invitrogen™

ChargeSwitch® gDNA Plant Kit

For purification of genomic DNA (gDNA) from plant samples

Catalog nos. CS18000 and CS18000-10

Version C
13 November 2006
25-0827

User Manual

Table of Contents

Kit Contents and Storage

Types of Kits

This manual is supplied with the following products.

Product	Catalog no.	Purification Protocol
ChargeSwitch® gDNA Plant Kit	CS18000	Manual
	CS18000-10	Automated

Shipping and Storage

All components of the ChargeSwitch® gDNA Plant Kit are shipped at room temperature.

Upon receipt, store components as follows:

- Store RNase A and ChargeSwitch® Lysis Buffer (L18) at 4°C
- Store the remaining kit components at room temperature

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

Kit Contents

The components supplied in the ChargeSwitch® gDNA (genomic DNA) Plant Kit are listed below.

The amount of reagents provided in the kits are sufficient to perform 96 (catalog no. CS18000) or 960 (catalog no. CS18000-10) purifications.

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

Components	Amount	
	CS18000	CS18000-10
ChargeSwitch® Magnetic Beads (25 mg/ml in 10 mM MES, pH 5.0, 10 mM NaCl, 0.1% Tween 20)	4 ml	40 ml
RNase A (5 mg/ml in 10 mM Tris-HCl, pH 8.5, 10 mM EDTA)	200 µl	2 ml
10% SDS in deionized water	10 ml	100 ml
ChargeSwitch® 10% Detergent (D1)	10 ml	100 ml
ChargeSwitch® Lysis Buffer (L18)	100 ml	2 x 500 ml
ChargeSwitch® Precipitation Buffer (N5)	38.5 ml	385 ml
ChargeSwitch® Wash Buffer (W12)	200 ml	2 x 1000 ml
ChargeSwitch® Elution Buffer (E6; 10 mM Tris-HCl, pH 8.5 with 1 mM EDTA)	14.5 ml	145 ml

Product Qualification

Quality Control

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.

Accessory Products

Additional Products

The table below lists additional products available from Invitrogen that may be used with the ChargeSwitch® gDNA Plant Kit.

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 website at www.invitrogen.com or call Technical Support (see page 23).

Product	Amount	Catalog no.
MagnaRack™ Magnetic Rack	1 rack	CS15000
96-well Magnetic Separator	1 rack	CS15096
96 Deep Well Block, 2.2 ml	1 case of 50	CS15196
ChargeSwitch® gDNA Micro Tissue Kit	50 purifications	CS11203
ChargeSwitch® gDNA Mini Tissue Kit	25 purifications	CS11204
ChargeSwitch® gDNA 10-20 µl Blood Kit	96 purifications	CS11010
ChargeSwitch® gDNA 50-100 µl Blood Kit	50 purifications	CS11000
ChargeSwitch® gDNA 1 ml Blood Kit	20 purifications	CS11001
ChargeSwitch® gDNA 1 ml Serum Kit	50 x 1 ml purifications	CS11040
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
Quant-iT™ DNA Assay Kit, High Sensitivity	1000 assays	Q33120
Quant-iT™ DNA Assay Kit, Broad-Range	1000 assays	Q33130
Quant-iT™ PicoGreen® dsDNA Assay	1 kit, 1 ml	P7589

E-Gel® Agarose Gels and DNA Ladders

E-Gel® Agarose Gels are bufferless pre-cast agarose gels designed for fast, convenient electrophoresis of DNA samples. E-Gel® Agarose Gels are available in different agarose percentages and well formats for your convenience. A large variety of DNA ladders is available from Invitrogen for sizing DNA. For details on these products, visit www.invitrogen.com or call Technical Support (page 23).

Overview

Introduction

The ChargeSwitch® gDNA Plant Kits allow rapid and efficient purification of genomic DNA (gDNA) from plant samples including leaves, tissues, and seeds.

After preparing the lysates, you can purify DNA in less than 15 minutes using the ChargeSwitch® Technology without the use of centrifugation or organic solvents. For more information about the Charge Switch® Technology, see below.

The kits are designed for manual processing of one or multiple samples (catalog no. CS18000) or for automated processing of multiple samples in 96-well format using liquid handling robots (catalog no. CS18000-10).

