Genomed

JetQuick® Genomic DNA Purification Kits

For purification of genomic DNA from blood, mammalian cells, tissue, swabs, fixed tissue, buffy coat, body fluids, bacteria, and yeast

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User Manual

Contents

Kit Contents and Storage

Types of Kits

This manual is supplied with the following products.

Product	Quantity	Cat. no.
JetQuick® Blood & Cell Culture DNA Miniprep Kit	50 preps	440 050
	250 preps	440 250
JetQuick® Blood & Cell Culture DNA Midiprep Kit	20 preps	441 020
	50 preps	441 050
JetQuick® Blood & Cell Culture DNA Maxiprep Kit	20 preps	442 020
	50 preps	442 050
JetQuick® Tissue DNA Miniprep Kit	50 preps	450 050
	250 preps	450 250

Intended Use

For research use only. Not intended for any human or animal diagnostic or therapeutic uses.

Shipping and Storage

Each kit is shipped at room temperature.

Upon receipt, store all components at room temperature.

Note: Genomed Protease, Proteinase K, and RNase A are stable when stored at room temperature. However, for optimal enzymatic performance, store the enzymes at 4°C upon arrival. For long-term storage, store Genomed Protease, Proteinase K, and RNase A at –20ºC.

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Kit Contents and Storage, Continued

JetQuick® Blood & Cell Culture DNA Kits

The components supplied in the JetQuick® Blood & Cell Culture DNA Kits are listed below.

Store all components at room temperature.

Item	Miniprep		Midiprep		Maxiprep	
	50 preps	**250 preps**	**20 preps**	**50 preps**	**20 preps**	**50 preps**
	440 050	**440 250**	**441 020**	**441 050**	**442 020**	**442 050**
Buffer K1	11 mL	55 mL	66 mL	165 mL	220 mL	550 mL
Buffer K2	10 mL	42.5 mL	68.5 mL	171 mL	68.5 mL	171 mL
Buffer KX	12 mL	61 mL	95 mL	228 mL	99 mL	243 mL
10 mM Tris-HCl, pH 8.5	22 mL	110 mL	35 mL	88 mL	44 mL	110 mL
RNase A (20 mg/mL)	650 µL	3 mL	3 mL	2 × 3 mL	3 × 3 mL	5 × 3.2 mL
Genomed Protease (lyophilized powder)	22 mg	5 × 22 mg	5 × 26 mg	320 mg	220 mg	560 mg
JetQuick® Spin Columns	50 each	250 each	20 each	50 each	20 each	50 each
JetQuick® Receiver Tubes (2.0 mL)	50 each	250 each	none	none	none	none

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Kit Contents and Storage, Continued

JetQuick® Tissue DNA Miniprep Kits

The components supplied in the JetQuick® Tissue DNA Miniprep Kits are listed below.

Store all components at room temperature.

Reagents	50 preps	250 preps
	450 050	**450 250**
Buffer T1	11 mL	55 mL
Buffer T2	11 mL	55 mL
Buffer T3	10 mL	42 mL
Buffer TX	12. mL	60 mL
10 mM Tris-HCl, pH 8.5	22 mL	110 mL
RNase A (20 mg/mL)	1.5 mL	2 × 3 mL
Proteinase K (lyophilized powder)	26 mg	5 × 26 mg
JetQuick® Spin Columns	50 each	250 each
JetQuick® Receiver Tubes (2.0 mL)	50 each	250 each

Introduction

System Overview

Introduction

JetQuick® Genomic DNA Purification Kits allow rapid and efficient purification of genomic DNA (gDNA). The kits are designed to efficiently isolate genomic DNA from a variety of samples including blood, mammalian cells and tissues, body fluid (*e.g.*, amniotic fluid, saliva, sperm, lymph), mouse/rat tail, swabs, bacteria, yeast, and fixed tissue. The isolated DNA is 20–50 kb in size and is suitable for PCR, restriction enzyme digestion, and Southern blotting.

How it Works

JetQuick® spin columns contain silica membranes that bind nucleic acids within columns under defined conditions. Using this system, purify DNA in the following manner:

1. Lyse nuclei and denature proteins (*e.g.*, nucleases, histones) in the presence of a cell lysis buffer.
2. Digest denatured proteins into smaller fragments and strip genomic DNA of all bound proteins with protease.
3. Remove residual RNA by digestion with RNase A prior to sample binding to the silica membrane.
4. Bind DNA to the membrane in the column in the presence of chaotropic salts. Remove impurities by thorough washing with Wash Buffers.
5. Elute gDNA in 10 mM Tris-HCl, pH 8.5.

Continued on next page

System Overview, Continued

Advantages

The advantages of using JetQuick® Genomic DNA Purification Kits are:

- Rapid and efficient purification of high-quality genomic DNA from a variety of samples such as mammalian cells and tissue, blood samples, any type of body fluid (*e.g.*, amniotic fluid, saliva, sperm, lymph), mouse tails, swabs, bacteria, yeast, and fixed tissue
- Simple lysis of cells and tissues with Proteinase K or Genomed Protease without the need for any mechanical lysis
- Minimal contamination from RNA
- Reliable performance of the purified DNA in PCR, restriction enzyme digestion, and Southern blotting

System Specifications

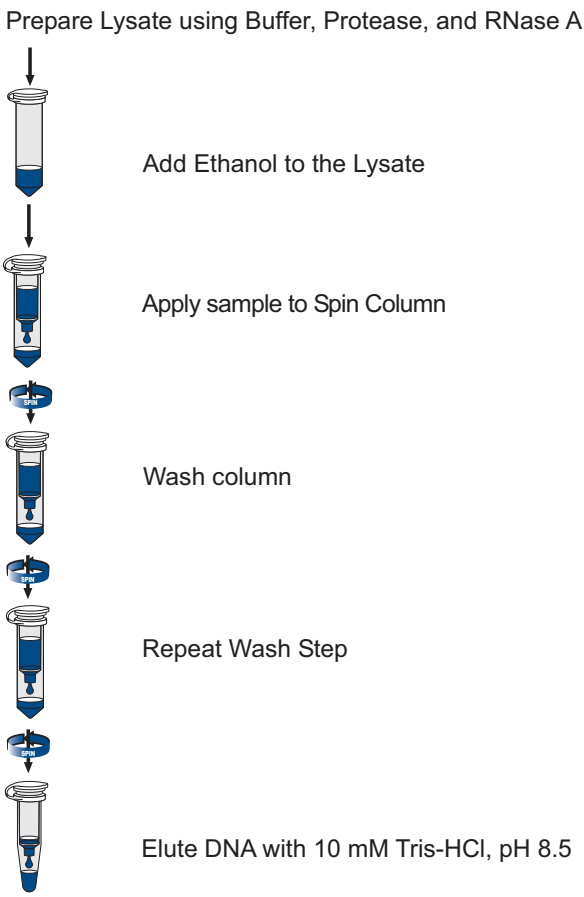
The table below lists the specifications for JetQuick® Genomic DNA Purification Kits.

Kit Type	**Blood**			**Tissue**
Kit Size	**Miniprep**	**Midiprep**	**Maxiprep**	**Miniprep**
Starting Material	200–1,000 µL whole blood	1–5 mL whole blood	5–20 mL whole blood	varies
Binding Capacity	50 µg	150 µg	600 µg	50 µg
Column Reservoir Capacity	800 µL	15 mL	15 mL	800 µL
Elution Volume	25–200 µL	500–800 µL	1–4 mL	25–200 µL
DNA Yield	85–95%	85–95%	85–95%	85–95%
DNA Size	up to 50 kb	up to 50 kb	up to 50 kb	up to 50 kb

Experimental Outline

Introduction

The figure below illustrates the basic steps necessary to use JetQuick® gDNA Purification Kits. For a more detailed overview of blood gDNA purification, refer to page 9 (**Purifying gDNA from Blood and Body Fluids Using Centrifugation**), or page 17 (**Purifying gDNA from Blood and Body Fluids Using Vacuum**).



Methods

General Information

Follow the recommendations below to obtain the best results:

- Maintain a sterile environment when handling DNA to avoid any contamination from DNases and ensure that no DNases are introduced into the sterile solutions of the kit
- Make sure all equipment that comes in contact with DNA is sterile, including pipette tips and microcentrifuge tubes
- Do not vortex the samples for more than 5–10 seconds at each vortexing step to avoid extensive shearing of DNA
- To minimize DNA degradation, perform lysate preparation steps quickly, and avoid repeated freezing and thawing of DNA samples
- Perform all centrifugation steps at room temperature
- Review **Elution Parameters** on pages 6–7 to determine the suitable elution volume for your requirements
- Perform a 5 minute incubation step with 10 mM Tris-HCl, pH 8.5
- If you are using water for elution, use sterile water, pH 7.0–8.5

Continued on next page

General Information, Continued

Before Starting

- Reconstitute **Buffers KX** and **K2** (JetQuick® Blood & Cell Culture DNA Kits) or **TX** and **T3** (JetQuick® Tissue DNA Miniprep Kits) with absolute ethanol as directed on the labels of the bottles. Mix well. Mark on each label that ethanol is added. Store the wash buffers with ethanol at room temperature.
- Resuspend **Genomed Protease** (JetQuick® Blood & Cell Culture DNA Kits) or **Proteinase K** (JetQuick® Tissue DNA Miniprep Kits) in double-distilled or Milli-Q®-grade water to a final concentration of 20 mg/mL. Store the reconstituted enzymes in single-use aliquots at −20°C. Avoid repeated freezing and thawing.

Safety Information

- Handle all blood and tissue samples in compliance with established institutional guidelines and take the appropriate precautions (wear a laboratory coat, disposable gloves, and eye protection) when handling blood and tissue samples. Since safety requirements for use and handling of blood and tissue samples may vary at individual institutions, consult the health and safety guidelines and/or officers at your institution.
- When processing blood and tissue samples, the eluates collected during wash steps contain biohazardous waste. Dispose the eluate and collection tubes/plates appropriately as biohazardous waste.
- Buffers K1, KX, T2, and TX contain guanidine hydrochloride. Always wear a laboratory coat, disposable gloves, and eye protection when handling buffers. Do not add bleach or acidic solutions directly to solutions containing guanidine hydrochloride or sample preparation waste as it forms reactive compounds and toxic gases when mixed with bleach or acids.

