illustra bacteria genomicPrep Mini Spin Kit

For the rapid extraction and purification of genomic DNA from various bacterial strains

Product booklet

Codes: 28-9042-58 (50 purifications)
28-9042-59 (250 purifications)

Page finder

1. Legal

Product use Restriction

The **illustra™ bacteria genomicPrep Mini Spin Kit** has been designed, developed, and sold **for research purposes only.** They are suitable **for *in vitro* use only.** No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is the responsibility of the user to verify the use of the illustra bacteria genomicPrep Mini Spin Kit for a specific application as the performance characteristics of this kit have not been verified for any specific bacterial strains.

Contact your GE Healthcare representative for the most current information (see back cover for contact details).

http://www.gelifesciences.com

Amersham Place, Little Chalfont,
Buckinghamshire. HP7 9NA UK.

2. Handling and storage

2.1. Safety warnings and precautions

Warning: For research use only.
Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals. All chemicals should be considered as potentially hazardous. Only persons trained in laboratory techniques and familiar with the principles of good laboratory practice should handle these products. Suitable protective clothing such as laboratory overalls, safety glasses, and gloves should be worn. Care should be taken to avoid contact with skin or eyes; if contact should occur, wash immediately with water. (See Material Safety Data Sheet and/or safety statements for specific recommendations).

Warning: This protocol requires the use of Ethanol.
The chaotrope in Lysis buffers type 3 & 4 is harmful if ingested, inhaled, or absorbed through the skin, and can cause nervous system disturbances, severe irritation, and burning. High concentrations are extremely destructive to the eyes, skin, and mucous membranes of the upper respiratory tract. Gloves should always be worn when handling this solution.

Use of this product with bacteria should be considered bio-hazardous. Follow appropriate safety procedures while using this kit and when handling DNA isolated from these sources.

Waste effluents from this kit should be decontaminated with bleach or detergent-based method. Decontamination with bleach may be reactive resulting in foam and emission of Ammonia gas and should be carried out in an exhaust hood.

Consult local safety regulations for safe disposal of all treated waste

2.2. Storage

All kit components should be stored at room temperature (20-25°C).
Once reconstituted, store Proteinase K at 4°C.

2.3. Expiry

For expiry date please refer to outer packaging label.
Reconstituted Proteinase K in DNase-free water is stable for 4 months when stored at 4°C or until expiry on outer packaging label.

3. Components

3.1. Kit contents

Identification	Pack Size	10 purifications	50 purifications	250 purifications
	Cat. No.	Sample Pack	28-9042-58	28-9042-59
	Proteinase K, lyophilized powder	1 vial (10 mg)	1 vial (30 mg)	2 vials (2 × 30 mg)
	Lysis buffer type 2	0.6 ml	3 ml	15 ml
	Lysis buffer type 3	0.11 ml	0.6 ml	2 × 1.5 ml
	Lysis buffer type 4	12 ml	60 ml	2 × 165 ml
	Wash buffer type 6	1.5 ml (Add 6 ml Absolute Ethanol before use)	6 ml (Add 24 ml Absolute Ethanol before use)	30 ml (Add 120 ml Absolute Ethanol before use)
	Elution buffer type 5	3 ml	12 ml	60 ml
	illustra bacteria mini columns	10	50	5 × 50
	Collection tubes	10	50	5 × 50

Refer to the Certificate of Analysis for a complete list of kit components. GE supplies a wide range of buffer types across the illustra nucleic acid purification and amplification range. The composition of each buffer has been optimized for each application and may vary between kits. Care must be taken to only use the buffers supplied in the particular kit you are using and not use the buffers supplied in other illustra kits e.g. the Lysis buffers type 2, 3 and 4 supplied in the illustra bacteria genomicPrep Mini Spin Kit are not the same as the Lysis buffer type 10 supplied in the illustra blood genomicPrep Mini Spin Kit.

