USER GUIDE

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ChargeSwitch® gDNA Buccal Cell Kits

For purification of genomic DNA from human buccal swabs

Catalog numbers CS11020, CS11021, CS11020-10, and CS11021-10

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Contents

Kit Contents and Storage

Types of Kits

This manual is supplied with the following products.

Product	Number of Purifications	Catalog No.
ChargeSwitch® gDNA Normalized Buccal Cell Kit	50 960	CS11020 CS11020-10
ChargeSwitch® gDNA Buccal Cell Kit	50 960	CS11021 CS11021-10

Shipping and Storage

All components of the ChargeSwitch® gDNA Buccal Cell Kits are shipped at room temperature. Upon receipt, store the Proteinase K at 2°C to 8°C. Store all other components at room temperature. All components are guaranteed stable for 6 months, if stored properly.

Contents

The components supplied in the ChargeSwitch® gDNA Buccal Cell Kits are listed in the following table.

Note: Some reagents in the kit may be provided in excess of the amount needed.

Components	Catalog No.			
	CS11020	CS11021	CS11020-10	CS11021-10
ChargeSwitch® Lysis Buffer (L11)	50 mL	50 mL	960 mL	960 mL
ChargeSwitch® Magnetic Beads	2 × 1 mL	2 × 1 mL	40 mL	40 mL
Proteinase K (20 mg/mL in 50 mM Tris-HCl, pH 8.5, 5 mM $CaCl_2$, 50% glycerol)	500 µL	500 µL	9.6 mL	9.6 mL
ChargeSwitch® Purification Buffer (N6)	5 mL	5 mL	100 mL	125 mL
ChargeSwitch® Wash Buffer (W12)	100 mL	100 mL	2 × 960 mL	2 × 960 mL
ChargeSwitch® Elution Buffer (E5; 10 mM Tris-HCl, pH 8.5)	15 mL	15 mL	2 × 100 mL	2 × 100 mL

Introduction

Overview

Introduction

The ChargeSwitch® gDNA Buccal Cell Kits allow rapid and efficient purification of genomic DNA from human buccal swabs. After preparing the lysates, you may purify DNA in less than 15 minutes using the ChargeSwitch® Technology. Depending on the kit used, samples may be handled individually or in an automated system using a liquid handling robot. For more information about the ChargeSwitch® Technology, see page 7.

Kit Usage

The ChargeSwitch® gDNA Buccal Cell Kits are designed to allow isolation of the following amounts of genomic DNA from human buccal cell swabs or pelleted cells from a mouthwash:

- **ChargeSwitch® gDNA Normalized Buccal Cell Kits:** Produce a normalized yield of genomic DNA at a concentration of 1–3 ng/µL in a total volume of 150 µL.
- **ChargeSwitch® gDNA Buccal Cell Kits:** Purify up to 6 µg of genomic DNA.

Note: Samples can be stored for up to 2 weeks at 2°C to 8°C before processing without a noticeable loss in DNA yield or quality. The purified genomic DNA is suitable for use in downstream applications such as PCR.

Important: The DNA yield varies and is dependent on several factors including the technique of the person taking the swab, whether the donor is a high or low shedder, and the type of swab used.

Overview, Continued

Advantages

Use of the ChargeSwitch® gDNA Buccal Cell Kits to isolate genomic DNA provides the following advantages:

- Uses 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 human buccal swabs in less than 15 minutes following sample preparation and lysis
- Simple lysis with Proteinase K without the need for any mechanical lysis
- Minimal contamination with RNA
- The purified genomic DNA demonstrates improved downstream performance in applications including PCR
- Includes a kit designed for automated processing of large numbers of samples in 96-well plates using a liquid handling robot

System Specifications

Starting Material:	Human buccal swabs
Elution Volume:	150 µL
DNA Yield:	1–3 ng/µL in 150 µL (Normalized Buccal Cell Kit) or up to 6 µg (Buccal Cell Kit)
DNA Size:	Varies (depends on quality of starting material

Overview, Continued

The ChargeSwitch® Technology

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

ChargeSwitch® Magnetic Bead Specifications

Bead Binding Capacity:	5–10 µg genomic DNA per mg
Bead Size:	<1 µm
Bead Concentration:	25 mg/mL (Buccal Cell Kits only) 3.125 mg/mL (Normalized Buccal Cell Kits only)
Storage Buffer:	10 mM MES, pH 5.0, 10 mM NaCl, 0.1% Tween® 20

