



PureLink™ Genomic DNA Kits

For purification of genomic DNA

Catalog nos. K1820-01, K1820-02, K1821-04

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User Manual

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Experienced Users' Mini Kit Procedure

Introduction

This quick reference sheet is included for experienced users of the PureLink™ Genomic DNA Mini Kit. For more details, refer to this manual.

Step	Procedure																								
Preparing lysates	<p>Prepare the lysate using an appropriate sample preparation protocol as follows:</p> <table border="1"> <thead> <tr> <th><u>Sample</u></th> <th><u>Amount</u></th> <th><u>Page no.</u></th> </tr> </thead> <tbody> <tr> <td>Mammalian cells, tissues and mouse tail</td> <td>5 x 10⁶ cells, up to 25 mg tissue (up to 10 mg spleen), 0.5-1 cm tail</td> <td>8</td> </tr> <tr> <td>Blood</td> <td>Up to 1 ml nonnucleated blood (need additional reagents, see page 9) 5-10 µl nucleated blood</td> <td>9</td> </tr> <tr> <td>Bacteria</td> <td>Up to 2 x 10⁹ cells</td> <td>10</td> </tr> <tr> <td>Yeast cells</td> <td>Up to 5 x 10⁷ cells</td> <td>11</td> </tr> <tr> <td>Buccal swab</td> <td>Human buccal swab</td> <td>11</td> </tr> <tr> <td>FFPE tissue</td> <td>1-8 sections of 5-15 µm thick of about 20-50 mm² area</td> <td>12</td> </tr> <tr> <td>Oragene™ preserved saliva</td> <td>Up to 1 ml</td> <td>13</td> </tr> </tbody> </table>	<u>Sample</u>	<u>Amount</u>	<u>Page no.</u>	Mammalian cells, tissues and mouse tail	5 x 10 ⁶ cells, up to 25 mg tissue (up to 10 mg spleen), 0.5-1 cm tail	8	Blood	Up to 1 ml nonnucleated blood (need additional reagents, see page 9) 5-10 µl nucleated blood	9	Bacteria	Up to 2 x 10 ⁹ cells	10	Yeast cells	Up to 5 x 10 ⁷ cells	11	Buccal swab	Human buccal swab	11	FFPE tissue	1-8 sections of 5-15 µm thick of about 20-50 mm ² area	12	Oragene™ preserved saliva	Up to 1 ml	13
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Binding DNA	<ol style="list-style-type: none"> 1. Remove a PureLink™ Spin Column in a Collection Tube from the package. 2. Load the lysate (~640 µl) with Lysis/Binding Buffer and ethanol prepared as described on pages 8-13 to the PureLink™ Spin Column. 3. Centrifuge the column at 10,000 × g for 1 minute at room temperature. 4. Discard the collection tube and place the spin column into a new collection tube. 5. Proceed to Washing DNA, below. 																								
Washing DNA	<ol style="list-style-type: none"> 1. Wash the column with 500 µl Wash Buffer 1 prepared with ethanol (page 15). 2. Centrifuge the column at 10,000 × g for 1 minute at room temperature. Discard the collection tube and place column into a new collection tube. 3. Wash the column with 500 µl Wash Buffer 2 prepared with ethanol (page 15). 4. Centrifuge the column at maximum speed for 3 minutes at room temperature. Discard the collection tube. 5. Proceed to Eluting DNA, below. 																								
Eluting DNA	<ol style="list-style-type: none"> 1. Place the spin column in a sterile 1.5-ml microcentrifuge tube. 2. Elute the DNA with 25-200 µl of PureLink™ Genomic Elution Buffer. See Elution Parameters (page 5) to choose a suitable elution volume for your needs. 3. Incubate the column at room temperature for 1 minute. 4. Centrifuge the column at maximum speed for 1 minute at room temperature. <i>The tube contains purified DNA.</i> 5. If desired, perform a second elution to increase recovery which lowers the overall concentration. <i>The tube contains purified DNA.</i> Remove and discard the column. 6. Use the purified gDNA for the desired downstream application. Store the purified gDNA at 4°C for short-term or -20°C for long-term storage. 																								