In order to avoid confusion and the accidental switching of buffers between kits, a numbering system has been adopted that relates to the entire range of buffers available in the illustra purification range. For example there are currently 14 Lysis buffers in the illustra range, 6 Wash buffers and 8 Elution buffers, denoted by Lysis buffer type 1–14, Wash buffer type 1–6 and Elution buffer type 1–8, respectively. Please ensure you use the correct type of Lysis, Wash and Elution buffer for your purification.

3.2. Materials supplied by user:

Disposables:
1.5 ml DNase free microcentrifuge tubes

Chemicals:
DNase-free Water
Absolute Ethanol
RNase A (20 mg/ml)
For Gram-positive bacteria only:
Lysozyme buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0, 0.1 M NaCl, 5% Triton X-100)
Lysozyme (10 mg/ml in 10 mM Tris-HCl, pH 8.0)

3.3. Equipment supplied by user:

Microcentrifuge that accommodates 1.5 ml microcentrifuge tubes.
Vortex mixer
Incubator or water bath for 55°C incubation and 70°C incubation.

4. Description

4.1. Introduction

The **illustra bacteria genomicPrep Mini Spin Kit** is designed for rapid extraction of genomic DNA from various bacterial strains. While the process is rapid, the protocols have also been designed to minimize shearing, resulting in high quality intact genomic DNA.

The kit utilizes Lysis buffers type 2 & 3 in combination with Proteinase K to release genomic DNA into solution from bacterial cells (1). The genomic DNA is de-proteinated in an extraction solution and is then bound onto a silica column in the presence of the chaotrope in Lysis buffer type 4 (2). Contaminants are removed during the Wash & Dry step and genomic DNA is eluted with pre-heated Elution buffer type 5.

The entire procedure can be completed in as little as 40 minutes to yield genomic DNA with a purity and quality that is compatible with most molecular biology techniques including cloning, restriction enzyme digestion, PCR amplification and genotyping applications.

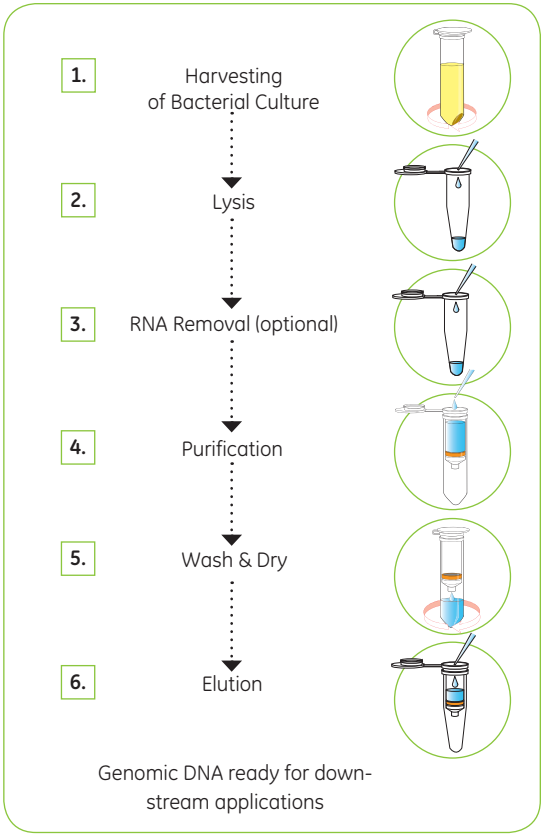
The Lysis buffers have been optimized to extract genomic DNA from several strains of Gram-negative bacteria such as *E. coli* DH5α, TOP10 and JM109, and Gram-positive bacteria such as *B. subtilis*, with yields ranging from 4–12 µg of genomic DNA per purification. Bacterial numbers ranging from 1–4 × 10^9 cells can be used for each purification. The kit is designed to reproducibly give consistent recovery of purified genomic DNA with high purity (A_{260}/A_{280} approximately 1.8).