The purified DNA is suitable for use in any downstream applications of choice such as PCR, restriction enzyme digestion, or Southern blotting.

The ChargeSwitch® Technology

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

In low pH conditions, the ChargeSwitch® Magnetic Beads have a positive charge that binds the negatively charged nucleic acid backbone (see 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 ChargeSwitch® Magnetic Beads 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.

Continued on next page

Overview, Continued

Advantages

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

- Uses magnetic bead-based technology to purify genomic DNA without the need for hazardous chemicals, centrifugation, or vacuum manifolds
- Rapid and efficient purification of genomic DNA from various plant samples in less than 15 minutes following sample preparation
- Minimal contamination with RNA
- Allows automated processing of large numbers of samples (catalog no. CS18000-10) in 96-well plates using a liquid handling robot
- Purified genomic DNA demonstrates improved performance in downstream applications including PCR, restriction enzyme digestion, and Southern blotting

System Specifications

Starting Material:	Up to 100 mg plant sample
Bead Binding Capacity:	1 mg bead binds 5-10 µg gDNA
Bead Size:	1 µm
Bead Concentration:	25 mg/ml
Bead Storage Buffer:	10 mM MES, pH 5.0, 10 mM NaCl, 0.1% Tween 20
Elution Volume:	150 µl
DNA Yield:	Up to 7 µg

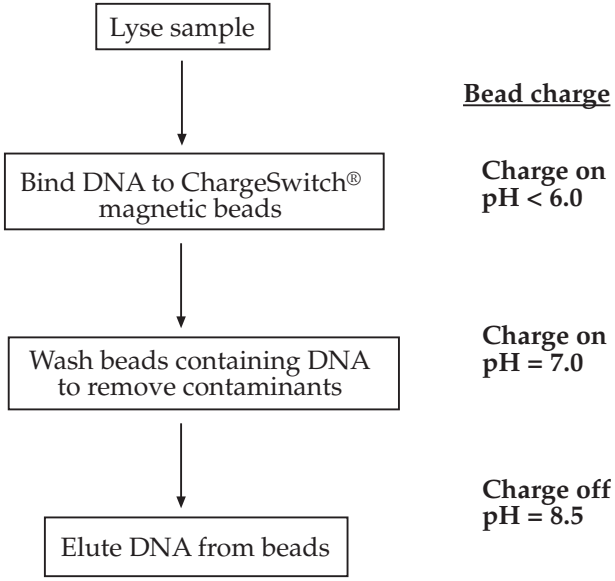
Automation

Use of the ChargeSwitch® gDNA Plant Kit (catalog no. CS18000-10) has been demonstrated on the Tecan Genesis® robotic workstation to purify gDNA in a fully automated system from large numbers of plant samples in a 96-well format. Other liquid handling robots are suitable provided that each robot is equipped with a gripper arm, a 96-well magnetic separator, and other additional hardware as described on page 13. 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 Support (page 23).

Experimental Overview

Experimental Outline

The figure below illustrates the basic steps necessary to purify genomic DNA using the ChargeSwitch® gDNA Plant Kits.



General Information

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

- Resuspend the ChargeSwitch® Magnetic Beads thoroughly before use.
- Maintain a sterile environment when handling DNA to avoid any contamination from DNases.
- Ensure that no DNases are introduced into the solutions supplied with the kit.
- Make sure all equipment that comes in contact with DNA is sterile including pipette tips and tubes.
- When removing supernatant, aspirate slowly to ensure that the pellet of beads is not disturbed.
- Make sure that the ChargeSwitch® Wash Buffer is removed completely before elution and the ChargeSwitch® Magnetic Beads are fully resuspended during the elution step.

Safety Information

Follow these safety guidelines when using the ChargeSwitch® Kits.

