DNA Hydration Solution (μl)<sup>§</sup></b>	5	20	50	100	500	1000
<b>Typical DNA yield (μg)<sup>¶</sup></b>	1.75	7	17.5	35	175	350

\* Cell number estimates assume an average of 7 x 10<sup>6</sup> white 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 (for example, 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) 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*			
	100–10,000	0.5–1 x 10 <sup>6</sup>	3–5 x 10 <sup>6</sup>	3–5 x 10 <sup>7</sup>
<b>Tube size (ml)</b>	0.6	1.5	1.5	15
<b>Cell Lysis Solution (μl)</b>	60	150	600	6000
<b>RNase A Solution (μl)</b>	0.50	0.75	3	30
<b>Protein Precipitation Solution (μl)</b>	20	50	200	2000
<b>Isopropanol (μl)<sup>†</sup></b>	60	150	600	6000
<b>70% ethanol (μl)</b>	60	150	600	6000
<b>DNA Hydration Solution (μl)<sup>‡</sup></b>	10	25	100	500
<b>Typical DNA yield (μg)<sup>§</sup></b>	0.0004–0.04	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 (for example, 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) 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
<b>Tube size (ml)</b>	1.5	15	15	50
<b>Cell Lysis Solution (ml)</b>	0.5	1	2.5	15
<b>RNase A Solution (μl)</b>	3	6	15	90
<b>Protein Precipitation Solution (μl)</b>	200	400	1000	6000
<b>Isopropanol (ml)*</b>	0.6	1.2	3	18
<b>70% ethanol (ml)</b>	0.6	1.2	3	18
<b>DNA Hydration Solution (μl)</b>	20	40	50	250

\* If the DNA yield is expected to be low (for example, 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) 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
<b>Tube size (ml)</b>	1.5	1.5	2	15
<b>Cell Lysis Solution (μl)</b>	100	600	750	6000
<b>RNase A Solution (μl)</b>	0.5	3	3.75	30
<b>Protein Precipitation Solution (μl)</b>	33	200	250	2000
<b>Isopropanol (μl)*</b>	100	600	750	6000
<b>70% ethanol (μl)</b>	100	600	750	6000
<b>DNA Hydration Solution (μl)†</b>	20	100	175	500
<b>Typical range of DNA yields (μg)‡</b>	0.3–8	5–8	12–100	50–800
<b>Typical DNA concentration (μg/ml)</b>	415	400	400	1000
<b>Expected DNA yield (μg)</b>	4.15	40	60	425

\* If the DNA yield is expected to be low (for example, 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) 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](http://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 Gentra Puregene Blood Kit

This protocol is for purification of genomic DNA from fresh or frozen samples of 300  $\mu$ l, 3 ml, or 10 ml whole blood using the Gentra 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  $\mu$ l 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 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  $\mu$ l, ▲ 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  $\mu$ l, ▲ 3 ml, or ● 10 ml whole blood or bone marrow, and mix by inverting 10 times.
3. Incubate ■ 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  $\times$  g, ▲ 2 min at 2000  $\times$  g, or ● 2 min at 2000  $\times$  g to pellet the white blood cells.

5. Carefully discard the supernatant by pipetting or pouring, leaving approximately ■ 10  $\mu$ l, ▲ 200  $\mu$ l, or ● 200  $\mu$ l 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  $\mu$ l, ▲ 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  $\mu$ l, ▲ 15  $\mu$ l, or ● 50  $\mu$ l 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  $\mu$ l, ▲ 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  $\times$  g, ▲ 5 min at 2000  $\times$  g, or ● 5 min at 2000  $\times$  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  $\mu$ l 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 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  $\times$  g, ▲ 3 min at 2000  $\times$  g, or ● 3 min at 2000  $\times$  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  $\mu$ l, ▲ 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  $\times$  g, ▲ 1 min at 2000  $\times$  g, or ● 1 min at 2000  $\times$  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 s, ▲ 1 min, or ● 10–15 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  $\mu$ l, ▲ 250  $\mu$ l, 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 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 Gentra Puregene Blood Kit

This protocol is for enhanced productivity purification of genomic DNA from 10 ml whole blood using the Gentra 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](http://www.qiagen.com)).

## Important point 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  $\times$  g to pellet the white blood cells.
4. Carefully discard the supernatant, leaving less than 200  $\mu$ l 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  $\times$  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.  
The pellet might be loose and easily dislodged.
14. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper for 1 min, taking care that the pellet remains in the tube.
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 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 Gentra Puregene Blood Kit

This protocol is for purification of genomic DNA from 10 ml compromised whole blood using the Gentra 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°C and 65°C for use in steps 7 and 19 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 133  $\mu$ l Glycogen Solution (cat. no. 158930). 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  $\times$  g.

The DNA will be visible as a small white pellet.  
The pellet might be loose and easily dislodged.
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 10 ml of 70% ethanol and invert several times to wash the DNA pellet.
16. Centrifuge for 1 min at 2000  $\times$  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. Allow to air dry for up to 5–10 min.