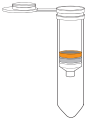
Gram-positive bacteria have a much thicker peptidoglycan layer than Gram-negative bacteria. It is necessary to pre-treat Gram-positive bacteria with lysozyme, as indicated in Section 5.3. Incubation time required with lysozyme may vary between species.

The kit contains sufficient reagents and columns for 50 (28-9042-58) or 250 (28-9042-59) purifications.

4.2. The basic principle

Use of the **illustra bacteria genomicPrep Mini Spin Kit** involves the following steps:



Step	Comments	Component
1. Harvesting of Bacterial Culture	Bacterial cells are pelleted and culture medium is removed.	
2. Lysis	Bacterial cells are lysed by the detergent and salt present in Lysis buffers type 2 & 3 and Proteinase K.	**Lysis buffer type 2** **Proteinase K** **Lysis buffer type 3**
3. RNA Removal (optional)	RNA is removed by RNase A.	
4. Purification	Chaotropic salt in Lysis buffer type 4 promotes the binding of genomic DNA to the novel silica membrane.	**Lysis buffer type 4** **illustra bacteria mini column & Collection tube** 
5. Wash & Dry	Lysis buffer type 4 containing a chaotropic salt removes protein and other contaminants from membrane-bound genomic DNA. Ethanolic Wash buffer type 6 removes residual salts and other contaminants and dries the silica membrane at the same time.	**Lysis buffer type 4** **Wash buffer type 6**
6. Elution	Genomic DNA is eluted in a low ionic strength buffer.	**Elution buffer type 5**

4.3. Product specifications

Sample Type:	Gram-negative Bacteria	Gram-positive Bacteria
Sample input	1–4 × 10^9 cells (A_{600} 1.0–4.0)	1–4 × 10^9 cells (A_{600} 1.0–4.0)
Elution volume	200 µl	200 µl
Yield* (from 2 × 10^9 cells)	4–12 µg	5–10 µg
Purity (A_{260}/A_{280})	> 1.7	> 1.7
Time/prep**	40 minutes	60 minutes
Product size	> 20 kbp	> 20 kbp

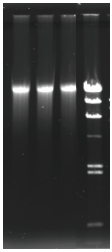
*Actual yields will vary depending on bacterial strain used and growth phase of bacteria.

**Actual time/prep will vary depending on user's experience with the protocol.

This kit is not suitable for the purification of RNA.

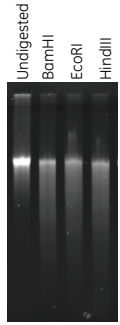
4.4. Typical output

Figure 1. Restriction endonuclease digestion of genomic DNA isolated from *E. coli* strain DH5α using the illustra bacteria genomicPrep Mini Spin Kit.



(a) Three individual high quality genomic DNA isolations

Lambda HindIII marker included for size comparison



(b) Restriction enzyme digestion

5. Protocol

Please refer to section 6.3 for Troubleshooting Guide.

Note: Columns and Buffers are NOT transferable between GE kits, e.g., the composition of the Lysis buffer type 4 in the bacteria genomicPrep Mini Spin Kit is not the same as the Lysis buffer type 10 in the blood genomicPrep Mini Spin Kit .

Use of icons

The Key below describes the purpose of the icons used throughout the protocol booklet.

This icon is used to highlight particularly critical steps within the protocol that must be adhered to. If this advice is not followed it will have a detrimental impact on results.

This icon is used to highlight technical tips that will enhance the description of the step. These tips may indicate areas of flexibility in the protocol or give a recommendation to obtain optimum performance of the kit.

5.1. Preparation of working solutions

Refer to section 3.2. Materials to be supplied by user

Proteinase K

Dissolve the supplied lyophilized **Proteinase K** in DNase-free water. Add 1.5 ml of DNase-free water to the vial of **Proteinase K** in kit 28-9042-58 or 1.5 ml to EACH vial of **Proteinase K** in kit 28-9042-59. Sample pack users, please add 500 µl DNase-free water to the supplied vial of **Protenase K**. Final concentration is 20 mg/ml. Vortex to dissolve. Once reconstituted, store **Proteinase K** at 4°C.

