USER GUIDE

invitrogen™
by life technologies™

ChargeSwitch®-Pro Filter Plasmid Midiprep and Maxiprep Kits

For Midiprep and Maxiprep purification of plasmid DNA from bacterial cells

Catalog numbers CS31104, CS31106, and CS31107

Revision date 9 October 2012
Publication Part number 100003539

MAN0001635

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

life technologies™

Table of Contents

Kit Contents and Storage

Shipping and Storage

All components are shipped at room temperature and stored at room temperature.

Do not freeze the columns. Freezing may damage the ChargeSwitch®-derivatized membrane in the columns.

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

Kit Contents

The components of each ChargeSwitch®-Pro Filter Plasmid Midiprep or Maxiprep Kit are listed in the following table:

25 Midiprep purifications (Cat. no. CS31104)

10 Maxiprep purifications (Cat. no. CS31106)

25 Maxiprep purifications (Cat. no. CS31107)

Component	Amounts/Kit		
	CS31104	**CS31106**	**CS31107**
ChargeSwitch®-Pro Resuspension Buffer (10 mM Tris-HCl, pH 8.5, 10 mM EDTA)	185 mL	85 mL	185 mL
ChargeSwitch®-Pro Lysis Buffer	185 mL	85 mL	185 mL
ChargeSwitch®-Pro Precipitation Buffer	185 mL	85 mL	185 mL
ChargeSwitch®-Pro RNase A	0.95 mL	0.45 mL	0.95 mL
ChargeSwitch®-Pro Wash Buffer 1	190 mL × 2	190 mL	190 mL × 2
ChargeSwitch®-Pro Wash Buffer 2	190 mL × 2	190 mL	190 mL × 2
ChargeSwitch®-Pro Elution Buffer (10 mM Tris-HCl, pH 8.5)	50 mL	50 mL	50 mL
ChargeSwitch®-Pro Filter Plasmid Midi Columns	25	—	—
ChargeSwitch®-Pro Filter Plasmid Maxi Columns	—	10	25

Introduction

Overview

Introduction

The ChargeSwitch®-Pro Filter Plasmid Midiprep and Maxiprep Kits contain all the components required for the rapid and efficient isolation of highly pure plasmid DNA from *E. coli* cells. The purification columns in the kit contain a novel ChargeSwitch®-derivatized membrane that is positively charged at low pH and neutral at pH 8.5, to bind and elute plasmid DNA without the use of harsh reagents.

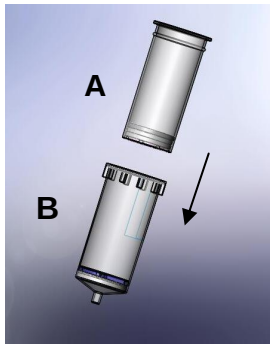
Using the kit, you prepare cell lysates with a modified alkaline lysis procedure and then purify the plasmid DNA using a simple centrifugation- or vacuum-based protocol.

Elute the DNA by raising the pH to 8.5 using a low-salt elution buffer. The purified plasmid DNA is ready for use in your downstream application of choice, including mammalian transfection, automated fluorescent DNA or manual sequencing, PCR, cloning, *in vitro* transcription, bacterial cell transformations, or restriction digestion.

Filter Column Assembly

The ChargeSwitch®-Pro Filter Plasmid Midiprep and Maxiprep Kits employ a nested column design consisting of an inner flat-bottomed column (labeled "A" in the following graphic), which fits into an outer luer-bottomed column (labeled "B" in the following graphic).

The inner column (Lysate Clarification Column) is used for rapid clarification of the bacterial lysate. The outer column (Binding Column) contains the ChargeSwitch® derivitized membrane which binds plasmid DNA from the clarified lysate.



Continued on next page

ChargeSwitch® Technology

ChargeSwitch® Technology provides a switchable surface that is charge dependent on the pH of the surrounding buffer to facilitate nucleic acid purification.

In low pH conditions, the ChargeSwitch® purification membrane has a positive charge that binds the negatively charged nucleic acid backbone. Proteins and other contaminants are not bound and are simply washed away in aqueous wash buffers.

To elute nucleic acids, the charge on the surface is neutralized by raising the pH to 8.5 using a low-salt elution buffer. Purified DNA elutes instantly into this elution buffer, and is ready for use in downstream applications of choice.

