invitrogen™
by life technologies™

iPrep™ ChargeSwitch® Forensic and Buccal Cell Kits

For purification of genomic DNA from forensic and buccal samples using the iPrep™ Instrument

Catalog nos. IS10002 and IS10003

Rev. date: 15 August 2010
Manual part no. 25-0964

MAN0000582

User Manual

Contents

Experienced Users Procedure

Introduction

This quick reference sheet is included for experienced users of the iPrep™ ChargeSwitch® Forensic and Buccal Cell Kits. For more details, refer to this manual.

Step	Procedure
Prepare lysate	1. Prepare **Lysis Mix: For each sample**, mix 1 mL ChargeSwitch® Lysis Buffer (L13 for Forensic Kit or L11 for Buccal Kit) and 10 µL Proteinase K to prepare the Lysis Mix. 2. Transfer the forensic sample or buccal swab sample to a sterile 1.5 mL microcentrifuge tube. 3. Add 1 mL Lysis Mix (see recipe, above) to the tube. Ensure the sample is completely immersed in the Lysis Mix. 4. Vortex or invert the tube for 10–15 seconds to mix. 5. Incubate as follows: • 1 hour at 55°C (for Forensic Kit) • 20 minutes at 37°C (for Buccal Cell Kit) 6. Remove any solid material from the lysate using sterile forceps. To avoid any loss in sample volume, press the solid material against the inside of the tube. Transfer the samples (~1 mL) to iPrep™ Sample and Elution Tubes.
Purification Protocol	1. Open the iPrep™ Card Slot and insert the iPrep™ Card: gDNA Forensic into the slot (arrow on the card is at the top and card label is facing your left side). 2. Turn **ON** the iPrep™ Instrument using the power switch on the left side of the instrument. The digital display shows the version for the iPrep™ instrument which changes in few seconds to display the Main menu. 3. Press **Start** to run a protocol. 4. Press **1** to select the Buccal protocol or press **2** to select Forensic protocol. 5. Open the iPrep™ instrument door and remove the iPrep™ Racks to set up the platform. 6. Remove the iPrep™ Cartridges from the box. To collect any solution from the foil, tap the cartridge to deposit the solution at the bottom of the tube.

Continued on next page

Experienced Users Procedure, Continued

Step	Procedure
Prepare tissue lysate	1. Load the desired number of cartridges on the iPrep™ Cartridge Rack. Insert the loaded iPrep™ Cartridge rack on to the iPrep™ Platform. 2. Load the iPrep™ Tip and Tube Rack as follows: • Load the first row (labeled as **E**) with 1–13 elution tubes **without caps** • Keep the second row (labeled as **T1**) empty • Load the third row (labeled as **T2**) with iPrep™ Tips in iPrep™ Tip Holders • Load the fourth row (labeled as **S**) with sample tubes **without caps** containing the lysate (~1 mL). 3. Read the sample and elution tube barcode, if needed. 4. Insert the iPrep™ Tip and Tube Rack on to the iPrep™ Platform. 5. Close the iPrep™ Door. Press **1** to continue. 6. Select the appropriate elution volume on the display. 7. Press **Start**. The automated purification protocol begins and various steps of the protocol including the approximate time remaining are displayed on the digital display. 8. At the end of the run, the instrument beeps briefly and the digital display shows **Protocol Finished** for 10 seconds. The Main menu appears after 10 seconds. 9. Open the instrument door. 10. Remove and cap the elution tubes containing the purified nucleic acid. Store the purified gDNA at 4ºC (short-term) or aliquot and store at –20ºC (long-term). 11. Discard the used cartridges, tips, and tubes into biohazard waste. Do not reuse the cartridges. 12. To purify more samples using the same iPrep™ Card, load the racks with new cartridges, tips, tubes, and samples, and start the protocol as described above. 13. If you are not using the instrument, close the instrument door and turn the power switch to **OFF**. 14. Remove and store the iPrep™ Card in the box.

Kit Contents and Storage

Types of Kits

This manual is supplied with the following products.

Product	Number of Purifications	Cat. no.
iPrep™ ChargeSwitch® Forensic Kit	52	IS10002
iPrep™ ChargeSwitch® Buccal Cell Kit	52	IS10003

Shipping and Storage

Each iPrep™ ChargeSwitch® Forensic Kit and iPrep™ ChargeSwitch® Buccal Cell Kit contains Reaction Cartridges and disposable plasticware (Box 1) and sample preparation reagents (Box 2).