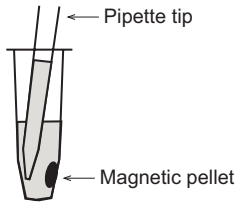
- Treat all reagents supplied in the kit as potential irritants.
- Always wear a suitable lab coat, disposable gloves, and protective goggles.
- If a spill of the buffers occurs, clean with a suitable laboratory detergent and water. If the liquid spill contains potentially infectious agents, clean the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

Continued on next page

Handling Magnetic Beads

Follow the recommendations below for best results:

- **Do not freeze ChargeSwitch® Magnetic Beads** as freezing damages the property of the beads for nucleic acid purifications. Store the beads at room temperature.
- Always keep the ChargeSwitch® Magnetic Beads in solution. **Do not allow the beads to dry**, including during washing procedures as this renders the beads non-functional.
- Before using the ChargeSwitch® Magnetic Beads, resuspend thoroughly in the storage buffer by vortexing before removal from the storage tube.
- During mixing steps, avoid forming bubbles by using an adjustable pipette set to a specific volume as directed in the protocol and by pipetting up and down gently with the pipet tip submerged in the solution.
- To aspirate the supernatant after bead washing, place the pipette tip away from the beads by angling the pipette such that the tip is pointed away from the pellet and carefully remove the supernatant without disturbing or removing any beads (see figure below).



- Discard ChargeSwitch® Magnetic Beads after use. **Do not reuse the beads**.

Sample Preparation

Introduction

Instructions for isolating genomic DNA (gDNA) from plant tissue are described below. Read the entire instructions before starting the purification procedure.

Plant Samples

The ChargeSwitch® gDNA Plant Kit is suitable for isolating plant gDNA from a large variety of plant samples including leaves, tissues, sprouts, shoots, and seeds.

To obtain high yield of DNA and minimize DNA degradation, use young plant samples such as leaves and freeze sample in liquid nitrogen immediately after collection.

For samples rich in polysaccharides and polyphenolics (such as pine, corn, cotton), prepare the lysate using Reagent A as described below and on the next page.

Materials Needed

- Plant samples (up to 100 mg)
- 1.5 ml microcentrifuge tubes and a microcentrifuge
- 96 x 2 ml deep well plate, page 13, (automated protocol)
- Tissue homogenizer, grinder, or mortar and pestle (depending on the sample)
- Adjustable pipettes and aerosol barrier pipette tips
- *Optional:* Reagent A, see below for recipe

Components supplied with the kit

- ChargeSwitch® Lysis Buffer (L18)
- RNase A
- ChargeSwitch® Precipitation Buffer (N5)
- 10% SDS

Preparing Reagent A

1. Prepare 10 ml Reagent A (300 mM $CaCl_2$, 15% polyvinylpyrolidone) **fresh** as follows for plant samples rich in polysaccharides and polyphenolics.

$CaCl_2$	0.441 g
Polyvinylpyrolidone (10,000 average MW)	1.5 g
ChargeSwitch® Lysis Buffer (L18)	10 ml

2. Mix well.
3. To 900 µl ChargeSwitch® Lysis Buffer (L18), add 100 µl Reagent A and use this buffer to prepare samples (see next page).

Continued on next page

Sample Preparation, Continued

Preparing Plant Lysate

Procedure to prepare lysate from up to 100 mg plant tissue:

1. Chill the Precipitation Buffer (N5) on ice.
2. For **hard plant tissue**, freeze the tissue in liquid nitrogen and grind frozen tissue to powder using mortar and pestle. Let liquid nitrogen evaporate before proceeding to Step 3.

   For **soft, non-fibrous plant tissue**, cut the tissue into small pieces.

   For **lyophilized samples**, proceed to Step 3.
3. At room temperature, add 1 ml ChargeSwitch® Lysis Buffer (L18) to the tissue from Step 2 **or** for samples rich in polysaccharides and polyphenolics, add 1 ml ChargeSwitch® Lysis Buffer (L18) containing Reagent A (see previous page for recipe) to the tissue from Step 2.

   Note: If the solution is very viscous, add more Lysis Buffer (L18). Also see Step 6 below.
4. Add 2 µl RNase A to the samples.
5. Prepare lysate by homogenizing the pieces of soft tissue with a tissue homogenizer or grinder, or by vortexing the ground tissue/lyophilized sample until sample is completely resuspended.
6. Add 100 µl 10% SDS to 1 ml plant lysate.

   If you varied the amount of ChargeSwitch® Lysis Buffer (L18) in Step 3, ensure to maintain a ratio of 10:1 for ChargeSwitch® Lysis Buffer (L18) to SDS.
7. Incubate at room temperature for 5 minutes.
8. Add 400 µl ChargeSwitch® Precipitation Buffer (N5) to the lysate. Mix by inversion or vortexing for 10 seconds until a precipitate forms.