Advantages of the Kit

The ChargeSwitch®-Pro Filter Plasmid Midiprep and Maxiprep Kits offer the following advantages:

- High-quality, high-yield plasmid DNA purification from *E. coli* without the use of ethanol, chaotropic salts, or organic solvents.
- Designed to isolate plasmid DNA from samples using a simple centrifugation or vacuum protocol following sample preparation.
- Reliable performance of the purified plasmid DNA in a variety of applications, including mammalian cell transfection, automated and manual sequencing, amplification reactions, *in vitro* transcription, bacterial cell transformation, cloning, and labeling.

Continued on next page

Note

The ChargeSwitch®-Pro Filter Plasmid Midiprep and Maxiprep Kits are compatible for use in isolation of plasmid from *endA*+ strains.

System Specifications

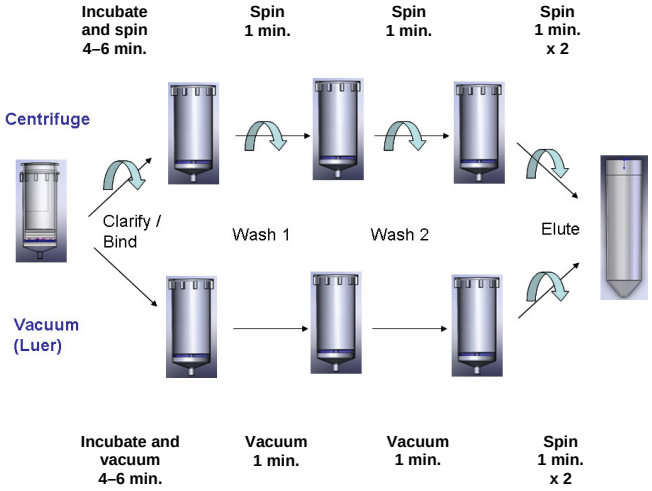
Parameter	Midiprep	Maxiprep
Starting Material (fresh, overnight LB culture)	25 mL	100 mL
Binding Capacity (per column)	300 µg	1 mg
Recommended Elution Volume	0.5–1 mL	1–2 mL
Typical DNA Yield	200 µg	800 µg
Plasmid Size	3–9 kb	3–9 kb
Purity OD 260/280	>1.8	>1.8
Purity OD 260/230	>1.8	>1.8
Endotoxin (EU/µg DNA)	≤1	≤1

Experimental Workflows

Workflow

Samples can be processed by centrifugation or by vacuum manifold. A vacuum manifold allows convenient processing of samples by reducing the number of handling and centrifugation steps. The following diagram illustrates the workflows for the kits using centrifugation (upper), and luer lock vacuum manifold (lower) protocols.

Centrifugation and Vacuum Workflow



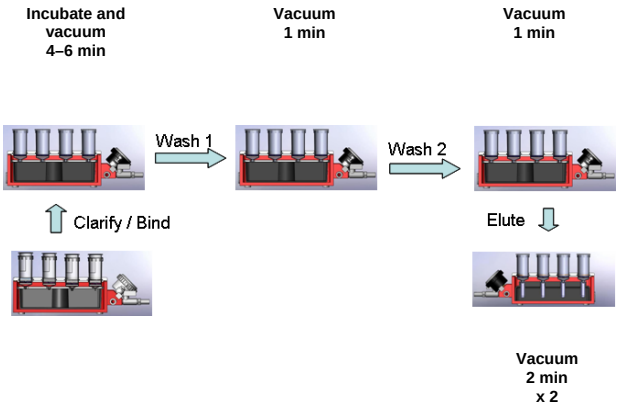
Continued on next page

Experimental Workflows, Continued

Workflow

The EveryPrep™ Universal Vacuum Manifold allows convenient processing of samples by reducing the number of handling steps, and supports vacuum assisted elution to eliminate centrifugation altogether. The following diagram illustrates the workflow for the kits using the EveryPrep™ Universal Vacuum Manifold.

EveryPrep™ Universal Vacuum Manifold Workflow



Methods

General Information

Introduction

Review the information in this section before starting. Guidelines are included for growing the bacterial culture.

Bacterial Cultures

- Grow transformed *E. coli* in LB medium with the appropriate antibiotic. **Do not** use richer medium like Terrific Broth to grow the *E. coli.*
- Use overnight bacterial cultures with an absorbance of 1–2 OD at 600 nm (A_{600}).
- Use 25 mL of bacterial culture for midipreps, and 100 mL of bacterial culture for maxipreps.
- For best results, use fresh overnight cultures, however, the kit can also be used to purify plasmid DNA from frozen cell pellets.