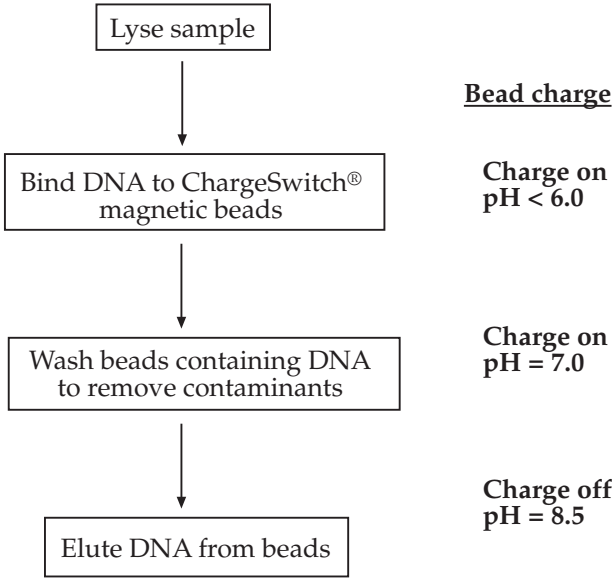
Automated Liquid Handling

Use of the ChargeSwitch® gDNA Buccal Cell Kits has been demonstrated on the Tecan Genesis® robotic workstation to purify DNA in a fully automated system from large numbers of buccal cell swabs in a 96-well format. Other liquid handling robots are suitable provided that each is equipped with a gripper arm, a 96-well magnetic separator, and other additional hardware as described on page 18. This manual provides general guidelines and a protocol that may be used to develop a script for your robot. For more information, see **www.lifetechnologies.com** or call Technical Support (page 35).

Experimental Outline

Introduction

The following workflow illustrates the basic steps necessary to purify genomic DNA from your buccal cell swab using one of the ChargeSwitch® gDNA Buccal Cell Kits.



Methods

General Information – Individual Samples

Introduction

This section provides general information needed to use the ChargeSwitch® gDNA Buccal Cell Kits (Cat. nos. CS11020 or CS11021) to process individual samples. If you are using a liquid handling robot to process large numbers of samples, see **General Information – Automated Sample Processing**, page 18.

User Supplied Materials

In addition to the reagents supplied with the kit, you need the following materials:

- A magnetic separation rack suitable for use with 1.5-mL microcentrifuge tubes (see the following image)
- Sterile, 1.5-mL microcentrifuge tubes
- Vortex mixer
- 20-µL, 200-µL, and 1-mL sterile, pipette tips
- Water bath at 37°C

MagnaRack™ Magnetic Separator

The MagnaRack™ magnetic separator available from Life Technologies (Cat. no. CS15000) is a two-piece magnetic separation rack for use in protocols with magnetic beads, and consists of a magnetic base station and a removable tube rack. The tube rack can hold up to 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 image). For more information, see **www.lifetechnologies.com** or call Technical Support (page 35).

General Information – Individual Samples, Continued

Safety Information

Follow the safety guidelines listed when using the ChargeSwitch® gDNA Buccal Cell Kits:

- 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.
- Dispose of biological samples and all liquid waste generated during the purification procedure as biohazardous waste.

Handling the ChargeSwitch® Magnetic Beads

Follow the guidelines listed when handling the ChargeSwitch® magnetic beads:

- Do not freeze the beads as this irreparably damages them. Store the beads at room temperature.
- Always keep the beads in solution. Do not allow them to dry out as this renders them non-functional.
- When using the beads, resuspend thoroughly in the storage buffer by vortexing before removal.
- Discard beads after use. Do not reuse.

General Information – Individual Samples, Continued

Elution Buffer

ChargeSwitch® Elution Buffer (E5; 10 mM Tris-HCl, pH 8.5) is supplied with the kit for eluting the DNA from the ChargeSwitch® Magnetic Beads. For best results, use Elution Buffer (E5) to elute the DNA. Alternatively, TE Buffer, pH 8.5–9.0 is acceptable. Note that the pH must be between 8.5–9.0 otherwise the DNA will not elute. **Do not use water for elution.**

The protocol suggests eluting the genomic DNA in 150 µL of ChargeSwitch® Elution Buffer (E5). When using the ChargeSwitch® gDNA Buccal Cell Kit, you may vary the amount of ChargeSwitch® Elution Buffer (E5) used to obtain genomic DNA in the desired final concentration. **For best results, always use a volume of ChargeSwitch® Elution Buffer (E5) that is equal to or greater than the volume of ChargeSwitch® Magnetic Beads used in the protocol.** If the volume of ChargeSwitch® Elution Buffer (E5) is lower than the volume of beads used, DNA elution is incomplete. You may need to perform a second elution to recover all DNA.

Important: When using the ChargeSwitch® gDNA Normalized Buccal Cell Kit, elute the genomic DNA in 150 µL of ChargeSwitch® Elution Buffer (E5). **Do not** vary the amount of ChargeSwitch® Elution Buffer (E5) used otherwise the yield will no longer be normalized at 1–3 ng/µL.

Isolating Genomic DNA from Individual Samples

Introduction

This section provides guidelines and instructions to isolate genomic DNA from human buccal swabs or pelleted cells from a mouthwash using the reagents supplied in the kit. Note that the protocol is optimized for efficient purification of DNA from small sample volumes. Depending on the volume of your sample, some further optimization of the protocol may be required.