Kit Contents and Storage

Types of Products

This manual is supplied with the following products:

Product	Quantity	Catalog No.
PureLink™ Genomic DNA Mini Kit	50 preps	K1820-01
	250 preps	K1820-02
PureLink™ 96 Genomic DNA Kit	4 x 96 preps	K1821-04

Shipping and Storage

All components of the PureLink™ Genomic DNA Kits are shipped at room temperature.

Upon receipt, store all components at room temperature.

Note: The Proteinase K solution and RNase A are stable for 1 year when stored at room temperature. For long-term storage (>1 year) or if room temperature is >25°C, store the Proteinase K solution and RNase A at 4°C.

PureLink™ Genomic DNA Mini Kit Contents

The components included in the PureLink™ Genomic DNA Mini Kits are listed below.

Component	K1820-01	K1820-02
	50 preps	250 preps
PureLink™ Genomic Lysis/Binding Buffer	10 ml	50 ml
PureLink™ Genomic Digestion Buffer	9 ml	45 ml
PureLink™ Genomic Wash Buffer 1	10 ml	50 ml
PureLink™ Genomic Wash Buffer 2	7.5 ml	37.5 ml
PureLink™ Genomic Elution Buffer (10 mM Tris-HCl, pH 9.0, 0.1 mM EDTA)	10 ml	50 ml
RNase A (20 mg/ml) in 50 mM Tris-HCl, pH 8.0, 10 mM EDTA	1 ml	5 ml
Proteinase K (20 mg/ml) in storage buffer (proprietary)	1 ml	5 ml
PureLink™ Spin Columns with Collection Tubes	50	5 x 50
PureLink™ Collection Tubes (2.0 ml)	100	5 x 100

Continued on next page

Introduction

Overview

Introduction

The PureLink™ Genomic DNA Kits allow rapid and efficient purification of genomic DNA. The kit is designed to efficiently isolate genomic DNA from mammalian cells and tissues, mouse/rat tail, blood samples, buccal swabs, bacteria, yeast, FFPE (formalin-fixed paraffin-embedded) tissue, and Oragene™ preserved saliva. After preparing the lysates, the DNA is rapidly purified from lysates using a spin column based centrifugation procedure or high throughput isolation using 96-well plates with a vacuum manifold or automated liquid handling workstations.

The isolated DNA is 20-50 kb in size and is suitable for PCR, restriction enzyme digestion, and Southern blotting.

System Overview

The PureLink™ Genomic DNA Kits are based on the selective binding of DNA to silica-based membrane in the presence of chaotropic salts.

The lysate is prepared from a variety of starting materials such as tissues, cells, or blood. The cells or tissues are digested with Proteinase K at 55°C using an optimized digestion buffer formulation that aids in protein denaturation and enhances Proteinase K activity. Any residual RNA is removed by digestion with RNase A prior to binding samples to the silica membrane.

The lysate is mixed with ethanol and PureLink™ Genomic Binding Buffer that allows high DNA binding PureLink™ Spin Column (Mini Kit) or Binding Plate (96 Kit). The DNA binds to the silica-based membrane in the column or plate and impurities are removed by thorough washing with Wash Buffers. The genomic DNA is then eluted in low salt Elution Buffer.

Advantages

The advantages of using PureLink™ Genomic DNA Kits are:

- Rapid and efficient purification of genomic DNA from a variety of samples such as mammalian cells and tissue, blood samples, mouse tails, buccal swabs, bacteria, yeast, FFPE tissue, and Oragene™ preserved saliva
 - Designed to rapidly purify high-quality DNA using spin column or 96-well plate format
 - Automation using standard robotic systems (96 kit) with no sample cross contamination
 - Simple lysis of cells and tissues with Proteinase K 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
-

Continued on next page

Methods

General Guidelines

Introduction

General guidelines for using the PureLink™ Genomic DNA Kits are described below. Review this section before starting the purification procedure.

Choose the appropriate purification protocol based on the type of kit you have purchased:

Kit	Page no.
PureLink™ Genomic DNA Mini Kits (K1820-01, K1820-02)	6
PureLink™ 96 Genomic DNA Kit (K1821-04)	17

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



- The PureLink™ Genomic Lysis/Binding Buffer and Wash Buffer 1 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.
- 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.



