USER GUIDE

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ChargeSwitch® gDNA Mini Bacteria Kit

For purification of genomic DNA (gDNA) from Gram positive and Gram negative bacteria

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For Research Use Only. Not for use in diagnostic procedures.

life technologies™

Contents

Kit Contents and Storage

Shipping and Storage

All components of the ChargeSwitch® gDNA (genomic DNA) Mini Bacteria Kit are shipped at room temperature.

Upon receipt, store components as follows:

- Store Proteinase K at 2°C to 8°C
- Mix RNase A in Resuspension Buffer (R4) as described on page 12 and store RNase A in Resuspension Buffer (R4) at 2°C to 8°C
- Store the remaining kit components at room temperature

All components are guaranteed stable for 6 months when stored properly.

Kit Contents

The components supplied in the ChargeSwitch® gDNA Mini Bacteria Kit are listed in the following table. The reagents supplied are sufficient to perform 50 purifications.
Note: Some reagents in the kit maybe provided in excess in the amount needed.

Component	Amount
ChargeSwitch® Magnetic Beads (25 mg/mL in 10 mM MES, pH 5.0, 10 mM NaCl, 0.1% Tween 20)	2 mL
Proteinase K (20 mg/mL in 50 mM Tris-HCl, pH 8.5, 5 mM $CaCl_2$, 50% glycerol)	0.5 mL
RNase A (5 mg/mL in 10 mM Tris-HCl, pH 8.5, 10 mM EDTA)	0.4 mL
ChargeSwitch® Resuspension Buffer (R4)	10 mL
ChargeSwitch® Lysis Buffer (L14)	25 mL
ChargeSwitch® Binding Buffer (B8)	20 mL
ChargeSwitch® Wash Buffer (W12)	100 mL
ChargeSwitch® Elution Buffer (E5; 10 mM Tris-HCl, pH 8.5)	10 mL

Introduction

Overview

Introduction

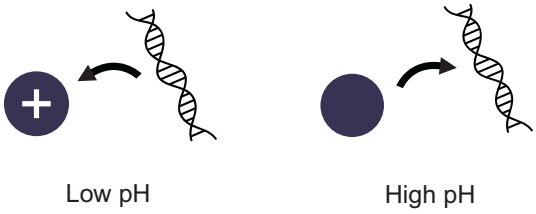
The ChargeSwitch® gDNA Mini Bacteria Kit allows rapid and efficient purification of up to 12 µg of genomic DNA (gDNA) from up to 0.5 mL of Gram positive or Gram negative bacterial culture, or from individual colonies picked from an agar plate.

After preparing the lysates, you can purify gDNA in less than 15 minutes using the ChargeSwitch® Technology. For more information about the Charge Switch® Technology, see the following section.

The ChargeSwitch® Technology

The ChargeSwitch® Technology (CST®) is a novel magnetic bead-based technology that provides a switchable surface which is charge dependent on the pH of the surrounding buffer to facilitate nucleic acid purification.

In low pH conditions, the ChargeSwitch® Magnetic Beads have a positive charge that binds the negatively charged nucleic acid backbone (see the following figure). Proteins and other contaminants are not bound and are simply washed away in an aqueous wash buffer. To elute nucleic acids, the charge on the surface of the bead is neutralized by raising the pH to 8.5 using a low salt elution buffer (see the following figure). Purified DNA elutes instantly into this elution buffer, and is ready for use in downstream applications.



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Overview, Continued

Advantages

Use of the ChargeSwitch® gDNA Mini Bacteria Kit to isolate genomic DNA provides the following advantages:

- A magnetic bead-based technology to isolate genomic DNA without the need for hazardous chemicals, centrifugation, or vacuum manifolds.
- Rapid and efficient purification of genomic DNA from bacterial culture in less than 15 minutes following sample preparation.
- Simple lysis of cells with Proteinase K and lysozyme without the need for any mechanical lysis.
- Minimal contamination with RNA.
- Purified genomic DNA that demonstrates improved downstream performance in applications including PCR, restriction enzyme digestion, and Southern blotting.