The pellet might be loose and easily dislodged.
18. Add 500  $\mu$ l DNA Hydration Solution and vortex for 5 s at medium speed to mix.
19. Incubate at 65°C for 1 h to dissolve the DNA.
20. 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 Buffy Coat Using the Gentra Puregene Blood Kit

This protocol is for purification of genomic DNA from buffy coat prepared from 3 ml whole blood using the Gentra Puregene Blood Kit.

## Things to do before starting

- Preheat water bath to 65°C for use in step 19 of the procedure.
- Buffy coat is a leukocyte-enriched fraction of whole blood. Preparing a buffy coat fraction from whole blood is simple and yields approximately 5–10 times more DNA than an equivalent volume of whole blood. Prepare buffy coat by centrifuging whole blood at 2500  $\times$  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. DNA may be purified from up to 200  $\mu$ l buffy coat preparation containing a maximum of  $1 \times 10^7$  white blood cells.
- Frozen buffy coat 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 8 of the procedure.

## Procedure

1. If the buffy coat preparation contains red blood cells, continue with step 2. Otherwise, pipet 3 ml Cell Lysis Solution into a 15 ml centrifuge tube, add 150–200  $\mu$ l sample, and continue with step 8.
2. Dispense 3 volumes RBC Lysis Solution into a 15 ml centrifuge tube (e.g., if processing 250  $\mu$ l buffy coat, dispense 750  $\mu$ l RBC Lysis Solution). Add 150–250  $\mu$ l buffy coat preparation.
3. Invert to mix, and incubate for 10 min at room temperature. Invert again at least once during the incubation.
4. Centrifuge for 5 min at 2000  $\times$  g.
5. Carefully discard the supernatant by pipetting or pouring, leaving approximately 100–200  $\mu$ l of the residual liquid and the 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 3 ml Cell Lysis Solution, and pipet up and down or vortex vigorously to lyse the cells.**

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 15  $\mu$ l RNase A Solution, and mix by inverting 25 times. Incubate for 15 min at 37°C. Then incubate for 3 min on ice to quickly cool the sample.**

**9. Add 1 ml Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.**

**10. Centrifuge for 5 min at 2000  $\times$  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 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.

**12. Mix by inverting gently 50 times.**

**13. Centrifuge for 3 min at 2000  $\times$  g.**

The DNA will 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 3 ml of 70% ethanol and invert several times to wash the DNA pellet.**

**16. Centrifuge for 1 min at 2000  $\times$  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. Allow to air dry for up to 15 min.**

The pellet might be loose and easily dislodged.

**18. Add 250  $\mu$ l DNA Hydration Solution and vortex for 5 s at medium speed to mix.**

**19. Incubate at 65°C for 1h to dissolve the DNA.**

**20. 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 a Buccal Brush Using the Gentra Puregene Buccal Cell Kit

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

## 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 $\mu$ l 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  $\mu$ l 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](http://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 $\mu$ l Puregene Proteinase K (cat. no. 158918), 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 $\mu$ l 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 $\mu$ l 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  $\mu$ l isopropanol and 0.5  $\mu$ l Glycogen Solution (cat. no. 158930) 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  $\mu$ l 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 up to 15 min.**

The pellet might be loose and easily dislodged.

**16. Add 20  $\mu$ l 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 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 Gentra Puregene Buccal Cell Kit

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

## Things to do before starting

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

## Procedure

- Dispense 10 ml Original Mint Scope® Mouthwash (Procter & Gamble) or Listerine® mouthwash (McNEIL-PPC, Inc) into a 50 ml centrifuge tube.
- 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.

- Centrifuge for 5 min at 2000 x g to pellet cells.

If the cell pellet is too loose, repeat the centrifugation.

- Carefully discard the supernatant by pipetting or pouring, leaving the pellet undisturbed.
- Add 1 ml Cell Lysis Solution, and mix by inverting 50 times.
- Incubate for 15 min at room temperature.

If the sample has a high protein content, and additional 20  $\mu$ l Cell Lysis Solution can be added. Note that the remainder of the protocol must be scaled up accordingly. Contact QIAGEN Technical Services for more information (see back cover or visit [www.qiagen.com](http://www.qiagen.com) ).

- Add 10  $\mu$ l Puregene Proteinase K (cat. no. 158918), and mix by inverting 3 times.
- Vortex vigorously at high speed for 20 s to mix.
- Incubate for 10 min at room temperature.
- Add 340  $\mu$ l Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.
- Incubate for 10 min on ice.

Incubation on ice is important to ensure a tight pellet in the next step.

- 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 5  $\mu$ l 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  $\times$  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  $\times$  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 1 min.