Follow the recommendations below to obtain the best results:

- Maintain a sterile environment when handling DNA to avoid any contamination from DNases
- 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

Continued on next page

General Guidelines, Continued

RNase A Digestion

RNase A digestion is performed during sample preparation to degrade RNA present in the sample and minimize RNA contamination in the purified DNA sample. RNA contamination also inflates the DNA content measured at 260 nm.

RNase A is supplied with the kit and an RNase 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 digestion step during sample preparation.

Proteinase K Digestion

The Proteinase K is used for efficient lysis of tissues/cells. Proteinase K digestion is performed using an optimized buffer formulation, PureLink™ Genomic Digestion Buffer, for optimal enzymatic activity.

Sample Amount

There are different protocols for preparing lysates depending on the starting material (sample). Based on your sample, choose an appropriate lysate preparation protocol from the table below.

The PureLink™ Genomic DNA Kits are suitable for isolating genomic DNA from a variety of samples using the recommended sample amount (see table below).

Note: If you start with less amount of sample, the yield of DNA may also be lower.

To obtain high yield of DNA and minimize DNA degradation, collect the sample and proceed immediately to sample preparation or freeze the sample in liquid nitrogen immediately after collection.

Sample	Amount
Mammalian cells	5 × 10 ⁶ cells (suspension or adherent cells)
Mammalian tissues	Up to 25 mg (up to 10 mg for spleen)
Mouse or rat tail	1 cm (mouse); 0.5 cm (rat)
Buccal swab	Human buccal swab
Nonnucleated whole blood (<i>e.g.</i> , human, mouse)	Up to 200 µl (single pass) Up to 1 ml (multiple pass, Mini Kit only)
Nucleated whole blood (<i>e.g.</i> , bird)	5-10 µl
Blood spot on paper	2-5 punches (2-3 mm in size)
Gram negative bacteria (<i>e.g.</i> , <i>E. coli</i>)	Up to 2 × 10 ⁹ cells
Gram positive bacteria (<i>e.g.</i> , Bacillus)	Up to 2 × 10 ⁹ cells
Yeast cells	Up to 5 × 10 ⁷ cells
FFPE tissue	1-8 sections of 5-15 µm thick with a tissue surface area of 20-50 mm ² .
Oragene™ preserved saliva	Up to 4 ml (Mini Kit); Up to 200 µl (96 Kit)

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General Guidelines, Continued

Whole Blood Sample

The PureLink™ Genomic DNA Kits are designed to purify genomic DNA from the following whole blood samples:

- Fresh or frozen whole blood
- Fresh or frozen whole blood collected in the presence of anti-coagulants such as EDTA or citrate
- Dried blood spots on paper such as FTA® card (Whatman) or S&S 903.

Elution Parameters

Elution Buffer

The genomic DNA is eluted using PureLink™ Genomic Elution Buffer (10 mM Tris-HCl, pH 9.0, 0.1 mM EDTA). Alternatively, Tris Buffer (10 mM Tris-HCl, pH 8.0-9.0) or sterile water can be used, if EDTA inhibits downstream reactions.

Elution Buffer Volume

The genomic DNA is eluted in 25-200 µl (Mini Kit) or 50-200 µl (96 Kit) of PureLink™ Genomic Elution Buffer. You can change the volume of elution buffer to obtain genomic DNA in the desired final concentration. Use the graph shown below to determine the most appropriate elution conditions for your application. For increased DNA yield, use a higher volume of elution buffer. For increased DNA concentration, use a lower volume of elution buffer.