Starting Material

Use this procedure to isolate genomic DNA from human buccal cell swabs or pelleted cells from a mouthwash. Process samples immediately after collection or store at 2°C to 8°C for up to 2 weeks. **Do not** store unprocessed samples at room temperature as buccal swabs contain bacteria and nucleases that will degrade DNA.

Important

The ChargeSwitch® Magnetic Beads supplied in the ChargeSwitch® gDNA Buccal Cell Kit differ in concentration from those supplied in the ChargeSwitch® gDNA Normalized Buccal Cell Kit. Although the purification protocol is identical for both kits, you **must** use the ChargeSwitch® Magnetic Beads supplied with each kit to obtain the DNA yields specified on page 5. **Do not** substitute ChargeSwitch® Magnetic Beads provided in the ChargeSwitch® gDNA Buccal Cell Kit for those in the ChargeSwitch® gDNA Normalized Buccal Cell Kit.

Isolating Genomic DNA from Individual Samples, Continued

Materials Needed

Components Not Supplied with the Kit

- Buccal swab(s)
- MagnaRack™ magnetic separator (Cat. no. CS15000)
- Sterile 1.5-mL microcentrifuge tubes
- Vortex mixer
- Sterile pipette tips (20 µL, 200 µL, and 1-mL)
- Water bath

Components Supplied with the Kit

- ChargeSwitch® Lysis Buffer (L11)
- Proteinase K
- ChargeSwitch® Magnetic Beads
- ChargeSwitch® Purification Buffer (N6)
- ChargeSwitch® Wash Buffer (W12)
- ChargeSwitch® Elution Buffer (E5) or TE Buffer (not supplied; 10 mM Tris-HCl, 1 mM EDTA, pH 8.5)

Isolating Genomic DNA from Individual Samples, Continued

Before Starting

Perform the following before beginning:

1. Set a water bath at 37°C.
2. Prepare a **Lysis Mix**: **For each sample**, mix 1 mL of ChargeSwitch® Lysis Buffer (L11) and 10 µL of Proteinase K to prepare the Lysis Mix. If you are isolating DNA from multiple samples, you may scale up the volume of reagents used and prepare a master Lysis Mix.
3. Vortex the tube containing the ChargeSwitch® Magnetic Beads to fully resuspend and evenly distribute the beads in the storage buffer.
4. Prepare a **Purification Mix**: **For each sample**, mix 40 µL of ChargeSwitch® Magnetic Beads (fully resuspended) and 100 µL of ChargeSwitch® Purification Buffer (N6) to prepare the Purification Mix. If you are isolating DNA from multiple samples, you may scale up the volume of reagents used and prepare a master Purification Mix.

Preparing the Lysate

Follow the procedure below to prepare a lysate from the human buccal cell swab.

1. Transfer the human buccal cell sample to a sterile microcentrifuge tube.
2. Add 1 mL of Lysis Mix (step 2, **Before Starting**) to the tube, making sure that the sample is completely immersed in the Lysis Mix.
3. Incubate the sample at 37°C for 20 minutes.
4. Proceed to **Binding DNA**, page 15.

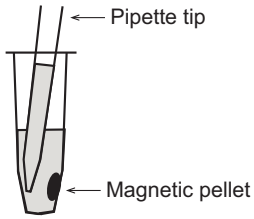
Isolating Genomic DNA from Individual Samples, Continued

Binding DNA

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

1. Transfer the digested supernatant (from Step 3, **Preparing the Lysate**) into a new, sterile microcentrifuge tube.
2. Gently pipet up and down the Purification Mix containing the ChargeSwitch® Magnetic Beads (from step 4, **Before Starting**, page 14) to fully resuspend the beads.
3. Add 140 µL of Purification Mix to the sample and pipet up and down gently 5 times to mix.

   Important: Use a 1 mL pipette tip set to 900 µL to mix the sample. Make sure that the tip is submerged, and pipet up and down gently to avoid forming bubbles.
4. Incubate at room temperature for 1 minute to allow the DNA to bind to the ChargeSwitch® Magnetic Beads.
5. Place the sample in the MagnaRack™ base station for 1 minute or until the beads have formed a tight pellet.
6. Without removing the tube from the MagnaRack™ base, carefully remove the supernatant and discard. Take care not to disturb the pellet of beads by angling the pipette such that the tip is pointed away from the pellet (see the following figure).



7. Proceed immediately to **Washing DNA**, page 16.

Isolating Genomic DNA from Individual Samples, Continued

Washing DNA

1. Without removing the tube from the MagnaRack™ base, add 1 mL of ChargeSwitch® Wash Buffer (W12) to the sample. Direct the ChargeSwitch® Wash Buffer over the pellet of magnetic beads in such a way that the beads are briefly resuspended in solution.
2. Leave the sample in the MagnaRack™ base station for 1 minute or until the beads have formed a tight pellet.
3. Without removing the tube from the MagnaRack™ base, carefully remove the supernatant and discard. Take care not to disturb the pellet of beads by angling the pipette such that the tip is pointed away from the pellet (see figure on page 15).
4. Repeat Steps 1–3.
5. Proceed to **Eluting DNA**.