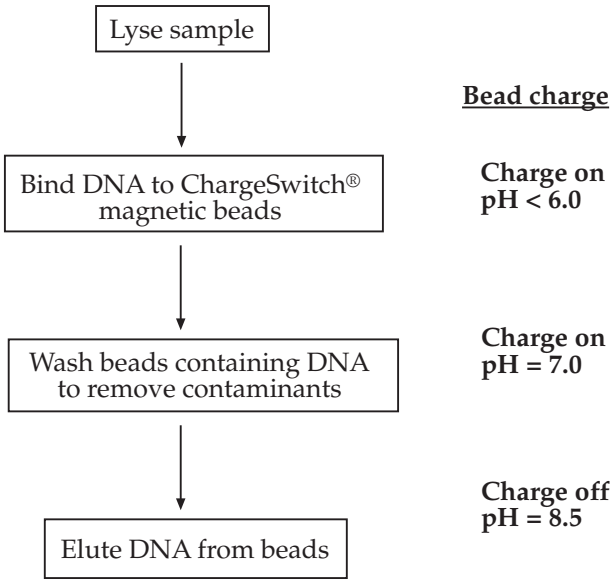
System Specifications

Starting Material:	0.5 mL liquid culture or 1 colony
Bead Binding Capacity:	1 mg beads bind 5–10 µg gDNA
Bead Size:	1 µm
Bead Concentration:	25 mg/mL
Bead Storage Buffer:	10 mM MES, pH 5.0, 10 mM NaCl, 0.1% Tween 20
Elution Volume:	200 µL
DNA Yield:	Up to 12 µg

Experimental Outline

Introduction

The following figure illustrates the basic steps necessary to purify genomic DNA using the ChargeSwitch® Kit.



Methods

General Information

Use the following recommendations to obtain the best results:

- Maintain a sterile environment when handling DNA to avoid any contamination from DNases.
- Ensure that no DNase is introduced into the solutions supplied with the kit.
- Make sure that all equipment coming in contact with DNA is sterile, including pipette tips and tubes.
- Perform the recommended wash steps during purification to obtain the best results.

Safety Information

Follow the provided safety guidelines when using the ChargeSwitch® gDNA Mini Bacteria Kit.

- Treat all reagents supplied in the kit as potential irritants.
- Always wear a suitable lab coat, disposable gloves, and protective goggles.
- If a spill of the buffers occurs, clean with a suitable laboratory detergent and water. If the liquid spill contains potentially infectious agents, clean the affected area first with laboratory detergent and water, then with 1% (v/v) sodium hypochlorite or a suitable laboratory disinfectant.

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MagnaRack™ Magnetic Rack

The MagnaRack™ Magnetic Rack available from Life Technologies (Cat. no. CS15000) is a two-piece magnetic separation rack for use in protocols with magnetic beads.

The MagnaRack™ Magnetic Rack consists of a magnetic base station and a removable tube rack. The tube rack can hold 24 microcentrifuge tubes. The tube rack fits onto the magnetic base station in two different positions associating the row of 12 neodymium magnets with a single row of 12 tubes for simple "on the magnet" and "off the magnet" sample processing (see the following figure). For more information, visit **www.lifetechnologies.com** or call Technical Support (page 20).

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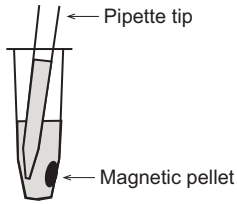
Handling Magnetic Beads

Follow the recommendations below for best results:

- During the mixing, washing, and resuspending steps of the ChargeSwitch® Magnetic Beads, mix beads by pipetting up and down gently to avoid forming bubbles as directed in the protocol.
- Do not allow the beads to dry as drying reduces the bead binding efficiency.
- To aspirate the supernatant after bead washing, place the pipette tip away from the beads by angling the pipette such that the tip is pointed away from the pellet (see the following figure) and carefully remove the supernatant without disturbing or removing any beads.



- **Do not freeze the magnetic beads**, as freezing damages the beads and frozen beads cannot be used for nucleic acid purifications.

Elution Buffer

The genomic DNA is eluted with Elution Buffer (E5; 10 mM Tris-HCl, pH 8.5). To obtain the best results, always use Elution Buffer (E5) to elute the DNA. If you wish to elute the DNA in any other buffer, be sure to use a buffer of **pH 8.5–9.0**. If the pH of the buffer is <8.5, the DNA will not elute.

Do not use water for elution.

The volume of elution buffer can be changed to obtain DNA in the desired final concentration. To obtain the best results, always use a volume of elution buffer that is equal or greater than the volume of beads used in the protocol. If the volume of elution buffer is lower than the volume of beads, DNA elution is incomplete and you may need to perform a second elution to recover all DNA.