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General Information, Continued

RNase A Digestion

- RNA contamination inflates the nucleic acid content measured at 260 nm.
- RNase A digestion is performed during sample preparation to degrade RNA present in the sample and minimize RNA contamination in the purified DNA sample.
- RNase A is supplied with the kit and an optional RNase A digestion step is included during sample preparation protocols.
- If RNA content of the sample is minimal (*e.g.*, mouse tail) and RNA contamination *does not* interfere with any downstream applications of the purified DNA, you may omit the RNase A digestion step during sample preparation.

Centrifugation

- Centrifuge Miniprep samples in a microcentrifuge.
- Centrifuge Midiprep and Maxiprep samples in a centrifuge with a swing-out rotor.

Elution Parameters

Elution Buffer

gDNA is eluted using 10 mM Tris-HCl, pH 8.5 (supplied with the kit). Alternatively, sterile water or TE can be used.

Elution Buffer Volume

For increased DNA yield, use a higher volume of elution buffer. For increased DNA concentration, use a lower volume of elution buffer.

Continued on next page

General Information, Continued

Number of Elutions

The absolute DNA yield can be increased by performing a second elution step. Use the same volume of buffer for both elution steps. To prevent dilution of the gDNA sample and to avoid contact of the spin column with the eluate, perform the two-elution steps using different tubes.

Prep Size	Volume of First Elution	DNA in 1st eluate	DNA in 2nd eluate
Miniprep	50 µL	80%	20%
	100 µL	90%	10%
Midiprep	0.3 mL	41%	59%
	0.5 mL	60%	40%
	0.8 mL	75%	25%
	1.0 mL	83%	17%
Maxiprep	1 mL	80%	20%
	2 mL	87%	13%
	3 mL	92%	8%
	4 mL	93%	7%

Purifying gDNA from Blood and Body Fluids Using Centrifugation

Introduction

The JetQuick® Blood & Cell Culture DNA Mini, Midi, and Maxiprep Kits are designed to purify gDNA from any type of body fluid (*e.g.*, amniotic fluid, saliva, sperm, lymph) and fresh or frozen whole blood, which may be collected in the presence of anti-coagulants such as EDTA or citrate. To obtain high-quality genomic DNA, follow the guidelines recommended on page 4. For a procedure using Vacuum manifold, see page 17.

Materials Needed

- 96–100% ethanol
- Sample for DNA isolation (see page 10)
- Sterile, DNase-free microcentrifuge tubes (Miniprep only)
- Sterile, DNase-free 50 mL tubes (Midiprep and Maxiprep)
- Water baths or heat blocks
- Microcentrifuge capable of centrifuging >12,000 × *g*
- Centrifuge with a swing-out rotor, capable of centrifuging 2,000–5,000 × *g* (Midiprep and Maxiprep only)
- *Optional*: Phosphate Buffered Saline (PBS, see page 57) or equivalent standard saline buffer for processing 1 mL Miniprep, 20 mL Maxiprep, or buffy coat samples
- *Optional*: White Blood Cell Buffer (see page 11) for processing 1 mL Miniprep or 20 mL Maxiprep samples

Components supplied with the JetQuick® Blood & Cell Culture DNA Mini, Midi, and Maxiprep Kits

- Buffers K1, K2, and KX
- Genomed Protease, lyophilized powder, see page 5
- RNase A (20 mg/mL)
- 10 mM Tris-HCl, pH 8.5
- JetQuick® Spin Columns
- JetQuick® Receiver Tubes (Miniprep Kits only)

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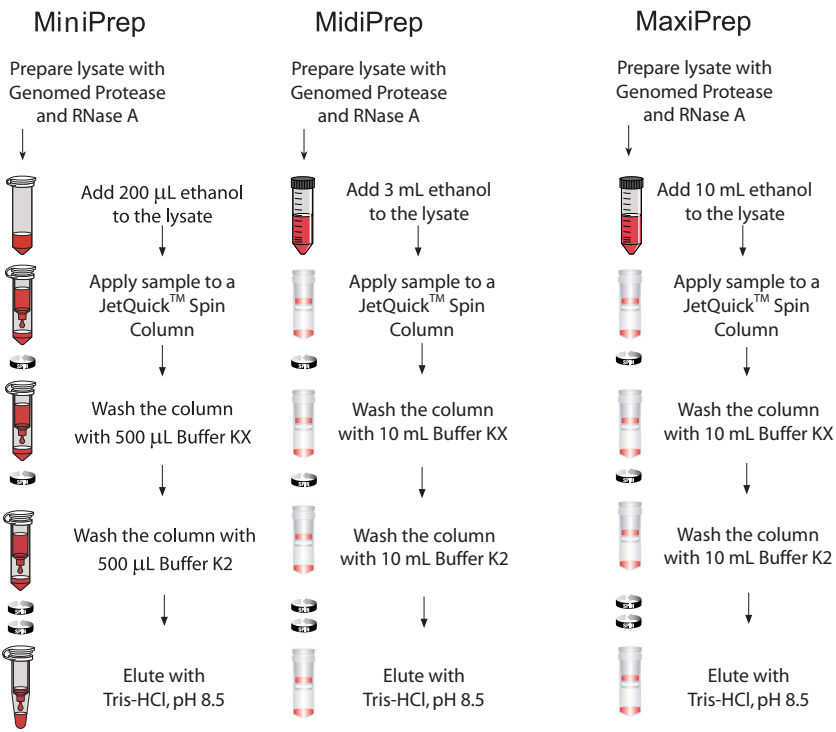
Purifying gDNA from Blood and Body Fluids Using Centrifugation, Continued

Experimental Outline

The figure below illustrates the basic steps necessary to purify gDNA using centrifugation with the JetQuick® Blood & Cell Culture DNA Purification Kits.



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Purifying gDNA from Blood and Body Fluids Using Centrifugation, Continued

Sample Volumes

The table below lists the appropriate procedure and the relevant page reference based on the starting sample volume.

Starting Volume	Prep Size	Protocol	Page no.
Up to 200 µL	Miniprep	Standard, no modification	12
300 µL – 1 mL	Miniprep	Requires lysate concentration	11
1–5 mL	Midiprep	Standard, no modification	12
6–10 mL	Maxiprep	Standard, no modification	12
11–20 mL	Maxiprep	Requires lysate concentration	11

- Blood from a healthy person contains on average 5×10^6 – 1×10^7 DNA-containing lymphocytes per mL. The values in the table are for nonnucleated blood (*e.g.*, human or mouse). Nucleated starting blood (*e.g.*, bird) volumes will be substantially lower (*e.g.*, 5–10 µL for Miniprep).
- Scale up or down the volumes of all buffers and components proportionally if using a non-standard starting volume (*e.g.,* standard starting volumes are 3 mL whole blood for Midiprep and 10 mL for Maxiprep).
- For body fluids, you may use the volumes indicated in the table above. However, if the body fluid sample is dilute or has few DNA-containing cells, concentrate the cells by centrifugation (12,000 × *g* for 30 seconds for Miniprep or 5,000 × *g* for 2 minutes for a Midiprep or Maxiprep). Following centrifugation, aspirate the supernatant and resuspend the cell pellet in 200 µL (Mini), 3 mL (Midi), or 10 mL (Maxi) PBS.

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Purifying gDNA from Blood and Body Fluids Using Centrifugation, Continued

Buffy Coat Samples

Use no more than 1 – 2 $\times 10^8$ cells per buffy coat preparation. Adjust the volume of buffy coat corresponding to this amount of cells with PBS, TBS or another standard saline buffer (to be provided by the user) to an overall volume of 3 mL (Midiprep) or 10 mL (Maxiprep).

Preparing Concentrated Lysates

If you are using standard starting blood volumes of up to 200 µL for Miniprep, 1–5 mL for Midiprep, or 6–10 mL for Maxiprep (see page 10), skip this section. To purify gDNA from 300 µL to 1 mL blood samples with the JetQuick® Blood Miniprep Kit or 11 to 20 mL blood samples with the JetQuick® Blood Maxiprep Kit:

1. Prepare WBC Buffer:

   10 mM Tris-HCl, pH 8.5
   5 mM $MgCl_2$
   320 mM Sucrose
   1% Triton® X-100

2. Place whole blood sample into a sterile harvesting tube.

Starting Materials	Miniprep	Maxiprep
Starting Sample Volume	300 µL – 1 mL	11–20 mL
Harvesting Tube Size	2 mL	50 mL

3. Add an equal volume of WBC Buffer to the blood sample. Mix thoroughly by inverting the tube several times.

4. Centrifuge using the following conditions:

Centrifugation	Miniprep	Maxiprep
Acceleration	12,000 × *g*	5,000 × *g*
Duration	30 seconds	2 minutes

Continued on next page

Purifying gDNA from Blood and Body Fluids Using Centrifugation, Continued

Preparing Concentrated Lysates, Continued

5. Aspirate the supernatant with a pipette. Do not disturb the white blood cell pellet which appears light red.
6. Resuspend the cell pellet in PBS or equivalent standard saline buffer. Mix by pulse-vortexing.

Reagent	Miniprep	Maxiprep
Volume PBS	180 µL	9.5 mL

7. Proceed immediately to **Preparing Lysate**, below.

Preparing Lysate

Prepare lysate from up to 200 µL (Mini), 1 to 5 mL (Midi), and 6 to 10 mL (Maxi) body fluid or blood samples (nucleated or nonnucleated) as described below. See page 10 for alternate starting volumes that require lysate concentration.

1. Set a water bath or heat block at 58°C (for Miniprep) or 70 °C (for Midiprep and Maxiprep).
2. Harvest cells by adding fresh or frozen blood, serum, plasma, or buffy coat to a clean, sterile tube. The table below indicates starting sample volumes.