Wash buffer type 6

Prior to use of this kit, add Absolute ethanol to the bottle containing **Wash buffer type 6**. Add 24 ml of Absolute ethanol to Wash buffer type 6 in kit 28-9042-58 or add 120 ml to Wash buffer type 6 in kit 28-9042-59. For the 10 purifications pack size, add 6 ml of Absolute ethanol to Wash buffer type 6 before use. Mix by inversion. Indicate on the label that this step has been completed. Store upright and airtight.

Elution buffer type 5

Heat **Elution buffer type 5** to 70°C in a water bath or heat-block prior to start of protocol.

RNase A

Prepare a stock solution of RNase A by re-suspending in DNase-free water (final concentration of 20 mg/ml) prior to use.
e.g., add 50 µl of DNase-free water to a vial containing 1 mg of RNase A.

Note: Users purifying genomic DNA from Gram-positive bacteria should also prepare the following materials:

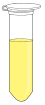
Lysozyme buffer -10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0, 0.1M NaCl, 5% Triton X-100.

Lysozyme - 10 mg/ml in 10 mM Tris-HCl, pH 8.0.

5.2. Protocol for purification of genomic DNA from **Gram-negative** bacteria

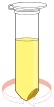
1. Harvesting of Bacterial Culture.

a. Transfer 1 ml overnight bacterial culture ($A_{600} \leq 4.0$) to a 1.5 ml microcentrifuge tube.



1 ml bacterial culture

b. Spin for 30 seconds at full speed (16 000 × g) in a microcentrifuge. Remove as much of the supernatant as possible by aspiration without disturbing the cell pellet .



30 seconds
16 000 × g

Note: Refer to Section 6.1. RPM calculation from RCF for determination of appropriate centrifugation speed for a specific rotor.

2. Lysis

a. Add 40 µl of **Lysis buffer type 2** and immediately mix by vortexing.

40 µl Lysis buffer type 2

Note: Mix until the bacterial cells are completely resuspended (no visible pellet).

b. Add 10 µl of **Proteinase K** (20 mg/ml) to the sample and mix by vortexing for 10 seconds.

10 µl Proteinase K

c. Add 10 µl of **Lysis buffer type 3** to the sample and mix by vortexing for 10 seconds

10 µl Lysis buffer type 3

d. Spin 5 seconds at 1 000 × g to collect sample in bottom of tube.

5 seconds
1 000 × g

Note: It is important to add the **Lysis buffer type 2, Proteinase K** and **Lysis buffer type 3** in the indicated sequence to obtain good genomic DNA purity.

e. Incubate the sample for 7 minutes at 55°C. — 7 minutes 55°C

f. Vortex and spin 5 seconds at 1 000 × g to collect sample in bottom of tube. Continue incubation for a further 8 minutes. — 5 seconds 1 000 × g 8 minutes 55°C

g. Spin 5 seconds 1 000 × g to collect sample in bottom of tube. — 5 seconds 1 000 × g

Note: A total incubation time of 15 minutes is required.

3. RNA Removal (optional)

a. Add 5 µl of **RNase A** (20 mg/ml) to the sample and mix by vortexing for 10 seconds. Spin 5 seconds at 1 000 × g to collect sample in bottom of tube. — 5 µl RNase A 5 seconds 1 000 × g

b. Incubate at 15 minutes at room temperature (RT). — 15 minutes RT

4. Purification

a. Add 500 µl of **Lysis buffer type 4** ▭ to the sample and mix by vortexing 10 seconds. — 500 µl Lysis buffer type 4

b. Incubate the sample for 5 minutes at room temperature. — 5 minutes RT

c. Vortex and spin for 5 seconds at 1 000 × g to collect sample in bottom of tube. Continue incubation for a further 5 minutes. — 5 seconds 1 000 × g 5 minutes RT

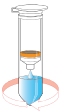
Note: A total incubation time of 10 minutes is required.

d. For each purification that is to be performed, place one bacteria mini column inside one Collection tube.

e. Apply each sample to a separate column.