Handling DNA

- Maintain a sterile environment when handling DNA to avoid any contamination from DNases.
- Ensure that no DNase is introduced into the solutions supplied with the kit.
- Make sure that all equipment coming in contact with DNA is sterile, including pipette tips and tubes.
- Store purified plasmid DNA at 4°C for immediate use or at –20°C for long-term storage.
- Avoid repeated freeze-thawing of purified DNA.

Handling the Columns

- **Do not freeze the columns.** Freezing may damage the CST-derivatized membrane.
- Discard the ChargeSwitch®-Pro Filter Plasmid Columns after use. Columns **cannot** be reused.

Continued on next page

Elution Buffer

For best results, use the ChargeSwitch®-Pro Elution Buffer provided in the kit. **Do not elute in water**. If it is necessary to elute in another buffer, use a buffer of **pH 8.5–9.0**. If the pH of the buffer is <8.5, the DNA will not elute efficiently.

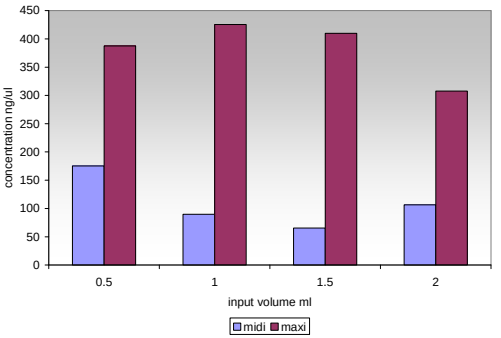
The volume of elution buffer can be varied to obtain plasmid DNA in the desired final concentration.

For increased DNA yield, use a higher elution buffer volume.

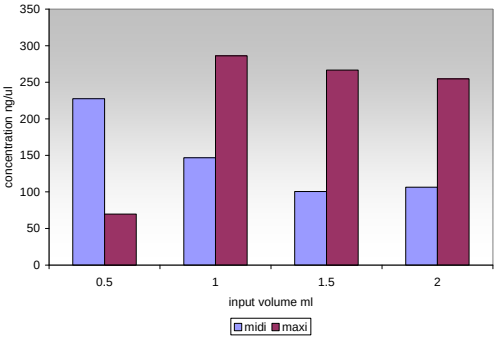
For increased DNA concentration, use a lower elution buffer volume.

The following graphs plot elution volume versus DNA concentration. It is designed to help you determine the most appropriate elution conditions for your application.

Elution Volume versus Concentration for Centrifuge Protocol



Elution Volume versus Concentration for Vacuum Protocol



Continued on next page

Safety Information

Follow the safety guidelines below when using the ChargeSwitch®-Pro Filter Plasmid Midiprep and Maxiprep Kits.

- 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.

Midiprep Procedure

Introduction

Protocols for isolating plasmid DNA from ≤25 mL overnight bacterial culture are described in this section.

Materials Needed

In addition to the materials supplied in the kit, you will need the following:

- ≤25 mL overnight bacterial culture (page 10)
- Sterile 50-mL centrifuge tubes (Life Technologies Cat. no. CS32000, or BD Falcon™ Cat. no. 352070)
- Swinging bucket centrifuge capable of >2250 × *g*
- Vacuum protocol only: Vacuum manifold and vacuum pump (capable of producing pressure of 15–20 in. Hg or –500 to –700 mbar)
  Note: Life Technologies produces the EveryPrep™ Universal Vacuum Manifold (see page 28 for ordering information), which is ideal for this application.
- Adjustable pipettes and aerosol barrier pipette tips

Before Starting

ChargeSwitch®-Pro Resuspension Buffer
Add the entire contents of supplied ChargeSwitch®-Pro RNase A to the ChargeSwitch®-Pro Resuspension Buffer. Mix well. Mark the box on the bottle to indicate that the RNase A has been added. Store the buffer with RNase A at room temperature.

ChargeSwitch®-Pro Lysis Buffer
Check the ChargeSwitch®-Pro Lysis Buffer for precipitates. If necessary, warm the buffer to 37ºC to dissolve any precipitate.

ChargeSwitch®-Pro Precipitation Buffer
If room temperature is >25°C, chilling the ChargeSwitch®-Pro Precipitation Buffer on ice before use may improve results.

