Invitrogen™
life technologies

Instruction Manual

ChargeSwitch® gDNA Blood Kits

For purification of genomic DNA from small volumes of human blood

Catalog nos. CS11000, CS11010, and CS11010-10

Version A

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25-0814

Table of Contents

Kit Contents and Storage

Types of Kits

This manual is supplied with the following products.

Product	Number of Purifications	Catalog no.
ChargeSwitch® gDNA 20 µl Blood Kit	96	CS11010
	960	CS11010-10
ChargeSwitch® gDNA 100 µl Blood Kit	50	CS11000

Shipping and Storage

All components of the ChargeSwitch® gDNA Blood Kits are shipped at room temperature. Upon receipt, store the Proteinase K at 4°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 Blood Kits are listed below.

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

Components	Catalog no.		
	CS11010	CS11000	CS11010-10
ChargeSwitch® Lysis Buffer (L12)	100 ml	100 ml	2 x 480 ml
ChargeSwitch® Magnetic Beads	2 x 1 ml	2 x 1 ml	2 x 10 ml
Proteinase K (20 mg/ml in 50 mM Tris-HCl, pH 8.5, 5 mM $CaCl_2$, 50% glycerol)	500 µl	500 µl	5 ml
ChargeSwitch® Purification Buffer (N5)	20 ml	20 ml	100 ml
ChargeSwitch® Wash Buffer (W12)	50 ml	100 ml	960 ml
ChargeSwitch® Elution Buffer (E5; 10 mM Tris-HCl, pH 8.5)	15 ml	15 ml	100 ml

Accessory Products

Additional Products

The table below lists additional products available from Invitrogen that may be used with the ChargeSwitch® gDNA Blood 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.invitrogen.com or call Technical Service (see page 29).

Product	Amount	Catalog no.
MagnaRack™	1 rack	CS15000
96-Well Magnetic Separator	1 rack	CS15096
ChargeSwitch® gDNA Micro Tissue Kit	50 purifications	CS11203
ChargeSwitch® gDNA Mini Tissue Kit	25 purifications	CS11204
ChargeSwitch® gDNA 1 ml Blood Kit	20 purifications	CS11001
ChargeSwitch® gDNA 1 ml Serum Kit	50 x 1 ml purifications	CS11040
ChargeSwitch® gDNA 50 µl Sheep Blood Kit	50 purifications	CS11300
ChargeSwitch® gDNA Mini Bacteria Kit	50 purifications	CS11301
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™ PicoGreen® dsDNA Quantitation Kit	200-2000 assays	P7589
Quant-iT™ PicoGreen® dsDNA Quantitation Reagent	200-200 assays (1 ml)	P7581

Introduction

Overview

Introduction

The ChargeSwitch® gDNA Purification Kits allow rapid and efficient purification of genomic DNA from small volumes of human blood. 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 3.

Intended Use for the Kits

The ChargeSwitch® gDNA Blood Kits are designed to allow isolation of genomic DNA from the following amounts of fresh or frozen, human blood treated with the anticoagulant EDTA or citrate. The purified genomic DNA is suitable for use in downstream applications including PCR, restriction enzyme digestion, and Southern blotting.

- ChargeSwitch® gDNA 20 µl Blood Kits: Purifies up to 600 ng of genomic DNA from 10-20 µl of human blood.
- ChargeSwitch® gDNA 100 µl Blood Kit: Purifies up to 3 µg of genomic DNA from 50-100 µl of human blood.

Important: Genomic DNA may be isolated from heparin-treated blood; however, the DNA **is not** suitable for use in downstream applications such as PCR due to the presence of the heparin.

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Overview, continued

Advantages

Use of the ChargeSwitch® gDNA Blood 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 blood samples 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, restriction enzyme digestion, and Southern blotting
- Includes a kit designed for automated processing of large numbers of 50-100 µl samples in 96-well plates using a liquid handling robot

System Specifications

Starting Material:	Varies; optimized for small sample volumes (*i.e.* 10-20 µl or 50-100 µl)
Elution Volume:	100 µl (20 µl kit) and 150 µl (100 µl kit)
DNA Yield:	Up to 600 ng (20 µl kit) and 3 µg (100 µl kit)
DNA Size:	Varies (depends on quality of starting material

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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 figure below). 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 figure below). Purified DNA elutes instantly into this elution buffer, and is ready for use in downstream applications.