Note: Do not overload the columns. The maximum column capacity is 720 µl.

f. Spin for 1 minute at 11 000 × g.



1 minute RT
11 000 × g

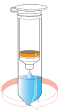
g. Discard the flowthrough by emptying the Collection tube. Place the column back inside the Collection tube.

5. Wash & Dry

a. Add 500 µl of **Lysis buffer type 4** ▭ to the column.

500 µl Lysis buffer type 4

b. Spin for 1 minute at 11 000 × g.



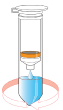
1 minute RT
1 minute
11 000 × g

c. Discard the flowthrough by emptying the collection tube. Place the column back inside the collection tube.

d. Add 500 µl of **Wash buffer type 6** ▭ to the column.

500 µl wash buffer type 6

e. Centrifuge for 3 minutes at 16 000 × g.



3 minutes
16 000 × g

f. Discard the Collection tube and transfer the column to a fresh DNase free 1.5 ml microcentrifuge tube (user supplied)

6. Elution

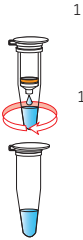
a. Apply 200 µl of pre-heated **Elution buffer type 5** directly to the top of glass fiber matrix in the column.

Note: Ensure that the Elution buffer type 5 is dispensed onto the center of the column.

Note: Change pipet tips between samples if applying Elution buffer type 5 to multiple samples, to reduce variation in volume of sample eluted.

b. Incubate the sample for 1 minute at room temperature.

c. Spin for 1 minute at 11 000 × g to recover the purified genomic DNA as flowthrough.

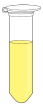


d. Proceed to downstream application. Store purified genomic DNA at -20°C

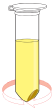
5.3. Protocol for purification of genomic DNA from **Gram-positive** bacteria

1. Harvesting of Bacterial Culture

a. Transfer 1 ml overnight bacterial culture ($A_{600} \leq 4.0$) to a 1.5 ml microcentrifuge tube.



b. Spin for 30 seconds at full speed (16 000 × g) in a microcentrifuge.
Remove as much of the supernatant as possible by aspiration without disturbing the cell pellet .



30 seconds
16 000 × g

Note: Refer to Section 6.1. RPM calculation from RCF for determination of appropriate centrifugation speed for a specific rotor.

2. Lysis

a. Add 40 µl of Lysozyme buffer and immediately mix by vortexing for 10 seconds.

40 µl Lysozyme buffer

Note: Mix until the bacteria cells are completely re-suspended.

b. Add 10 µl of **Lysozyme** (10 mg/ml) to the sample and mix by vortexing for 10 seconds.

10 µl Lysozyme

c. Incubate the sample for 5 minutes at room temperature

5 minutes RT

d. Vortex and spin for 5 seconds at 1 000 × g to collect sample in bottom of tube.Continue incubation for a further 5 minutes.

5 seconds
1 000 × g
5 minutes RT

Note: A total incubation time of 10 minutes is required.

e. Add 10 µl of **Proteinase K** (20 mg/ml) to the sample.

10 µl Proteinase K

f. Mix by vortexing for 10 seconds. Spin 5 seconds 1 000 × g to collect sample in bottom of tube.

5 seconds
1 000 × g

g. Incubate the sample for 7 minutes at 55°C. — 7 minutes 55°C

h. Vortex and spin for 5 seconds at 1 000 × g to collect sample in bottom of tube. Continue incubation for a further 8 minutes. — 5 seconds 1 000 × g 8 minutes 55°C

Note: A total incubation time of 15 minutes is required. — 5 seconds 1 000 × g

i. Spin 5 seconds 1 000 × g to collect sample in bottom of tube.