Eluting DNA

1. Remove the tube containing the pelleted magnetic beads from the MagnaRack™ base station (Step 4, **Washing DNA**). There should be no supernatant in the tube.
2. Add 150 µL of ChargeSwitch® Elution Buffer (E5) (or TE Buffer, pH 8.5) to the tube, and pipet up and down gently 10 times to resuspend the magnetic beads.

   Important: Do not use water for elution. The DNA will not elute due to the poor buffering capacity of water.
3. Incubate at room temperature for 1 minute.
4. Place the sample in the MagnaRack™ base station for 1 minute or until the beads have formed a tight pellet.
5. Without removing the tube from the MagnaRack™ base, 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 (see figure on page 15).

   Note: If the eluate containing the DNA is discolored, repeat Steps 4–5.
6. Discard the used magnetic beads. Do not reuse the beads.

Isolating Genomic DNA from Individual Samples, Continued

Storing DNA

Store the purified DNA at –20°C or use immediately for PCR or other appropriate downstream application. Avoid repeatedly freezing and thawing DNA.

Quantitating DNA Yield

To quantitate yield of your DNA, we recommend using the Quant-iT™ DNA Assay Kit, High Sensitivity (Cat. no. Q33120) available from Life Technologies. This kit contains a state-of-the-art quantitation reagent, pre-diluted standards, and a pre-made buffer to allow sensitive and accurate fluorescence-based quantitation of dsDNA. For more information about the Quant-iT™ DNA Assay Kit, see **www.lifetechnologies.com** or call Technical Support (page 35).

General Information – Automated Sample Processing

Introduction

This section provides general information to use the ChargeSwitch® gDNA Buccal Cell Kits (Cat. nos. CS11020-10 and CS11021-10) to process large numbers of samples in 96-well format using an automated liquid handling robot. If you wish to process small numbers of samples individually, see **General Information – Individual Samples**, page 9.

Hardware Requirements

The ChargeSwitch® chemistry is ideal for purification of DNA using a liquid handling robot, avoiding the need for centrifugation steps or the use of ethanol or chaotropic salts. You will need to have the following hardware to perform automated processing of buccal swabs using one of the ChargeSwitch® gDNA Buccal Cell Kits:

- Any liquid handling robotic workstation with a gripper arm
- Appropriate tips for liquid dispensing and aspiration (see **Tip Selection** for factors to consider)
- 96-well Magnetic Separator (see page 19)
- Shaker
- Incubator, heat block, or water bath for heating samples
- 96 × 2.2 mL deep-well plate(s) (Cat. no. CS15196)
- 96 × 300 µL U-Bottomed microtiter plate (Greiner, Cat. no. 650201)

For an example of how to set up the deck, see page 20.

Tip Selection

You may use any tips of choice to dispense and aspirate liquid during the purification procedure. Consider the following factors when choosing an appropriate tip to use:

- Fixed vs. disposable tips
- Tip size vs. head size
- Conductive or non-conductive
- Sterile or non-sterile
- Filtered or non-filtered

General Information – Automated Sample Processing, Continued

96-well Magnetic Separator

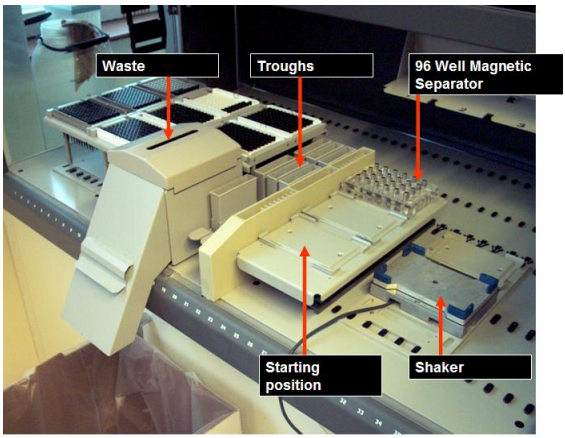
The 96-well Magnetic Separator available from Life Technologies (Cat. no. CS15096) is a magnetic separation rack that can hold up to 96 samples in a deep-well plate. The deep-well plate fits onto the magnetic base station, associating the array of 24 neodymium magnets with the samples for 'on the magnet' and 'off the magnet' sample processing (see the following images). For more information, see **www.lifetechnologies.com** or call Technical Support (page 35).

General Information – Automated Sample Processing, Continued

Deck Set Up

Once you have the required hardware, you will need to configure the deck of your liquid handling robot appropriately to process samples. You may use any suitable configuration of your choice. An example is provided here.