Low pH High pH

ChargeSwitch® Magnetic Bead Specifications

Bead Binding Capacity:	5-10 µg genomic DNA per mg
Bead Size:	< 1 µm
Bead Concentration:	25 mg/ml
Storage Buffer:	10 mM MES, pH 5.0, 10 mM NaCl, 0.1% Tween 20

Automated Liquid Handling

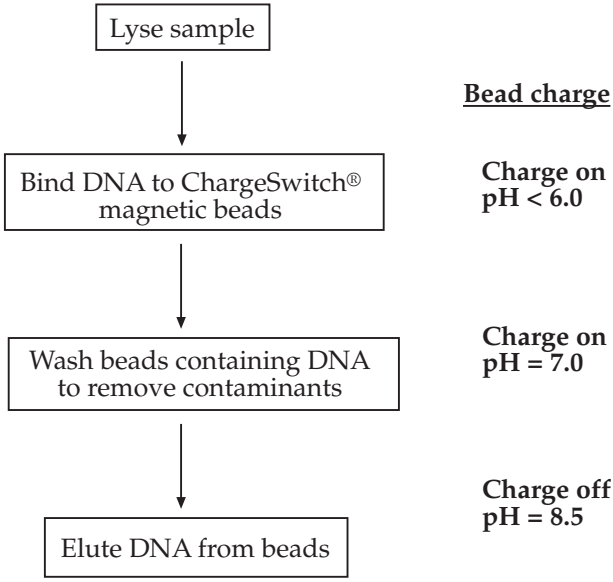
Use of the ChargeSwitch® gDNA 20 µl Blood Kit has been demonstrated on the Tecan Genesis® robotic workstation to purify DNA in a fully automated system from large numbers of 10-20 µl blood samples in a 96-well format. Other liquid handling robots are suitable provided that each is equipped with a gripper arm, a 96-well magnetic separator, and other additional hardware as described on page 19. This manual provides general guidelines and a protocol that may be used to develop a script for your robot. For more information, see www.invitrogen.com or call Technical Service (page 29).

Genesis® is a registered trademark of Tecan AG Group

Experimental Outline

Introduction

The figure below illustrates the basic steps necessary to purify genomic DNA from your blood sample using the ChargeSwitch® gDNA Blood Kit.



Methods

General Information – Individual Samples

Introduction

This section provides general information needed to use the ChargeSwitch® gDNA 20 µl or 100 µl Blood Kits (Catalog nos. CS11010 or CS11000) 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 14.

Processing Samples

When isolating genomic DNA using the kit, you may process blood samples in individual microcentrifuge tubes or in 96-well format. Depending on the option chosen, you will need to have specific supplies and equipment (see below and the next page).

User Supplied Materials

In addition to the reagents supplied with the kit, you need to have the following materials on hand before beginning:

- A magnetic separation rack suitable for use with 1.5 ml microcentrifuge tubes or 96-well plates (see next page)
- Sterile, 1.5 ml microcentrifuge tubes (if processing a few samples)
- 96 x 2 ml deep well plate (if processing samples in 96-well format; Greiner, Catalog no. 780270, Abgene, Catalog no. AB-0932, or equivalent)
- 96 x 300 µl U-Bottomed microtiter plate (if processing samples in 96-well format; Greiner, Catalog no. 650201 or equivalent)
- Vortex mixer
- 20 µl, 200 µl, and 1 ml sterile, pipette tips

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General Information – Individual Samples, continued

MagnaRack™

The MagnaRack™ available from Invitrogen (Catalog 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 figure below). For more information, see www.invitrogen.com or call Technical Service (page 29).

96-Well Magnetic Separator

The 96-well Magnetic Separator available from Invitrogen (Catalog 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 figures below). For more information, see www.invitrogen.com or call Technical Service (page 29).

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General Information – Individual Samples, continued

Safety Information

Follow the safety guidelines below when using the ChargeSwitch® gDNA Blood 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.
- Dispose of biological samples and all liquid waste generated during the purification procedure as biohazardous waste.

Handling the ChargeSwitch® Magnetic Beads

Follow the guidelines below 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.

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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 100 µl (10-20 µl sample) or 150 µl (50-100 µl sample) of ChargeSwitch® Elution Buffer (E5). 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.