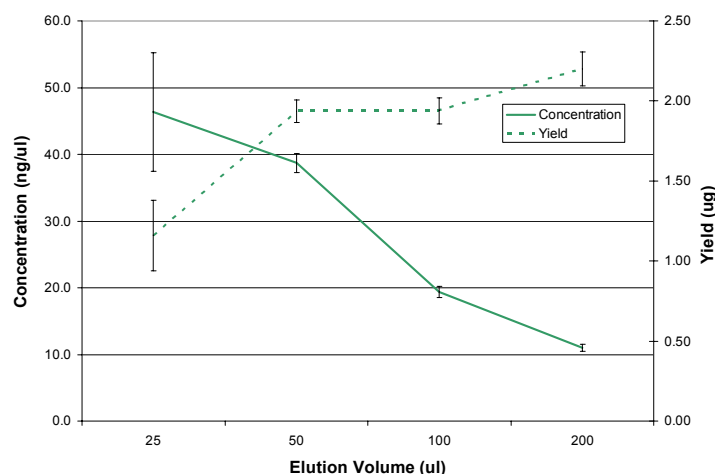


Figure Legend: Genomic DNA was purified from 100 µl blood samples with the PureLink™ Genomic DNA Mini Kit using different elution volumes.

Number of Elutions

Using 50 µl and 100 µl elution buffer volume, the first elution recovers ~80% and 90% of bound genomic DNA, respectively. To maximize genomic DNA recovery, you may perform a second elution to recover the remaining 10-15% gDNA. Perform the second elution using the same volume of buffer used for first elution. To prevent dilution of the gDNA sample and also avoid contact of the spin column with the eluate, perform the two-elution steps using different tubes.

Note: Sufficient PureLink™ Genomic Elution Buffer is included to perform up to 2 x 100 µl elution per sample. If you wish to perform >2 x 100 µl elution per sample, you need to purchase additional PureLink™ Genomic Elution Buffer (page x) available separately.

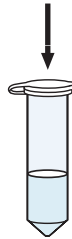
Purification Procedure Using Mini Kit

Experimental Overview

Introduction

The flow chart for purifying genomic DNA using the PureLink™ Genomic DNA Mini Kits is shown below.

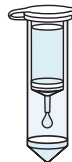
Prepare lysate using
Digestion Buffer and
Proteinase K



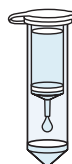
Add Lysis/Binding Buffer
and ethanol to the lysate



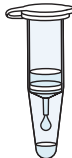
Apply sample to a
PureLink™ Spin Column



Wash the column
with Wash Buffer 1



Wash the column
with Wash Buffer 2



Elute DNA with
Elution Buffer

Preparing Lysates–Mini Kit

Introduction

Instructions for preparing lysates from mammalian cells and tissues, mouse tail, buccal swabs, blood, bacteria, yeast, FFPE tissues, and Oragene™ preserved saliva are described below.

To obtain high-quality genomic DNA, follow the guidelines recommended on page 3.



Important

Make sure there is no precipitate visible in PureLink™ Genomic Digestion Buffer or PureLink™ Genomic Lysis/Binding Buffer. If any precipitate is visible in the buffers, warm the buffers at 37°C for 3-5 minutes and mix well to dissolve the precipitate before use.

Materials Needed

- 96-100% ethanol
- Sample for DNA isolation (see page 4 for recommended starting amount)
- Phosphate Buffered Saline (PBS) for mammalian cell lysate (page x)
- Lysozyme and Lysozyme Digestion Buffer (25 mM Tris-HCl, pH 8.0, 2.5 mM EDTA, 1% Triton X-100) for bacterial cell lysate
- Zymolase Buffer (1 M sorbitol, 10 mM sodium EDTA, 14 mM β -mercaptoethanol) and Zymolase (lyticase) enzyme for yeast lysate
- CitriSolv™ Clearing Agent (Fisher catalog. no. 22-143-975) or xylene for FFPE tissue
- 3 M sodium acetate (pH 5-5.5) and 2.8 ml isopropanol for Oragene™ samples
- Sterile, DNase-free microcentrifuge tubes
- Water baths or heat blocks

Components supplied with the kit

- PureLink™ Genomic Lysis/Binding Buffer
 - PureLink™ Genomic Digestion Buffer
 - Proteinase K (20 mg/ml)
 - RNase A (20 mg/ml)
-

Continued on next page

Preparing Lysates—Mini Kit, Continued

Gram Negative Bacterial Cell Lysate

Prepare Gram negative bacterial cell lysate as described below.