Location	Trough Contents	Plate
1	—	96-well Deep-well plate #1
2	Lysis Mix (i.e. ChargeSwitch® Lysis Buffer (L11) + Proteinase K)	—
3	Purification Mix (i.e. ChargeSwitch® Purification Buffer (N6) + ChargeSwitch® Magnetic Beads)	—
4	ChargeSwitch® Wash Buffer (W12)	—
5	ChargeSwitch® Elution Buffer (E5)	—
6	—	—
7	—	96-well Deep-well plate #2
8	Waste	—
9	—	96-well Magnetic Separator
10	—	Shaker
11	—	96-well Sample Tray
12	ChargeSwitch® Lysis Buffer (L11)	—
13	—	96-well U-bottomed microtiter plate (for final elution)



General Information – Automated Sample Processing, Continued

Primary Liquid Handling Parameters

The table below lists the primary liquid handling parameters required to isolate DNA using the automated protocol. Use the parameters and guidelines provided, as well as the protocol on pages 25–26 to program your robot.

Parameter	Aim	Guidelines
[Magnetic Bead Preparation]	To resuspend beads prior to mixing with solution	• Only required once • Beads stay in suspension for up to 45 minutes
[Mixing #1]	Used to mix beads or bead/DNA pellet with buffer	• Aspirate/dispense at 400–500 µL • Aspirate/dispense position fixed 1–2 mm above the well bottom • Use tips/volume setting at 80 µL volume
[Dispense liquid]	Normal liquid parameters for adding a reagent to each well	• Aspirate/dispense at 300–400 µL • Use multi-dispense if appropriate to save time
[Transfer supernatant to waste]	To remove and discard supernatant	• Aspirate slowly at 50–100 µL/second • Aspirate off the entire liquid volume using liquid detect and tracking or setting fixed height 1 mm above the well bottom • Do not disturb pellet • Dispense to waste
[Transfer supernatant to another plate]	To transfer supernatant to another plate	• Aspirate slowly at 50–100 µL/second • Aspirate off the entire liquid volume using liquid detect and tracking or setting fixed height 1 mm above the well bottom • Do not disturb pellet • Dispense slowly at 50–100 µL/second • Avoid splashing
[Final DNA Elution]	To dispense the eluate containing DNA	• Dispense at 10 µL/second • Aspirate from position fixed 1 mm above the well bottom • Avoid bead carry-over • Dispense into new plate at 2 mm above the well bottom

Automated Genomic DNA Isolation

Introduction

This section provides a general protocol for automated isolation of genomic DNA from human buccal cell swabs in a 96-well format using the ChargeSwitch® gDNA Buccal Cell Kit (Cat. no. 11021-10). Use this general protocol to develop the script for your liquid handling robot.

Note: If you are using the ChargeSwitch® gDNA Normalized Buccal Cell Kit (Cat. no. 11020-10), follow the protocol on pages 27–30.

Important

The ChargeSwitch® Magnetic Beads supplied in the ChargeSwitch® gDNA Buccal Cell Kit differ in concentration from those supplied in the ChargeSwitch® gDNA Normalized Buccal Cell Kit. Use **only** the ChargeSwitch® Magnetic Beads supplied in the ChargeSwitch® gDNA Buccal Cell Kit in this protocol.

Materials Needed

Components Not Supplied with the Kit

- Liquid handling robot configured to process samples in 96-well plates
- Buccal swabs
- 96 × 2.2 mL deep-well plate(s) (Cat. no. CS15196)
- 96 × 300 µL U-bottomed microtiter plate
- Optional: 96 × 2 mL glass-filled, polypropylene, Unifilter® Microplate (Whatman, Cat. no. 7720-7235; see **Using a Filter Plate** on the following page)

Components Supplied with the Kit

- ChargeSwitch® Lysis Buffer (L11)
- Proteinase K
- ChargeSwitch® Magnetic Beads
- ChargeSwitch® Purification Buffer (N6)
- ChargeSwitch® Wash Buffer (W12)
- ChargeSwitch® Elution Buffer (E5) or TE Buffer (not supplied; 10 mM Tris-HCl, 1 mM EDTA, pH 8.5)

Automated Genomic DNA Isolation, Continued

Important Guidelines

To maximize DNA yield, follow these recommendations when processing your samples:

- Ensure that the robotic tips enter the wells of the plates without interfering with the pellet of beads.
- When removing supernatant, leave samples on the 96-well Magnetic Separator and aspirate slowly to ensure that the pellet of beads is not disturbed.
- When resuspending pelleted ChargeSwitch® Magnetic Beads, make sure that all beads are fully resuspended to maximize DNA recovery.
- To maximize DNA yield, make sure that all Wash Buffer is removed before elution.
- To maximize DNA yield, make sure that the beads are fully resuspended during the elution step.

Lysate Volume

The first step of the genomic DNA isolation protocol requires addition of Lysis Mix to the sample. You may add either 1 mL or 1.4 mL of Lysis Mix to the sample. Note that some of the Lysis Mix may be absorbed by the swab, resulting in a lower volume of lysate being available for purification. To maximize DNA yield, we recommend using 1.4 mL of Lysis Mix (Step 2, **Automated Protocol**, page 25). To prepare Lysis Mix, see the following page.