Isolating Genomic DNA Using the Mini Bacteria Kit

Introduction

Instructions for isolating genomic DNA from 0.5 mL bacterial culture or a single colony as described in this section.

The procedure is designed for isolating genomic DNA using the ChargeSwitch® Magnetic Beads procedure in a total time of **~15 minutes** after sample preparation.

Materials Needed

Components not supplied with the kit

- A fresh solution of lysozyme (e.g. Sigma Aldrich Cat no. L7651), 50 mg/mL in water
- For purification of DNA from staphylococci, a fresh solution of lysostaphin (e.g. Sigma Aldrich Cat no. L6876), 1 mg/mL in water or 0.1 M NaCl
- MagnaRack™ Magnetic Rack (see page 9)
- Sterile 1.5-mL microcentrifuge tubes
- Vortex mixer
- Adjustable pipettes and sterile tips
- Water baths, heating blocks, or incubators

Components supplied with the kit

- RNase A in Resuspension Buffer (R4), see next page
- Proteinase K
- ChargeSwitch® Magnetic Beads
- ChargeSwitch® Binding Buffer (B8)
- ChargeSwitch® Lysis Buffer (L14)
- ChargeSwitch® Wash Buffer (W12)
- ChargeSwitch® Elution Buffer (E5)

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Isolating Genomic DNA Using the Mini Bacteria Kit, Continued

Before Starting

- Add the entire content of supplied RNase A to the Resuspension Buffer (R4). Mix well. Mark the bottle label to indicate that RNase A is added. Store the buffer with RNase at 4°C.
- Add 5 µL of fresh lysozyme solution (50 mg/mL) to 100 µL Resuspension Buffer with RNase A for each sample.

  Lysozyme is used to lyse the bacterial cell wall.
- For staphylococci, also add 1 µL of fresh lysostaphin solution (1 mg/mL) to each 100 µL Resuspension Buffer with RNase A for each sample.

  Lysostaphin is required to lyse the cell wall of some species of staphylococci but does not adversely affect lysis of other bacteria. Other lytic enzymes may be required for the lysis of other bacteria.

Bacterial Cell Lysis

1. Set a water bath or heating block at 37°C, 55°C, and 80°C.
2. Harvest cells from up to 0.5 mL overnight bacterial culture by centrifugation.

   Alternatively, you can mix 100 µL "whole" overnight culture or a colony from an agar plate with Resuspension Buffer (R4) with RNase A as described in Step 3 of this protocol.
3. Resuspend the cell pellet in 100 µL of Resuspension Buffer (R4) containing RNase A and 5 µL of lysozyme solution (50 mg/mL) by pipetting up and down gently several times or by vortexing. Ensure that the cells are evenly distributed.

   For some species of staphylococci, add 1 µL of 1 mg/mL lysostaphin.
4. Incubate the sample for 10 minutes at 37°C.
5. During the incubation, premix 500 µL Lysis Buffer (L14) with 10 µL Proteinase K solution for each sample.
6. Add 500 µL Lysis Buffer/Proteinase K mixture from Step 5 of this protocol to each sample (from Step 4) and invert the capped tube 6 times to mix the sample.
7. Incubate the sample as follows:
   - Gram negative bacteria, incubate for 1–1.5 hour at 80°C
   - Gram positive bacteria, incubate for 10 minutes at 55°C

Continued on next page

Isolating Genomic DNA Using the Mini Bacteria Kit, Continued

Binding DNA

Use the following procedure to bind DNA to the ChargeSwitch® Magnetic Beads.

1. Vortex the tube containing the ChargeSwitch® Magnetic Beads to fully resuspend and evenly distribute the beads in the storage buffer.
2. Add 40 µL ChargeSwitch® Magnetic Beads to the sample from Step 7 of the **Bacterial Cell Lysis** protocol and mix well.
3. Pipet up and down gently to mix without forming bubbles.
4. Add 300 µL Binding Buffer (B8) and mix using 5–6 short pulses (1–2 seconds) on a vortex mixer.