3. RNA Removal (optional)

a. Add 5 µl of RNase A (20 mg/ml) to the sample and mix by vortexing for 10 seconds. — 5 µl RNase A

b. Spin for 5 seconds at 1 000 × g to collect sample in bottom of tube. — 5 seconds 1 000 × g

c. Incubate the sample for 15 minutes at room temperature (RT). — 15 minutes RT

4. Purification

a. Add 500 µl of **Lysis buffer type 4** ▭ to the sample and mix by vortexing 10 seconds. — 500 µl Lysis buffer type 4

b. Incubate the sample for 5 minutes at room temperature. — 5 minutes RT

c. Vortex, spin 5 seconds 1 000 × g to collect sample in bottom of tube and continue incubation for a further 5 minutes. — 5 seconds 1 000 × g 5 minutes RT

Note: A total incubation time of 15 minutes is required.

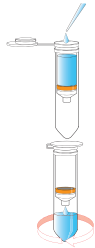
d. For each purification that is to be performed, place one bacteria Mini

Column inside one Collection tube.

e. Apply each sample to a separate column.

Note: Do not overload the columns. The maximum column loading capacity is 720 µl.

f. Spin for 1 minute at 11 000 × g.

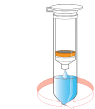


g. Discard the flowthrough by emptying the Collection tube. Place the column back inside the Collection tube.

5. Wash & Dry

a. Add 500 µl of **Lysis buffer type 4** ▭ to the column.

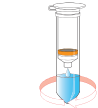
b. Centrifuge for 1 minute at 11 000 × g.



c. Discard the flowthrough by emptying the Collection tube. Place the column back inside the Collection tube.

d Add 500 µl of **Wash buffer type 6** ▭ to the column.

e. Spin for 3 minutes at full speed (16 000 × g).



f. Discard the Collection tube and transfer the column to a fresh DNase free 1.5 ml microcentrifuge tube (user supplied),

6. Elution

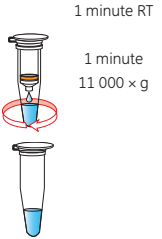
a. Apply 200 µl of pre-heated **Elution buffer type 5** directly to the top of glass fiber matrix in the column.

Note: Ensure that the Elution buffer type 5 is dispensed onto the center of the column.

Note: Change pipet tips between samples if applying Elution buffer type 5 to multiple samples, to reduce variation in volume of sample eluted.

b. Incubate the sample at room temperature for 1 minute.

c. Spin for 1 minute at 11 000 × g to recover the purified genomic DNA as flowthrough.



d. Proceed to downstream application. Store purified genomic DNA at -20°C.

6. Appendices

6.1 RPM calculation from RCF

The appropriate centrifugation speed for a specific rotor can be calculated from the following formula:

$RPM = 1\,000 \times \sqrt{(RCF/1.12r)}$

Where RCF = relative centrifugal force; r = radius in mm measured from the center of the spindle to the bottom of the rotor bucket; and RPM = revolutions per minute.
E.g. if an RCF of 735 × g is required using a rotor with a radius of 73 mm, the corresponding RPM would be 3 000.

6.2. Yield and purity estimation of genomic DNA

Purified genomic DNA concentration should be determined by UV spectrophotometry (A_{260}) and through comparison with a known standard by agarose gel electrophoresis. The reliable UV spectrophotometric range should be determined for each spectrophotometer. Generally, for spectrophotometers with a 1 cm path length, A_{260} readings that lie between 0.1 and 1.0 can be trusted and therefore appropriate dilutions (5 to 50 ng/ml) should be analyzed. For Nano-Drop™ spectrophotometers, absorbance readings between 1 and 10 are reliable.

The UV spectrophotometric ratios A_{260}:A_{280} provide information regarding the purity of genomic DNA. A purity ratio of 1.7 to 1.9 indicates that the genomic DNA is pure for all standard molecular biology applications. If the ratio is lower than 1.7, the purified genomic DNA might contain some protein impurities. Similarly, if the ratio is higher than 1.9, the genomic DNA might contain some RNA impurities.