Continued on next page

Preparing the Bacterial Lysate

1. Harvest up to 25 mL of overnight bacterial culture by centrifugation at >4000 × *g* for 10 minutes.
2. Resuspend the cell pellet in 5 mL of ChargeSwitch®-Pro Resuspension Buffer premixed with RNase A (see **Before Starting,** page 13). Invert the tube or vortex until any remaining cell clumps are dispersed.
3. Add 5 mL of ChargeSwitch®-Pro Lysis Buffer. Mix by inverting capped tube at least 10 times until the lysate becomes homogenous. **Do not vortex,** as this may result in shearing of the genomic DNA.

   Note: Due to the reduced volume of solution used in the ChargeSwitch®-Pro Filter Midiprep System, thorough mixing of the lysate is required to maximize release of plasmid DNA.
4. Incubate at room temperature for 5 minutes. The lysate becomes clear and viscous. **Do not incubate longer than 5 minutes.**
5. Add 5 mL of ChargeSwitch®-Pro Precipitation Buffer. Immediately mix by inversion 6–10 times until the precipitate that forms becomes homogeneous. **Do not vortex**.
6. Proceed immediately to **Centrifugation Protocol**, next page; **EveryPrep™ Universal Vacuum Manifold Protocol**, page 16; or **Vacuum Protocol**, page 18.

Continued on next page

Centrifugation Protocol

Use the following procedure to bind, wash, and elute plasmid DNA using a swinging bucket centrifuge. See page 16 for a protocol using the EveryPrep™ Universal Vacuum Manifold, and page 18 for a vacuum-based protocol.

1. Place the assembled ChargeSwitch®-Pro Filter Midi Column into a 50-mL conical tube (not provided).
2. Carefully transfer the lysate mixture from Step 5, **Preparing the Bacterial Lysate**, onto the column.
3. Incubate for 2–3 minutes at room temperature to allow the precipitate to float to the surface.
4. Centrifuge the column at >2250 × *g* for 2–3 minutes.
5. Remove the column assembly and decant the flow-through from the 50-mL conical tube.
6. Remove the inner Lysate Clarification Column from the column assembly (see page 5) and discard. Re-insert the Binding Column into the 50-mL conical tube.
7. Add 15 mL of ChargeSwitch®-Pro Wash Buffer 1 to the column.
8. Centrifuge at >2250 × *g* for 1 minute.
9. Remove the column and discard the flow-through from the 50-mL conical tube. Re-insert the column into the 50-mL conical tube.
10. Add 15 mL of ChargeSwitch®-Pro Wash Buffer 2 to the column.
11. Centrifuge at >2250 × *g* for 1 minute.
12. Remove the column from the tube. Discard the flow-through *and* the 50-mL conical tube.
13. Insert the column into a clean 50-mL conical tube.
14. Add 0.5–1 mL of ChargeSwitch®-Pro Elution Buffer onto the column and incubate for 1 minute.
15. Centrifuge at >2250 × *g* for 1 minute.
16. Transfer the eluate back onto the same Binding Column and replace it in the same 50-mL conical tube. Centrifuge at >2250 × *g* for 1 minute.
17. The eluate contains the purified plasmid DNA. Store plasmid DNA at 4° C for immediate use or at –20° C for long-term storage.

EveryPrep™ Universal Vacuum Manifold Protocol

Follow the procedure below to bind, wash, and elute plasmid DNA using the EveryPrep™ Universal Vacuum Manifold. Use a vacuum pump capable of producing pressure of 15–20 in. Hg or –500 to –700 mbar. Refer to the manual for the EveryPrep™ Universal Vacuum Manifold for details, and alternative protocols.

1. Place the Waste Tray in one chamber of the manifold and cover it with the Waste Cover.
2. Seat the Midi/Maxi Elution Top Plate above the Waste Cover and insert the required number of assembled ChargeSwitch®-Pro Midi Filter Columns firmly into the plate.
3. Block the remaining holes with the provided stoppers.
4. Carefully transfer the supernatant from Step 5, **Preparing the Bacterial Lysate**, onto the columns.
5. Incubate for 2–3 minutes at room temperature to allow the precipitate to float to the surface.
6. Apply maximum vacuum pressure (15–20 in. Hg) until the liquid has passed through the column (approximately 2–3 minutes).
7. Release the vacuum. Gently remove the inner Lysate Clarification Column (see page 5) and discard.
8. Add 15 mL of ChargeSwitch®-Pro Wash Buffer 1 to the column.
9. Apply maximum vacuum pressure until the liquid has passed through the column (approximately 1 minute). Release the vacuum.
10. Add 15 mL of ChargeSwitch®-Pro Wash Buffer 2 to the column.
11. Apply maximum vacuum pressure until the liquid has passed through the column (approximately 1 minute). Release the vacuum.