   Note: The Binding Buffer (B8) lowers the pH of the solution. The ChargeSwitch® Magnetic Beads now have a positive charge and will bind DNA. The beads may clump together.
5. Incubate at room temperature for 1 minute.
6. Place the sample on the MagnaRack™ Magnetic Rack for 1 minute or until the beads have formed a tight pellet.
7. Without removing the tube from the MagnaRack™, carefully remove and discard the supernatant without disturbing the pellet of beads by angling the pipette such that the tip is pointed away from the pellet (see figure on page 10).
8. Proceed immediately to **Washing DNA**, page 14.

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Isolating Genomic DNA Using the Mini Bacteria Kit, Continued

Washing DNA

1. Remove the tube containing the pelleted magnetic beads from the MagnaRack™ (Step 7, **Binding DNA**).
2. Add 1 mL Wash Buffer (W12) to the tube and pipet up and down gently 3 times using a 1-mL pipette tip to mix the sample without forming bubbles.
3. Place the sample on the MagnaRack™ Magnetic Rack for 1 minute or until the beads have formed a tight pellet.
4. Without removing the tube from the MagnaRack™, carefully remove and discard the supernatant without disturbing the pellet of beads by angling the pipette such that the tip is pointed away from the pellet.
5. Remove the tube containing the pelleted magnetic beads from the MagnaRack™.
6. Add 1 mL Wash Buffer (12) to the tube and mix by pipetting up and down gently 3 times using a 1-mL pipette tip.
7. Place the sample on the MagnaRack™ Magnetic Rack for 1 minute or until the beads have formed a tight pellet.
8. Without removing the tube from the MagnaRack™, carefully remove and discard the supernatant without disturbing the pellet of beads by angling the pipette such that the tip is pointed away from the pellet.
9. Proceed immediately to **Eluting DNA**, page 15.

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Isolating Genomic DNA Using the Mini Bacteria Kit, Continued

Eluting DNA

1. Remove the tube containing the pelleted magnetic beads from the MagnaRack™.
2. Add 200 µL Elution Buffer (E5; 10 mM Tris-HCl, pH 8.5) to the tube and pipet up and down gently 5–10 times to mix the sample without forming bubbles.

   Note: See page 10 for more information on elution buffer volume.
3. Incubate at room temperature for 5 minutes.

   Tip: For maximum yield, mix the suspension of beads (by pipetting up and down gently) half way through the incubation.
   For higher DNA yields, perform elution at 55°C to 65°C.
4. Place the sample in the MagnaRack™ for 1 minute or until the beads have formed a tight pellet.
5. Without removing the tube from the MagnaRack™, carefully remove the **supernatant containing the DNA** to a sterile microcentrifuge tube. Take care not to disturb the pellet of beads by angling the pipette such that the tip is pointed away from the pellet.

   Note: If the supernatant containing the DNA is discolored, repeat Steps 4–5 of this protocol.
6. Discard the used magnetic beads. **Do not** re-use the magnetic beads.

Storing DNA

- Store the purified DNA at –20°C, or use DNA for the desired downstream application.
- To avoid repeated freezing and thawing of DNA, store the purified DNA at 4°C for short-term use, or aliquot the DNA and store at –20°C for long-term storage.

DNA Quantitation

DNA Yield

After using the ChargeSwitch® gDNA Mini Bacteria Kit, you may estimate the yield of purified genomic DNA by checking the UV absorbance (A_{260}/A_{280}) or using the Quant-iT™ Kits.

UV Absorbance

1. Measure the A_{260} of the solution using a spectrophotometer blanked against 10 mM Tris-HCl, pH 8.5.
2. Calculate the amount of DNA using formula:

$$\text{DNA } (\mu g) = A_{260} \times 50\ \mu g/(A_{260} \times 1\ mL) \times \text{dilution factor} \times \text{total sample volume (mL)}$$

For DNA, A_{260} = 1 for a 50 µg/mL solution measured in a cuvette with an optical path length of 1 cm.

Note: Any contamination from RNA will inflate the DNA content measured at 260 nm. To avoid any interference from RNA, use the Quant-iT™ Kits.

Quant-iT™ DNA Assay Kits

The Quant-iT™ Kits (see page 19 for ordering information) provide a rapid, sensitive, and specific fluorescent method for dsDNA quantitation. The kit contains a state-of-the-art quantitation reagent, DNA standards for standard curve, and a pre-made buffer to allow fluorescent DNA quantitation using standard fluorescent microplate readers or fluorometers.