1 OD unit (A_{260}) is equivalent to approximately 50 µg/ml double-stranded DNA.

Yield = A_{260} × 50 µg/ml × 0.2 ml = the total µg of purified genomic DNA in the sample.

6.3. Troubleshooting guide

This guide may be helpful in the first instance, however if problems persist or for further information please contact GE technical services. Visit http://www.gelifesciences.com for contact information. Alternatively log onto http://www.gelifesciences.com/illustra

Problem: DNA yield is low

Possible cause	Suggestions
The bacteria was not fully lysed	• After addition of Lysis buffer type 2, vortex sample until no cell clumps are visible. Do not proceed to addition of Proteinase K until you are confident that this step has been completed. • Make sure the correct volumes of Lysis buffers type 2–4 were added.
Elution buffer type 5 was not heated to 70ºC	• After addition of pre-heated Elution buffer type 5 incubate at room temperature for 1 minute. Yield may be increased by incubating at 70ºC for 5 minutes.

Problem: DNA sample floats out of the well when loading a gel

Possible cause	Suggestions
The Wash buffer type 6 was not completely removed before Elution step	• Perform an extra one minute spin at 16 000 × g in a clean Collection tube.
The Collection tube was not emptied prior to commencing Wash & Dry step	• This may cause the Collection tube to overfill when the Wash & Dry step is performed.

Problem: DNA fails to digest

Possible cause	Suggestions
EDTA present in Elution buffer type 5 inhibiting digestion	• Use DNase free water or 10 mM Tris-HCl (pH 8.0) to elute sample, instead of Elution buffer type 5.

6.4. Related products

A full range of molecular biology reagents can be found in the GE catalog and on the website
http://www.gelifesciences.com/illustra

If you need further information, GE technical services are happy to assist. Visit http://www.gelifesciences.com for contact information.

Application	Product	Product Code	Pack Size
DNA purification from PCR and enzymes (enzyme removal, buffer exchange, primer removal), 100bp–10kbp size range	illustra GFX™ PCR DNA & Gel Band Purification Kit	28-9034-70	100 purifications
Preparation of PCR products for automated sequencing One-tube, one-step rapid method	ExoSAP-IT™	US78201	500 rxns
Purification of oligonucleotides (buffer exchange and de-salt). Spin column format	illustra MicroSpin™ G-25 Columns	27-5325-01	50 purifications
Dye terminator removal from automated sequencing reactions	AutoSeq G-50	27-5340-01	50 purifications

Application	Product	Product Code	Pack Size
Preparation of circular DNA templates for cycle sequencing in 4–6 hours	illustra TempliPhi™ 100 Amplification Kit	25-6400-10	100 reactions
Genomic DNA amplification 4–7 µg in 1.5 hours	illustra GenomiPhi™ V2 DNA Amplification Kit	25-6600-30	25 reactions
Genomic DNA amplification 40–50 µg from nanograms of source material	illustra GenomiPhi HY DNA Amplification Kit	25-6600-22	25 reactions
PCR	illustra Hot Start Master Mix	25-1500-01	100 reactions
	illustra Ready-To-Go™ PCR Beads	27-9558-01	100 reactions
Blunt-Ended PCR Cloning	Blunt-Ended PCR Cloning Kit	RPN5110	40 reactions
DNA Ligation	DNA Ligation system	RPN1507	50 Reactions
Agarose gel analysis	100 Base-Pair Ladder	27-4007-01	100 µl
Agarose gel analysis	KiloBase DNA Marker	27-4004-01	50 µg

7. References

1. Vogelstein, B. and Gillespie, D., *Proc. Natl. Acad.* Sci. USA 76, 615 (1979).

2. Marko, M. A., Chipperfield, R. and Birnboim, H. C., Anal. *Biochem.* 121, 382 (1982).

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28-9042-58

29275349 AD 06-2017

Quick Reference Protocol Card

illustra™ bacteria genomicPrep Mini Spin Kit

28-9042-58 (50 purifications)
28-9042-59 (250 purifications)