The pellet might be loose and easily dislodged.
21. Add 400  $\mu$ l 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 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 Gentra Puregene Blood Kit

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

## Important point before starting

- In some steps of the procedure, one of two choices can be made. Choose ■ if processing 50  $\mu$ l body fluid samples; choose ▲ if processing 1 ml body fluid samples.

## 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  $\times g$  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  $\mu$ l 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  $\mu$ l or ▲ 1 ml body fluid, and mix by pipetting up and down.
  - 1b. Dispense ■ 550  $\mu$ l 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 ■ 50  $\mu$ l 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  $\mu$ l or ▲ 30  $\mu$ l Puregene Proteinase K (cat. no. 158918), 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  $\mu$ l or ▲ 30  $\mu$ l 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  $\mu$ l 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  $\times$  g or ▲ 10 min at 2000  $\times$  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  $\mu$ l isopropanol into a clean 1.5 ml 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  $\mu$ g or ▲ <10  $\mu$ g), add ■ 0.5  $\mu$ l or ▲ 10  $\mu$ l Glycogen Solution (cat. no. 158930).
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  $\times$  g or ▲ 10 min at 2000  $\times$  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  $\mu$ l 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  $\times$  g or ▲ 1 min at 2000  $\times$  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 up to 15 min.

The pellet might be loose and easily dislodged.

16. Add ■ 10  $\mu$ l or ▲ 100  $\mu$ l 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 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 Gentra Puregene Cell Kit

This protocol is for purification of genomic DNA from  $1\text{--}2 \times 10^6$  or  $1\text{--}2 \times 10^7$  cultured cells using the Gentra Puregene Cell Kit.

## Important point before starting

- In some steps of the procedure, one of two choices can be made. Choose ■ if processing  $1\text{--}2 \times 10^6$  cells; choose ▲ if processing  $1\text{--}2 \times 10^7$  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  $\mu\text{l}$  suspension containing up to  $2 \times 10^7$  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  $\times g$  in a 1.5 ml microcentrifuge tube for 5 seconds. Remove the supernatant, leaving 200  $\mu\text{l}$  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 steps 1a (for cells grown in suspension) or 1b (for cells grown in a monolayer).
  - 1a Cells grown in suspension (do not use more than  $2 \times 10^7$  cells): Determine the number of cells. Centrifuge the appropriate number of cells for 5 min at 300  $\times g$  in a 1.5 ml microcentrifuge tube. Remove the supernatant leaving behind 200  $\mu\text{l}$  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  $2 \times 10^7$  cells): Cells grown in a monolayer can be detached from the culture flask by either trypsinization or using a cell scraper.

### To trypsinize cells:

Determine the number of 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 transfer the appropriate number of cells (maximum  $2 \times 10^7$  cells) to a 1.5 ml microcentrifuge tube. Centrifuge for 5 min at  $300 \times g$ . Remove the supernatant leaving behind 200  $\mu$ l 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  $2 \times 10^7$  cells) to a 1.5 ml microcentrifuge tube and centrifuge for 5 min at  $300 \times g$ . Remove the supernatant leaving behind 200  $\mu$ l residual fluid. Thoroughly suspend the pellet in the residual fluid by pipetting up and down until the cells are resuspended. Continue with step 2.

2. Add ■ 1–2  $\times 10^6$  or ▲ 1–2  $\times 10^7$  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  $\times g$  or ▲ 3 min at 500  $\times g$  to pellet cells.
4. Carefully discard the supernatant by pipetting or pouring, leaving approximately ■ 20  $\mu$ l or ▲ 200  $\mu$ l residual liquid.
5. Vortex the tube vigorously to resuspend the cells in the residual supernatant. Vortexing greatly facilitates cell lysis in the next step.
6. Add ■ 300  $\mu$ l 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).

7. Optional: If RNA-free DNA is required, add ■ 1.5  $\mu$ l or ▲ 15  $\mu$ l 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.
8. Add ■ 100  $\mu$ l or ▲ 1 ml Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.

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

9. **Centrifuge ■ for 1 min at 13,000–16,000  $\times g$  or ▲ 10 min at 2000  $\times g$ .**

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  $\mu l$  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.

11. **Mix by inverting gently 50 times.**

12. **Centrifuge for ■ 1 min at 13,000–16,000  $\times g$  or ▲ 3 min at 2000  $\times g$ .**

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  $\mu l$  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  $\times g$  or ▲ 1 min at 2000  $\times g$ .**

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 up to 15 min.**

The pellet might be loose and easily dislodged.

17. **Add ■ 50  $\mu l$  or ▲ 250  $\mu l$  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 1–2 x 10<sup>6</sup> Fixed Cells Using the Gentra Puregene Tissue Kit

This protocol is for purification of genomic DNA from 1–2 x 10<sup>6</sup> cells fixed in methanol–acetic acid, 70% ethanol, or 95% ethanol using the Gentra 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

- Transfer 1–2 x 10<sup>6</sup> cells fixed in methanol–acetic acid (3:1), 70% ethanol, or 95% ethanol to a 1.5 ml microcentrifuge tube.