Troubleshooting

Introduction

Refer to the following table to troubleshoot problems that you may encounter when purifying genomic DNA with the kit.

Problem	Cause	Solution
Low DNA yield	Incomplete lysis	• Decrease the amount of starting material used. • Be sure to add lysozyme and Proteinase K during lysis. You may need to add lysostaphin for efficient lysis of some staphylococci.
	Improper handling ChargeSwitch® Magnetic Beads added	Vortex the tube containing the ChargeSwitch® Magnetic Beads to fully resuspend the beads in solution before adding them to your sample.
	Pellet of beads disturbed or lost during binding or washing steps	• Keep the sample in the MagnaRack™ Magnetic Rack when removing supernatant during the binding or washing steps. • Remove the supernatant without disturbing the pellet of beads by angling the pipette tip away from the pellet.
	Incorrect elution conditions	• After adding ChargeSwitch® Elution Buffer to the sample, pipet up and down to resuspend the magnetic beads before incubation. • Incubate the sample at 55°C to 65°C to improve the yield. • Do not use water to elute DNA. Use Elution Buffer (E5) or if you are using a buffer for elution, ensure the pH of the buffer is 8.5–9.0.

Continued on next page

Troubleshooting, Continued

Problem	Cause	Solution
No DNA recovered	Water used for elution	Do not use water for elution. Use ChargeSwitch® Elution Buffer (E5). The elution buffer **must** have a pH of 8.5–9.0 or the DNA will remain bound to the ChargeSwitch® Magnetic Beads.
	ChargeSwitch® Magnetic Beads stored or handled improperly	• Store beads at room temperature. **Do not freeze** the beads as they will become irreparably damaged. • Make sure that the beads are in solution at all times and do not allow the beads to dry. Dried beads are non-functional.
Eluate containing DNA is discolored	Magnetic pellet disturbed during elution	Place the sample in the MagnaRack™ until the beads form a tight pellet. Remove the eluate to a sterile microcentrifuge tube, taking care not to disturb the bead pellet.
RNA contamination	Forgot to add RNase A	Perform RNase A digestion prior to binding the DNA to the magnetic beads.
DNA is sheared or degraded	Lysate mixed too vigorously or small pipette tips used during mixing	• Use a 1-mL pipette tip and pipet up and down gently to mix without forming bubbles. • Make sure that the pipette tip is submerged in the solution during mixing.
	DNA repeatedly frozen and thawed	Aliquot DNA and store at 4°C or –20°C. Avoid repeated freezing and thawing.
	DNA contaminated with DNases	Maintain a sterile environment while working (i.e. wear gloves and use DNase-free reagents).

Appendix

Accessory Products

Additional Products

The following table lists additional products available from Life Technologies that may be used with the ChargeSwitch® gDNA Mini Bacteria Kit.

In addition, the table lists other ChargeSwitch® gDNA Kits that are available for purification of genomic DNA from other sources. For more information about these and other ChargeSwitch® gDNA Kits, visit **www.lifetechnologies.com** or call Technical Support (see page 20).

Product	Quantity	Cat. no.
MagnaRack™ Magnetic Rack	1 rack	CS15000
ChargeSwitch® gDNA 50–100 µL Blood Kit	50 purifications	CS11000
ChargeSwitch® gDNA 0.2–1 mL Serum Kit	50 purifications	CS11040
ChargeSwitch® gDNA Mini Tissue Kit	25 purifications	CS11204
ChargeSwitch® gDNA Micro Tissue Kit	50 purifications	CS11203
ChargeSwitch® gDNA Normalized Buccal Cell Kit	50 purifications	CS11020
ChargeSwitch® gDNA Buccal Cell Kit	50 purifications	CS11021
ChargeSwitch® Forensic DNA Purification Kit	100 purifications	CS11200
Quant-iT™ DNA Assay Kit, High Sensitivity	1,000 assays	Q33120
Quant-iT™ DNA Assay Kit, Broad-Range	1,000 assays	Q33130
Quant-iT™ PicoGreen® dsDNA Assay	1 kit, 1 mL	P7589

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.

A large variety of DNA ladders are available from Life Technologies for sizing DNA.

For more product information visit **www.lifetechnologies.com** or call Technical Support (see page 20).

Technical Support

Obtaining Support

For the latest services and support information for all locations, go to **www.lifetechnologies.com**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com)**
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/support**.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Purchaser Notification

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