Upon receipt, store Box 1 at room temperature and Box 2 at 4°C. See below for kit contents.

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

Box 1 Contents

The components supplied in the iPrep™ ChargeSwitch® Forensic and Buccal Cell Kit (Box 1) are listed below.

Store Box 1 at room temperature. Do not freeze the iPrep™ Cartridge Kit.

Sufficient reagents are supplied to perform 52 purifications.

Reagents	Amount
iPrep™ Forensic Cartridge Kit (Cat. no. IS10002)	1 kit
iPrep™ Buccal Cell Cartridge Kit (Cat. no. IS10003)	1 kit
iPrep™ Sample and Elution Tubes	2 × 52 tubes
iPrep™ Tips and Tip Holders	1 bag with 52 tips and holders

Continued on next page

Kit Contents and Storage, Continued

iPrep™ Forensic Cartridge Kit Contents

Each iPrep™ Forensic Cartridge has 12 positions with 10 sealed wells and two heating positions (position 12 with an empty well and position 11 to add an empty or reagent filled tube).

Positions 1–5 contain wells filled with reagents while positions 6–10 contain empty wells for this protocol.

The components supplied in each well of the iPrep™ Forensic Cartridge Kit (Box 1) are listed below.

Do not freeze the iPrep™ Forensic Cartridge Kit.

Reagent	Well no.
ChargeSwitch® Magnetic Beads (13.5 mg/mL in 10 mM potassium acetate, pH 4.0, 6.6 mM KCl)	1
ChargeSwitch® Binding Buffer (N5)	2
ChargeSwitch® Wash Buffer (W12)	3
ChargeSwitch® Wash Buffer (W12)	4
ChargeSwitch® Elution Buffer (E5, 10 mM Tris-HCl, pH 8.5)	5
Empty	6–10

Box 2 Contents for Forensic Kit

The components supplied in the iPrep™ ChargeSwitch® Forensic Kit (Box 2) are listed below.

Store Box 2 at 4°C.

Sufficient reagents are supplied to perform 52 purifications.

Reagents	Amount
Proteinase K (20 mg/mL in 50 mM Tris-HCl, pH 8.0, 5 mM $CaCl_2$, 50% glycerol)	550 µL
ChargeSwitch® Lysis Buffer (L13)	55 mL

Continued on next page

Kit Contents and Storage, Continued

iPrep™ Buccal Cartridge Kit Contents

Each iPrep™ Buccal Cartridge has 12 positions with 10 sealed wells and two heating positions (position 12 with an empty well and position 11 to add an empty or reagent filled tube).

Positions 1–5 contain wells filled with reagents while positions 6–10 contain empty wells for this protocol.

The components supplied in each well of the iPrep™ Buccal Cartridge Kit (Box 1) are listed below.

Do not freeze the iPrep™ Buccal Cartridge Kit.

Reagent	Well no.
ChargeSwitch® Magnetic Beads (27 mg/mL in 10 mM potassium acetate, pH 4.0, 6.6 mM KCl)	1
ChargeSwitch® Binding Buffer (N6)	2
ChargeSwitch® Wash Buffer (W12)	3
ChargeSwitch® Wash Buffer (W12)	4
ChargeSwitch® Elution Buffer (E5, 10 mM Tris-HCl, pH 8.5)	5
Empty	6–10

Box 2 Contents for Buccal Kit

The components supplied in the iPrep™ ChargeSwitch® Buccal Cell Kit (Box 2) are listed below.

Store Box 2 at 4°C.

Sufficient reagents are supplied to perform 52 purifications.

Reagents	Amount
Proteinase K (20 mg/mL in 50 mM Tris-HCl, pH 8.0, 5 mM $CaCl_2$, 50% glycerol)	550 µL
ChargeSwitch® Lysis Buffer (L11)	55 mL

Intended Use

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

Introduction

Product Overview

Introduction

The ChargeSwitch® Forensic and Buccal Cell Kits allow rapid, automated purification of genomic DNA from forensic samples and human buccal swabs using the ChargeSwitch® Technology with the iPrep™ Purification Instrument.

After preparing the lysates, genomic DNA is purified within 20 minutes.

For more information about the ChargeSwitch® Technology, see page 3.

Intended Use for the Kits

Forensic Kit

The iPrep™ ChargeSwitch® Forensic Kit is designed to allow isolation of genomic DNA from the following forensic samples. The purified genomic DNA is suitable for use in short tandem repeat (STR) analysis.