1. Set a water bath or heat block at 55°C.
 2. Harvest up to 2×10^9 Gram negative (~1 ml of overnight *E. coli* culture) by centrifugation. If you are using a frozen cell pellet, proceed to Step 3.
 3. Resuspend the cell pellet in 180 μ l PureLink™ Genomic Digestion Buffer. Add 20 μ l Proteinase K (supplied with the kit) to lyse the cells. Mix well by brief vortexing.
 4. Incubate the tube at 55°C with occasional vortexing until lysis is complete (30 minutes to up to 4 hours).
 5. Add 20 μ l RNase A (supplied with the kit) to the lysate, mix well by brief vortexing, and incubate at room temperature for 2 minutes.
 6. Add 200 μ l PureLink™ Genomic Lysis/Binding Buffer and mix well by vortexing to obtain a homogenous solution.
 7. Add 200 μ l 96-100% ethanol to the lysate. Mix well by vortexing for 5 seconds to yield a homogenous solution.
Note: When processing multiple samples, you may prepare a master Buffer/ethanol Mix by mixing 200 μ l Lysis/Binding Buffer and 200 μ l 96-100% ethanol for each sample.
 8. Proceed to **Binding DNA** (page 15).
-

Gram Positive Bacterial Cell Lysate

Prepare Gram positive bacterial cell lysate as described below.

1. Set two water baths or heat blocks at 37°C and 55°C, respectively.
 2. Prepare Lysozyme Digestion Buffer (see recipe on page 7). To ~200 μ l Lysozyme Digestion Buffer/sample, add **fresh** Lysozyme to obtain a final Lysozyme concentration of 20 mg/ml.
 3. Harvest up to 2×10^9 Gram positive cells by centrifugation. If you are using a frozen cell pellet, proceed to Step 3.
 4. Resuspend the cell pellet in 180 μ l Lysozyme Digestion Buffer containing Lysozyme from Step 2. Mix well by brief vortexing.
 5. Incubate at 37°C for 30 minutes.
 6. Add 20 μ l Proteinase K (supplied with the kit). Mix well by brief vortexing.
 7. Add 200 μ l PureLink™ Genomic Lysis/Binding Buffer and mix well by brief vortexing.
 8. Incubate at 55°C for 30 minutes.
 9. Add 200 μ l 96-100% ethanol to the lysate. Mix well by vortexing for 5 seconds to yield a homogenous solution.
 10. Proceed to **Binding DNA** (page 15).
-

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Purification Procedure Using Spin Columns

Introduction

The purification procedure is designed for purifying genomic DNA using a spin column-based centrifugation procedure in a total time of **10-15 minutes**.

Materials Needed

- Lysates prepared as described on pages 8-13
- Sterile, DNase-free 1.5 ml microcentrifuge tubes for elution
- Microcentrifuge capable of centrifuging $>10,000 \times g$
- Optional: sterile water, pH 7.0-8.5, if you are using water for elution

Components supplied with the kit

- PureLink™ Genomic Wash Buffers 1 and 2
 - PureLink™ Genomic Elution Buffer
 - PureLink™ Spin Columns in Collection Tubes
 - PureLink™ Collection Tubes
-



Follow the recommendations below to obtain the best results:

- Perform all centrifugation steps at room temperature
 - Review **Elution Parameters** on page 5 to determine the suitable elution volume for your requirements
 - Perform a 1 minute incubation step with PureLink™ Genomic Elution Buffer
 - Be sure to perform the recommended wash steps to obtain the best results
 - If you are using water for elution, always use sterile water, pH 7.0-8.5
-

Before Starting

Add 96–100% ethanol to PureLink™ Genomic Wash Buffer 1 and PureLink™ Genomic Wash Buffer 2 according to instructions on each label. Mix well. Mark on the labels that ethanol is added. Store both wash buffers with ethanol at room temperature.