Continued on next page

EveryPrep™ Universal Vacuum Manifold Protocol, continued

12. Place the Elution Rack into the Elution Chamber. The Elution Rack should contain a clean 2 mL elution tube in each position corresponding to a ChargeSwitch®-Pro Filter Midi Column.
13. Transfer the Midi/Maxi Elution Top Plate so that it is positioned over the Elution Chamber.
14. Add 0.5–1 mL of ChargeSwitch®-Pro Elution Buffer onto each column and incubate for 1 minute.
15. Apply a vacuum of 5–10 in. Hg for 2 minutes to allow the elution buffer to completely pass through the column.
16. Release the vacuum, and transfer the eluate back onto the same Binding Column.
17. Apply a vacuum of 5–10 in. Hg for 2 minutes.
18. Remove the Midi/Maxi Elution Top Plate, and discard the columns.
19. The eluate contains the purified plasmid DNA. Store purified plasmid DNA at 4°C for immediate use or at –20°C for long-term storage.

Vacuum Protocol

Follow the procedure below to bind, wash, and elute plasmid DNA using a vacuum manifold and pump. Use a vacuum pump capable of producing pressure of 15–20 in. Hg or –500 to –700 mbar. See page 15 for a centrifuge-based protocol.

1. Insert the assembled ChargeSwitch®-Pro Filter Midi Column into the luer extension of a vacuum manifold.
2. Carefully transfer the supernatant from Step 5, **Preparing the Bacterial Lysate**, onto the column.
3. Incubate 2–3 minutes at room temperature to allow precipitate to float to the surface.
4. Apply maximum vacuum pressure (15–20 in. Hg) until the liquid has passed through the column (approximately 2–3 minutes).
5. Release the vacuum. Gently remove the inner Lysate Clarification Column (see page 5) and discard.
6. Add 15 mL of ChargeSwitch®-Pro Wash Buffer 1 to the column.
7. Apply maximum vacuum pressure until the liquid has passed through the column (approximately 1 minute). Release the vacuum.
8. Add 15 mL of ChargeSwitch®-Pro Wash Buffer 2 to the column.
9. Apply maximum vacuum pressure until the liquid has passed through the column (approximately 1 minute). Release the vacuum.
10. Remove the column from the manifold and insert it into a clean 50-mL conical tube.
11. Add 0.5–1 mL of ChargeSwitch®-Pro Elution Buffer onto the column and incubate for 1 minute.
12. Centrifuge at >2250 × *g* in a swinging bucket centrifuge for 1 minute.
13. Transfer the eluate back onto the same Binding Column and replace it in the same 50-mL conical tube. Centrifuge at >2250 × *g* for 1 minute.
14. The eluate contains the purified plasmid DNA. Store plasmid DNA at 4° C for immediate use or at –20° C for long-term storage.

Maxiprep Procedure

Introduction

Protocols for isolating plasmid DNA from 100 mL overnight bacterial culture are described in this section.

Materials Needed

In addition to the materials supplied in the kit, you will need the following:

- Up to 100 mL overnight bacterial culture (page 10)
- Sterile 50-mL centrifuge tubes (Life Technologies Cat. no. CS32000, or BD Falcon™ Cat. no. 352070)
- Swinging bucket centrifuge capable of >2250 × *g*
- Vacuum protocol only: Vacuum manifold and vacuum pump (capable of producing pressure of 15–20 in. Hg or –500 to –700 mbar)
  Note: Life Technologies produces the EveryPrep™ Universal Vacuum Manifold (see page 28 for ordering information), which is ideal for this application.
- Adjustable pipettes and aerosol barrier pipette tips

Before Starting

ChargeSwitch®-Pro Resuspension Buffer
Add the entire contents of supplied ChargeSwitch®-Pro RNase A to the ChargeSwitch®-Pro Resuspension Buffer. Mix well. Mark the box on the bottle to indicate that the RNase A has been added. Store the buffer with RNase A at room temperature.

ChargeSwitch®-Pro Lysis Buffer
Check the ChargeSwitch®-Pro Lysis Buffer for precipitates. If necessary, warm the buffer to 37ºC to dissolve any precipitate.