Starting Materials	Miniprep	Midiprep	Maxiprep
Starting Sample Volume	200 µL	3 mL	10 mL
Harvesting Tube Size	1.5 mL	50 mL	50 mL

Continued on next page

Purifying gDNA from Blood and Body Fluids Using Centrifugation, Continued

Preparing Lysate, Continued

3. Add the reagents in the order listed in the table below and mix thoroughly by vortexing or inverting the tube. Do not add Genomed Protease directly to Buffer K1. First mix the suspended cells with the enzyme, and then add Buffer K1.

Reagent / Operation	Miniprep	Midiprep	Maxiprep
Genomed Protease	20 µL	300 µL	500 µL
RNase A *(optional)*	10 µL	100 µL	300 µL
Mix sample	vortex	vortex	vortex
Buffer K1	200 µL	3 mL	10 mL
Mix sample	vortex	vortex	vortex

4. Incubate the sample for 10 minutes at the temperature indicated in the table below to degrade protein:

Incubation	Miniprep	Midiprep	Maxiprep
Temperature	58ºC	70°C	70°C

Note: The optimal incubation temperature for Genomed Protease is 58°C. However, to reach the catalytic temperature range as quickly as possible, incubate Midiprep and Maxiprep samples at 70°C. If incubation at 58°C is desired, extend the incubation time to 20 minutes.

5. Add ethanol and mix by vortexing.

Reagent	Miniprep	Midiprep	Maxiprep
96–100% Ethanol	200 µL	3 mL	10 mL

Note: Mix the sample immediately to prevent precipitation of nucleic acids due to high local alcohol concentrations.

6. Proceed immediately to **Purifying DNA**, next page.

Continued on next page

Purifying gDNA from Blood and Body Fluids Using Centrifugation, Continued

Purifying DNA

1. Assemble a JetQuick® Spin Column with an appropriate receiver tube:

Spin Column Assembly	Miniprep	Midiprep	Maxiprep
Receiver Tube size	2 mL, provided in the kit	50 mL	50 mL

Note: For 50 mL tubes, loosely attach a cap to allow ventilation during centrifugation.

2. Apply the sample from Step 5, previous page, into the JetQuick® Spin Column.

   Note: For Maxiprep starting blood volumes >5 mL, load 15 mL blood lysate onto the JetQuick® Maxi-Spin column with two successive rounds of centrifugation.

3. Centrifuge samples using the following conditions:

Centrifugation	Miniprep	Midiprep	Maxiprep
Acceleration	10,000 × *g*	2,000 × *g*	2,000 × *g*
Duration	1 minute	3 minutes	3 minutes

4. Discard the flow-through.
5. Add reconstituted Buffer KX (page 5) to the column.

Reagent	Miniprep	Midiprep	Maxiprep
Buffer KX	500 µL	10 mL	10 mL

6. Centrifuge using the following conditions:

Centrifugation	Miniprep	Midiprep	Maxiprep
Acceleration	10,000 × *g*	5,000 × *g*	5,000 × *g*
Duration	1 minute	2 minutes	2 minutes

Continued on next page

Purifying gDNA from Blood and Body Fluids Using Centrifugation, Continued

Purifying DNA, Continued

7. Discard the flow-through and reassemble the spin column.
8. Add Buffer K2 prepared with ethanol (page 5) to the column.

Reagent	Miniprep	Midiprep	Maxiprep
Buffer K2	500 µL	10 mL	10 mL

9. Centrifuge using the conditions described in Step 6.
10. Discard the flow-through and re-assemble the spin column using the same receiver tube. Centrifuge again to clear the silica membrane of any residual liquid. Discard the receiver tube.

Centrifugation	Miniprep	Midiprep	Maxiprep
Acceleration	12,000 × *g*	5,000 × *g*	5,000 × *g*
Duration	1 minute	10 minutes	10 minutes

11. Place the JetQuick® Spin Column into a new, sterile elution tube (not supplied).

Spin Column Assembly	Miniprep	Midiprep	Maxiprep
Elution tube size	1.5 mL	50 mL	50 mL

Continued on next page

Purifying gDNA from Blood and Body Fluids Using Centrifugation, Continued

Purifying DNA, Continued

12. Add prewarmed (70ºC) 10 mM Tris-HCl, pH 8.5, or water to the column. See **Elution Parameters** (pages 6–7) to determine the suitable elution volume for your application. Incubate at room temperature for the time specified in the table below.

Elution	Miniprep	Midiprep	Maxiprep
10 mM Tris-HCl, pH 8.5	25–200 µL	500–800 µL	1–4 mL
Incubation Time	2 minutes	5 minutes	5 minutes

13. Centrifuge the column at room temperature to elute the DNA.

Centrifugation	Miniprep	Midiprep	Maxiprep
Acceleration	10,000 × *g*	5,000 × *g*	5,000 × *g*
Time	2 minutes	2 minutes	2 minutes

14. *Optional:* To recover more DNA, perform a second elution in another sterile tube. Centrifuge the column as directed in Step 13.

    The tube contains purified DNA. Remove and discard the column. Combine the contents of the elution tubes only if overall yield is of greater importance than maintaining the higher concentration obtained in the first eluate (depending on your application).

Storing DNA

To avoid repeated freezing and thawing of DNA,

- Store the purified DNA at 4°C for immediate use or
- Aliquot the DNA and store at –20°C for long-term storage.

Purifying gDNA from Blood and Body Fluids Using Vacuum

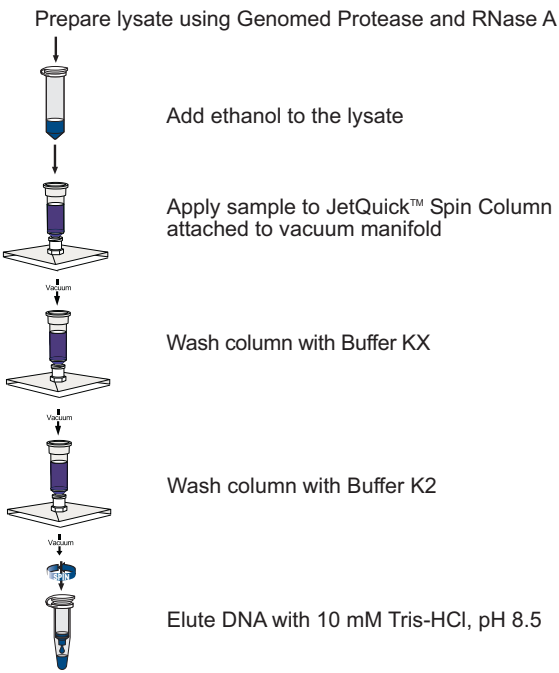
Introduction

The procedure for purifying genomic DNA from blood and any type of body fluid (*e.g.*, amniotic fluid, saliva, sperm, lymph) using JetQuick® Blood & Cell Culture DNA Kits with a vacuum manifold is described below.

To obtain high-quality genomic DNA, follow the guidelines recommended on page 4.

Experimental Outline

The figure below illustrates the basic steps necessary to purify gDNA using vacuum with the JetQuick® Blood & Cell Culture DNA Kits.



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Purifying gDNA from Blood and Body Fluids Using Vacuum, Continued

Materials Needed

- 96–100% ethanol
- Sample for DNA isolation (see page 10)
- Sterile, DNase-free microcentrifuge tubes
- Water baths or heat blocks
- Microcentrifuge capable of centrifuging >12,000 × *g*
- Vacuum manifold, see page 57

Components supplied with the JetQuick® Blood & Cell Culture DNA Kits

- Buffers K1, K2, and KX
- Genomed Protease, lyophilized powder, see page 5
- RNase A (20 mg/mL)
- 10 mM Tris-HCl, pH 8.5
- JetQuick® Spin Columns
- JetQuick® Receiver Tubes

Preparing Lysate

Prepare lysate from up to 200 µL (Mini), 1 to 5 mL (Midi), or 6 to 10 mL (Maxi) blood or body fluid samples. See page 10 for detailed starting sample volume information.

1. Set a water bath or heat block at 58°C (Mini) or 70 °C (Midi and Maxi).
2. Harvest cells by adding fresh or frozen blood, body fluid, serum, plasma, or buffy coat to a clean, sterile tube.

Starting Materials	Miniprep	Midiprep	Maxiprep
Starting Sample Volume	200 µL	3 mL	10 mL
Harvesting Tube Size	1.5 mL	50 mL	50 mL

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Purifying gDNA from Blood and Body Fluids Using Vacuum, Continued

Preparing Lysate, Continued

3. Add the reagents in the order listed in the table below and mix thoroughly by vortexing or inverting the tube. Do not add Genomed Protease directly to Buffer K1. First mix the suspended cells with the enzyme, and then add Buffer K1.

Reagent / Operation	Miniprep	Midiprep	Maxiprep
Genomed Protease	20 µL	300 µL	500 µL
RNase A (optional)	10 µL	100 µL	300 µL
Mix sample	vortex	vortex	vortex
Buffer K1	200 µL	3 mL	10 mL
Mix sample	vortex	vortex	vortex

4. Incubate the sample for 10 minutes at the temperature indicated in the table below to degrade protein:

Incubation	Miniprep	Midiprep	Maxiprep
Temperature	58ºC	70°C	70°C

Note: The optimal incubation temperature for Genomed Protease is 58°C. However, to reach the catalytic temperature range as quickly as possible, incubate Midiprep and Maxiprep samples at 70°C. If incubation at 58°C is desired, extend the incubation time to 20 minutes.

5. Add ethanol and mix by vortexing.

Reagent	Miniprep	Midiprep	Maxiprep
96–100% Ethanol	200 µL	3 mL	10 mL

Note: Mix the sample immediately to prevent precipitation of nucleic acids due to high local alcohol concentrations.

6. Proceed immediately to **Purifying DNA** (next page).

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Purifying gDNA from Blood and Body Fluids Using Vacuum, Continued

Purifying DNA

1. Attach vacuum manifold (such as the EveryPrep™ Universal Vacuum Manifold, see page 57) to a vacuum source. Attach as many JetQuick® Spin Columns as necessary to the female Luer inlets on the vacuum manifold.
2. Load the blood lysate from Step 6, previous page, into the JetQuick® Spin Columns.
3. Apply vacuum (–200 to –650 mbar) until all liquid is pulled through the column. Turn off the vacuum source.
4. Add reconstituted Buffer KX (page 5) to the column.