   If different amounts of ml ChargeSwitch® Lysis Buffer (L18) are used, maintain a ratio of 10:4 for ChargeSwitch® Lysis Buffer (L18) to ChargeSwitch® Precipitation Buffer (N5).
9. Centrifuge at maximum speed for 5 minutes at room temperature to produce a clear lysate.
10. Transfer the clear lysate to a new, sterile 1.5 ml microcentrifuge tube for manual purification or a 96 x 2 ml deep well plate (page 15) for automated purification.
11. Proceed to **Purification Procedure**—**Manual Protocol** (next page) or **Purification Procedure**—**Automated Protocol** (page 13).

Purification Procedure—Manual Protocol

Introduction

This procedure is designed for purifying genomic DNA from plant samples using microcentrifuge tubes. If you are using an automated liquid handling system to process the samples, proceed with the **Purification Procedure—Automated Protocol**, page 13.

Use this **Manual Protocol** with the ChargeSwitch® gDNA Plant Kit, catalog no. CS18000.

MagnaRack™

The MagnaRack™ Magnetic Rack available from Invitrogen (catalog no. CS15000) is a two-piece magnetic separation rack for use in protocols with magnetic beads. The MagnaRack™ Magnetic Rack consists of a magnetic base station and a removable tube rack. The tube rack can hold 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, visit www.invitrogen.com or call Technical Support (page 23).

Continued on next page

Purification Procedure—Manual Protocol, Continued

Materials Needed

You will need the following items:

- Plant sample lysate (see **Sample Preparation**, page 6)
- MagnaRack™ (previous page)
- 1.5 ml microcentrifuge tubes
- Adjustable pipettes and aerosol barrier pipette tips

Components supplied with the kit

- ChargeSwitch® Magnetic Beads
- ChargeSwitch® 10% Detergent (D1)
- ChargeSwitch® Wash Buffer (W12)
- ChargeSwitch® Elution Buffer (E6)

Continued on next page

Purification Procedure—Manual Protocol, Continued

Binding DNA

Follow the protocol below to bind genomic DNA to ChargeSwitch® Magnetic Beads. The volumes given are on a per sample basis.

Start with lysed plant samples (page 6)

1. **Thoroughly** vortex the tube containing the ChargeSwitch® Magnetic Beads to fully resuspend the beads in the storage buffer.
2. Add 100 µl ChargeSwitch® 10% Detergent (D1) to the ~l.2 ml lysate (from Step 10, page 7).
3. Add 40 µl resuspended ChargeSwitch® Magnetic Beads.
4. Mix gently by pipetting up and down 5 times using a 1 ml pipette tip set to 900 µl without forming any bubbles to evenly distribute the ChargeSwitch® Magnetic Beads within the solution.

   Important: Avoid forming bubbles by ensuring that the pipette tip is submerged during mixing, and by pipetting up and down gently.
5. Incubate at room temperature for 1 minute.
6. Place tubes on the MagnaRack™ ('on the magnet' position) until the ChargeSwitch® have formed a tight pellet.
7. Without removing the tubes from the magnet, carefully aspirate and discard the supernatant without disturbing the pellet of beads by angling the pipette such that the tip is pointed away from the pellet (see figure on page 5).
8. Proceed immediately to **Washing DNA**, next page.

Continued on next page

Purification Procedure—Manual Protocol, Continued

Washing DNA

1. Remove tubes from the magnet.
2. Add 1 ml ChargeSwitch® Wash Buffer (W12) to the tube and gently pipet up and down 5 times to resuspend the beads using a 1 ml adjustable pipette set to 900 µl to mix without forming bubbles.
3. Place tubes on the magnet until the beads have formed a tight pellet and the supernatant is clear.
4. Without removing the tubes from the magnet, carefully aspirate and discard the supernatant without disturbing the pellet of beads by angling the pipette such that the tip is pointed away from the pellet.
5. Remove tubes from the magnet.
6. Add 1 ml ChargeSwitch® Wash Buffer (W12) to the tube and gently pipet up and down 5 times to resuspend the beads using a 1 ml adjustable pipette set to 900 µl to mix without forming bubbles.
7. Place tubes on the magnet until the beads have formed a tight pellet and the supernatant is clear.
8. Without removing the tubes from the magnet, carefully aspirate and discard the supernatant without disturbing the pellet of beads by angling the pipette such that the tip is pointed away from the pellet.
9. Proceed immediately to **Eluting DNA**, next page.