ChargeSwitch®-Pro Precipitation Buffer
If room temperature is >25°C, chilling the ChargeSwitch®-Pro Precipitation Buffer on ice before use may improve results.

Continued on next page

Preparing the Bacterial Lysate

1. Harvest up to 100 mL of overnight bacterial culture by centrifugation at >2250 × *g* for 10 minutes.
2. Resuspend the cell pellet in 7 mL of ChargeSwitch®-Pro Resuspension Buffer premixed with RNase A (see **Before Starting,** page 19). Invert the tube or vortex until any remaining cell clumps are dispersed.
3. Add 7 mL of ChargeSwitch®-Pro Lysis Buffer. Mix by inverting capped tube at least 10 times until the lysate becomes homogenous. **Do not vortex,** as this may result in shearing of the genomic DNA.

   Note: Due to the reduced volume of solution used in the ChargeSwitch®-Pro Filter Midiprep System, thorough mixing of the lysate is required to maximize release of plasmid DNA.
4. Incubate at room temperature for 5 minutes. The lysate becomes clear and viscous. **Do not incubate longer than 5 minutes.**
5. Add 7 mL of ChargeSwitch®-Pro Precipitation Buffer. Immediately mix by inversion 6–10 times until the precipitate that forms becomes homogeneous. **Do not vortex**.
6. Proceed immediately to **Centrifugation Protocol**, next page; **EveryPrep™ Universal Vacuum Manifold Protocol**, page 22; or **Vacuum Protocol**, page 24.

Continued on next page

Centrifugation Protocol

Use the following procedure to bind, wash, and elute plasmid DNA using a swinging bucket centrifuge. See page 24 for a vacuum-based protocol.

1. Place the assembled ChargeSwitch®-Pro Filter Maxi Column into a 50-mL conical tube (not provided).
2. Carefully transfer the lysate mixture from Step 5, **Preparing the Bacterial Lysate**, onto the column.
3. Incubate for 2–3 minutes at room temperature to allow the precipitate to float to the surface.
4. Centrifuge the column at >2250 × *g* for 2–3 minutes.
5. Remove the column assembly and decant the flow-through from the 50-mL conical tube.
6. Remove the inner Lysate Clarification Column from the column assembly (see page 5) and discard. Re-insert the Binding Column into the 50-mL conical tube.
7. Add 15 mL of ChargeSwitch®-Pro Wash Buffer 1 to the column.
8. Centrifuge at >2250 × *g* for 1 minute.
9. Remove the column and discard the flow-through from the 50-mL conical tube. Re-insert the column into the 50-mL conical tube.
10. Add 15 mL of ChargeSwitch®-Pro Wash Buffer 2 to the column.
11. Centrifuge at >2250 × *g* for 1 minute.
12. Remove the column from the tube. Discard the flow-through *and* the 50-mL conical tube.
13. Insert the column into a clean 50-mL conical tube.
14. Add 1–2 mL of ChargeSwitch®-Pro Elution Buffer onto the column and incubate for 1 minute.
15. Centrifuge at >2250 × *g* for 1 minute.
16. Transfer the eluate back onto the same Binding Column and replace it in the same 50-mL conical tube. Centrifuge at >2250 × *g* for 1 minute.
17. The eluate contains the purified plasmid DNA. Store plasmid DNA at 4° C for immediate use or at –20° C for long-term storage.

EveryPrep™ Universal Vacuum Manifold Protocol

Use the following procedure to bind, wash, and elute plasmid DNA using the EveryPrep™ Universal Vacuum Manifold. Use a vacuum pump capable of producing pressure of 15–20 in. Hg or –500 to –700 mbar. Refer to the manual for the EveryPrep™ Universal Vacuum Manifold for details, and alternative protocols.

1. Place the Waste Tray in one chamber of the manifold and cover it with the Waste Cover.
2. Seat the Midi/Maxi Elution Top Plate above the Waste Cover and insert the required number of assembled ChargeSwitch®-Pro Filter Maxi Columns firmly into the plate.
3. Block the remaining holes with the provided stoppers.
4. Carefully transfer the supernatant from Step 5 **Preparing the Bacterial Lysate**, onto the column.
5. Incubate for 2–3 minutes at room temperature to allow the precipitate to float to the surface.
6. Apply maximum vacuum pressure (15–20 in. Hg) until the liquid has passed through the column (approximately 2–3 minutes).
7. Release the vacuum. Gently remove the inner Lysate Clarification Column (see page 5) and discard.
8. Add 15 mL of ChargeSwitch®-Pro Wash Buffer 1 to the column.
9. Apply maximum vacuum pressure until the liquid has passed through the column (approximately 1 minute). Release the vacuum.
10. Add 15 mL of ChargeSwitch®-Pro Wash Buffer 2 to the column.
11. Apply maximum vacuum pressure until the liquid has passed through the column (approximately 1 minute). Release the vacuum.

