



PureLink® HiPure Plasmid DNA Purification Kits

**For Mini, Midi, and Maxi preparation of
Plasmid DNA**

Catalog numbers K2100-02, K2100-03, K2100-04, K2100-05,
K2100-06, and K2100-07

Revision Date 2 May 2011
Publication Part Number 25-0787

MAN0000486

Contents

Kit Contents and Storage	v
Description of the System.....	1
About the Kit.....	1
Experimental Overview	4
Methods.....	5
Before Starting	5
Miniprep Procedure.....	8
Midiprep Procedure.....	11
Maxiprep Procedure	15
Estimating DNA Yield and Quality	18
Expected Results.....	19
Appendix	20
Procedure for BAC DNA	20
Procedure for Bacmid DNA	24
Procedure for Cosmid DNA.....	26
Procedure for ssM13 DNA.....	28
Troubleshooting	31
Accessory Products.....	33
Technical Support	34
Purchaser Notification.....	36

Kit Contents and Storage

Types of Products

This manual is supplied with the following products:

Product	Quantity	Catalog no.
PureLink® HiPure Plasmid Miniprep Kit	25 preps	K2100-02
	100 preps	K2100-03
PureLink® HiPure Plasmid Midiprep Kit	25 preps	K2100-04
	50 preps	K2100-05
PureLink® HiPure Plasmid Maxiprep Kit	10 preps	K2100-06
	25 preps	K2100-07

Shipping and Storage

All components of the PureLink® HiPure Plasmid DNA Purification Kits are shipped at room temperature.

Upon receipt, store all components at room temperature.

Contents

The components included in the PureLink® HiPure Plasmid DNA Purification Kits are listed below.

Component	Miniprep		Midiprep		Maxiprep	
	Cat. no. K2100-02	Cat. no. K2100-03	Cat. no. K2100-04	Cat. no. K2100-05	Cat. no. K2100-06	Cat. no. K2100-07
Resuspension Buffer (R3)	10 mL	50 mL	100 mL	200 mL	100 mL	250 mL
RNase A	100 µL	550 µL	550 µL	1.5 mL	550 µL	1.5 mL
Lysis Buffer (L7)	10 mL	50 mL	100 mL	200 mL	100 mL	250 mL
Precipitation Buffer (N3)	10 mL	40 mL	100 mL	200 mL	100 mL	250 mL
Equilibration Buffer (EQ1)	50 mL	250 mL	250 mL	2 × 250 mL	300 mL	2 × 400 mL
Wash Buffer (W8)	125 mL	500 mL	500 mL	2 × 500 mL	2 × 300 mL	3 × 500 mL
Elution Buffer (E4)	25 mL	90 mL	125 mL	250 mL	200 mL	400 mL
TE Buffer (TE)	15 mL	15 mL	15 mL	30 mL	30 mL	30 mL
HiPure Columns	25 each	100 each	25 each	50 each	10 each	25 each
Column Holders	—	—	5 each	10 each	3 each	5 each

Continued on next page

Kit Contents and Storage, Continued

Buffer Composition The composition of buffers included in the PureLink® HiPure Plasmid Purification Kits is listed below.

Buffer	Composition
Resuspension Buffer (R3)	50 mM Tris-HCl, pH 8.0 10 mM EDTA
RNase A	20 mg/mL in Resuspension Buffer (R3)
Lysis Buffer (L7)	0.2 M NaOH 1% (w/v) SDS
Precipitation Buffer (N3)	3.1 M Potassium acetate, pH 5.5
Equilibration Buffer (EQ1)	0.1 M Sodium acetate, pH 5.0 0.6 M NaCl 0.15% (v/v) Triton® X-100
Wash Buffer (W8)	0.1 M Sodium acetate, pH 5.0 825 mM NaCl
Elution Buffer (E4)	100 mM Tris-HCl, pH 8.5 1.25 M NaCl
TE Buffer (TE)	10 mM Tris-HCl, pH 8.0 0.1 mM EDTA

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

Description of the System

About the Kit

System Summary

The PureLink® HiPure Plasmid Purification Kits allow you to isolate high yields of highly pure plasmid DNA. The kits are designed to efficiently isolate plasmid DNA from *E. coli* in 1.5–2 hours using anion-exchange columns, without the use of any organic solvents or cesium chloride (CsCl). The isolated plasmid DNA is of high purity, equivalent to two passes through CsCl gradients, and contains low endotoxin levels (page 19).