Isolating Genomic DNA from 10-20 µl Blood Samples

Introduction

This section provides guidelines and instructions to isolate genomic DNA from 10-20 µl samples of human blood. Note that the protocol is optimized for efficient purification of DNA from these sample volumes. If you wish to isolate genomic DNA from 50-100 µl samples of human blood, use the protocol on pages 14-18.

Starting Material

Use this procedure to isolate genomic DNA from human blood samples that have been treated as follows:

- Volume: 10-20 µl of human blood
- Treatment: EDTA- or citrate-treated
- Sample state: Fresh or frozen

Materials Needed

Have the following materials on hand before beginning:

- Blood sample(s)
- MagnaRack™ (Catalog no. CS15000) or 96-well Magnetic Separator (Catalog no. CS15096)
- Sterile 1.5 ml microcentrifuge tubes
- 96 x 2 ml deep well plate (if processing samples in 96-well format)
- 96 x 300 µl U-bottomed microtiter plate (if processing samples in 96-well format)
- Vortex mixer
- Sterile pipette tips (20 µl, 200 µl, and 1 ml)

Components Supplied with the Kit

- ChargeSwitch® Lysis Buffer (L12)
- Proteinase K
- ChargeSwitch® Magnetic Beads
- ChargeSwitch® Purification Buffer (N5)
- ChargeSwitch® Wash Buffer (W12)
- ChargeSwitch® Elution Buffer (E5) or TE Buffer (not supplied; 10 mM Tris-HCl, 1 mM EDTA, pH 8.5)

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Isolating Genomic DNA from 10-20 µl Blood Samples, continued

Before Starting

Perform the following before beginning:

1. Prepare a **Lysis Mix**: **For each sample**, mix 0.5 ml of ChargeSwitch® Lysis Buffer (L12) and 5 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.
2. Vortex the tube containing the ChargeSwitch® Magnetic Beads to fully resuspend and evenly distribute the beads in the storage buffer.
3. Prepare a **Purification Mix**:**For each sample**, mix 20 µl of ChargeSwitch® Magnetic Beads (fully resuspended; see above) and 100 µl of ChargeSwitch® Purification Buffer (N5) 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 10-20 µl blood sample.

1. Transfer the 10-20 µl blood sample to a sterile microcentrifuge tube (or a 96 x 2 ml deep well plate).
2. Add 0.5 ml of Lysis Mix (see above) to the sample and pipet up and down gently 5 times to mix.

   Important: Use a 1 ml pipette tip set to 450 µl to mix the sample. Make sure that the tip is submerged, and pipet up and down gently to avoid forming bubbles.
3. Incubate the sample at room temperature for 10 minutes or until the sample is clear with no visible lumps.
4. Proceed to **Binding DNA**, next page.

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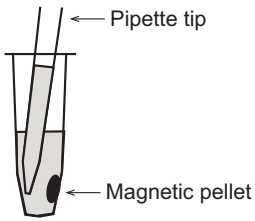
Isolating Genomic DNA from 10-20 µl Blood Samples, continued

Binding DNA

Follow the procedure below to bind the DNA to the ChargeSwitch® Magnetic Beads.

1. Gently pipet up and down the Purification Mix containing the ChargeSwitch® Magnetic Beads (see previous page) to fully resuspend the beads.
2. Add 120 µl of ChargeSwitch® Purification Mix to the digested sample (from Step 3, previous page) and pipet up and down gently 5 times to mix.

   Important: Use a 1 ml pipette tip set to 550 µl to mix the sample. Make sure that the tip is submerged, and pipet up and down gently to avoid forming bubbles.
3. Incubate at room temperature for 1 minute to allow the DNA to bind to the ChargeSwitch® Magnetic Beads.
4. Place the sample in the MagnaRack™ (or 96-Well Magnetic Separator if using a 96-well deep well plate) for 1 minute or until the beads have formed a tight pellet.
5. Without removing the sample from the MagnaRack™, 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 below).



6. Remove the sample containing the pelleted magnetic beads from the MagnaRack™. There should be no supernatant in the tube.
7. Add 500 µl of ChargeSwitch® Lysis Buffer (L12; **without** Proteinase K) to the tube and pipet up and down gently 3 times to mix. Use a 1 ml pipette tip set to 450 µl.