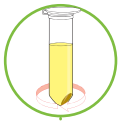
A Protocol for purification of genomic DNA from Gram-NEGATIVE bacteria

- Ensure appropriate volume of Absolute ethanol added to Wash buffer type 6
- Ensure Elution buffer type 5 pre-heated to 70°C buffer type 2.
- Prepare RNase A & Proteinase K solution

:Add :Spin :Incubate

1. Harvesting of Bacterial Culture

- 1 ml bacterial culture
- 30 seconds 16 000 × g; discard supernatant



2. Lysis

- 40 µl Lysis buffer type 2; vortex
- 10 µl Proteinase K; vortex
- 10 µl Lysis buffer type 3; vortex; 5 seconds 1 000 × g
- 7 minutes 55°C; vortex; 5 seconds 1 000 × g
- 8 minutes 55°C; 5 seconds 1 000 × g

3. RNA Removal (optional)

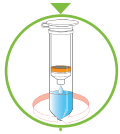
- 5 µl RNase A; vortex; 5 seconds 1 000 × g
- 15 minutes room temperature

4. Purification

- 500 µl Lysis buffer type 4; vortex
- 5 minutes room temperature; 5 seconds 1 000 × g
- 5 minutes room temperature
- Transfer supernatant to bacteria mini Column inside Collection tube
- 1 minute 11 000 × g; discard flowthrough

5. Wash & Dry

- 500 µl Lysis buffer type 4
- 1 minute 11 000 × g; discard flowthrough
- 500 µl Wash buffer type 6
- 3 minutes 16 000 × g
- Transfer bacteria mini column to a new DNase-free microcentrifuge tube



6. Elution

- 200 µl pre-heated Elution buffer type 5
- Incubate: 1 minute room temperature
- 1 minute 11 000 × g
- Retain flowthrough; store purified genomic DNA at -20°C

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Quick Reference Protocol Card

illustra bacteria genomicPrep Mini Spin Kit

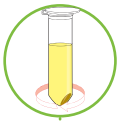
28-9042-58 (50 purifications)
28-9042-59 (250 purifications)

B Protocol for purification of genomic DNA from Gram-POSITIVE bacteria

- Ensure appropriate volume of Absolute ethanol added to Wash buffer type 6
- Ensure Elution buffer type 5 pre-heated to 70°C
- Prepare RNase A, Proteinase K & lysozyme solutions and Lysozyme buffer

:Add :Spin :Incubate

1. Harvesting of Bacterial Culture



- 1 ml bacterial culture
- 30 seconds 16 000 × g; discard supernatant

2. Lysis

- 40 µl Lysozyme buffer; vortex
- 10 µl Lysozyme (10 mg/ml); vortex
- 5 minutes room temperature; vortex; 5 seconds 1 000 × g
- 5 minutes room temperature; 5 seconds 1 000 × g
- 10 µl Proteinase K; vortex; 5 seconds 1 000 × g
- 7 minutes 55°C; vortex; 5 seconds 1 000 × g
- 8 minutes 55°C; 5 seconds 1 000 × g

3. RNA Removal (optional)

- 5 µl RNase A; vortex; 5 seconds 1 000 × g
- 15 minutes room temperature

4. Purification

- 500 µl Lysis buffer type 4; vortex
- 5 minutes room temperature; vortex, 5 seconds 1 000 × g
- 5 minutes room temperature
- Transfer supernatant to bacteria mini column inside Collection tube
- 1 minute 11 000 × g; discard flowthrough

5. Wash & Dry

- 500 µl Lysis buffer type 4
- 1 minute 11 000 × g; discard flowthrough
- 500 µl Wash buffer type 6
- 3 minutes 16 000 × g
- Transfer bacteria mini column to a new DNase-free microcentrifuge tube

6. Elution

- 200 µl pre-heated Elution buffer type 5
- Incubate: 1 minute room temperature
- 1 minute 11 000 × g
- Retain flowthrough; store purified genomic DNA at -20°C

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