Continued on next page

EveryPrep™ Universal Vacuum Manifold Protocol, continued

12. Place the Elution Rack into the Elution Chamber. The Elution Rack should contain a clean 2 mL elution tube in each position corresponding to a ChargeSwitch®-Pro Filter Maxi Column.
13. Transfer the Midi/Maxi Elution Top Plate so that it is positioned over the Elution Chamber.
14. Add 1–1.5 mL of ChargeSwitch®-Pro Elution Buffer onto each column and incubate for 1 minute.
15. Apply a vacuum of 5–10 in. Hg for 2 minutes to allow the elution buffer to completely pass through the column.
16. Release the vacuum, and transfer the eluate back onto the same Binding Column.
17. Apply a vacuum of 5–10 in. Hg for 2 minutes.
18. Remove the Midi/Maxi Elution Top Plate, and discard the columns.
19. The eluate contains the purified plasmid DNA. Store purified DNA at 4° C for immediate use or at –20° C for long-term storage.

Vacuum Protocol

Follow the procedure below to bind, wash, and elute the DNA using a vacuum manifold and pump. Use a vacuum pump capable of producing pressure of 15–20 in. Hg or –500 to –700 mbar. See page 21 for a centrifuge-based protocol.

1. Insert the assembled ChargeSwitch®-Pro Filter Maxi Column into the luer extension of a vacuum manifold.
2. Carefully transfer the supernatant from Step 5, page 20, onto the column.
3. Incubate for 2–3 minutes at room temperature to allow the precipitate to float to the surface.
4. Apply maximum vacuum pressure (15–20 in. Hg) until the liquid has passed through the column (approximately 2–3 minutes).
5. Release the vacuum. Gently remove the inner Lysate Clarification Column (see page 5) and discard.
6. Add 15 mL of ChargeSwitch®-Pro Wash Buffer 1 to the column.
7. Apply maximum vacuum pressure until the liquid has passed through the column (approximately 1 minute). Release the vacuum.
8. Add 15 mL of ChargeSwitch®-Pro Wash Buffer 2 to the column.
9. Apply maximum vacuum pressure until the liquid has passed through the column (approximately 1 minute). Release the vacuum.
10. Remove the column from the manifold and insert it into a clean 50-mL conical tube.
11. Add 1–2 mL of Elution Buffer onto the column and incubate for 1 minute.
12. Centrifuge at >2250 × *g* in a swinging bucket centrifuge for 1 minute.
13. Transfer the eluate back onto the same Binding Column and replace it in the same 50-mL conical tube. Centrifuge at >2250 × *g* for 1 minute.
14. The eluate contains the purified plasmid DNA. Store purified plasmid DNA at 4°C for immediate use or at –20°C for long-term storage.

Analyzing Plasmid DNA Yield and Quality

Plasmid 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. 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 the dilution buffer.
2. Calculate the concentration of DNA using the formula:

   DNA (µg/mL) = A_{260} × 50 × 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

Quant-iT™ Kits from Life Technologies provide a rapid, sensitive, and specific fluorescent method for dsDNA quantitation. Each kit contains a state-of-the-art quantitation reagent and a pre-made buffer to allow fluorescent DNA quantitation using standard fluorescent microplate readers/fluorometers or the Qubit® Quantitation Fluorometer. Visit **www.lifetechnologies.com** for more information.

Note: We recommend using a known quantity of plasmid DNA as a standard when calculating yield using Quant-iT™ Kits. The non-supercoiled DNA standard provided in these kits typically fluoresces more brightly than supercoiled plasmid DNA, which may lead to inaccuracies in quantitation.

Plasmid DNA Quality

Typically, plasmid DNA isolated using the ChargeSwitch®-Pro Filter Plasmid Midiprep and Maxiprep Kits have an A_{260}/A_{280} ratio of 1.7–2.0 when samples are diluted in Tris-HCl pH 7.5, indicating that the DNA is free of contaminants that could interfere with downstream applications. Absence of contaminating RNA may be confirmed by agarose gel electrophoresis.