- Centrifuge at 13,000–16,000 x g for 5 s to pellet cells.

- 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.

- 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.

- Complete cell lysis by following step 5a or 5b below:

- 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.

- If maximum yield is required, add 1.5 µl Puregene Proteinase K (cat. no. 158918) 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.

- Add 1.5 µl RNase A Solution, and mix the sample by inverting the tube 25 times.

- Incubate at 37°C for 15 min.

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

- Incubate for 1 min on ice to quickly cool the sample.

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

- 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  $\mu$ l 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  $\mu$ g), add 0.5  $\mu$ l Gycogen Solution (cat. no. 158930).
13. Mix by inverting gently 50 times.
14. Centrifuge for 1 min at 13,000–16,000  $\times g$ .  
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  $\mu$ l of 70% ethanol and invert several times to wash the DNA pellet.
17. Centrifuge for 1 min at 13,000–16,000  $\times g$ .
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 up to 15 min.  
The pellet might be loose and easily dislodged.
19. Add 50  $\mu$ l 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 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 Tissue Using the Gentra 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 Gentra 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 $\mu$ l 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 $\mu$ l or ▲ 15 $\mu$ l Puregene 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 $\mu$ l or ▲ 15 $\mu$ l 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 $\mu$ l 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 $\times$ g or ▲ 10 min at 2000 $\times$ 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  $\mu$ l 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  $\mu$ g) add ■ 0.5  $\mu$ l Glycogen Solution (cat. no. 158930).
8. Mix by inverting gently 50 times.
9. Centrifuge for ■ 1 min at 13,000–16,000  $\times$  g or ▲ 3 min at 2000  $\times$  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  $\mu$ l 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  $\times$  g or ▲ 1 min at 2000  $\times$  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 up to 15 min.

The pellet might be loose and easily dislodged.
14. Add ■ 50  $\mu$ l or ▲ 150  $\mu$ l 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 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 Fixed Tissue Using the Gentra Puregene Tissue Kit

This protocol is for purification of genomic DNA from 5–10 mg fixed tissue using the Gentra 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  $\mu$ l 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  $\mu$ l Puregene 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  $\mu$ l Puregene 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  $\mu$ l 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  $\mu$ l Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.
8. Centrifuge for 3 min at 13,000–16,000  $\times g$ .

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  $\mu$ l 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.

10. If the DNA yield is expected to be low (<10  $\mu$ g), add 0.5  $\mu$ l Gycogen Solution (cat. no. 158930).

11. Mix by inverting gently 50 times.

12. Centrifuge for 5 min at 13,000–16,000  $\times g$ .

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  $\mu$ l ml of 70% ethanol and invert several times to wash the DNA pellet.

15. Centrifuge for 1 min at 13,000–16,000  $\times g$ .

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 up to 10 min.  
The pellet might be loose and easily dislodged.

17. Add 20  $\mu$ l 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 FFPE Tissue Using the Gentra Puregene Tissue Kit

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

## 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  $\mu$ l 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  $\times$  g to pellet the tissue.**
4. **Carefully discard the supernatant.**
5. **Add 300  $\mu$ l xylene and incubate for 5 min with gentle shaking at room temperature.**
6. **Centrifuge for 1–3 min at 13,000–16,000  $\times$  g to pellet the tissue.**
7. **Carefully discard the supernatant.**
8. **Add 300  $\mu$ l xylene and incubate for 5 min with gentle shaking at room temperature.**
9. **Centrifuge for 1–3 min at 13,000–16,000  $\times$  g to pellet the tissue.**
10. **Carefully discard the supernatant.**
11. **Add 300  $\mu$ l 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  $\times$  g to pellet the tissue.**
13. **Carefully discard the supernatant.**
14. **Add 300  $\mu$ l 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  $\times$  g to pellet the tissue.**
16. **Carefully discard the supernatant.**
17. **Add 300  $\mu$ l Cell Lysis Solution, and homogenize using 30–50 strokes with a microcentrifuge tube pestle.**

18. Add 1.5  $\mu$ l Puregene 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  $\mu$ l Puregene 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  $\mu$ l 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  $\mu$ l Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.
23. Centrifuge for 3 min at 13,000–16,000  $\times$  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  $\mu$ l 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  $\mu$ g), add 0.5  $\mu$ l Glycogen Solution (cat. no. 158930).
26. Mix by inverting gently 50 times.
27. Centrifuge for 5 min at 13,000–16,000  $\times$  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  $\mu$ l ml of 70% ethanol and invert several times to wash the DNA pellet.
30. Centrifuge for 1 min at 13,000–16,000  $\times$  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 up to 10 min.