Reagent	Miniprep	Midiprep	Maxiprep
Buffer KX	500 µL	10 mL	10 mL

5. Apply vacuum (–200 to –650 mbar) until all liquid has been pulled through the column. Turn off vacuum source.
6. Add Buffer K2 prepared with ethanol (page 5) to the column and repeat Step 5.

Reagent	Miniprep	Midiprep	Maxiprep
Buffer K2	500 µL	10 mL	10 mL

7. To remove last traces of ethanol, turn on the vacuum again and pull air through the spin columns on the vacuum manifold until all residual ethanol is removed from the silica membrane.
8. Place the JetQuick® Spin Column into a new, sterile elution tube (not supplied).

Spin Column Assembly	Miniprep	Midiprep	Maxiprep
Elution tube size	1.5 mL	50 mL	50 mL

Continued on next page

Purifying gDNA from Blood and Body Fluids Using Vacuum, Continued

Purifying DNA, Continued

9. Add prewarmed (70ºC) 10 mM Tris-HCl, pH 8.5, or water to the column. See **Elution Parameters** (pages 6–7) to determine the suitable elution volume for your application. Incubate at room temperature for the time specified in the table below.

Elution	Miniprep	Midiprep	Maxiprep
10 mM Tris-HCl, pH 8.5	25–200 µL	500–800 µL	1–4 mL
Incubation Time	2 minutes	5 minutes	5 minutes

10. Centrifuge the column at room temperature to elute the DNA.

Centrifugation	Miniprep	Midiprep	Maxiprep
Acceleration	10,000 × *g*	5,000 × *g*	5,000 × *g*
Time	2 minutes	2 minutes	2 minutes

The tube contains purified genomic DNA.

11. *Optional:* To recover more DNA, perform a second elution in another sterile tube. Centrifuge the column as directed in Step 10.

    The tube contains purified DNA. Remove and discard the column. Combine the contents of the elution tubes only if overall yield is of greater importance than maintaining the higher concentration obtained in the first eluate (depending on your application).

Storing DNA

To avoid repeated freezing and thawing of DNA,

- Store the purified DNA at 4°C for immediate use or
- Aliquot the DNA and store at –20°C for long-term storage.

Purifying gDNA from Mammalian Cells

Introduction

The procedure for purifying genomic DNA from mammalian cells using JetQuick® Blood & Cell Culture DNA Mini, Midi, and Maxiprep Kits is described below.

To obtain high-quality genomic DNA, follow the guidelines recommended on page 4.

Materials Needed

- 96–100% ethanol
- Sample for DNA isolation (see next page)
- Phosphate Buffered Saline (PBS) (page 57)
- Sterile, DNase-free microcentrifuge tubes (Miniprep only)
- Sterile, DNase-free 50-mL tubes (Midiprep and Maxiprep)
- Centrifuge with swing-out rotor (Midiprep and Maxiprep)
- Microcentrifuge capable of centrifuging at >12,000 × *g*
- Water baths or heat blocks

Components supplied with the JetQuick® Blood & Cell Culture DNA Mini, Midi, and Maxiprep Kits

- Buffers K1, K2, and KX
- Genomed Protease, lyophilized powder, see **Before Starting**, page 5
- RNase A (20 mg/mL)
- 10 mM Tris-HCl, pH 8.5
- JetQuick® Spin Columns
- JetQuick® Receiver Tubes (Miniprep Kits only)

Continued on next page

Purifying gDNA from Mammalian Cells,
Continued

Starting Material

The table below lists the amount of starting material recommended for each purification kit.

Prep Size	Number of cells
Miniprep	5×10^6 to 1×10^7
Midiprep	7.5×10^7 to 1×10^8
Maxiprep	2.5×10^8 to 5×10^8

Preparing Cell Lysate

Prepare lysate from mammalian cells as described below.

1. Set a water bath or heat block at 58°C (Miniprep) or 70°C (Midiprep and Maxiprep).
2. Harvest cells into the appropriate tube (see table below).
   - For cells grown in a monolayer, remove the growth medium from the culture plate and harvest cells by trypsinization or use a cell scraper according to established protocols. Transfer cells into a harvesting tube (see table below).
   - For cells grown in suspension, transfer cells into a harvesting tube.

Starting Materials	Miniprep	Midiprep	Maxiprep
Harvesting tube	1.5 mL	50 mL	50 mL

3. Centrifuge at 300–350 × *g* for 5 minutes to pellet cells.
4. Remove the growth medium completely, without disturbing the cell pellet, and resuspend the cells in PBS.

Reagent	Miniprep	Midiprep	Maxiprep
PBS	200 µL	3 mL	10 mL

Continued on next page

Purifying gDNA from Mammalian Cells,
Continued

Preparing Cell Lysate, Continued

5. Add the reagents in the order listed in the table below and mix thoroughly by vortexing or inverting the tube. Do not add Genomed Protease directly to Buffer K1. First mix the suspended cells with the enzyme, and then add Buffer K1.

Reagent / Operation	Miniprep	Midiprep	Maxiprep
Genomed Protease (20 mg/mL)	20 µL	300 µL	500 µL
Optional: RNase A (20 mg/mL)	10 µL	100 µL	300 µL
Mix sample	vortex	vortex	vortex
Buffer K1	200 µL	3 mL	10 mL
Mix sample	vortex	vortex	vortex

6. Incubate the sample for 10 minutes to degrade protein:

Incubation	Miniprep	Midiprep	Maxiprep
Temperature	58ºC	70°C	70°C

Note: The optimal incubation temperature for Genomed Protease is 58°C. However, to reach the catalytic temperature range as quickly as possible, incubate Midiprep and Maxiprep samples at 70°C. If incubation at 58°C is desired, extend the incubation time to 20 minutes.

7. Add ethanol and mix well by vortexing.

Reagent	Miniprep	Midiprep	Maxiprep
96–100% Ethanol	200 µL	3 mL	10 mL

Note: Mix the sample immediately to prevent precipitation of nucleic acids due to high local alcohol concentrations.

8. Proceed immediately to **Purifying DNA** (next page).

Continued on next page

Purifying gDNA from Mammalian Cells,
Continued

Purifying DNA

1. Assemble a JetQuick® Spin Column with an appropriate receiver tube:

Spin Column Assembly	Miniprep	Midiprep	Maxiprep
Receiver Tube	2 mL	50 mL	50 mL

Note: For the Midiprep and Maxiprep, loosely attach the cap to the tube to allow ventilation during the centrifugation.

2. Apply the sample from Step 7, previous page, into the JetQuick® Spin Column.
3. Centrifuge samples using the following conditions:

Centrifugation	Miniprep	Midiprep	Maxiprep
Acceleration	10,000 × *g*	2,000 × *g*	2,000 × *g*
Time	1 minute	3 minutes	3 minutes

4. Discard the flow-through.
5. Add Buffer KX with ethanol (page 5) to the column.

Reagent	Miniprep	Midiprep	Maxiprep
Buffer KX	500 µL	10 mL	10 mL

6. Centrifuge using the following conditions:

Centrifugation	Miniprep	Midiprep	Maxiprep
Acceleration	10,000 × *g*	5,000 × *g*	5,000 × *g*
Time	1 minute	2 minutes	2 minutes

7. Discard the flow-through and reassemble the spin column.

Continued on next page

Purifying gDNA from Mammalian Cells,
Continued

Purifying DNA,
Continued

8. Add Buffer K2 reconstituted with ethanol (see page 5) to the column.

Reagent	Miniprep	Midiprep	Maxiprep
Buffer K2	500 µL	10 mL	10 mL

9. Centrifuge using the conditions described in Step 6.
10. Discard the flow-through and re-assemble the spin column using the same receiver tube. Centrifuge again to clear the silica membrane of any residual liquid. Discard the receiver tube.

Centrifugation	Miniprep	Midiprep	Maxiprep
Acceleration	12,000 × *g*	5,000 × *g*	5,000 × *g*
Time	1 minute	10 minutes	10 minutes

11. Place the JetQuick® Spin Column into a new, sterile elution tube.

Spin Column Assembly	Miniprep	Midiprep	Maxiprep
Elution tube size	1.5 mL	50 mL	50 mL

12. Add prewarmed (70ºC) 10 mM Tris-HCl, pH 8.5, or prewarmed water to the column. See **Elution Parameters** (pages 6–7) to determine the suitable elution volume for your application. Incubate at room temperature for the time specified in the table below.

Elution	Miniprep	Midiprep	Maxiprep
10 mM Tris-HCl, pH 8.5	25–200 µL	500–800 µL	1–4 mL
Incubation time	2 minutes	5 minutes	5 minutes

Continued on next page

Purifying gDNA from Mammalian Cells,
Continued

Purifying DNA, Continued

13. Centrifuge the column at room temperature to elute the DNA.

Centrifugation	Miniprep	Midiprep	Maxiprep
Acceleration	10,000 × *g*	5,000 × *g*	5,000 × *g*
Time	2 minutes	2 minutes	2 minutes

14. *Optional:* To recover more DNA, perform a second elution step in another sterile tube. Centrifuge the column as directed in Step 13.

    The tube contains purified DNA. Remove and discard the column. Combine the contents of the elution tubes only if overall yield is of greater importance than maintaining the higher concentration obtained in the first eluate (depending on your application).

Storing DNA

To avoid repeated freezing and thawing of DNA,

- Store the purified DNA at 4°C for immediate use or
- Aliquot the DNA and store at –20°C for long-term storage.

Purifying gDNA from Tissue

Introduction

The procedure for purifying gDNA from mammalian tissues, including mouse tail, using JetQuick® Tissue DNA Miniprep Kits is described below. The protocol was adapted from the method published by Bowtell (see Bowtell, D.L.L. 1987. Rapid isolation of eukaryotic DNA. *Anal. Biochem.* 162: 463).

To obtain high-quality genomic DNA, follow the guidelines recommended on page 4.