The PureLink® HiPure Plasmid DNA Purification Kits are available in three formats that allow you to purify plasmid DNA using different starting culture volumes (page 5).

The HiPure Technology

The HiPure technology is based on anion-exchange chromatography. The technology uses a patented resin composed of small particles with a uniform pore size, to provide high yields and reproducible performance.



The spacer arm with increased length provides improved DNA binding efficiency. The unique patented ion-exchange moiety provides high efficiency for separation of DNA from cellular contaminants including RNA.

Continued on next page

About the Kit, Continued

System Overview

The PureLink® HiPure Plasmid DNA Purification Kits use a patented anion-exchange resin to purify plasmid DNA to a level equivalent to two passes through CsCl gradients. The patented resin provides excellent capacity with fast flow rates, high resolution, high yield, and efficient endotoxin removal.

To purify plasmid DNA, you will harvest *E. coli* cells, resuspend the cells in Resuspension Buffer (R3) with RNase A, and then lyse the cells with Lysis Buffer (L7). Then, you will add Precipitation Buffer (N3) to the lysate and clarify the lysate using a centrifuge. Next, you will pass the clear lysate through a pre-packed anion exchange column. The negatively charged phosphates on the backbone of the DNA interact with the positive charges on the surface of the resin. The temperature, salt concentration, and pH of the solutions influence binding. Under moderate salt conditions, plasmid DNA remains bound to the resin while RNA, proteins, carbohydrates and other impurities are washed away with Wash Buffer (W8). After washing, you will elute the plasmid DNA under high salt conditions with the Elution Buffer (E4). Last, you will desalt and concentrate the DNA with an alcohol precipitation step. You can complete the entire protocol in 1.5–2 hours.

Advantages

The advantages of using PureLink® HiPure Plasmid DNA Purification Kits are:

- Purify all types and sizes of plasmid DNA, including BAC, bacmids, and ssM13 DNAs (see page 20)
 - Purify high-quality plasmid DNA suited for mammalian transfections
 - High yield of plasmid DNA
 - Reliable performance of the purified plasmid DNA in a variety of applications (see **Downstream Applications**, page 3)
-

Continued on next page

About the Kit, Continued

System Specifications

Specification*	Miniprep	Midiprep	Maxiprep
Starting culture volume	1–3 mL	15–25 mL	100–200 mL
Column Binding Capacity**	30 µg	350 µg	850 µg
Column Reservoir Capacity	2.5 mL	10 mL	60 mL
Elution Volume	0.9 mL	5 mL	15 mL
DNA Recovery	90–95%	90–95%	90–95%
Expected DNA Yield***	≤30 µg	100–350 µg	500–850 µg

* Specifications and results are based on high copy number plasmids.