The pellet might be loose and easily dislodged.
32. Add 20  $\mu$ l 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 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 Gentra Puregene Mouse Tail Kit

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

## Things to do before starting

- Preheat water baths to 55°C and 65°C for use in steps 4 and 16 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  $\mu$ l Cell Lysis Solution into a 1.5 ml microcentrifuge tube and add the tissue from the previous step.**
3. **Add 1.5  $\mu$ l Puregene 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  $\mu$ l RNase A Solution (cat. no. 158922), 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  $\mu$ l Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.**
7. **Centrifuge for 3 min at 13,000–16,000  $\times$  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  $\mu$ l isopropanol into a clean 1.5 ml microcentrifuge tube and add the supernatant from the previous step by pouring carefully.**
9. **Mix by inverting gently 50 times.**

**10. Centrifuge for 1 min at 13,000–16,000  $\times$  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  $\mu$ l of 70% ethanol and invert several times to wash the DNA pellet.**
13. **Centrifuge for 1 min at 13,000–16,000  $\times$  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 up to 10 min.  
The pellet might be loose and easily dislodged.
15. Add 50  $\mu$ l 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 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 Gentra Puregene Yeast/Bact. Kit

This protocol is for purification of genomic DNA from fresh or frozen samples of 1 ml overnight yeast cultures (approximately  $1\text{--}2 \times 10^8$  cells) using the Gentra Puregene Yeast/Bact. 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  $1\text{--}2 \times 10^8$  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  $\times g$  to pellet cells.
4. Carefully discard the supernatant by pipetting or pouring.
5. Add 300  $\mu\text{l}$  Cell Suspension Solution, and pipet up and down.
6. Add 1.5  $\mu\text{l}$  Lytic Enzyme Solution, and mix by inverting 25 times. Incubate for 30 min at 37°C.
7. Centrifuge for 1 min at 13,000–16,000  $\times g$  to pellet cells.
8. Carefully discard the supernatant by pipetting or pouring.
9. Add 300  $\mu\text{l}$  Cell Lysis Solution, and pipet up and down to lyse the cells.
10. Add 100  $\mu\text{l}$  Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.
11. Centrifuge for 3 min at 13,000–16,000  $\times g$ .

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  $\mu\text{l}$  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  $\times g$ .  
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  $\mu$ l of 70% ethanol and invert several times to wash the DNA pellet.
17. Centrifuge for 1 min at 13,000–16,000  $\times$  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 up to 10 min.

The pellet might be loose and easily dislodged.
19. Add 50  $\mu$ l DNA Hydration Solution and vortex for 5 s at medium speed to mix.
20. Add 1.5  $\mu$ l 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 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-Negative Bacteria Using the Gentra Puregene Yeast/Bact. Kit

This protocol is for purification of genomic DNA from fresh or frozen samples of 0.5 ml Gram-negative bacterial cultures using the Gentra Puregene Yeast/Bact. 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  $\times 10^9$  cells/ml. Due to the small genome size of Gram-negative bacteria, up to 3  $\times 10^9$  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  $\times g$  for 1 min. Remove the supernatant, leaving 200  $\mu l$  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  $\mu l$  of the culture (containing approximately 0.5–1.5  $\times 10^9$  cells) to a 1.5 ml microcentrifuge tube on ice.**
3. **Centrifuge for 5 s at 13,000–16,000  $\times g$  to pellet cells.**
4. **Carefully discard the supernatant by pipetting or pouring.**
5. **Add 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  $\mu l$  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  $\mu l$  Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.**
9. **Centrifuge for 3 min at 13,000–16,000  $\times g$ .**

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  $\mu$ l 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  $\times$  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  $\mu$ l of 70% ethanol and invert several times to wash the DNA pellet.
15. Centrifuge for 1 min at 13,000–16,000  $\times$  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 up to 15 min.  
The pellet might be loose and easily dislodged.
17. Add 50  $\mu$ l 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 Gentra Puregene Yeast/Bact. Kit

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

## Things to do before starting

- Preheat water baths to 37°C for use in steps 6 and 10, 65°C for use in step 22, and 80°C for use in step 9 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.**
2. **Transfer 500  $\mu$ l of the cell culture (containing approx.  $0.5\text{--}1.5 \times 10^9$  cells) to a 1.5 ml microcentrifuge tube on ice.**  
Longer centrifuge times may be necessary for some species to form a tight pellet.
3. **Centrifuge for 5 s at 13,000–16,000  $\times g$  to pellet cells.**
4. **Carefully discard the supernatant by pipetting or pouring.**
5. **Add 300  $\mu$ l Cell Suspension Solution, and pipet up and down.**
6. **Add 1.5  $\mu$ l Lytic Enzyme Solution, and mix by inverting 25 times. Incubate for 30 min at 37°C.**
7. **Centrifuge for 1 min at 13,000–16,000  $\times g$  to pellet cells.**
8. **Carefully discard the supernatant with a pipet.**
9. **Add 300  $\mu$ l 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.
10. **Add 1.5  $\mu$ l RNase A Solution, and mix by inverting 25 times. Incubate for 15–60 min at 37°C.**
11. **Incubate for 1 min on ice to quickly cool the sample.**
12. **Add 100  $\mu$ l 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.
13. **Centrifuge for 3 min at 13,000–16,000  $\times g$ .**  
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.