Continued on next page

Purification Procedure—Manual Protocol, Continued

Eluting DNA

1. Remove the tube containing the pelleted magnetic beads from the magnet. There should be no supernatant in the tube.
2. Add 150 µl of ChargeSwitch® Elution Buffer (E6).

   Note: You may vary the elution volume (50-100 µl) to suit the application. Do not use volumes <50 µl for eluting DNA.
3. Pipet up and down gently 15-30 times using an adjustable pipette set to 100 µl (adjust to any variations on the ChargeSwitch® Elution Buffer volume used) until ChargeSwitch® Magnetic Beads are completely dispersed and no clumps are visible.

   Note: If a few grainy clumps remain, proceed to the next step. This will not adversely affect the result.
4. Incubate at room temperature for 1 minute.
5. Place tubes on the magnet until the beads have formed a tight pellet and the supernatant is clear.
6. Without removing the tubes from the magnet, carefully transfer the **supernatant containing the DNA** to a sterile microcentrifuge tube without disturbing the pellet by angling the pipette such that the tip is pointed away from the pellet.

   If the supernatant containing the DNA is discolored, repeat Steps 6-7.
7. Discard the used ChargeSwitch® Magnetic Beads. **Do not** reuse the ChargeSwitch® Magnetic Beads.

For DNA quantitation refer to page 20.

Storing DNA

Store the purified gDNA at -20°C or use immediately for the desired downstream application. Avoid repeated freezing and thawing of gDNA.

Purification Procedure—Automated Protocol

Introduction

This section provides general information for using the ChargeSwitch® gDNA Plant Kit (catalog no. CS18000-10) to process large numbers of samples in 96-well format using an automated liquid handling robot and magnetic separator.

If you wish to process small numbers of samples individually, see **Purification Procedure—Manual Protocol** (page 8).

Hardware Requirements

The ChargeSwitch® chemistry is ideal for purification of DNA using liquid handling robots, by avoiding the need for centrifugation steps, vacuum manifolds or the use of ethanol or chaotropic salts. You will need the following hardware to perform automated processing of plant samples using the ChargeSwitch® gDNA Plant Kit:

- Any liquid handling robotic workstation with a gripper arm
- Appropriate tips for liquid dispensing and aspiration
- 96-well magnetic separator (see next page)
- Plate shaker capable of 1500-1800 rpm (optional—for use with deep well plates, if desired)

Continued on next page

Purification Procedure—Automated Protocol, Continued

96-Well Magnetic Separator

The 96-well Magnetic Separator available from Invitrogen (catalog no. CS15096) is a magnetic separation rack suitable for use in protocols with magnetic beads. The rack can hold up to 96 samples in a deep well plate. The deep well plate fits onto the magnetic separator, associating the array of 24 neodymium magnets with the samples for simple processing of magnetic samples (see figures below). For more information, see www.invitrogen.com or contact Technical Support (page 23).

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

Continued on next page

Purification Procedure—Automated Protocol, Continued

Materials Needed

You will need the following materials:

- Plant sample lysates (see **Sample Preparation for Manual and Automated Protocol**, page 6)
- Liquid handling robot configured to process samples in 96-well plates
- 96-well Magnetic Separator (see previous page)
- 96 x 2 ml deep well plate (page vi)
- 96 x 300 µl U-bottom microplate (Greiner, catalog no. 650201)

Components supplied with the kit

- ChargeSwitch® Magnetic Beads
- ChargeSwitch® 10% Detergent (D1)
- ChargeSwitch® Wash Buffer (W12)
- ChargeSwitch® Elution Buffer (E6)