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Isolating Genomic DNA from 10-20 µl Blood Samples, continued

Binding DNA, continued

8. Add 50 µl of ChargeSwitch® Purification Buffer (N5) and pipet up and down gently 3 times to mix. Use a 1 ml pipette tip set to 500 µl.
9. Incubate at room temperature for 1 minute.
10. Place the sample in the MagnaRack™ (or 96-Well Magnetic Separator if appropriate) for 1 minute or until the beads have formed a tight pellet.
11. Without removing the sample from the MagnaRack™, 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 11).
12. Proceed immediately to **Washing DNA**, below.

Washing DNA

1. Remove the sample containing the pelleted magnetic beads from the MagnaRack™ (Step 11, previous page). There should be no supernatant in the tube.
2. Add 500 µl of ChargeSwitch® Wash Buffer (W12) to the sample and pipet up and down gently twice to resuspend the magnetic beads.

   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.
3. Place the sample in the MagnaRack™ for 1 minute or until the beads have formed a tight pellet.
4. Without removing the sample from the MagnaRack™, 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 11).
5. Proceed to **Eluting DNA**, next page.

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Isolating Genomic DNA from 10-20 µl Blood Samples, continued

Eluting DNA

1. Remove the sample containing the pelleted magnetic beads from the MagnaRack™ (Step 4, previous page). There should be no supernatant in the tube.
2. Add 100 µl of ChargeSwitch® Elution Buffer (E5) (or TE Buffer, pH 8.5) to the sample 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™ for 3 minutes 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 (or a 96 x 300 µl U-bottomed microtiter plate). 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 11).
6. Discard the used magnetic beads. Do not reuse the beads.

Storing DNA

Store the purified DNA at -20°C or use immediately for downstream analysis. Avoid repeatedly freezing and thawing DNA.

Quantitating DNA Yield

To quantitate the yield of your DNA, we recommend using the Quant-iT™ PicoGreen® dsDNA Quantitation Kit (Catalog no. P7589) available from Invitrogen. This kit contains the reagents necessary to allow sensitive and accurate fluorescence-based detection of as little as 25 pg/ml of dsDNA using the Quant-iT™ PicoGreen® dsDNA Quantitation Reagent. For more information about the Quant-iT™ PicoGreen® dsDNA Quantitation Kit, see www.invitrogen.com or call Technical Service (page 29).

Isolating Genomic DNA from 50-100 µl Blood Samples

Introduction

This section provides guidelines and instructions to isolate genomic DNA from 50-100 µl samples of human blood. Note that the protocol is optimized for efficient purification of DNA from these sample volumes. If you wish to isolate genomic DNA from 10-20 µl samples of human blood, use the protocol on pages 9-13.

Starting Material

Use this procedure to isolate genomic DNA from human blood samples that have been treated as follows:

- Volume: 50-100 µl of human blood
- Treatment: EDTA- or citrate-treated
- Sample state: Fresh or frozen

Materials Needed

Have the following materials on hand before beginning:

- Blood sample(s)
- MagnaRack™ (Catalog no. CS15000) or 96-well Magnetic Separator (Catalog no. CS15096)
- Sterile 1.5 ml microcentrifuge tubes
- 96 x 2 ml deep well plate (if processing samples in 96-well format)
- 96 x 300 µl U-bottomed microtiter plate (if processing samples in 96-well format)
- Vortex mixer
- Sterile pipette tips (20 µl, 200 µl, and 1 ml)

Components Supplied with the Kit

- ChargeSwitch® Lysis Buffer (L12)
- Proteinase K
- ChargeSwitch® Magnetic Beads
- ChargeSwitch® Purification Buffer (N5)
- ChargeSwitch® Wash Buffer (W12)
- ChargeSwitch® Elution Buffer (E5) or TE Buffer (not supplied; 10 mM Tris-HCl, 1 mM EDTA, pH 8.5)

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Isolating Genomic DNA from 50-100 µl Blood Samples, continued

Before Starting

Perform the following before beginning:

1. Prepare a **Lysis Mix**: **For each sample**, mix 1 ml of ChargeSwitch® Lysis Buffer (L12) 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.
2. Vortex the tube containing the ChargeSwitch® Magnetic Beads to fully resuspend and evenly distribute the beads in the storage buffer.
3. Prepare a **Purification Mix**:**For each sample**, mix 40 µl of ChargeSwitch® Magnetic Beads (fully resuspended; see above) and 200 µl of ChargeSwitch® Purification Buffer (N5) 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 50-100 µl blood sample.