14. Pipet 300  $\mu$ l 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.
15. Mix by inverting gently 50 times.
16. Centrifuge for 1 min at 13,000–16,000  $\times$  g.  
The DNA will be visible as a small white pellet.
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 300  $\mu$ l of 70% ethanol and invert several times to wash the DNA pellet.
19. Centrifuge for 1 min at 13,000–16,000  $\times$  g.  
The pellet might be loose and easily dislodged.
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 up to 15 min.
21. Add 100  $\mu$ l DNA Hydration Solution and vortex 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 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. 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](http://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 Puregene Proteinase K (cat. no. 158918) to a final concentration of 100 µg/ml 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.

### Protein pellet soft, loose, or absent

- a) Sample was not cooled sufficiently before adding Protein Precipitation Solution
- b) Protein Precipitation Solution was not mixed uniformly with the cell lysate
- c) Centrifuge speed set incorrectly

To obtain a tight protein pellet be sure that the sample is cooled to room temperature or below ( $\leq 20\text{--}22^\circ\text{C}$ ) prior to adding Protein Precipitation Solution. To obtain a tight protein pellet:

- Revortex the sample 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.

Be sure to vortex vigorously for the full 20 s as specified in the protocol.

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  $2000 \times g$ . If a g-force of  $2000 \times g$  cannot be attained by your centrifuge, increase centrifugation time to achieve the same total g-force. For example,  $2000 \times g$  for 10 min is equivalent to a total g-force of  $20,000 \times g \times \text{time (min)}$ . If your centrifuge only achieves  $1600 \times g$ , centrifuge at  $1600 \times g$  for 12.5 min [ $(1600 \times g) (12.5 \text{ min}) = 20,000 \times g \times \text{min}$ ].

**Note:**  $2000 \times g$  and 2000 rpm are not equivalent. Use this equation to check if your rpm is set correctly  $g(\text{rcf}) = 1.12 \times r \times (\text{rpm}/1000)^2$  where  $r$  is the radius of the rotor in mm.

### Samples are slow to rehydrate

- a) Samples were not mixed during the hydration step
- b) The DNA pellet was dried too long prior to adding DNA Hydration Solution
- c) Protein contamination in the rehydrated DNA sample

Incubate with gentle shaking to facilitate hydration of the DNA.

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.

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 62.

### $A_{260}/A_{280}$ too high

RNA contamination

Ratios above 2.0 may indicate the presence of RNA. RNA can be removed 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 64.

### 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.

---

## Comments and suggestions

---

b) DNA is sheared

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.

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) per 300 µl isopropanol.

### Comments and suggestions

---

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.

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 Puregene Proteinase K (cat. no. 158918) to a final concentration of 100 µg/ml 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) 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 65.

### 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.

#### 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; i.e., 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 Puregene Proteinase K (cat. no. 158918). Add Puregene Proteinase K to the Cell Lysis Solution to a final concentration of 100 µg/ml 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 ml 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  $\times$  g. If a g-force of 2000  $\times$  g cannot be attained by your centrifuge, increase centrifugation time to achieve the same total g-force.

For example, 2000  $\times$  g for 10 min is equivalent to a total g-force of 20,000  $\times$  g  $\times$  time (min). If your centrifuge only achieves 1600  $\times$  g, centrifuge at 1600  $\times$  g for 12.5 min  $[(1600 \times g) (12.5 \text{ min}) = 20,000 \times g \times \text{min}]$ .

**Note:** 2000  $\times$  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.

### 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
- b) Cell clumps are present after adding Cell Lysis Solution to the sample

Remove fixative completely after centrifuging to pellet the cells; do not wash cells with PBS as this will result in a greatly reduced yield.

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 Puregene Proteinase K (cat. no. 158918) to a final concentration of 100  $\mu$ g/ml and incubating at 55°C until cells are completely lysed (1 h to overnight).

## **Appendix A: Protocol for Purifying Viral DNA**

The Puregene procedure can be used to purify viral DNA.

The whole blood and buffy coat protocols (pages 19–27) or the cultured cell protocol (page 34) should be used to purify viral DNA that is white blood cell-associated or cell-associated.

The body fluid protocol (page 32) 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. 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.

Absorbance readings at 260 nm should lie between 0.1 and 1.0 to be accurate. Sample dilution should be adjusted accordingly. Use elution buffer or water (as appropriate) to dilute samples and to calibrate the spectrophotometer. Measure the absorbance at 260 and 280 nm, or scan absorbance from 220–320 nm (a scan will show if there are other factors affecting absorbance at 260 nm). Both DNA and RNA are measured with a spectrophotometer. To measure only DNA, a fluorometer must be used.