Materials Needed

- 96–100% ethanol
- Sample for DNA isolation (see below for recommended starting amount)
- Sterile, DNase-free microcentrifuge tubes
- Water baths or heat blocks
- Microcentrifuge capable of centrifuging >12,000 × *g*

Components supplied with the JetQuick™ Tissue DNA Miniprep Kit

- Buffers T1, T2, T3, and TX
- Proteinase K, lyophilized powder, see page 5
- RNase A (20 mg/mL)
- 10 mM Tris-HCl, pH 8.5
- JetQuick® Spin Columns
- JetQuick® Receiver Tubes

Starting Material

The table below lists the amount of starting material recommended based on tissue type.

Tissue	Amount
Brain, Lung, Heart, Kidney	25–30 mg
Liver, Spleen	10–20 mg
Mouse Tail	0.8–1.2 cm

Continued on next page

Purifying gDNA from Tissue, Continued

Preparing Tissue Lysate

Check Buffer T1 for precipitates. If present, warm the solution briefly at 37°C to dissolve the precipitate.

1. Set one water bath or heat block at 55°C and another at 70°C.
2. Mince the tissue sample with a scalpel or freeze the tissue in liquid nitrogen, and grind it into a fine powder with a mortar and pestle.
3. Add 200 µL Buffer T1 to the minced tissue sample in a 1.5 mL microcentrifuge tube. Mix thoroughly by inverting the tube several times.
4. Add 20 µL Proteinase K (20 mg/mL) to the tube. Mix thoroughly by inverting the tube several times.

   Note: When processing multiple samples, you may prepare a master Digestion Buffer Mix by pre-mixing 200 µL Buffer T1 and 20 µL Proteinase K for each sample.
5. Incubate at 55°C with occasional vortexing until mixture is clear, indicating complete lysis (1–2 hours). For mouse tails or larger tissue pieces, incubate overnight.
6. To remove any particulate materials, centrifuge the lysate at 10,000 × *g* for 5 minutes at 4ºC. Transfer clear supernatant to a new, sterile microcentrifuge tube.
7. *Optional:* Add 20 µL RNase A (20 mg/mL) to the lysate and mix well by brief vortexing.
8. Add 200 µL Buffer T2 and mix well by vortexing.
9. Incubate at 70ºC for 10 minutes. Cool the mixture at room temperature for 1 minute.
10. Add 200 µL 96–100% ethanol to the lysate. Mix immediately and thoroughly by vortexing for 5 seconds.

    Note: When processing multiple samples, you may prepare a Master Buffer/Ethanol Mix by pre-mixing 200 µL Buffer T2 and 200 µL 96–100% ethanol for each sample.
11. Proceed immediately to **Purifying DNA** (next page).

Continued on next page

Purifying gDNA from Tissue, Continued

Purifying DNA

1. Assemble a JetQuick® Spin Column with a 2 mL Receiver Tube. Apply the sample from Step 10, previous page, into the JetQuick® Spin Column.

   Note: Processing too many cells may lead to a high DNA content in the sample, so that DNA may already be partially precipitated. If any precipitated matter is present, co-transfer it into the spin column.
2. Centrifuge the sample for 1 minute at 10,000 × *g*.
3. Discard the flow-through and re-assemble the JetQuick® Spin Column into the Receiver Tube.
4. Add 500 µL Buffer TX with ethanol (see page 5) to the column.
5. Centrifuge column at room temperature at 10,000 × *g* for 1 minute.
6. Discard the flow-through and re-assemble the JetQuick® Spin Column.
7. Add 500 µL Buffer T3 with ethanol (page 5) to the column.
8. Centrifuge column at room temperature at 10,000 × *g* for 1 minute.
9. Discard the flow-through and re-assemble the JetQuick® Spin Column using the same 2 mL Receiver Tube. Centrifuge the column again at 12,000 × *g* for 1 minute at room temperature to clear the silica membrane of any residual liquid. Discard the 2 mL Receiver Tube.
10. Place the JetQuick® Spin Column into a sterile 1.5-mL microcentrifuge tube.
11. Add 25–200 µL of prewarmed (65 ºC–70ºC) 10 mM Tris-HCl, pH 8.5, or prewarmed water to the column. See **Elution Parameters** (pages 6–7) to determine the suitable elution volume for your application.

    Note: Make certain that the elution buffer covers the entire membrane.

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Purifying gDNA from Tissue, Continued

Purifying DNA, Continued

12. Incubate at room temperature for 5 minutes.
13. Centrifuge the column at 12,000 × *g* for 2 minutes at room temperature.

    The tube contains purified genomic DNA.
14. *Optional:* To recover more DNA, perform a second elution step in another sterile 1.5 mL microcentrifuge tube.
15. Centrifuge the column at 12,000 × *g* for 2 minutes at room temperature.

    The tube contains purified DNA. Remove and discard the column. Combine the contents of the elution tubes only if overall yield is of greater importance than maintaining the higher concentration obtained in the first eluate (depending on your application).

Storing DNA

To avoid repeated freezing and thawing of DNA,

- Store the purified DNA at 4°C for immediate use or
- Aliquot the DNA and store at –20°C for long-term storage.

Purifying gDNA from Swabs

Introduction

The procedure for purifying genomic DNA from buccal, nasal, pharyngeal, and vaginal swabs using JetQuick® Blood & Cell Culture DNA Miniprep Kits is described below.

To obtain high-quality genomic DNA, follow the guidelines recommended on page 4.

Materials Needed

- 96–100% ethanol
- Swab Sample
- Phosphate Buffered Saline (PBS) or equivalent saline buffer (i.e. TBS) (see page 57 for ordering information)
- Sterile, DNase-free microcentrifuge tubes
- Microcentrifuge capable of centrifuging at >12,000 × *g*
- Water baths or heat blocks

Components supplied with the JetQuick® Blood & Cell Culture DNA Miniprep Kit

- Buffers K1, K2, and KX
- Genomed Protease, lyophilized powder, see **Before Starting**, page 5
- RNase A (20 mg/mL)
- 10 mM Tris-HCl, pH 8.5
- JetQuick® Spin Columns
- JetQuick® Receiver Tubes

Note

The swab protocol utilizes an increased volume of Buffer K1. Therefore, the standard "50 prep" kit contains sufficient Buffer K1 to process 18 swab samples and the "250 prep" JetQuick® Blood & Cell Culture DNA Miniprep Kit contains sufficient Buffer K1 to process 90 samples. To utilize all other components included in the JetQuick® Blood & Cell Culture DNA Miniprep Kits, purchase additional Buffer K1 separately (see page 57).

Continued on next page

Purifying gDNA from Swabs, Continued

Human Buccal Swab Lysate

1. Collect the buccal swab with a suitable tool, such as a T-swab Kit (Isohelix), Dacron swab (Fitzco), C.E.P. Omni Swab (Whatman), or Cotton swab (Puritan Hardwood Products) according to standard collection procedures. Nasal, pharyngeal, or vaginal swabs can be collected in a similar way.
2. Set a water bath or heat block at 58°C.
3. Place the buccal swab into a capped 2 mL microcentrifuge tube (not provided with the kit).
   - For C.E.P. Omni swabs, press the stem end toward the swab to eject it into the microcentrifuge tube.
   - For swabs from other suppliers, snap or cut the swab at the break point.

   The swab should fit entirely inside the tube so that the cap may close.
4. Add 600 µL of PBS, Tris Buffered Saline (TBS), or equivalent standard saline buffer. Mix by vortexing.
5. Add 20 µL Genomed Protease (20 mg/mL) and 10 µL RNase A (20 mg/mL, *optional*) to the sample and mix very thoroughly by vortexing or inverting the tube.
6. Add 600 µL Buffer K1 and mix very thoroughly by vortexing or inverting the tube.
7. Incubate at 58ºC for 10 minutes.
8. Add 600 µL of 96–100% ethanol.
9. Mix well by vortexing to obtain a homogenous solution.

   Note: Mix the sample immediately to prevent the precipitation of nucleic acids due to high local alcohol concentrations.
10. Proceed immediately to **Purifying DNA,** next page.

Continued on next page

Purifying gDNA from Swabs, Continued

Purifying DNA

1. Assemble a JetQuick® Spin Column with a 2 mL Receiver Tube. Apply 700 µL of the sample from Step 9, previous page, into the JetQuick® Spin Column.
2. Centrifuge the sample for 1 minute at 10,000 × *g*.
3. Discard the flow-through.

   Note: Repeat Steps 1–3 until all liquid from Step 9 has been processed over the JetQuick® Spin Column. Squeeze out residual liquid from the swab and discard the swab.
4. Add 500 µL Buffer KX with ethanol (page 5) to the column.
5. Centrifuge the column at room temperature at 10,000 × *g* for 1 minute.
6. Discard the flow-through and re-assemble the JetQuick® Spin Column.
7. Add 500 µL Buffer K2 with ethanol (page 5) to the column.
8. Centrifuge the column at room temperature at 10,000 × *g* for 1 minute.
9. Discard the flow-through and re-assemble the JetQuick® Spin Column using the same 2 mL Receiver Tube. Centrifuge the column again at 12,000 × *g* for 1 minute at room temperature to clear the silica membrane of any residual liquid. Discard the receiver tube.
10. Place the JetQuick® Spin Column into a sterile 1.5 mL microcentrifuge tube.
11. Add 25–200 µL of prewarmed (65 ºC –70ºC) 10 mM Tris-HCl, pH 8.5, or prewarmed water to the column. See **Elution Parameters** (pages 6–7) to determine the suitable elution volume for your application.
12. Incubate at room temperature for 2 minutes.

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Purifying gDNA from Swabs, Continued

Purifying DNA, Continued

13. Centrifuge the column at 10,000 × *g* for 2 minutes at room temperature.

    The tube contains purified genomic DNA.

14. *Optional:* To recover more DNA, perform a second elution step in another sterile 1.5 mL microcentrifuge tube.

15. Centrifuge the column at 10,000 × *g* for 2 minutes at room temperature.

    The tube contains purified DNA. Remove and discard the column. Combine the contents of the elution tubes only if overall yield is of greater importance than maintaining the higher concentration obtained in the first eluate (depending on your application).