1. Transfer the 50-100 µl blood sample to a sterile microcentrifuge tube or a 96 x 2 ml deep well plate.
2. Add 1 ml of Lysis Mix (see above) 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.
3. Incubate the sample at room temperature for 10 minutes or until the sample is clear with no visible lumps.
4. Proceed to **Binding DNA**, next page.

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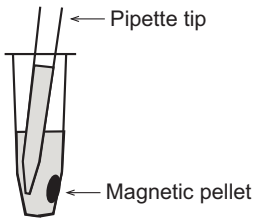
Isolating Genomic DNA from 50-100 µl Blood Samples, continued

Binding DNA

Follow the procedure below to bind the DNA to the ChargeSwitch® Magnetic Beads.

1. Gently pipet up and down the Purification Mix containing the ChargeSwitch® Magnetic Beads (see previous page) to fully resuspend the beads.
2. Add 240 µl of ChargeSwitch® Purification Mix to the digested sample (from Step 3, previous page) 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.
3. Incubate at room temperature for 1 minute to allow the DNA to bind to the ChargeSwitch® Magnetic Beads.
4. Place the sample in the MagnaRack™ (or 96-Well Magnetic Separator) for 1 minute or until the beads have formed a tight pellet.
5. Without removing the sample from the MagnaRack™, 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 below).



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

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Isolating Genomic DNA from 50-100 µl Blood Samples, continued

Washing DNA

1. Remove the sample containing the pelleted magnetic beads from the MagnaRack™ (Step 5, previous page). There should be no supernatant in the tube.
2. Add 1 ml of ChargeSwitch® Wash Buffer (W12) to the sample and pipet up and down gently twice to resuspend the magnetic beads. Use a 1 ml pipette tip set to 900 µl to mix.
3. Place the sample in the MagnaRack™ for 1 minute or until the beads have formed a tight pellet.
4. Without removing the sample from the MagnaRack™, 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 11).
5. Remove the sample containing the pelleted magnetic beads from the MagnaRack™. There should be no supernatant in the tube.
6. Add 1 ml of ChargeSwitch® Lysis Buffer (L12; **without** Proteinase K) to the tube and pipet up and down gently 3 times to mix. Use a 1 ml pipette tip set to 900 µl to mix.
7. Add 50 µl of ChargeSwitch® Purification Buffer (N5) and pipet up and down gently 3 times to mix. Use a 1 ml pipette tip set to 900 µl to mix.
8. Incubate at room temperature for 1 minute.
9. Place the sample in the MagnaRack™ for 1 minute or until the beads have formed a tight pellet.
10. Without removing the sample from the MagnaRack™, 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 11).
11. Remove the tube containing the pelleted magnetic beads from the MagnaRack™.
12. Add 1 ml of ChargeSwitch® Wash Buffer (W12) to the tube and pipet up and down gently twice to resuspend the magnetic beads. Use a 1 ml pipette tip set to 900 µl to mix.

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Isolating Genomic DNA from 50-100 µl Blood Samples, continued

Washing DNA, continued

13. Place the sample in the MagnaRack™ for 1 minute or until the beads have formed a tight pellet.
14. Without removing the sample from the MagnaRack™, 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 11).
15. Proceed to **Eluting DNA**, below.

Eluting DNA

1. Remove the sample containing the pelleted magnetic beads from the MagnaRack™ (Step 14, above). 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™ for 3 minutes 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 (or a 96 x 300 µl U-bottomed microtiter plate). 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 11).
6. Discard the used magnetic beads. Do not reuse the beads.

Storing DNA

Store the purified DNA at -20°C or use immediately for downstream analysis. Avoid repeatedly freezing and thawing DNA.

Quantitating DNA Yield

To quantitate yield of your DNA, use the Quant-iT™ PicoGreen® dsDNA Quantitation Kit (Catalog no. P7589). For more information, see page 13.

General Information – Automated Sample Processing

Introduction

This section provides general information to use the ChargeSwitch® gDNA 20 µl Blood Kit (Catalog no. CS11010-10) 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 5.