### Determination of DNA length

The length of genomic DNA can be determined by pulsed-field gel electrophoresis (PFGE) through an agarose gel. The DNA should be concentrated by alcohol\* precipitation and reconstituted by gentle agitation in approximately 30  $\mu$ l TE buffer, pH 8.0,\* for at least 30 minutes at 60°C. Avoid drying the DNA pellet for more than 10 minutes at room temperature since over-dried genomic DNA is very difficult to redissolve. Load 3–5  $\mu$ g DNA per well. Standard PFGE conditions are as follows:

- 1% agarose gel in 0.5x TBE electrophoresis buffer\*
- Switch intervals: 5–40 s
- Run time: 17 h
- Voltage: 170 V

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

## 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
Puregene Proteinase K	100 μg/ml final concentration	3	6	15	30
Protein Precipitation Solution	2 volumes	200	400	1000	2000
Isopropanol	6 volumes	600	1200	3000	6000
Glycogen Solution	33.3 μg/ml final concentration	1	2	5	10
70% ethanol	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, Puregene Proteinase K (cat. no. 158918) 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 ml 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).
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  $\times$  g (microcentrifuge tube) or for 1 min at 2000  $\times$  g (15 ml 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 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 nm 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 $\mu$ l sample ( $\mu$ l)
Protein Precipitation Solution	0.5 volumes	50
96–100% ethanol	2 volumes	200
Glycogen Solution	33.3 $\mu$ g/ml final concentration	0.5
70% ethanol	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).
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  $\times g$  (microcentrifuge tube) or for 10 min at 2000  $\times g$  (15 ml 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  $\times g$  (microcentrifuge tube) or for 2 min at 2000  $\times g$  (15 ml 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 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 64.

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).**
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  $\times$  g (microcentrifuge tube) or for 10 min at 2000  $\times$  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  $\times$  g (microcentrifuge tube) or for 2 min at 2000  $\times$  g (15 ml 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 tube cap is tightly closed to avoid leakage.**

## Ordering Information

Product	Contents	Cat. no.
<b>Gentra Puregene Blood Kit — for purification of archive-quality DNA from whole blood and bone marrow</b>		
Gentra Puregene Blood Kit (3 ml)	For 3 ml blood: RBC Lysis Solution, RNase A Solution, and Reagents	158422
Gentra Puregene Blood Kit (30 ml)	For 30 ml blood: RBC Lysis Solution, RNase A Solution, and Reagents	158445
Gentra Puregene Blood Kit (120 ml)	For 120 ml blood: RBC Lysis Solution, RNase A Solution, and Reagents	158467
Gentra Puregene Blood Kit (1000 ml)	For 1000 ml blood: RBC Lysis Solution, and Reagents	158389
Gentra Puregene Blood Kit Plus (1000 ml)	For 1000 ml blood: RBC Lysis Solution, RNase A Solution, and Reagents	158489
<b>Gentra Puregene Buccal Cell Kit — for purification of archive-quality DNA from buccal cells</b>		
Gentra Puregene Buccal Cell Kit (10)	For 10 preps: 10 Buccal Collection Brushes, RNase A Solution, Glycogen Solution, and Reagents	158822
Gentra Puregene Buccal Cell Kit (100)	For 100 preps: 100 Buccal Collection Brushes, RNase A Solution, Glycogen Solution, and Reagents	158845
Gentra Puregene Buccal Cell Kit (400)	For 400 preps: 400 Buccal Collection Brushes, RNase A Solution, Glycogen Solution, and Reagents	158867
<b>Gentra Puregene Cell Kit — for purification of archive-quality DNA from cell cultures and cell suspensions</b>		
Gentra Puregene Cell Kit ( $2 \times 10^7$ )	For $2 \times 10^7$ cells: RNase A Solution and Reagents	158722
Gentra Puregene Cell Kit ( $2 \times 10^8$ )	For $2 \times 10^8$ cells: RNase A Solution and Reagents	158745
Gentra Puregene Cell Kit ( $8 \times 10^8$ )	For $8 \times 10^8$ cells: Reagents	158767
Gentra Puregene Cell Kit Plus ( $6.7 \times 10^9$ )	For $6.7 \times 10^9$ cells: RNase A Solution and Reagents	158788