Troubleshooting

Introduction

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

Problem	Cause	Solution
Low plasmid DNA yield	Poor quality of starting material or incomplete lysis	• Ensure media is completely removed after cell harvest. • If the cell lysate is too viscous, reduce the amount of cells used per sample. Attempting to lyse too many cells may result in incomplete lysis. See the culture volume recommendations on page 10. • Check the growth conditions of the cell culture to ensure plasmid propagation. Use a high copy number plasmid if possible. • Cell cultures that are overgrown (e.g., grown >16 hours) may begin to lyse, resulting in reduced yields and contaminating genomic DNA. • Ensure complete resuspension of the bacterial cell pellet. Decrease the amount of starting material used. • Chill the Precipitation Buffer on ice before use to improve the precipitation efficiency and plasmid DNA yield. • Mix lysate thoroughly (>10 inversions) to ensure complete lysis. • Increase the incubation time during lysis but do not exceed 5 minutes.
	Elution conditions require optimization	If you are using a different buffer for elution, ensure that the pH of the buffer is 8.5–9.0.

Continued on next page

Problem	Cause	Solution
Low plasmid DNA yield, continued	ChargeSwitch®-derivatized membrane is not functional	**Do not freeze the columns.** Store the columns at room temperature. Do not re-use the columns.
	Cell cultures are overgrown	Cells grown more than 16 hours may begin to lyse, resulting in reduced yields. Do not use richer media.
	Quantitation is inaccurate: Supercoiled plasmid DNA fluoresces less than the DNA standard provided in quantitation kit	We recommend using plasmid DNA as a standard when calculating yield using a fluorescence-based DNA quantitation kit. Such kits typically provide non-supercoiled DNA as a standard, which fluoresces more brightly than supercoiled plasmid DNA, leading to inaccuracies in quantitation.
Lysate Clarification Column clogging	Too much precipitate	• Incubate the lysate for 2–3 minutes to allow the precipitate to float. • Cell culture may be overgrown. See page 10 for details on culturing cells.
Genomic DNA contamination	Genomic DNA sheared during handling	Gently invert the tubes to mix after adding buffers. **Do not vortex** as it can shear the genomic DNA. To efficiently precipitate the genomic DNA away from the plasmid DNA, the genomic DNA must be intact.
RNA contamination	Insufficient RNase treatment	• Ensure RNase A is added to the resuspension buffer. • Add additional RNAse A to 100 µL/mL if buffer has been stored for >6 months. • Ensure sufficient mixing after addition of lysis buffer to allow proper RNase A digestion.
Plasmid DNA degradation	Incorrect lysis procedure	Incubate the lysate at room temperature for no longer than 5 minutes, because it might begin to denature the DNA.

Appendix

Accessory Products

Additional Products

The following table lists additional products available from Life Technologies that may be used with the ChargeSwitch®-Pro Filter Plasmid Midiprep and Maxiprep Kits.

A large selection of Life Technologies products is available for cleanup of DNA and RNA from various sources. For more information, visit **www.lifetechnologies.com** or contact Technical Support (page 29).

Product	Amount	Catalog No.
EveryPrep™ Universal Vacuum Manifold	1 unit	K211101
ChargeSwitch®-Pro Filter Collection Tubes	25 tubes	CS32000
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
Luria Broth Base (Miller's LB Broth Base)®, powder	2.5 kg	12795-084
Ampicillin	200 mg	11593-019
Carbenicillin, Disodium Salt	5 g	10177-012
One Shot® TOP10 Chemically Competent *E. coli*	10 reactions	C4040-10
	20 reactions	C4040-03
	40 reactions	C4040-06
One Shot® TOP10 Electrocomp™ *E. coli*	10 reactions	C4040-50
	20 reactions	C4040-52
ChargeSwitch® PCR Cleanup Kit	100 preps	CS12000
PureLink® PCR Purification Kit	50 preps	K3100-01
PureLink® Gel Extraction Kit	50 preps	K2100-12

Technical Support

Obtaining Support

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

At the website, you can:

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

Safety Data Sheets (SDS)

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

Certificate of Analysis

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

Limited Product Warranty

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

Purchaser Notification

Limited Use Label License No. 358: Research Use Only

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

Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA
Phone +1 760 603 7200 | Toll Free in USA 800 955 6288

For support visit
www.lifetechnologies.com/support or email techsupport@lifetech.com

www.lifetechnologies.com

life technologies™