Hardware Requirements

The ChargeSwitch® chemistry is ideal for purification of DNA using open liquid handling robots, 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 10-20 µl blood samples using the ChargeSwitch® gDNA 20 µl Blood Kit:

- Any liquid handling robotic workstation with a gripper arm
- Appropriate tips for liquid dispensing and aspiration (see below for factors to consider)
- 96-Well Magnetic Separator (see page 6)
- Shaker
- 96 x 2 ml deep well plate(s) (Greiner, Catalog no. 780270 or Abgene, Catalog no. AB-0932)
- 96 x 300 µl U-Bottomed microtiter plate (Greiner, Catalog 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

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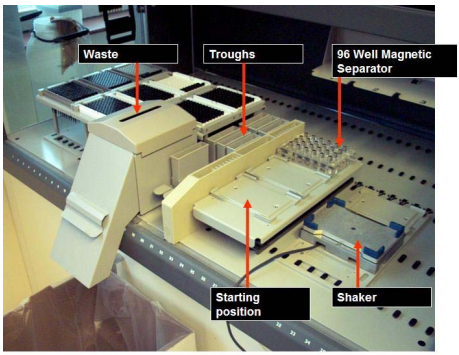
General Information – Automated Sample Processing, continued

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 below.

Location	Trough Contents	Plate
1		96-well sample tray start position
2	Purification Mix (*i.e.* ChargeSwitch® Purification Buffer (N5) + ChargeSwitch® Magnetic Beads)	
3	Lysis Mix (*i.e.* ChargeSwitch® Lysis Buffer (L12) + Proteinase K)	
4	ChargeSwitch® Purification Buffer (N5)	
5	ChargeSwitch® Lysis Buffer (L12)	
6	ChargeSwitch® Wash Buffer (W12)	
7	ChargeSwitch® Elution Buffer (E5)	
8	Waste	
9		96-Well Magnetic Separator
10		Shaker
11		96-well Sample Tray
12	ChargeSwitch® Lysis Buffer (L12)	
13		96-well U-bottomed microtiter plate (for final elution)



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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 24-25 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 10-20 µl blood samples in a 96-well format. Use this general protocol to develop the script for your liquid handling robot.

Materials Needed

Have the following materials on hand before beginning:

- Liquid handling robot configured to process samples in 96-well plates
- 10-20 µl blood samples
- 96 x 2 ml deep well plate(s)
- 96 x 300 µl U-bottomed microtiter plate

Components Supplied with the Kit

- ChargeSwitch® Lysis Buffer (L12)
- Proteinase K
- ChargeSwitch® Magnetic Beads
- ChargeSwitch® Purification Buffer (N5)
- ChargeSwitch® Wash Buffer (W12)
- ChargeSwitch® Elution Buffer (E5) or TE Buffer (not supplied; 10 mM Tris-HCl, 1 mM EDTA, pH 8.5)

continued on next page

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.

Before Starting

Perform the following before beginning:

- Prepare **Lysis Mix: For each sample**, mix 0.5 ml of ChargeSwitch® Lysis Buffer (L12) and 5 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 50 µl of ChargeSwitch® Purification Buffer (N5) and 20 µl of ChargeSwitch® Magnetic Beads (make sure that the beads are thoroughly resuspended) to prepare the Purification Mix. Scale up the volume of reagents used (based on number of samples) to prepare a master mix.

continued on next page

Automated Genomic DNA Isolation, continued

Automated Protocol

Follow the protocol below to isolate genomic DNA from 10-20 µl blood samples. The volumes given are on a per sample basis.

1. Start with 96 x 10-20 µl blood samples in a 96 x 2 ml deep well plate.
2. Add 500 µl of Lysis Mix (see previous page) and incubate at room temperature for 10 minutes. Once during the incubation, pipet up and down gently 15 times to mix. Set the pipette tip to 350 µl and avoid forming bubbles.
3. Add 70 µl of Purification Mix (see previous page; make sure that the beads are thoroughly resuspended)
4. Shake at medium fast speed (*e.g.* pulse, 10 seconds) to evenly distribute the magnetic beads within the solution.
5. Shake samples rapidly for 20 seconds to mix.
6. Wait for 30 seconds.
7. Move samples to the 96-Well Magnetic Separator.
8. Wait for 90 seconds.
9. Slowly aspirate all of the supernatant and discard, leaving behind the pellet of beads.
10. Remove samples from the 96-Well Magnetic Separator.
11. Add 500 µl of ChargeSwitch® Lysis Buffer (L12; no Proteinase K) and shake samples rapidly for 20 seconds to evenly distribute the magnetic beads within the solution.
12. Add 50 µl of ChargeSwitch® Purification Buffer (N5) and shake at medium speed for 20 seconds to mix. Samples should appear clear, with no brown flecks.
13. Wait for 30 seconds.
14. Move samples to the 96-Well Magnetic Separator.
15. Wait for 60 seconds.
16. Slowly aspirate all of the supernatant and discard, leaving behind the pellet of beads. Proceed to the next page.