# Ordering Information

Product	Contents	Cat. no.
Gentra Puregene Cell Kit (6.7 x 10 <sup>9</sup> )	For 6.7 x 10 <sup>9</sup> cells: RNase A Solution and Reagents	158388
<b>Gentra Puregene Tissue Kit — for purification of archive-quality DNA from tissue</b>		
Gentra Puregene Tissue Kit (100 mg)	For 100 mg tissue: RNase A Solution, Puregene Proteinase K, and Reagents	158622
Gentra Puregene Tissue Kit (4 g)	For 4 g tissue: RNase A Solution, Puregene Proteinase K, and Reagents	158667
Gentra Puregene Tissue Kit (33 g)	For 33 g tissue: RNase A Solution, Puregene Proteinase K, and Reagents	158689
<b>Gentra Puregene Mouse Tail Kit — for purification of archive-quality DNA from mouse tails</b>		
Gentra Puregene Mouse Tail Kit (100 mg)	For 100 mg mouse tails: Puregene Proteinase K and Reagents	158222
Gentra Puregene Mouse Tail Kit (4 g)	For 4 g mouse tails: Puregene Proteinase K and Reagents	158267
<b>Gentra Puregene Yeast/Bact. Kit — for purification of archive-quality DNA from yeast and bacteria</b>		
Gentra Puregene Yeast/Bact. Kit (5 ml)	For 5 ml bacterial cultures or 10 ml yeast cultures: Cell Suspension Solution, Lytic Enzyme Solution, RNase A Solution, and Reagents	158522
Gentra Puregene Yeast/Bact. Kit (200 ml)	For 200 ml bacterial cultures or 400 ml yeast cultures: Cell Suspension Solution, Lytic Enzyme Solution, RNase A Solution, and Reagents	158567
<b>Accessories</b>		
RBC Lysis Solution (450 ml)	450 ml RBC Lysis Solution	158902
RBC Lysis Solution (1000 ml)	1000 ml RBC Lysis Solution	158904
Cell Lysis Solution (125 ml)	125 ml Cell Lysis Solution	158906
Cell Lysis Solution (1000 ml)	1000 ml Cell Lysis Solution	158908
Protein Precipitation Solution (50 ml)	50 ml Protein Precipitation Solution	158910

## Ordering Information

Product	Contents	Cat. no.
Protein Precipitation Solution (350 ml)	350 ml Protein Precipitation Solution	158912
DNA Hydration Solution (100 ml)	100 ml DNA Hydration Solution	158914
DNA Hydration Solution (500 ml)	500 ml DNA Hydration Solution	158916
Puregene Proteinase K (650 µl)	650 µl Puregene Proteinase K	158918
Puregene Proteinase K (5 ml)	5 ml Puregene Proteinase K	158920
RNase A Solution (650 µl)	650 µl RNase A Solution	158922
RNase A Solution (5 ml)	5 ml RNase A Solution	158924
Cell Suspension Solution (125 ml)	125 ml Cell Suspension Solution	158926
Lytic Enzyme Solution (650 µl)	650 µl Lytic Enzyme Solution	158928
Glycogen Solution (500 µl)	500 µl Glycogen Solution	158930
Clotspin Baskets (50)	50 Clotspin Baskets	158932

## Notes

## Notes

## Notes

[www.qiagen.com](http://www.qiagen.com)

**Australia** = Orders 03-9840-9800 = Fax 03-9840-9888 = Technical 1-800-243-066

**Austria** = Orders 0800/28-10-10 = Fax 0800/28-10-19 = Technical 0800/28-10-11

**Belgium** = Orders 0800-79612 = Fax 0800-79611 = Technical 0800-79556

**Canada** = Orders 800-572-9613 = Fax 800-713-5951 = Technical 800-DNA-PREP (800-362-7737)

**China** = Orders 021-51345678 = Fax 021-51342500 = Technical 021-51345678

**Denmark** = Orders 80-885945 = Fax 80-885944 = Technical 80-885942

**Finland** = Orders 0800-914416 = Fax 0800-914415 = Technical 0800-914413

**France** = Orders 01-60-920-926 = Fax 01-60-920-925 = Technical 01-60-920-930 = Offers 01-60-920-928

**Germany** = Orders 02103-29-12000 = Fax 02103-29-22000 = Technical 02103-29-12400

**Hong Kong** = Orders 800 933 965 = Fax 800 930 439 = Technical 800 930 425

**Ireland** = Orders 1800-555-049 = Fax 1800-555-048 = Technical 1800-555-061

**Italy** = Orders 02-33430411 = Fax 02-33430426 = Technical 800-787980

**Japan** = Telephone 03-5547-0811 = Fax 03-5547-0818 = Technical 03-5547-0811

**Luxembourg** = Orders 8002-2076 = Fax 8002-2073 = Technical 8002-2067

**The Netherlands** = Orders 0800-0229592 = Fax 0800-0229593 = Technical 0800-0229602

**Norway** = Orders 800-18859 = Fax 800-18817 = Technical 800-18712

**South Korea** = Orders 1544 7145 = Fax 1544 7146 = Technical 1544 7145

**Sweden** = Orders 020-790282 = Fax 020-790582 = Technical 020-798328

**Switzerland** = Orders 055-254-22-11 = Fax 055-254-22-13 = Technical 055-254-22-12

**UK** = Orders 01293-422-911 = Fax 01293-422-922 = Technical 01293-422-999

**USA** = Orders 800-426-8157 = Fax 800-718-2056 = Technical 800-DNA-PREP (800-362-7737)