- Dried blood spots on paper and clothing
- Hair follicles and hair shafts
- Cigarette butts
- Semen
- "Touch DNA" (*e.g.* tools, mobile phones, and microscopes)

Note: These kits are also suitable for mitochondrial DNA (mtDNA) extraction from hair follicles for single nucleotide polymorphism (SNP) analysis.

Buccal Cell Kit

The iPrep™ ChargeSwitch® Buccal Cell Kit is designed for isolating 1–3 µg genomic DNA from human buccal cell swabs. Samples can be stored for up to 2 weeks at 4°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.

Continued on next page

Product Overview, Continued

Advantages

Use of the iPrep™ ChargeSwitch® Forensic and 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 automated purification of genomic DNA from up to 12 samples and a control in less than 20 minutes following sample lysis using the iPrep™ Instrument
- Pre-filled reagent cartridges provide easy set up and consistent results
- Minimal contamination with RNA
- The purified genomic DNA demonstrates improved performance in downstream applications
- Includes a flexible protocol to facilitate genomic DNA isolation from a large variety of forensic sample types

System Specifications

Starting Material:	Varies; optimized for samples with low DNA content (Forensic Kit)
	Human buccal swabs (Buccal Cell Kit)
Elution Volume:	75 µL or 150 µL
DNA Yield:	Varies (depends on sample size and type)
DNA Size:	Varies (depends on quality of starting material)

Continued on next page

Product 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 nucleic 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:	27 mg/mL (Buccal Kit)
	13.5 mg/mL (Forensic Kit)
Storage Buffer:	10 mM potassium acetate, pH 4.0, 6.6 mM KCl

iPrep™ Purification Instrument

Introduction

The iPrep™ Purification Instrument is a benchtop, automated nucleic acid purification instrument with integrated Magnetic and Syringe unit capable of purifying nucleic acids from up to 13 samples (12 samples and one control). Each iPrep™ Instrument consists of the Magnetic and Syringe Unit, and a platform. A pre-programmed iPrep™ Protocol Card controls the purification parameters such as buffer volumes, mixing steps, and incubation time.

For more details on the iPrep™ Purification Instrument, see the manual supplied with the instrument.

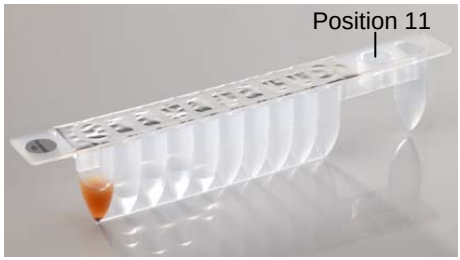
iPrep™ Reaction Cartridge

The iPrep™ Reaction Cartridges are supplied with the iPrep™ Kits and are designed to fit onto the iPrep™ Cartridge Rack in only one orientation. Each cartridge is pre-filled with reagents required for the iPrep™ ChargeSwitch® Forensic and Buccal Cell protocol.

Each cartridge has 12 positions with 10 sealed wells and two heating positions (position 12 with an empty well and position 11 to add an empty or reagent filled tube). For the iPrep™ ChargeSwitch® Forensic and Buccal Cell Kits, positions 1–5 contain wells filled with reagents and wells 6-10 are empty.

Cartridge Specifications:

Material:	Polypropylene cartridge sealed with laminated aluminum foil
Max Volume:	1,000 µL/well
Dimension:	5.9 inches (l) × 1.2 inches (w) × 0.7 inches (d)



Continued on next page

iPrep™ Purification Instrument, Continued

iPrep™ Tips and Tip Holders

The iPrep™ Tips and Tip Holders are included with the iPrep™ Kits and are placed on the iPrep™ Tip and Tube Rack as described on page 16. While assembling the tips on the rack, always insert the iPrep™ Tips into the iPrep™ Tip Holders using gloved hands. Always use the tips with the holders to prevent any contamination.

Tip Specifications:

Tip Material:	Polypropylene with filter barriers
Tip Holder Material:	Polypropylene
Volume:	5–1,000 µL
Tip Dimension:	3.9 inches (l) × 0.43 inches (d)

iPrep™ Tip Holder

iPrep™ Tip

iPrep™ Tubes

Two sets of iPrep™ Tubes are required for the purification protocol. The iPrep™ Sample and Elution Tubes are included with each iPrep™ Kit and placed on the iPrep™ Tip and Tube Rack as described on page 14.