Storing DNA

To avoid repeated freezing and thawing of DNA,

- Store the purified DNA at 4°C for immediate use or
- Aliquot the DNA and store at −20°C for long-term storage

Purifying gDNA from Paraffin Embedded Tissue

Introduction

The procedure for purifying gDNA from fixed tissues using the JetQuick® Tissue DNA Miniprep Kit is described below.

The fixative used influences the yield and quality of the purified DNA. Fixatives such as alcohols or formalin are easiest to handle. For tissues fixed with paraffin, a pre-treatment of the sample is necessary (see page 37). Fixatives that cause cross-linking (i.e., osmic acid) are not recommended as it can be difficult to obtain amplifiable DNA from tissues fixed with these agents.

To obtain high-quality genomic DNA, follow the guidelines recommended on page 4.

Materials Needed

- Xylene or PBS
- 96–100% ethanol
- Sample for DNA isolation
- Sterile, DNase-free microcentrifuge tubes
- Water baths or heat blocks
- Microcentrifuge capable of centrifuging >12,000 × *g*

Components supplied with the JetQuick® Tissue DNA Miniprep Kit

- Buffers T1, T2, T3, and TX
- Proteinase K, lyophilized powder, see **Before Starting**, page 5
- RNase A (20 mg/mL)
- 10 mM Tris-HCl, pH 8.5
- JetQuick® Spin Columns
- JetQuick® Receiver Tubes

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Purifying gDNA from Paraffin Embedded Tissue, Continued

Note

An RNase A digestion step is normally not necessary for fixed tissues. Due to the age of the sample and prior treatments most of the RNA is already degraded.

Xylene Extraction

It may **not be necessary** to remove the paraffin by xylene extraction before processing. The paraffin melts during the 56°C incubation and does not affect the JetQuick® gDNA purification procedure. However, xylene extraction is necessary for some types of paraffin-embedded samples. You may try omitting the xylene-extraction protocol, since it makes the isolation procedure much simpler.

Formalin/ Alcohol Fixed Tissues

Wash formalin-/**alcohol** fixed tissue (up to 30 mg tissue) twice with PBS and proceed immediately to **Preparing Fixed Tissue Lysate** (page 38).

Formalin-Fixed, Paraffin-Embedded Tissue

1. Set a water bath or heat block at 37°C.
2. Place a small section of FFPE tissue (no more than 30 mg) in a sterile microcentrifuge tube.
3. Add 1.2 mL xylene to the sample and vortex vigorously for a few seconds.

   Note: Use appropriate precautions while using xylene and dispose of xylene in compliance with established institutional guidelines. CitriSolv™ Clearing Agent (Fisher catalog no. 22-143-975) is a biodegradable alternative to xylene for paraffin extraction.
4. Centrifuge at 12,000 × *g* for 5 minutes at room temperature. Carefully remove the supernatant without disturbing the pellet.

Continued on next page

Purifying gDNA from Paraffin Embedded Tissue, Continued

Formalin-Fixed, Paraffin-Embedded Tissue, Continued

5. Add 1.2 mL 96–100% ethanol and vortex gently to remove residual xylene from the tissue pellet.
6. Centrifuge at 12,000 × *g* for 5 minutes at room temperature. Carefully remove the supernatant.
7. Repeat ethanol extraction (Steps 5–6) once.
8. Incubate the tubes with the lid open at 37ºC for 10 to 15 minutes to evaporate residual ethanol.
9. Proceed immediately to **Preparing Fixed Tissue Lysate**, below.

Before Starting

Check Buffer T1 for precipitates. If present, warm the solution briefly at 37°C to dissolve the precipitate.

Preparing Fixed Tissue Lysate

Following fixative-removal from the tissue sample, prepare the lysate according to the following procedure:

1. Set one water bath or heat block at 56°C and another at 70°C.
2. Add 200 µL Buffer T1 to the tissue sample. Mix thoroughly by inverting the tube several times.
3. Add 20 µL Proteinase K (20 mg/mL). Mix well by inverting the tube several times.

   Note: When processing multiple samples, you may prepare a master Digestion Buffer Mix by pre-mixing 200 µL Buffer T1 and 20 µL Proteinase K for each sample.
4. Incubate at 56°C for 1 hour to overnight until mixture is clear, indicating complete tissue digestion. Mix the sample several times during the incubation for efficient digestion.

Continued on next page

Purifying gDNA from Paraffin Embedded Tissue, Continued

Preparing Fixed Tissue Lysate, Continued

5. Centrifuge the lysate at 10,000 × *g* for 3 minutes at 4ºC to remove any particulate materials. Transfer lysate to a new, sterile microcentrifuge tube.
6. Add 200 µL Buffer T2 to the cleared lysate and mix well by vortexing. Incubate at 70ºC for 10 minutes. Cool the mixture at room temperature for 1 minute.
7. Add 200 µL 96–100% ethanol to the lysate. Mix immediately and thoroughly by vortexing for 5 seconds.

   Note: When processing multiple samples, you may prepare a Master Buffer/Ethanol Mix by pre-mixing 200 µL Buffer T2 and 200 µL 96–100% ethanol for each sample.
8. Proceed immediately to **Purifying DNA** (below).

Purifying DNA

1. Assemble a JetQuick® Spin Column with a 2 mL Receiver Tube. Apply the sample from Step 7, above, into the JetQuick® Spin Column.

   Note: Processing too many cells may lead to a high DNA content in the sample, so that DNA may already be partially precipitated. If any precipitated matter is present, co-transfer it into the spin column.
2. Centrifuge the sample for 1 minute at 10,000 × *g*.
3. Discard the flow-through and re-assemble the spin column into the 2 mL Receiver Tube.
4. Add 500 µL Buffer TX with ethanol (see page 5) to the JetQuick® Spin Column.
5. Centrifuge the JetQuick® Spin Column at room temperature at 10,000 × *g* for 1 minute.
6. Discard the flow-through and re-assemble the JetQuick® Spin Column.

Continued on next page

Purifying gDNA from Paraffin Embedded Tissue, Continued

Purifying DNA, Continued

7. Add 500 µL Buffer T3 with ethanol (page 5) to the JetQuick® Spin Column.
8. Centrifuge the JetQuick® Spin Column at room temperature at 10,000 × *g* for 1 minute.
9. Discard the flow-through and re-assemble the spin column using the same receiver tube. Centrifuge the column again at 12,000 × *g* for 1 minute at room temperature to clear the silica membrane of any residual liquid. Discard the 2 mL Receiver Tube.
10. Place the JetQuick® Spin Column into a sterile 1.5-mL microcentrifuge tube.
11. Add 25–100 µL of prewarmed (65 ºC–70ºC) 10 mM Tris-HCl, pH 8.5, or prewarmed water to the column. See **Elution Parameters** (pages 6–7) to determine the suitable elution volume for your application.

    Note: Make certain that the elution buffer covers the entire membrane.
12. Incubate at room temperature for 5 minutes.
13. Centrifuge the column at 12,000 × *g* for 2 minutes at room temperature.

    The tube contains purified DNA. Remove and discard the column. Combine the contents of the elution tubes only if overall yield is of greater importance than maintaining the higher concentration obtained in the first eluate (depending on your application).

Storing DNA

To avoid repeated freezing and thawing of DNA,

- Store the purified DNA at 4°C for immediate use or
- Aliquot the DNA and store at –20°C for long-term storage.

Purifying gDNA from Bacteria

Introduction

The procedure for purifying gDNA from various sources of bacterial species using the JetQuick® Tissue DNA Miniprep Kit is described below. Bacterial gDNA may be purified from biological fluids, from eye, nasal, or pharyngeal swabs or from bacterial cultures, see table below.

For the isolation of gDNA from eye, nasal, or pharyngeal swabs or from gram-positive cultures, additional buffers and enzymes are necessary (see relevant protocols for more information).

Materials Needed

- PBS and fungicide (for **Harvesting Bacteria from Swabs**)
- Lysozyme Digestion Buffer (for **Harvesting Gram Positive Bacteria**)
- 96–100% ethanol
- Sample for DNA isolation
- Sterile, DNase-free microcentrifuge tubes
- Water baths or heat blocks
- Microcentrifuge capable of centrifuging >12,000 × *g*

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Purifying gDNA from Bacteria, Continued

Components supplied with the JetQuick® Tissue DNA Miniprep Kit

- Buffers T1, T2, T3, and TX
- Proteinase K, lyophilized powder, see page 5
- RNase A (20 mg/mL)
- 10 mM Tris-HCl, pH 8.5
- JetQuick® Spin Columns and JetQuick® Receiver Tubes

Harvesting Bacteria from Biological Fluids

1. Harvest bacteria by centrifugation at 12,000 × *g* for 3 minutes. Remove the clarified supernatant completely with a pipette.
2. Resuspend the cell pellet in 200 µL Buffer T1.
3. Proceed immediately to **Preparing Bacterial Lysate**, page 44.

Harvesting Bacteria from Swabs

1. Place sample in 2 mL PBS containing fungicide.
2. Incubate at room temperature for several hours.
3. Harvest bacteria by centrifugation at 12,000 × *g* for 3 minutes. Remove the clarified supernatant completely with a pipette.
4. Resuspend the cell pellet in 200 µL Buffer T1.
5. Proceed immediately to **Preparing Bacterial Lysate,** page 44.

Continued on next page

Purifying gDNA from Bacteria, Continued

Harvesting Gram Negative Bacteria

1. Harvest bacteria:
   - For suspension cultures, centrifuge at 12,000 × *g* for 3 minutes. Remove the clarified supernatant completely with a pipette.
   - For plate cultures, remove bacterial cells from culture plate with an inoculation loop.
2. Suspend the bacterial cells in 200 µL Buffer T1 with vigorous stirring or vortexing.
3. Proceed immediately to **Preparing Bacterial Lysate,** next page.

Harvesting Gram Positive Bacteria

Gram-positive bacteria require a pre-incubation with specific enzymes such as Lysozyme or Lysostaphin (*Staphylococcus* specific) to disrupt the rigid multilayered murein cell wall. For these species, prepare the cell lysate as described below.