Binding DNA

1. Remove a PureLink™ Spin Column in a Collection Tube from the package.
 2. Add the lysate (~640 μ l) prepared with PureLink™ Genomic Lysis/Binding Buffer and ethanol to the PureLink™ Spin Column.
 3. Centrifuge the column at $10,000 \times g$ for 1 minute at room temperature.
Note: If you are processing $>200 \mu$ l starting material such as blood, buccal swabs, or Oragene™ preserved saliva, you need to perform multiple loading of the lysate by transferring any remaining lysate to the same PureLink™ Spin Column (above) and centrifuge at $10,000 \times g$ for 1 minute.
 4. Discard the collection tube and place the spin column into a clean PureLink™ Collection Tube supplied with the kit.
 5. Proceed to **Washing DNA**, next page.
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Purification Procedure Using Spin Columns, Continued

Washing DNA

1. Add 500 μ l Wash Buffer 1 prepared with ethanol (page 15) to the column.
 2. Centrifuge column at room temperature at $10,000 \times g$ for 1 minute.
 3. Discard the collection tube and place the spin column into a clean PureLink™ collection tube supplied with the kit.
 4. Add 500 μ l Wash Buffer 2 prepared with ethanol (page 15) to the column.
 5. Centrifuge the column at maximum speed for 3 minutes at room temperature. Discard collection tube.
 6. Proceed to **Eluting DNA**, below.
-

Eluting DNA

1. Place the spin column in a sterile 1.5-ml microcentrifuge tube.
 2. Add 25-200 μ l of PureLink™ Genomic Elution Buffer to the column. See **Elution Parameters** (page 5) to choose the suitable elution volume for your needs.
 3. Incubate at room temperature for 1 minute. Centrifuge the column at maximum speed for 1 minute at room temperature.
The tube contains purified genomic DNA.
 4. To recover more DNA, perform a second elution step using the same elution buffer volume as first elution in another sterile 1.5 ml microcentrifuge tube.
 5. Centrifuge the column at maximum speed for 1.5 minutes at room temperature.
The tube contains purified DNA. Remove and discard the column.
-

Storing DNA

- Store the purified DNA at -20°C or use DNA for the desired downstream application.
 - For long-term storage, store the purified DNA in PureLink™ Genomic Elution Buffer at -20°C as DNA stored in water is subject to acid hydrolysis.
 - 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.
-

Expected Results

DNA Yield

The yield of genomic DNA obtained from various samples using the PureLink™ Genomic DNA Mini Kits is listed below. The DNA quantitation was performed using UV absorbance at 260 nm. The yield is the total yield from 2 x 200 µl elutions.

Material	Amount	DNA Yield
<i>E.coli</i> cells	2×10^9	10-30 µg
HeLa cells	5×10^6	20-40 µg
293F cells	5×10^6	15-30 µg
Human Blood	200 µl	3-10 µg
Mouse Tail	1-1.2 cm	5-25 µg
Mouse Brain	25 mg	10-30 µg
Mouse Liver	25 mg	10-30 µg
Mouse Spleen	10 mg	10-40 µg

Note: The DNA yield varies with the sample and DNA content of the sample.

DNA Quality

Genomic DNA isolated from various samples was analyzed by agarose gel electrophoresis on a 1% E-Gel® agarose gel.

Samples on the gel are:

Lane M: 1 Kb Plus DNA Ladder

Lane 1: 200 ng DNA isolated from Gram positive bacteria (2×10^9 cells)

Lane 2: 200 ng DNA isolated from Gram negative bacteria, *E. coli* (2×10^9 cells)

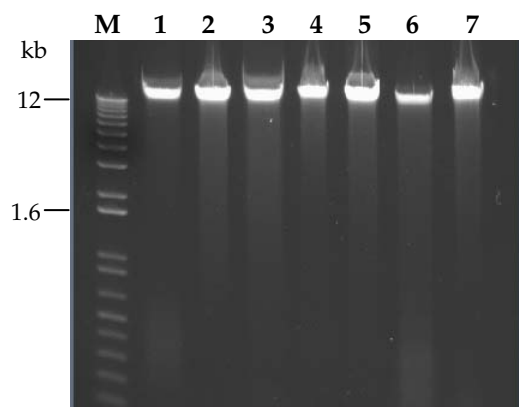
Lane 3: 200 ng DNA isolated from human 293F (5×10^6 cells)

Lane 4: 200 ng DNA isolated from human whole blood (200 µl)

Lane 5: 200 ng DNA isolated from rat brain tissue (20 mg)

Lane 6: 200 ng DNA isolated from human saliva (200 µl of Oragene™ sample)

Lane 7: 200 ng DNA isolated from rat liver tissue (20 mg)



Troubleshooting

Introduction

Refer to the table below to troubleshoot any problems you may encounter with the PureLink™ Genomic DNA Kits.