Using a Filter Plate

To maximize recovery volume and minimize contaminant transfer during lysate preparation, you may prepare lysates in a 96 × 2 mL filter plate (see Steps 1–2, **Automated Protocol**, page 25), then directly filter the supernatant into a 96 × 2.2 mL deep-well plate to perform the remainder of the purification procedure. We recommend using the 96 × 2 mL glass-filled polypropylene Unifilter® Microplate from Whatman (Cat. no. 7720-7235). Other 96 × 2 mL filter plates are suitable.

Automated Genomic DNA Isolation,
Continued

Before Starting

Perform the following before beginning:

- Prepare **Lysis Mix:** For **each sample**, mix 1 mL of ChargeSwitch® Lysis Buffer (L11) and 10 µL of Proteinase K to prepare the Lysis Mix. If lysing in 1.4 mL volume, mix 1.4 mL of ChargeSwitch® Lysis Buffer (L11) and 10 µL of Proteinase K for each sample. Scale up the volume of reagents used (based on number of samples) to prepare a master mix.
- Prepare **Purification Mix**: For **each sample**, mix 100 µL of ChargeSwitch® Purification Buffer (N5) and 40 µL of ChargeSwitch® Magnetic Beads to prepare the Purification Mix (make sure that the beads are thoroughly resuspended). If lysing in 1.4 mL volume, mix 140 µL of ChargeSwitch® Purification Buffer (N5) and 40 µL of ChargeSwitch® Magnetic Beads for each sample. Scale up the volume of reagents used (based on number of samples) to prepare a master mix.

Automated Genomic DNA Isolation,
Continued

Automated Protocol

Follow this protocol to isolate genomic DNA from buccal swabs. The volumes given are on a per sample basis.

1. Start with 96 buccal cell samples in a 96 × 2.2 mL deep-well plate or 96 × 2 mL filter plate.
2. Add 1 mL (or 1.4 mL) of Lysis Mix (see **Lysate Volume**, page 23) and incubate at 37°C for 20 minutes (use a heating block).

   Note: Optimal incubation parameters (i.e. time) vary depending on the sample and automation plasticware, and should be determined empirically.
3. After incubation, transfer or filter as appropriate, as much of the lysate as possible to a 96 × 2.2 mL deep-well plate, without interfering with the samples.
4. Add 140 µL (or 180 µL if 1.4 mL of Lysis Mix used) of Purification Mix (see **Before Starting**, page 24; make sure that the beads are thoroughly resuspended).
5. Shake at medium fast speed (e.g. pulse, 10 seconds) to evenly distribute the magnetic beads within the solution.
6. Wait for 10 seconds.
7. Move samples to the 96-well Magnetic Separator.
8. Wait for 60–90 seconds.
9. Slowly aspirate all of the supernatant and discard, leaving behind the pellet of beads.
10. While samples are still on the 96-well Magnetic Separator, add 1 mL of ChargeSwitch® Wash Buffer (W12).
11. Wait for 60 seconds or until the beads have formed a tight pellet.
12. Slowly aspirate all of the supernatant and discard, leaving behind the pellet of beads.
13. While samples are still on the 96-well Magnetic Separator, add 1 mL of ChargeSwitch® Wash Buffer (W12).

Automated Genomic DNA Isolation, Continued

Automated Protocol, Continued

14. Wait for 30–60 seconds.
15. Slowly aspirate all of the supernatant and discard, leaving behind the pellet of beads.
16. Move samples to the shaker.
17. Add 150 µL of ChargeSwitch® Elution Buffer (E5).

    Note: You may vary elution volume depending on your needs. Do not elute in volumes <60 µL as the DNA may not completely elute from the beads.
18. Shake rapidly for 1–2 minutes to completely disperse the beads within the solution.
19. Move samples to the 96-well Magnetic Separator.
20. Wait for 1 minute.
21. Slowly aspirate **supernatant containing the DNA** to a 96 × 300 µL U-bottomed microtiter plate.

Storing DNA

Store the purified DNA at –20°C or use immediately for downstream applications such as PCR. Avoid repeatedly freezing and thawing DNA.

Quantitating DNA Yield

To quantitate yield of your DNA, use the Quant-iT™ DNA Assay Kit, High Sensitivity (Cat. no. Q33120). For more information, see page 17.

Automated Genomic DNA Isolation – Normalized Kit

Introduction

This section provides a general protocol for automated isolation of genomic DNA from human buccal cell swabs in a 96-well format using the ChargeSwitch® gDNA Normalized Buccal Cell Kit (Cat. no. 11020-10). Use this general protocol to develop the script for your liquid handling robot.

Note: If you are using the ChargeSwitch® gDNA Buccal Cell Kit (Cat. no. 11021-10), follow the guidelines and protocol on pages 22–26.