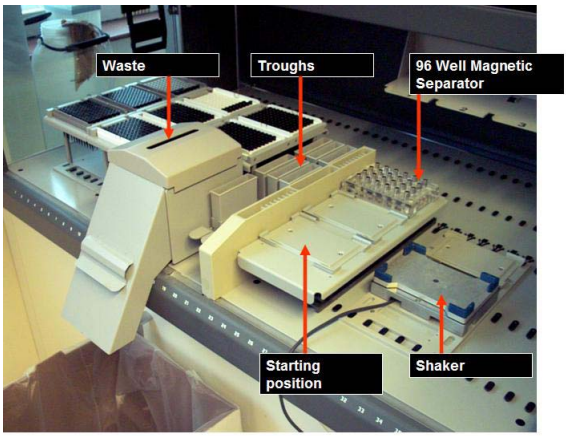
Continued on next page

Purification Procedure—Automated Protocol, 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	ChargeSwitch® Detergent (D1)	
3	ChargeSwitch® Magnetic Beads	
4	ChargeSwitch® Wash Buffer (W12)	
5	Waste	
6		96-Well Magnetic Separator
7		Shaker
8		96-well sample tray
9	ChargeSwitch® Elution Buffer (E6)	
10		96-well U-bottom microtiter plate (for final elution)



Continued on next page

Purification Procedure—Automated Protocol, Continued

To maximize DNA yield using the automated protocol, follow these important recommendations below when processing 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, ensure that all beads are fully resuspended to maximize DNA recovery.
- To maximize DNA yield, make sure that all Wash Buffer is removed from the beads before adding the Elution Buffer and the beads are fully resuspended during the elution step.

Continued on next page

Purification Procedure—Automated Protocol, Continued

Automated Protocol

Follow the protocol below to purify genomic DNA from plant samples. The volumes given are on a per sample basis.

The protocol below describes the use of 96-well deep-well plates (page 15) and can also be used with microcentrifuge tubes.

Start with plant lysates prepared as described on page 6.

1. Vortex the tube containing the ChargeSwitch® Magnetic Beads to fully resuspend and evenly distribute the beads in the storage buffer.
2. Add 100 µl 10% ChargeSwitch® Detergent (D1) to the ~l.2 ml lysate (from Step 10, page 7).
3. Add 40 µl resuspended ChargeSwitch® Magnetic Beads.
4. Shake at medium fast speed (*e.g.* pulse, 10 seconds) to evenly distribute the ChargeSwitch® Magnetic Beads within the solution. You may also mix by gently pipetting up and down 5 times using a 1 ml pipette tip set to 900 µl without forming any bubbles.
5. Incubate at room temperature for 1 minute.
6. Move the plate to the 96-well Magnetic Separator.
7. Wait for 60-90 seconds.
8. Slowly aspirate all of the supernatant and discard, leaving behind the pellet of beads.
9. Remove the plate from the 96-well Magnetic Separator.
10. Add 1 ml ChargeSwitch® Wash Buffer (W12) to the samples.
11. Shake at medium speed (*e.g.* pulse, 10 seconds) to evenly distribute the magnetic beads within the solution or mix by gently pipetting up and down 5 times.
12. Move the plate to the 96-well Magnetic Separator.
13. Wait for 60-90 seconds.
14. Slowly aspirate all of the supernatant and discard, leaving behind the pellet of beads.

Continued on next page

Purification Procedure—Automated Protocol, Continued

Automated Protocol, continued

Protocol continued from previous page.

15. Remove the plate from the 96-well Magnetic Separator.
16. Add 1 ml ChargeSwitch® Wash Buffer (W12) to the samples.
17. Shake at medium speed (*e.g.* pulse, 10 seconds) to evenly distribute the magnetic beads within the solution or mix by gently pipetting up and down 5 times.
18. Move the plate to the 96-well Magnetic Separator.
19. Wait for 60-90 seconds.
20. Slowly aspirate all of the supernatant and discard, leaving behind the pellet of beads.
21. Remove plate from the 96-well Magnetic Separator.
22. Add 150 µl of ChargeSwitch® Elution Buffer (E6).

    Note: You may vary the elution volume (50-100 µl) to suit the application. Do not use volumes <50 µl for eluting DNA.
23. Shake the plate rapidly for 1 minute or mix by pipetting up and down until ChargeSwitch® Magnetic Beads are completely dispersed and no clumps are visible.
24. Incubate at room temperature for 1 minute.