Problem	Cause	Solution
Low DNA yield	Incomplete lysis	<ul style="list-style-type: none"> Decrease the amount of starting material used. Be sure to add Proteinase K during lysis. For tissues, cut the tissue into smaller pieces and ensure the tissue is completely immersed in the Digestion Buffer to obtain optimal lysis. If incomplete lysis is observed, increase the digestion time or amount of Proteinase K used for lysis.
	Poor quality of starting material	Be sure to use fresh sample and process immediately after collection or freeze the sample 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.
	PureLink™ Spin Column or Binding Plate is clogged	Make sure that the lysate is clear when the lysate is loaded on to the spin column or plate. Remove any particulate or viscous material by centrifugation prior to loading the lysate on to the spin column or plate.
	Incorrect binding conditions	<ul style="list-style-type: none"> Be sure to add PureLink™ Lysis/Binding Buffer and 96-100% ethanol to the lysate prior to loading the samples on the spin column or Binding Plate. Mix the sample properly with Binding Buffer and ethanol by vortexing. Avoid overloading the column or plate.
	Ethanol not added to Wash Buffers 1 and 2	Be sure to add 96–100% ethanol to Wash Buffers 1 and 2 as indicated on the label.
	Incorrect elution conditions	<ul style="list-style-type: none"> Add elution buffer and perform incubation for 1 minute with elution buffer before centrifugation. To recover more DNA, perform a second elution step.
	DNA is sheared or degraded	<ul style="list-style-type: none"> Avoid repeated freezing and thawing of samples to prevent any DNA damage. Maintain a sterile environment while working to avoid any contamination from DNases.

Troubleshooting, Continued

Problem	Cause	Solution
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	<ul style="list-style-type: none"> • Be sure to add ethanol to the lysate prior to loading the lysate on to the spin column or plate. 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 or plate. • If the problem persists, perform an additional wash step with 500 μl Wash Buffer 1 to obtain a total of two 500 μl wash steps with Wash Buffer 1 followed by a single 500 μl wash with Wash Buffer 2.
RNA contamination	Silica membrane binds total nucleic acid present in the sample	Perform RNase digestion step during sample preparation.
Inhibition of downstream enzymatic reactions	Presence of ethanol in purified DNA	<p>Traces of ethanol from the Wash Buffer 2 can inhibit downstream enzymatic reactions.</p> <ul style="list-style-type: none"> • To remove Wash Buffer 2 from spin columns, discard Wash Buffer 2 flow through. Place the spin column into the Wash Tube and centrifuge the spin column at maximum speed for 2-3 minutes to completely dry the column. • To remove any traces of Wash Buffer 2 from the Binding Plate and dry the membrane, centrifuge the plate stack at $>2250 \times g$ for 15 minutes or apply vacuum for 10 minutes. The plate can also be warmed at 70°C for 10 minutes to evaporate any ethanol.
	Presence of salt in purified DNA	<ul style="list-style-type: none"> • Use the correct order of Wash Buffers for washing. Always wash with Wash Buffer 1 followed by washing with Wash Buffer 2. • Always maintain a ratio of 1:1:1 for Sample:Binding Buffer:Ethanol.
Low elution volume or sample cross-contamination	Incorrect vacuum pressure	<ul style="list-style-type: none"> • Make sure the vacuum manifold is sealed tightly and there is no leakage. A vacuum pressure of -6 to -12 inches Hg (-200 to -400 mbar or -150 to -300 mm Hg) is required to obtain the best results. • To avoid any cross contamination and ensure proper contact between the PureLink™ Genomic Binding Plate and elution plate, raise the elution plate in the vacuum manifold using adaptors as described on page 30.

Appendix

Technical Support

World Wide Web



Visit the Invitrogen website at www.invitrogen.com for:

- Technical resources, including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, etc.
 - Complete technical support contact information
 - Access to the Invitrogen Online Catalog
 - Additional product information and special offers
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Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our web page (www.invitrogen.com).

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MSDS Requests

MSDSs (Material Safety Data Sheets) are available on our website at www.invitrogen.com/msds.