Important

The ChargeSwitch® Magnetic Beads supplied in the ChargeSwitch® gDNA Normalized Buccal Cell Kit differ in concentration from those supplied in the ChargeSwitch® gDNA Buccal Cell Kit. Use **only** the ChargeSwitch® Magnetic Beads supplied in the ChargeSwitch® gDNA Normalized Buccal Cell Kit in this protocol.

Materials Needed

Components Not Supplied with the Kit

- Liquid handling robot configured to process samples in 96-well plates
- Buccal swabs
- 96 × 2.2 mL deep-well plates (Cat. no. CS15196)
- 96 × 300 µL U-bottomed microtiter plate
- Optional: 96 × 2 mL glass-filled, polypropylene, Unifilter® Microplate (Whatman, Cat. no. 7720-7235; see **Using a Filter Plate**, page 28)

Components Supplied with the Kit

- ChargeSwitch® Lysis Buffer (L11)
- Proteinase K
- ChargeSwitch® Magnetic Beads
- ChargeSwitch® Purification Buffer (N6)
- ChargeSwitch® Wash Buffer (W12)
- ChargeSwitch® Elution Buffer (E5) or TE Buffer (not supplied; 10 mM Tris-HCl, 1 mM EDTA, pH 8.5)

Automated Genomic DNA Isolation – Normalized Kit, Continued

Important Guidelines

To maximize DNA yield, follow these recommendations when processing your samples:

- Ensure that the robotic tips enter the wells of the plates without interfering with the pellet of beads.
- When removing supernatant, leave samples on the 96-well Magnetic Separator and aspirate slowly to ensure that the pellet of beads is not disturbed.
- When resuspending pelleted ChargeSwitch® Magnetic Beads, make sure that all beads are fully resuspended to maximize DNA recovery.
- To maximize DNA yield, make sure that all Wash Buffer is removed before elution.
- To maximize DNA yield, make sure that the beads are fully resuspended during the elution step.

Using a Filter Plate

Some of the Lysis Mix may be absorbed by the sample, resulting in a lower volume of lysate being available for purification. To maximize recovery volume and minimize contaminant transfer during lysate preparation, you may prepare lysates in a 96 × 2 mL filter plate (see Steps 1–2, **Automated Protocol**, page 29), then directly filter the supernatant into a 96 × 2.2 mL deep-well plate to perform the remainder of the purification procedure. We recommend using the 96 × 2 mL glass-filled polypropylene Unifilter® Microplate from Whatman (Cat. no. 7720-7235). Other 96 × 2 mL filter plates are suitable.

Automated Genomic DNA Isolation – Normalized Kit, Continued

Before Starting

Perform the following before beginning:

- Prepare **Lysis Mix:** For **each sample**, mix 1 mL of ChargeSwitch® Lysis Buffer (L11) and 10 µL of Proteinase K to prepare the Lysis Mix. Scale up the volume of reagents used (based on number of samples) to prepare a master mix.
- Prepare **Purification Mix**: For **each sample**, mix 100 µL of ChargeSwitch® Purification Buffer (N5) and 40 µL of ChargeSwitch® Magnetic Beads to prepare the Purification Mix (make sure that the beads are thoroughly resuspended). Scale up the volume of reagents used (based on number of samples) to prepare a master mix.

Automated Protocol

Follow this protocol to isolate genomic DNA from buccal swabs. The volumes given are on a per sample basis.

1. Start with 96 buccal cell samples in a 96 × 2.2 mL deep-well plate or 96 × 2 mL filter plate.
2. Add 1 mL of Lysis Mix (see **Before Starting**, page 29) and incubate at 37°C for 20 minutes (use a heating block).

   Note: Optimal incubation parameters (i.e. time) vary depending on the sample and automation plasticware, and should be determined empirically.
3. After incubation, transfer or filter as appropriate, as much of the lysate as possible to a 96 × 2.2 mL deep-well plate, without interfering with the samples.
4. Add 140 µL of Purification Mix (see **Before Starting**, page 29; make sure that the beads are thoroughly resuspended).
5. Shake at medium fast speed (e.g. pulse, 10 seconds) to evenly distribute the magnetic beads within the solution.
6. Wait for 10 seconds.
7. Move samples to the 96-well Magnetic Separator.
8. Wait for 60–90 seconds.
9. Slowly aspirate all of the supernatant and discard, leaving behind the pellet of beads.