1. Set a water bath or heat block at 37ºC.
2. Prepare Lysozyme Digestion Buffer:

   20 mM Tris-HCl, pH 8.0
   2 mM EDTA,
   1.2% Triton® X-100

   To 200 µL Lysozyme Digestion Buffer/sample, add **fresh** Lysozyme or Lysostaphin to obtain a final Lysozyme concentration of 20 mg/mL or Lysostaphin concentration of 200 µg/mL.
3. Pellet up to 2 × 10^9 Gram positive cells by centrifugation at 12,000 × *g* for 3 minutes.
4. Resuspend the cell pellet in 200 µL Lysozyme Digestion Buffer with Lysozyme or Lysostaphin from Step 2.
5. Incubate at 37ºC for 30 minutes.
6. Proceed immediately to **Preparing Bacterial Lysate,** next page.

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Purifying gDNA from Bacteria, Continued

Preparing Bacterial Lysate

1. Set one water bath or heat block at 56°C and another at 70°C.
2. Add 20 µL Proteinase K (20 mg/mL) to the to the sample from Step 3 (**Harvesting Bacteria from Biological Fluids**, page 42 or **Harvesting Gram Negative Bacteria**, page 43) or Step 5 (**Harvesting Bacteria from Swabs**, page 42 or **Harvesting Gram Positive Bacteria**, page 43). Mix thoroughly by inverting the tube several times.
3. Incubate at 56°C with occasional vortexing until mixture is clear, indicating lysis is complete (1 to 2 hours).

   Note: Reduce incubation time to 30 minutes for Gram Positive Bacterial Cell Lysates.
4. To remove any particulate materials, centrifuge the lysate at 10,000 × *g* for 3 minutes at 4ºC. Transfer supernatant to a new, sterile microcentrifuge tube.
5. *Optional:* Add 20 µL RNase A (20 mg/mL) to the lysate and mix well by brief vortexing.
6. Add 200 µL Buffer T2 and mix well by vortexing.
7. Incubate at 70ºC for 10 minutes. Cool the mixture at room temperature for 1 minute.
8. Add 200 µL 96–100% ethanol to the lysate. Mix immediately and thoroughly by vortexing for 5 seconds.

   Note: When processing multiple samples, you may prepare a Master Buffer/Ethanol Mix by pre-mixing 200 µL Buffer T2 and 200 µL 96–100% ethanol for each sample.
9. Proceed immediately to **Purifying DNA**, next page.

Continued on next page

Purifying gDNA from Bacteria, Continued

Purifying DNA

1. Assemble a JetQuick® Spin Column with a 2 mL Receiver Tube. Apply the sample from Step 8, previous page, into the JetQuick® Spin Column.

   Note: Processing too many cells may lead to a high DNA content in the sample, so that DNA may already be partially precipitated. If any precipitated matter is present, co-transfer it into the spin column.
2. Centrifuge the sample for 1 minute at 10,000 × *g*.
3. Discard the flow-through and re-assemble the JetQuick® Spin Column into the Receiver Tube.
4. Add 500 µL Buffer TX with ethanol (see page 5) to the column.
5. Centrifuge the column at room temperature at 10,000 × *g* for 1 minute.
6. Discard the flow-through and re-assemble the JetQuick® Spin Column.
7. Add 500 µL Buffer T3 with ethanol (page 5) to the column.
8. Centrifuge the column at room temperature at 10,000 × *g* for 1 minute.
9. Discard the flow-through and re-assemble the JetQuick® Spin Column using the same 2 mL Receiver Tube. Centrifuge the column again at 12,000 × *g* for 1 minute at room temperature to clear the silica membrane of any residual liquid. Discard the 2 mL Receiver Tube.
10. Place the JetQuick® Spin Column into a sterile 1.5-mL microcentrifuge tube.
11. Add 25–200 µL of prewarmed (65 ºC–70ºC) 10 mM Tris-HCl, pH 8.5, or prewarmed water to the column. See **Elution Parameters** (pages 6–7) to determine the suitable elution volume for your application.

    Note: Make certain that the elution buffer comes into contact with the entire membrane.

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Purifying gDNA from Bacteria, Continued

Purifying DNA, Continued

12. Incubate at room temperature for 5 minutes.
13. Centrifuge the column at 12,000 × *g* for 2 minutes at room temperature.

    The tube contains purified genomic DNA.
14. *Optional:* To recover more DNA, perform a second elution step in another sterile 1.5 mL microcentrifuge tube.
15. Centrifuge the column at 12,000 × *g* for 2 minutes at room temperature.

    The tube contains purified DNA. Remove and discard the column. Combine the contents of the elution tubes only if overall yield is of greater importance than maintaining the higher concentration obtained in the first eluate (depending on your application).

Storing DNA

To avoid repeated freezing and thawing of DNA,

- Store the purified DNA at 4°C for immediate use or
- Aliquot the DNA and store at –20°C for long-term storage.

Purifying gDNA from Yeast

Introduction

The procedure for purifying gDNA from yeast using the JetQuick® Tissue DNA Miniprep Kit is described below. The protocol has been modified from the publication: Boeke, J.D., Garfinkel, D.J., Styles, C.A., and Fink, G.R. 1985. Ty elements transpose through an RNA intermediate. *Cell* 40:491.

To obtain high-quality genomic DNA, follow the guidelines recommended on page 4.

Materials Needed

- Buffer Y1
- 96–100% ethanol
- Sample for DNA isolation
- Sterile, DNase-free microcentrifuge tubes
- Water baths or heat blocks
- Microcentrifuge capable of centrifuging >12,000 × *g*

Components supplied with the JetQuick® Tissue DNA Miniprep Kit

- Buffers T1, T2, T3, and TX
- Proteinase K, lyophilized powder, see page 5
- RNase A (20 mg/mL)
- 10 mM Tris-HCl, pH 8.5
- JetQuick® Spin Columns
- JetQuick® Receiver Tubes

Continued on next page

Purifying gDNA from Yeast, Continued

Zymolyase

Preparing the cell lysate includes an incubation step with Zymolyase (see Step 5, below). Zymolyase digests cell walls of yeast cells enzymatically during incubation. An equivalent enzyme to Zymolyase is Lyticase (Sigma, Cat. no. L2524). Use concentrated Zymolyase, but dilute Lyticase from the stock solution in distilled water to a final concentration of 1,000 U/mL. Perform Lyticase incubations for at least 30 minutes at 30°C. Store stock solutions of both enzymes in aliquots at −20°C and use only once.

Preparing Yeast Lysate

Check Buffer T1 for precipitates. If present, warm the solution briefly at 37°C to dissolve the precipitate. Prepare lysate from yeast as described below.

1. **Prepare Buffer Y1**:
   0.9 M sorbitol
   0.1 M EDTA (pH 7.5)
   14 mM β-mercaptoethanol
2. Set a water bath or heat block at 56°C.
3. Grow the Saccharomyces culture to saturation in YPD or YEPD. Harvest by centrifugation at 3,000–5,000 × *g* for 5 to 10 minutes at 4°C. Discard the supernatant and resuspend in 2 mL buffer Y1 (see Step 1).
4. Centrifuge again at 3,000–5,000 × *g* for 5–10 minutes at 4°C. Discard the supernatant. Resuspend the cells in 1 mL Buffer Y1.
5. Add 20 units of Zymolyase 100T (MP Biomedicals; 100,000 units/g) and incubate at 37°C for 20 to 30 minutes.

   Note: Monitor spheroplast formation by examining detergent sensitivity: dilute a small sample of cells in 1% SDS. Spheroplasting is sufficient when greater than 90% of the cells burst when examined under the microscope.

Continued on next page

Purifying gDNA from Yeast, Continued

Preparing Yeast Lysate, Continued

6. Centrifuge the spheroplasts at 5,000 × *g* for 10 minutes at 4°C. Discard the supernatant.
7. Resuspend the spheroplasts in 200 µL Buffer T1 in a 1.5 mL microcentrifuge tube. Mix thoroughly by inverting the tube several times.
8. Add 20 µL Proteinase K (20 mg/mL) to the tube. Mix thoroughly by inverting the tube several times.

   Note: When processing multiple samples, you may prepare a master Digestion Buffer Mix by combining 200 µL Buffer T1 and 20 µL Proteinase K for each sample.
9. Incubate with occasional vortexing until lysis is complete: 1 to 2 hours or overnight at 56ºC.
10. To remove any particulate materials, centrifuge the lysate at 10,000 × *g* for 3 minutes at 4ºC. Transfer supernatant to a new, sterile microcentrifuge tube.
11. *Optional:* Add 20 µL RNase A (20 mg/mL) to the lysate and mix well by brief vortexing.
12. Add 200 µL Buffer T2 and mix well by vortexing.
13. Incubate at 70ºC for 10 minutes. Then cool the mixture at room temperature for 1 minute.
14. Add 200 µL 96–100% ethanol to the lysate. Mix immediately and thoroughly by vortexing for 5 seconds.

    Note: When processing multiple samples, you may prepare a Master Buffer/Ethanol Mix by pre-mixing 200 µL Buffer T2 and 200 µL 96–100% ethanol for each sample.
15. Proceed immediately to **Purifying DNA**, next page.

Continued on next page

Purifying gDNA from Yeast, Continued

Purifying DNA

1. Assemble a JetQuick® Spin Column with a 2 mL Receiver Tube. Apply the sample from Step 14, previous page, into the JetQuick® Spin Column.

   Note: Processing too many cells may lead to a high DNA content in the sample, so that DNA may already be partially precipitated. If any precipitated matter is present, co-transfer it into the spin column.
2. Centrifuge the sample for 1 minute at 10,000 × *g*.
3. Discard the flow-through and re-assemble the spin column into the 2 mL Receiver Tube.
4. Add 500 µL Buffer TX with ethanol (see page 5) to the column.
5. Centrifuge the column at 10,000 × *g* for 1 minute at room temperature.
6. Discard the flow-through and re-assemble the spin column.
7. Add 500 µL Buffer T3 with ethanol (page 5) to the column.
8. Centrifuge the column at 10,000 × *g* for 1 minute at room temperature.
9. Discard the flow-through and re-assemble the JetQuick® Spin Column using the same 2 mL Receiver Tube. Centrifuge the column again at 12,000 × *g* for 1 minute at room temperature to clear the silica membrane of any residual liquid. Discard the receiver tube.
10. Place the JetQuick® Spin Column into a sterile 1.5-mL microcentrifuge tube.
11. Add 25–200 µL of prewarmed (65 ºC–70ºC) 10 mM Tris-HCl, pH 8.5, or prewarmed water to the column. Refer to pages 6–7 to determine elution volume.
12. Incubate at room temperature for 5 minutes.