Tube Specifications:

Material:	Polypropylene
Capacity:	1.5 mL
Style:	Tubes with caps
Dimensions:	1.7 inches (l) × 0.4 inches (d)

Continued on next page

iPrep™ Purification Instrument, Continued

iPrep™ Card

To use the iPrep™ ChargeSwitch® Forensic and Buccal Cell Kits with the iPrep™ Purification Instrument, you need to purchase the iPrep™ Card: gDNA Forensic (page 23).

The iPrep™ Card: gDNA Forensic (includes buccal protocol) is pre-programmed with the purification protocol (for forensic and buccal) that directs the volume of reagents used and incubation time.

Always store the card in the box, protected from light.

To avoid damaging the card:

- Do not drop or bend the card
- Do not wipe or clean the card using volatile chemicals such as alcohol or equivalent
- Do not expose the card to water

iPrep™ Platform

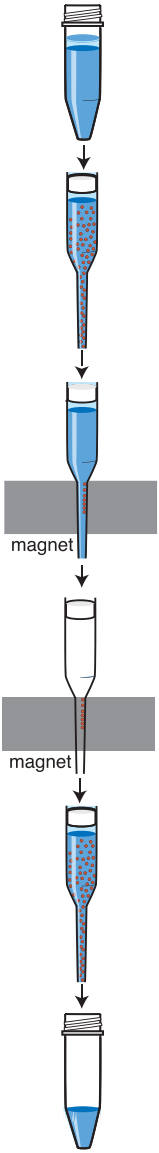
The platform on the iPrep™ Instrument allows the placement of iPrep™ Tip and Tube Rack, and iPrep™ Cartridge Rack that are filled with plastic disposables and reagent cartridges required for the purification protocol.

Set up the platform as shown in the figure on page 16 for the iPrep™ ChargeSwitch® Forensic and Buccal Cell Kits.

Experimental Outline

Introduction

The figure below illustrates the basic steps necessary to purify genomic DNA using the iPrep™ ChargeSwitch® Forensic and Buccal Cell Kits.



Sample Lysis

DNA binds to ChargeSwitch® Magnetic beads at pH <6.0

Magnetic separation of beads in tips

Separation of liquid solution from beads

ChargeSwitch® Magnetic beads are washed (pH = 7.0)

Purified DNA is eluted from beads at pH > 8.5

Methods

General Information

User Supplied Materials

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

- iPrep™ Purification Instrument (page 23)
- iPrep™ Card: gDNA Forensic (includes buccal protocol, page 23)
- Sterile, 1.5 mL elution tubes or you may use iPrep™ Tubes
- Water bath or heat block
- Forensic samples (for Forensic Kit)
- Buccal swabs (for Buccal Cell Kit)

Follow the recommendations below to obtain the best results:

- Maintain a sterile environment when handling DNA to avoid any contamination from DNases
- Ensure that no DNases are introduced into the sterile solutions of the kit
- Do not vortex the samples for more than 5–10 seconds during sample lysis to avoid extensive shearing of DNA
- To minimize DNA degradation, perform lysate preparation steps quickly, and avoid repeated freezing and thawing of DNA samples
- Do not freeze the beads as this irreparably damages them. Store the beads at room temperature.
- When using the beads from the Reaction Cartridges, collect any solution from the foil by tapping the cartridge to deposit the solution at the bottom of the tube. Do not allow the beads to dry out as this renders them non-functional.
- Discard Reaction Cartridges, iPrep™ Tips, and iPrep™ Tip Holders after use. Do not reuse.

Continued on next page

General Information, Continued

Safety Information

Follow the safety guidelines below when using the iPrep™ ChargeSwitch® Forensic and 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.

Preparing Forensic Samples

Introduction

Instructions are included in this section to prepare lysates from forensic samples (see page 1 for a list of sample types) using the reagents supplied in the iPrep™ ChargeSwitch® Forensic Kit (page 23).

The protocol is optimized for efficient purification of DNA from small sample volumes. Depending on the volume and type of your sample, some further optimization of the protocol may be required.

Handling Samples

Follow these general guidelines when handling forensic samples:

- Process samples immediately after collection or store at 4°C for up to 2 weeks
- Where possible and appropriate, cut up the sample into small pieces (*i.e.* ~0.5 cm^2 or 0.25 cm^3) to facilitate processing.
- Avoid overloading the sample tube to allow efficient mixing of Lysis Mix with the sample.
- When dealing with blood-stained items, take care to ensure that the amount of blood processed is kept to a minimum (≤ 20 µL blood spot). Processing of larger heavily blood-stained items may result in contamination of the purified DNA with heme.