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Automated Genomic DNA Isolation, continued

Automated Protocol, continued

17. Remove samples from the 96-Well Magnetic Separator.
18. Add 500 µl of ChargeSwitch® Wash Buffer (W12).
19. Shake at medium speed (*e.g.* pulse, 10 seconds) to evenly distribute the magnetic beads within the solution.
20. Move samples to the 96-Well Magnetic Separator.
21. Wait for 60 seconds.
22. Slowly aspirate all of the supernatant and discard, leaving behind the pellet of beads.
23. Leave samples on the 96-Well Magnetic Separator for the second wash.
24. Add 500 µl of ChargeSwitch® Wash Buffer (W12).
25. Wait for 30-60 seconds.
26. Slowly aspirate all of the supernatant and discard, leaving behind the pellet of beads.
27. Move samples to the shaker.
28. Add 100 µl of Elution Buffer. Pipet up and down gently 50 times to mix (set the pipette tip to 75 µl).
29. Shake rapidly for 1-2 minutes to completely disperse the beads within the solution.
30. Move samples to the 96-Well Magnetic Separator.
31. Wait for 1 minute.
32. Slowly aspirate **supernatant containing the DNA** to a 96 x 300 µl U-bottomed microtiter plate.

Storing DNA

Store the purified DNA at -20°C or use immediately for downstream analysis. Avoid repeatedly freezing and thawing DNA.

Quantitating DNA Yield

To quantitate yield of your DNA, use the Quant-iT™ PicoGreen® dsDNA Quantitation Kit (Catalog no. P7589). For more information, see page 13.

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	• Decrease the amount of starting material used. • Be sure to add Proteinase K during lysis. • Increase the length of incubation at room temperature.
	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™ 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.
	Incomplete dissociation of DNA from the ChargeSwitch® Magnetic Beads	Perform additional mixing of the suspension of beads (by pipetting up and down).

continued on next page

Troubleshooting, continued

Problem	Cause	Solution
Low DNA yield, continued	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 to elute DNA. 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.
	Added Lysis Buffer containing Proteinase K during second rebinding step	Use ChargeSwitch® Lysis Buffer (L12) without Proteinase K during the second rebinding step.
	Didn't add Purification Buffer during second rebinding step	You must add ChargeSwitch® Purification Buffer (N5) during the second rebinding step to adjust the pH of the solution to allow binding of DNA to the beads.

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Troubleshooting, continued

Problem	Cause	Solution
No DNA recovered, continued	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.
DNA is sheared or degraded	Lysate mixed too vigorously or small pipette tips used during mixing	• Use a 1 ml pipette tip set to 900 µl to mix the sample. • Pipet up and down gently to mix.
	Bubbles formed during mixing steps	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

Technical Service

World Wide Web

Visit the Invitrogen Web Resource using your World Wide Web browser. At the site, you can:

- Get the scoop on our hot new products and special product offers
- View and download vector maps and sequences
- Download manuals in Adobe® Acrobat® (PDF) format
- Explore our catalog with full color graphics
- Obtain citations for Invitrogen products
- Request catalog and product literature

Once connected to the Internet, launch your Web browser (Internet Explorer 5.0 or newer or Netscape 4.0 or newer), then enter the following location (or URL):

http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

Contact Us

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

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Technical Service, continued

MSDS Requests

To request an MSDS, visit our Web site at **www.invitrogen.com.** On the home page, go to 'Technical Resources', select 'MSDS', and follow instructions on the page.

Limited Warranty

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Purchaser Notification and Product Qualification

Purchaser Notification

Limited Use Label License No. 265: ChargeSwitch® Technology

The use of this product may be covered by European Patent No. EP1036082B1 and foreign equivalents.

Product Qualification

Each kit is functionally tested to ensure conformance with the most current approved product specifications. Current specifications consist of tests for:

- Bead size, charge, and binding capacity
- Nucleic acid quality and quantity
- Buffer turbidity, volume, and absence of RNases and DNases
- Kit packaging and labeling accuracy

For individual lot test results and more information, visit www.invitrogen.com to download the Certificate of Analysis.

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