Continued on next page

Purifying gDNA from Yeast, Continued

Purifying DNA, Continued

13. Centrifuge the column at 12,000 × *g* for 2 minutes at room temperature.

    The tube contains purified genomic DNA.

14. *Optional:* To recover more DNA, perform a second elution step in another sterile 1.5 mL microcentrifuge tube.

15. Centrifuge the column at 12,000 × *g* for 2 minutes at room temperature.

    The tube contains purified DNA. Remove and discard the column. Combine the contents of the elution tubes only if overall yield is of greater importance than maintaining the higher concentration obtained in the first eluate (depending on your application).

Storing DNA

To avoid repeated freezing and thawing of DNA,

- Store the purified DNA at 4°C for immediate use or
- Aliquot the DNA and store at –20°C for long-term storage.

Examples of Expected Results

Blood gDNA Miniprep

Genomic DNA from various samples was isolated using a JetQuick® Blood & Cell Culture DNA Miniprep Kit and analyzed by agarose gel electrophoresis on a 1% TAE gel.

Samples on the gel are:

Lanes 1, 10: Lambda *Hin*dIII/*Eco*RI

Lane 2: DNA isolated from fresh EDTA-blood

Lane 3: DNA isolated from EDTA-blood stored at 4ºC for 2 days

Lane 4: DNA isolated from frozen EDTA-blood

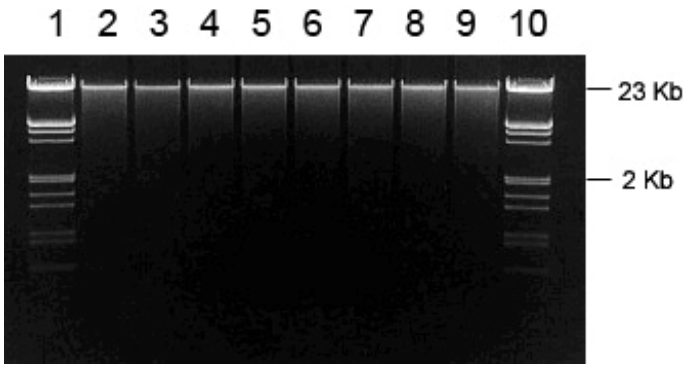
Lane 5: DNA isolated from fresh citrate-blood

Lane 6: DNA isolated from citrate-blood stored at 4ºC for 2 days

Lane 7: DNA isolated from frozen citrate-blood

Lane 8: DNA isolated from fresh heparin-blood

Lane 9: DNA isolated from frozen heparin-blood



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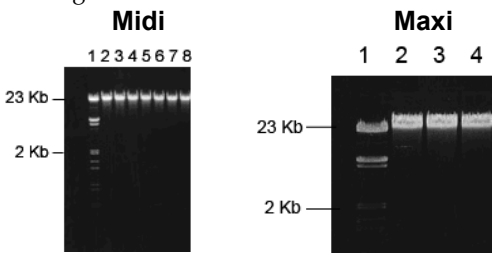
Examples of Expected Results, Continued

Blood gDNA Midi- and Maxiprep

Genomic DNA isolated from various samples was analyzed by agarose gel electrophoresis on a 0.8% TAE gel. 500 ng (Midiprep) or 700 ng (Maxiprep) blood gDNA per sample from 7 (Midiprep) or 3 (Maxiprep) blood samples prepared in parallel with the JetQuick® Blood & Cell Culture DNA Midiprep or Maxiprep Kits. Lambda DNA digested with *Eco*RI / *Hin*dIII was used as a DNA size marker in lane 1 for both gels.



Tissue gDNA Miniprep

Genomic DNA isolated from various tissue samples using a JetQuick® Tissue DNA Miniprep Kit was analyzed by agarose gel electrophoresis on a 1% TAE gel.

Lane contents are as follows:

Lanes 1, 8: Lambda *Hin*dIII/*Eco*RI

Lane 2: DNA isolated from bovine liver

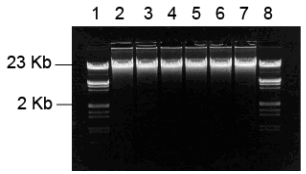
Lane 3: DNA isolated from bovine spleen

Lane 4: DNA isolated from bovine pancreas

Lane 5: DNA isolated from porcine kidney

Lane 6: DNA isolated from porcine spleen

Lane 7: DNA isolated from chicken liver



Troubleshooting

Introduction

Refer to the table below to troubleshoot problems that you may encounter when using the JetQuick® genomic DNA Purification kits.

Problem	Cause	Solution
Low DNA yield	Incomplete lysis	• Decrease the amount of starting material used. • Add Genomed Protease or Proteinase K during lysis. • Increase the digestion time or amount of Genomed Protease or Proteinase K used for lysis.
	Poor quality of starting material	Use fresh samples and process immediately after collection or freeze the samples at –80°C or in liquid nitrogen. The yield and quality of DNA isolated is dependent on the type and age of the starting material.
	Incorrect binding conditions	Add 96–100% ethanol to the lysate prior to loading the samples on the spin column. Mix the sample by vortexing. Avoid overloading the column.
	Ethanol not added to Buffers KX and K2 (JetQuick® Blood & Cell Culture DNA Kits) or Buffers TX and T3 (JetQuick® Tissue DNA Miniprep Kits)	Add 96–100% ethanol to Wash Buffers KX and K2 or Buffers TX and T3 as indicated on the label.

Continued on next page

Troubleshooting, Continued

Problem	Cause	Solution
Low DNA yield	Incorrect elution conditions	Add 10 mM Tris-HCl, pH 8.5 and perform incubation with buffer before centrifugation. To recover more DNA, perform a second elution step.
	DNA is sheared or degraded	Avoid repeated freezing and thawing of samples to prevent any DNA damage. Maintain a sterile environment while working to avoid any contamination from DNases.
Dark colored eluate or discolored membrane (mammalian tissue, mouse tails, or blood samples only)	Pigments from tissues or heme from blood bind to the silica matrix and co-elute with DNA	Be sure to add ethanol to the lysate prior to loading the lysate on the JetQuick® Spin Columns. The ethanol prevents the pigments from sticking on the silica matrix. Perform centrifugation of the lysate at a higher speed and longer time prior to loading the lysate on to the column.
RNA contamination	Silica membrane binds total nucleic acid present in the sample	Perform RNase A digestion step during sample preparation.

Continued on next page

Troubleshooting, Continued

Problem	Cause	Solution
Inhibition of downstream enzymatic reactions	Presence of ethanol in purified DNA	Traces of ethanol from the Wash Buffer K2 (JetQuick® Blood & Cell Culture DNA Kits) or T3 (JetQuick® Tissue DNA Miniprep Kits) can inhibit downstream enzymatic reactions. To remove Wash Buffers K2 or T3 from JetQuick® Spin Column, discard Wash Buffer flow-through. Centrifuge the JetQuick® Spin Column at maximum speed for up to 15 minutes or incubate the spin columns for 10 minutes at 70°C in an incubator to evaporate residual ethanol.
	Presence of salt in purified DNA	Use the correct order of Wash Buffers for washing. Maintain a ratio of 1:1:1 for Sample:Binding Buffer (K1 or T2):Ethanol.
Low elution volume or sample cross-contamination (applies to **Purifying gDNA from Blood and Body Fluids Using Vacuum only**)	Incorrect vacuum pressure	Make sure the vacuum manifold is sealed tightly without leakage. Maintain the vacuum pressure at –6 to –12 inches Hg (–200 to –400 mbar or –150 to –300 mm Hg) to obtain the best results.

Appendix

Accessory Products

Additional Products

The table below lists additional products available from Genomed or Invitrogen that may be used with the JetQuick® genomic DNA Purification Kits.

Product	Amount	Cat. no.
Products below are available from Genomed. See www.genomed-dna.com		
Buffer K1	200 mL	K1-200
Buffer K2	150 mL	K2-500
Buffer KX	216 mL	KX-500
Buffer T1	200 mL	T1-200
Buffer T2	200 mL	T2-200
Buffer T3	150 mL	T3-500
Buffer TX	216 mL	TX-500
Proteinase K	40 mg	GN-PK-040
	200 mg	GN-PK-200
Ribonuclease A	50 mg	GN-RN-50
	100 mg	GN-RN-100
Products below are available from Invitrogen. See www.invitrogen.com		
Phosphate Buffered Saline (PBS), 1X	500 mL	10010-023
EveryPrep™ Universal Vacuum Manifold	1 unit	K211101

E-Gel® Agarose Gels and DNA Ladders

E-Gel® Agarose Gels are bufferless, pre-cast agarose gels designed for fast, convenient electrophoresis of DNA samples. E-Gel® agarose gels are available in different agarose percentages and well formats for your convenience.

DNA ladders are available from Invitrogen for sizing DNA. For more details on these products, visit www.invitrogen.com.

Technical Support

World Wide Web

Visit the website at **www.genomed-dna.com** for:

- Technical resources, including manuals, SDSs, FAQs
- Complete technical support contact information
- Access to the Online Catalog
- Additional product information and special offers

Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on **www.genomed-dna.com**.

GENOMED GmbH
Poststr. 22
D-32584 Löhne
Germany
Tel: +49-(0)5732-904700
Fax: +49-(0)5732-9047010
E-mail: techservice@genomed-dna.com

Purchaser Notification

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Continued on next page

Purchaser Notification, Continued

Limited Use Label License No. 5: Invitrogen Technology

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Corporate Headquarters
Genomed GmbH
Poststr. 22
D-32584 Löhne, Germany
Tel:+49-(0)5732-904700
Fax:+49-(0)5732-9047010
techservice@genomed-dna.com

www.genomed-dna.com