Starting Material– Common Sample Types

Use this procedure to isolate genomic DNA from any of the forensic sample types listed on page 1. When processing the following common sample types, we recommend using these amounts of starting material:

- Dried blood spots: Process 1–5 punched-out circles from the dried blood spot (paper hole punch, ~5 mm diameter)
- Hair follicles: Process the root of the end of the human hair
- Cigarette butts: Cut ~8 mm from the end of the cigarette butt, and place end down in the tube

Continued on next page

Preparing Forensic Samples, Continued

Materials Needed

You will need the following materials:

- Forensic sample(s)
- ChargeSwitch® Lysis Buffer (L13), supplied in the kit
- Proteinase K (supplied in the kit)
- Sterile 1.5 mL microcentrifuge tubes or you may use iPrep™ Tubes
- Sterile forceps
- Vortex mixer
- Water bath or heat block set to 55ºC

Before Starting

Perform the following before beginning:

- Set a water bath at 55°C.
- **Prepare Lysis Mix: For each sample**, mix 1 mL ChargeSwitch® Lysis Buffer (L13) and 10 µL 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.

Preparing the Lysate

Follow the procedure below to prepare a lysate from the sample.

1. Transfer the forensic sample to a sterile 1.5 mL microcentrifuge tube or an iPrep™ Tube.
2. Add 1 mL Lysis Mix (see recipe, above) to the tube. Ensure the sample is completely immersed in Lysis Mix.
3. Vortex or invert the tube for 10–15 seconds to mix.
4. Incubate for 1 hour at 55°C.

   Note: Vortex or invert the tube during this period.
5. Remove any solid material from the lysate using sterile forceps. To avoid any loss in sample volume, press the solid material against the inside of the tube. Transfer the samples (~1 mL) to the iPrep™ Sample and Elution Tubes.
6. Proceed immediately to the **Genomic DNA Purification Protocol**, page 14.

Preparing Human Buccal Cell Samples

Introduction

Instructions are included in this section to prepare lysates from human buccal cell sample using the reagents supplied in the iPrep™ ChargeSwitch® Buccal Cell Kit (see page 23 for purchasing).

Starting Material

Use this procedure to isolate genomic DNA from human buccal cell swabs. Process samples immediately after collection or store at 4°C for up to 2 weeks. **Do not** store unprocessed samples at room temperature as buccal swabs may contain bacteria and nucleases that can degrade DNA.

Materials Needed

You will need the following materials:

- Buccal swab(s)
- ChargeSwitch® Lysis Buffer (L11), supplied in the kit
- Proteinase K (supplied in the kit)
- Sterile 1.5 mL microcentrifuge tubes or you may use iPrep™ Tubes
- Sterile forceps
- Vortex mixer
- Water bath or heat block set to 37ºC

Before Starting

Perform the following before beginning:

- Set a water bath at 37°C.
- **Prepare Lysis Mix: For each sample**, mix 1 mL ChargeSwitch® Lysis Buffer (L11) and 10 µL 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.

Continued on next page

Preparing Human Buccal Cell Samples,
Continued

Preparing the Lysate

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

1. Transfer the buccal swab sample to a sterile 1.5 mL microcentrifuge tube.
2. Add 1 mL Lysis Mix (see recipe, previous page) to the tube. Ensure the sample is completely immersed in the Lysis Mix.
3. Vortex or invert the tube for 10–15 seconds to mix.
4. Incubate at 37°C for 20 minutes.

   Note: Vortex or invert the tube during this period.
5. Remove any solid material from the lysate using sterile forceps. To avoid any loss in sample volume, press the solid material against the inside of the tube. Transfer the samples (~1 mL) to the iPrep™ Sample and Elution Tubes.
6. Proceed immediately to the **Genomic DNA Purification Protocol**, next page.

Genomic DNA Purification Protocol

Introduction

Instructions are included in this section to purify genomic DNA from forensic and buccal cell samples using the iPrep™ Purification Instrument.

Materials Needed

You will need the following materials:

- Lysates (pages 10–12)
- iPrep™ Purification Instrument (page 23)
- iPrep™ Card: gDNA Forensic (includes buccal protocol, page 23)

Components Supplied with the Kit

- iPrep™ Forensic Cartridge Kit (for Forensic Kit only)
- iPrep™ Buccal Cell Cartridge Kit (for Buccal Cell Kit only)
- iPrep™ Sample and Elution Tubes
- iPrep™ Tips and Tip Holders

Before Starting

Perform the following before starting:

- Prepare the lysates from the forensic or buccal swabs as described
- Ensure that you have the iPrep™ Card: gDNA Forensic (page 23) to run the protocol
- Make sure the iPrep™ Purification Instrument is unpacked and installed

Continued on next page

Genomic DNA Purification Protocol,
Continued

Purification Protocol

Purify genomic DNA from the lysate using the iPrep™ Purification Instrument as described below.