    Note: If <150 µl ChargeSwitch® Elution Buffer is used for elution, increase the incubation time to 5 minutes.
25. Move plate to the 96-well Magnetic Separator.
26. Wait for 1 minute.
27. Slowly aspirate and transfer **supernatant containing the DNA** to a 96 x 300 µl U-bottom microplate without disturbing the pellet.
28. Discard the used ChargeSwitch® Magnetic Beads. **Do not** reuse the ChargeSwitch® Magnetic Beads.

For DNA quantitation refer to next page.

Storing DNA

Store the purified gDNA at -20°C or use immediately for the desired downstream application. Avoid repeated freezing and thawing of gDNA.

DNA Quantitation

DNA Yield

Perform DNA quantitation using UV absorbance at 260 nm or Quant-iT™ Kits.

UV Absorbance

1. Prepare a dilution of the DNA solution in 10 mM Tris-HCl, pH 7.5. Mix well. Measure the absorbance at 260 nm (A_{260}) of the dilution in a spectrophotometer (using a cuvette with an optical path length of 1 cm) blanked against 10 mM Tris-HCl pH 7.5
2. Calculate the concentration of DNA using the formula:

   DNA (µg/ml) = $A_{260} \times 50$ x dilution factor

   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™ Kits

The Quant-iT™ Kits (see page vi for ordering information) provide a rapid, sensitive, and specific fluorescent method for dsDNA quantitation. The kit contains a state-of-the-art quantitation reagent, DNA standards for standard curve, and a pre-made buffer to allow fluorescent DNA quantitation using standard fluorescent microtiter plate readers/fluorometers, or the Qubit™ Quantitation Fluorometer available from Invitrogen.

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	Reduce the amount of starting material. Be sure to add SDS during lysis.
	Poor quality of starting material	Use fresh sample and process immediately after collection, or freeze the sample at -80°C or in liquid nitrogen. The yield and quality of DNA isolated depends on the type and age of the starting material.
	Incorrect handling of ChargeSwitch® Magnetic Beads	Vortex the tube containing the ChargeSwitch® Magnetic Beads to fully resuspend the beads before adding them to your sample.
	Pellet of beads disturbed or lost during binding or washing steps	• Keep the sample on the MagnaRack™ or on the 96-well Magnetic Separator when removing supernatant during the binding or washing steps. • Remove the supernatant without disturbing the pellet of beads.
	Incorrect elution conditions	• After adding ChargeSwitch® Elution Buffer (E6) to the sample, pipet up and down to resuspend the ChargeSwitch® Magnetic Beads before incubation. • Do not use water to elute DNA. Use ChargeSwitch® Elution Buffer (E6).

Continued on next page

Troubleshooting, Continued

Problem	Cause	Solution
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 ChargeSwitch® Elution Buffer (E6).
	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 are not dried. Dried beads are non-functional.
Eluate containing DNA is discolored	Magnetic pellet disturbed during elution	• Place the sample on the MagnaRack™ or on the 96-well Magnetic Separator until the beads form a tight pellet. • Remove the eluate to a sterile microcentrifuge tube or sterile microtiter plate, without disturbing the bead pellet.
DNA is sheared or degraded	Lysate mixed too vigorously	• Use appropriate pipette tip set to a volume, lower than the total volume of the solution 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 (*e.g.* wear gloves and use DNase-free reagents).

Technical Support

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:
Invitrogen Corporation
1600 Faraday Avenue
Carlsbad, CA 92008 USA
Tel: 1 760 603 7200
Tel (Toll Free): 1 800 955 6288
Fax: 1 760 602 6500
E-mail:
tech_support@invitrogen.com

European Headquarters:
Invitrogen Ltd
Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF, UK
Tel: +44 (0) 141 814 6100
Tech Fax: +44 (0) 141 814 6117
E-mail:
eurotech@invitrogen.com

MSDS Requests

MSDSs (Material Safety Data Sheets) are available on our website at www.invitrogen.com/msds.

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, 1600 Faraday Avenue, Carlsbad, California 92008. Phone (760) 603-7200. Fax (760) 602-6500. Email: outlicensing@invitrogen.com

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

Tecan Genesis® is a registered trademark of Tecan Group AG.

invitrogen™

Corporate Headquarters
Invitrogen Corporation
1600 Faraday Avenue
Carlsbad, CA 92008
T: 1 760 603 7200
F: 1 760 602 6500
E: tech.service@invitrogen.com

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