Automated Genomic DNA Isolation – Normalized Kit, Continued

Automated Protocol, Continued

10. Remove samples from the 96-well Magnetic Separator.
11. Add 500 µL of ChargeSwitch® Wash Buffer (W12).
12. Shake at medium fast speed (e.g. pulse, 10 seconds) to evenly distribute the magnetic beads within the solution.
13. Move samples to the 96-well Magnetic Separator.
14. Wait for 60–90 seconds.
15. Slowly aspirate all of the supernatant and discard, leaving behind the pellet of beads.
16. While samples are still on the 96-well Magnetic Separator, add 500 µL of ChargeSwitch® Wash Buffer (W12).
17. Wait for 30–60 seconds.
18. Slowly aspirate all of the supernatant and discard, leaving behind the pellet of beads.
19. Move samples to the shaker.
20. Add 150 µL of ChargeSwitch® Elution Buffer (E5).
21. Shake rapidly for 1–2 minutes to completely disperse the beads within the solution.
22. Move samples to the 96-well Magnetic Separator.
23. Wait for 1 minute.
24. Slowly aspirate **supernatant containing the DNA** to a 96 × 300 µL U-bottomed microtiter plate.

Storing DNA

Store the purified DNA at –20°C or use immediately for downstream applications such as PCR. Avoid repeatedly freezing and thawing DNA.

Quantitating DNA Yield

To quantitate the yield of your DNA, use the Quant-iT™ DNA Assay Kit, High Sensitivity (Cat. no. Q33120). For more information, see page 17.

Troubleshooting

Introduction

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

Problem	Cause	Solution
Low DNA yield	Incomplete lysis	• Be sure to add Proteinase K during lysis. • Increase the length of incubation at 37°C.
	Poor quality of starting material	Process samples immediately after collection or store the sample at 2°C to 8°C. Do not store samples at room temperature as bacteria and nucleases present in the sample will degrade the DNA.
	Insufficient amount of ChargeSwitch® Magnetic Beads added	• Vortex the tube containing the ChargeSwitch® Magnetic Beads to fully resuspend the beads in solution before preparing the Purification Mix. • Before adding Purification Mix to your sample, make sure that the beads are fully resuspended.
	Pellet of beads disturbed or lost during binding or washing steps	• Keep the sample in the MagnaRack™ base or 96-well Magnetic Separator 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.
	Bubbles formed during mixing steps	Make sure that the pipette tip is submerged in the solution during mixing.

Troubleshooting, Continued

Problem	Cause	Solution
Low DNA yield, continued	Incomplete dissociation of DNA from the ChargeSwitch® Magnetic Beads	Perform additional mixing of the suspension of beads (by pipetting up and down).
	Incorrect elution conditions	• After adding ChargeSwitch® Elution Buffer (E5) to the sample, pipet up and down to fully resuspend the magnetic beads before incubation. • Do not use water for elution. Use ChargeSwitch® Elution Buffer (E5) or TE, pH 8.5.
	Lysate mixed too vigorously or small pipette tips used during mixing	• Use the appropriate pipette tip set to a volume lower than the total volume of solution in the sample. • Pipet up and down gently to mix.
No DNA recovered	Water used for elution	Do not use water for elution. The elution buffer **must** have a pH = 8.5–9.0 or the DNA will remain bound to the ChargeSwitch® Magnetic Beads. Use Elution Buffer (E5) or TE, pH 8.5.
	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 become dried. Dried beads are non-functional.
	Purification Mix did not contain ChargeSwitch® Magnetic Beads	Purification Mix should contain ChargeSwitch® Purification Buffer (N6) + ChargeSwitch® Magnetic Beads.

Troubleshooting, Continued

Problem	Cause	Solution
DNA is degraded	Buccal swabs stored at room temperature	Process buccal swabs immediately after collection or store at 2°C to 8°C.
DNA yields vary widely between samples	Variability in sample collection	DNA yields can vary depending on swabbing technique, whether the donor is a high or low shedder, and the type of swab used.

Appendix

Accessory Products

Additional Products

The following table lists additional products available from Life Technologies that may be used with the ChargeSwitch® gDNA Buccal Cell Kits. In addition, the table lists a selection of ChargeSwitch® gDNA Kits that are available for purification of genomic DNA from other sources. For more information about these and other ChargeSwitch® gDNA Kits, refer to our Web site at **www.lifetechnologies.com** or call Technical Support (see page 35).

Product	Amount	Catalog No.
MagnaRack™ Magnetic Separator	1 rack	CS15000
96-well Magnetic Separator	1 rack	CS15096
96 Deep Well Block	50/case	CS15196
ChargeSwitch® gDNA Micro Tissue Kit	50 purifications	CS11203
ChargeSwitch® gDNA Mini Tissue Kit	25 purifications	CS11204
ChargeSwitch® gDNA 50–100 µL Blood Kit	50 purifications	CS11000
ChargeSwitch® gDNA 0.2–1 mL Serum Kit	50 purifications	CS11040
ChargeSwitch® gDNA 50 µL Sheep Blood Kit	50 purifications	CS11300
ChargeSwitch® gDNA Mini Bacteria Kit	50 purifications	CS11301
ChargeSwitch® Forensic DNA Purification Kit	100 purifications	CS11200
Quant-iT™ DNA Assay Kit, High Sensitivity	1000 assays	Q33120

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

Purchaser Notification

Limited Use Label License No. 358: Research Use Only
The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact **outlicensing@lifetech.com** or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

Notes

Notes

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