For details on using the iPrep™ Purification Instrument, refer to the manual supplied with the instrument.

Insert the iPrep™ Card: gDNA Forensic (available separately from Invitrogen, page 23) prior to turning on the instrument.

1. Ensure the power switch on the iPrep™ Instrument is on the **OFF** position.
2. Open the iPrep™ Card Slot and insert the iPrep™ Card: gDNA Forensic into the slot in the correct orientation (arrow on the card is at the top and card label is facing your left side).
3. Using the power switch located on the left side of the instrument, turn **ON** the instrument.

   If the card is fully inserted in the correct orientation, all axes return to their original positions automatically. The digital display shows the version for the iPrep™ which changes in a few seconds to display the Main menu.
4. Press **Start** to run a protocol.
5. Press **1** to select the Buccal protocol for buccal samples or Press **2** to select the Forensic protocol for forensic samples.
6. Open the iPrep™ instrument door and remove the iPrep™ Cartridge Rack and iPrep™ Tip and Tube Rack to set up the platform.
7. Remove the iPrep™ Cartridges from the box. To collect any solution from the foil, tap the cartridge to deposit the solution at the bottom of the tube.
8. Load the desired number of cartridges on the iPrep™ Cartridge Rack. Insert the loaded rack on the iPrep™ Platform.

   Note: You can load 1–13 cartridges on the rack depending on the number of samples that you wish to process. If you are loading less than 13 cartridges, ensure that the remaining plastic ware (tips and tubes) are also loaded in the same order as the cartridges.

Continued on next page

Genomic DNA Purification Protocol,
Continued

Purification Protocol, Continued

Procedure continued from previous page

9. Load the iPrep™ Tip and Tube Rack as follows (see figure below):
   - Load the first row (labeled as **E**) with 1–13 elution tubes **without caps** (you may place the caps on the rack as shown in the figure below)
   - Keep the second row (labeled as **T1**) empty
   - Load the third row (labeled as **T2**) with iPrep™ Tips in the iPrep™ Tip Holders
   - Load the fourth row (labeled as **S**) with sample tubes **without caps** containing the lysate (~1 mL)

10. Read the sample and elution tube barcode if needed.
11. Load the iPrep™ Tip and Tube Rack on the iPrep™ Platform.
12. Close the iPrep™ instrument door.
13. Press **1** to continue.
14. Select the appropriate elution volume on the display.
15. Ensure that you have loaded the cartridges, tubes, and tips in the appropriate positions, and the sample and elution tubes **do not have any caps**.

Continued on next page

Genomic DNA Purification Protocol,
Continued

Purification Protocol, Continued

16. Press **Start**. The automated purification protocol begins and various steps of the protocol including the approximate time remaining are displayed.

    Important: Do not open the door once the protocol has begun.

    To pause the protocol, press the **Stop** key. To resume the protocol after a pause, press the **Start** key. To cancel/stop the protocol, press the **Stop** key twice. For details, see the iPrep™ Instrument manual.

17. At the end of the run, the instrument beeps briefly and the digital display shows **Protocol Finished** for 10 seconds. The Main menu appears after 10 seconds.
18. Open the instrument door. Remove and cap the elution tubes containing the purified nucleic acid. Store the purified gDNA as described below.
19. Discard the used cartridges, tips, and tubes into biohazard waste. Do not reuse the cartridges.
20. To purify more samples using the same iPrep™ protocol card, load the racks with new cartridges, tips, tubes, and samples, and start the protocol as described above.
21. If you are not using the instrument, close the instrument door and turn the power switch to **OFF**.
22. Remove the iPrep™ Card and store the card in the box, protected from light.

Storing DNA

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

DNA Quantitation and Analysis

Quantitating DNA Yield

You may estimate the yield of purified genomic DNA by checking the UV absorbance at 260 nm or using one of the Quant-iT™ DNA Assay 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 the formula:

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

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

Quant-iT™ DNA Assay Kits

The Quant-iT™ DNA Assay Kits (see page 23 for ordering information) provide a rapid, sensitive, and accurate method for dsDNA quantitation with minimal interference from RNA, protein, ssDNA (primers), or other common contaminants that affect UV absorbance. Each kit contains a state-of-the-art quantitation reagent, pre-diluted standards for a standard curve, and a pre-made buffer to allow fluorescence-based DNA quantitation. For more information, see www.invitrogen.com or call Technical Support (page 24).

Note

Since the yield of DNA isolated from forensic samples varies depending on the quality of the starting material, it may be difficult to quantitate DNA yield in samples with very low DNA yield.

We recommend analyzing the DNA from forensic samples using STR (short tandem repeat) analysis as generation of STR profiles is the main downstream application for genomic DNA isolated using forensic samples.

Continued on next page

DNA Quantitation and Analysis, Continued

Analyzing DNA Quality

Typically, DNA isolated using the iPrep™ ChargeSwitch® Forensic and Buccal Cell Kits has an A_{260}/A_{280} >1.70 when samples are diluted in Tris-HCl (pH 7.5) indicating that the DNA is reasonably clean of proteins that could interfere with downstream applications.

Purified gDNA may be analyzed by agarose gel electrophoresis to check the DNA quality (usually a single band at >40 kb with no smearing) and confirm the absence of contaminating RNA.

Genomic DNA isolated with the iPrep™ ChargeSwitch® Buccal Cell Kit is usually >40 kb in size.

Note: Size usually varies and is dependent on the quality of the starting material. For buccal samples, the DNA usually appears fragmented with many fragments at <40 kb. This is because the buccal cells are apoptotic and DNA is sometimes degraded by nucleases from bacteria present in the mouth that appear in the buccal swab sample during collection. See next page for an example DNA quality isolated from buccal cell samples.

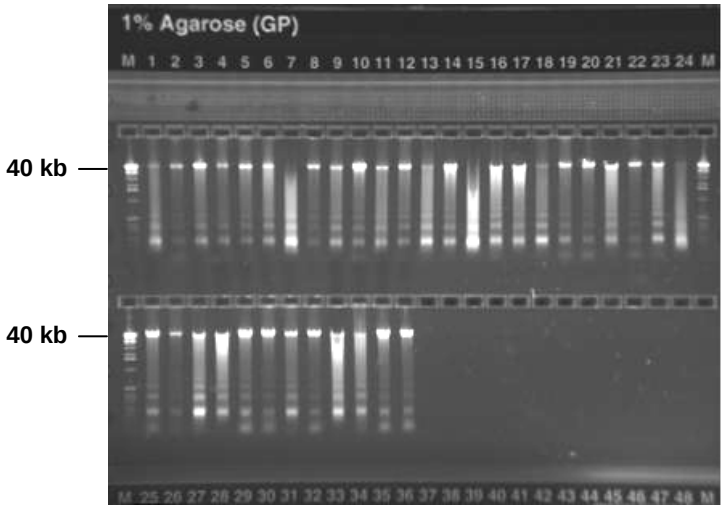
Expected Results

Example of DNA Quality

Genomic DNA (10 µL) isolated from 36 human buccal swab samples was analyzed by agarose gel electrophoresis on a 1% E-Gel® *48* gel.

Samples on the gel are:

Lanes 1–36:	Human buccal swab samples
Lanes M:	1 Kb DNA Extension Ladder (0.5 µg/lane)



Troubleshooting

Introduction

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

To troubleshoot problems with the iPrep™ Purification Instrument, refer to the manual supplied with the instrument.

Observation	Cause	Solution
Low DNA yield	Incomplete lysis	• Decrease the amount of starting material used. • Be sure to add Proteinase K during lysis. • Make sure that the sample is completely immersed in the Lysis Buffer. • Increase the length of incubation at 37ºC or 55°C.
	Poor quality of starting material	Be sure to process sample immediately after collection or store the sample at 4°C. The yield and quality of DNA isolated depends on the starting material. You may need to increase the amount of starting material used or use an alternative sample.
	Insufficient amount of ChargeSwitch® Magnetic Beads added	During shipping, some ChargeSwitch® Magnetic bead solution may adhere to the sealing foil of the cartridge. To collect any bead solution from the foil, tap the cartridge to deposit the bead solution at the bottom of the tube.

Continued on next page

Troubleshooting, Continued

Observation	Cause	Solution
No DNA recovered	ChargeSwitch® Magnetic Beads stored or handled improperly	• Store cartridge containing the beads at room temperature. Do not freeze the cartridge as the beads may be irreparably damaged. • Make sure that the beads are in solution at all times and do not dry. Dried beads are non-functional.
Eluate containing DNA is discolored	• Magnetic beads present in the eluate • DNA contaminated with heme	• Remove any magnetic beads using a magnetic separator (MagnaRack™ separator is available from Invitrogen, see page 23) or centrifuge the sample in a microcentrifuge for 1 minute at maximum speed. • Minimize the amount of blood or blood-stained sample used (≤20 µL blood spot).
DNA is sheared or degraded	Lysate mixed too vigorously	Vortex gently to avoid DNA damage.
	Bubbles formed during mixing steps	Make sure that the sample volume is at least 600 µL to prevent excessive bubble formation during mixing.
	Purified DNA repeatedly frozen and thawed	Aliquot purified DNA and store at 4°C (short-term) or –20°C (long-term). 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 table below lists additional products available from Invitrogen that may be used with iPrep™ ChargeSwitch® Forensic and Buccal Cell Kits.

For more information about these products, visit www.invitrogen.com or call Technical Support (page 24).

Product	Amount	Cat. no.
iPrep™ Purification Instrument	1 unit	IS10000
iPrep™ Card: gDNA Forensic (includes buccal protocol)	1 card	IS10011
iPrep™ Card: gDNA Tissue	1 card	IS10013
iPrep™ Card: Viral DNA/RNA	1 card	IS10016
iPrep™ Card: gDNA Blood	1 card	IS10012
iPrep™ Card: Buffy Coat	1 card	IS10015
iPrep™ ChargeSwitch® Forensic Kit	1 kit (52 purifications)	IS10002
iPrep™ ChargeSwitch® Buccal Cell Kit	1 kit (52 purifications)	IS10003
iPrep™ ChargeSwitch® gDNA Tissue Kit	1 kit (52 purifications)	IS10004
iPrep™ Small Tips	1 bag of 52 tips	IS10111
iPrep™ Tip and Tube Rack	1 rack	IS10101
iPrep™ Cartridge Rack	1 rack	IS10102
Quant-iT™ PicoGreen® dsDNA Assay Kit	1 kit	P7589
Quant-iT™ DNA Assay Kit, High Sensitivity	1000 assays	Q33120
Quant-iT™ DNA Assay Kit, Broad-Range	1000 assays	Q33130
Qubit® Fluorometer	1 each	Q32857
MagnaRack™ Magnetic Separator	1 rack	CS15000

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. In addition, a large variety of DNA ladders is available from Invitrogen for sizing DNA. For more information about these products, see www.invitrogen.com or call Technical Support (page 24).

Technical Support

World Wide Web

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

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

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).

Corporate Headquarters:	**European Headquarters:**
5791 Van Allen Way	Inchinnan Business Park
Carlsbad, CA 92008 USA	3 Fountain Drive
Tel: 1 760 603 7200	Paisley PA4 9RF, UK
Tel (Toll Free): 1 800 955 6288	Tel: +44 (0) 141 814 6100
Fax: 1 760 602 6500	Tech Fax: +44 (0) 141 814 6117
E-mail: tech_support@invitrogen.com	E-mail: eurotech@invitrogen.com

Continued on next page

Technical Support, Continued

SDS Requests

SDSs (Safety Data Sheets) are available on our website at www.invitrogen.com/sds.

Limited Warranty

Invitrogen is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about an Invitrogen product or service, contact our Technical Support Representatives.

Invitrogen warrants that all of its products will perform according to specifications stated on the certificate of analysis. The company will replace, free of charge, any product that does not meet those specifications. This warranty limits Invitrogen Corporation's liability only to the cost of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. Invitrogen reserves the right to select the method(s) used to analyze a product unless Invitrogen agrees to a specified method in writing prior to acceptance of the order.

Invitrogen makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore Invitrogen makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, please report it to our Technical Support Representatives.

Invitrogen assumes no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose.

Purchaser Notification

Limited Use Label License No. 5: Invitrogen Technology

The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of patents owned or controlled by Invitrogen Corporation which cover this product based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Licensing Department, Invitrogen Corporation, 5791 Van Allen Way, Carlsbad, California 92008. Phone (760) 603-7200. Fax (760) 602-6500. Email: outlicensing@invitrogen.com

invitrogen™
by life technologies™

Corporate Headquarters
5791 Van Allen Way
Carlsbad, CA 92008
T: 1 760 603 7200
F: 1 760 602 6500
E: tech_support@invitrogen.com

For country-specific contact information visit our web site at **www.invitrogen.com**