** Binding capacity depends on plasmid copy number, type and size, and volume of bacterial culture used.

*** DNA yield depends on plasmid copy number, type and size; bacterial strain; and growth conditions.

Downstream Applications

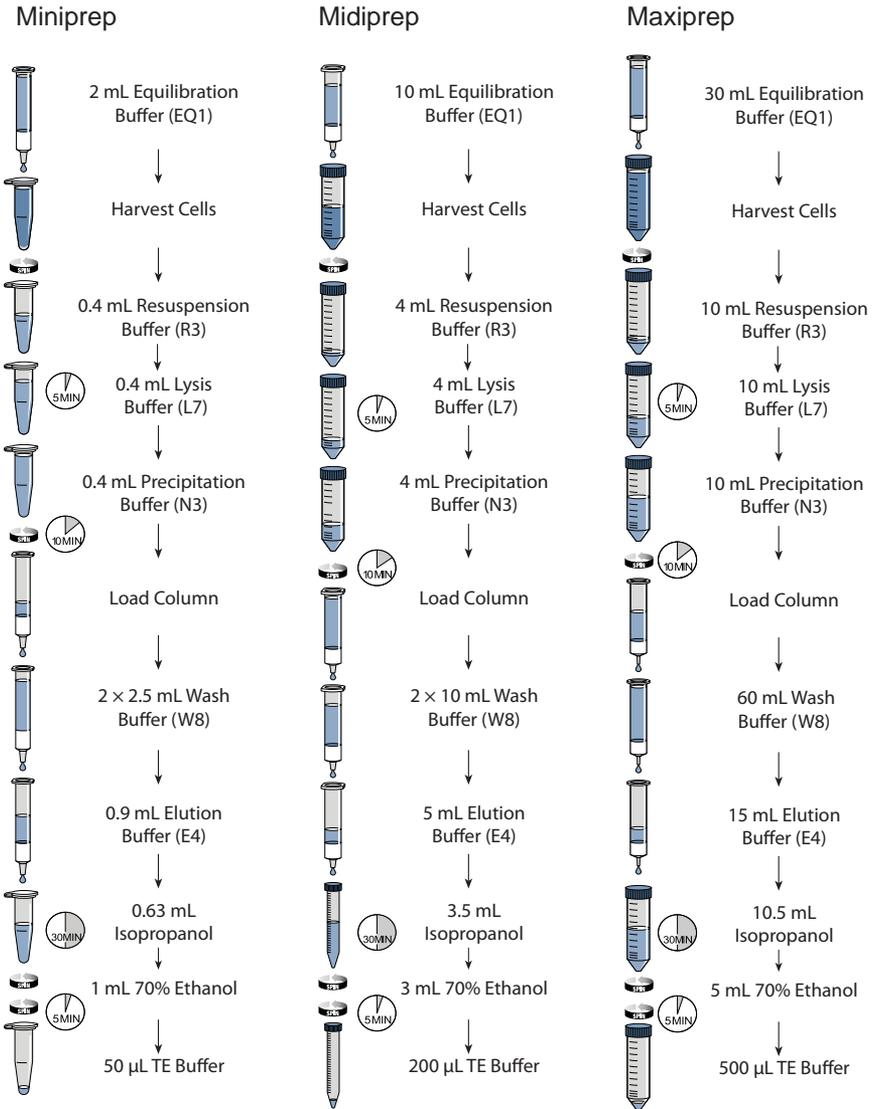
The purified DNA is ultrapure and suitable for downstream applications, including those requiring the highest purity, such as:

- Transfection of mammalian cells
 - Automated and manual DNA sequencing
 - PCR amplification
 - *In vitro* transcription
 - Bacterial cell transformation
 - Cloning
 - Labeling
-

Experimental Overview

Introduction

The flow chart for purifying plasmid DNA using the PureLink® HiPure Plasmid DNA Purification Kits is shown below.



Methods

Before Starting

Introduction

Review the information in this section before starting. Guidelines are included for growing the overnight cell culture and for determining the appropriate amounts of starting material based on the plasmid copy number used.



Some buffers in the PureLink® HiPure Plasmid DNA Purification Kit contain hazardous chemicals.

Always wear a laboratory coat, disposable gloves, and eye protection when handling the buffers.

Bacterial Cultures

Grow transformed *E. coli* cells overnight in LB (Luria-Bertani) medium with the appropriate antibiotic. Harvest the bacterial culture in transition between exponential phase and stationary phase. The culture should have a cell density of $\sim 10^9$ cells/mL or an optical density of 2.0 at 600 nm (OD_{600}).

Plasmid Type and Copy Number

Use PureLink® HiPure Plasmid DNA Purification Kits for purifying all types of plasmid DNA, including BAC (page 20), bacmids (page 24), and ssM13 DNAs (page 28).

High copy number plasmids provide the best results, with a typical yield of 2–6 μg DNA/mL from an overnight culture. Yields from low copy number plasmids are dependent upon culture conditions and vector/host strain combinations.

When isolating low copy number plasmids, use a higher volume of cell culture, as directed in the protocol.

Recommended volumes of cell culture for plasmid DNA purification are listed in the table below.

Plasmid Copy Number	Miniprep	Midiprep	Maxiprep*
High-copy number plasmid	1–3 mL	15–25 mL	100–200 mL
Low-copy number plasmid	10–15 mL	25–100 mL	250–500 mL

*When performing Maxipreps of low copy number plasmids from bacterial cultures of >200 mL, double the volumes of Resuspension Buffer (R3), Lysis Buffer (L7), and Precipitation Buffer (N3) indicated in the protocol (page 16).

Continued on next page

Before Starting, Continued



Note

If the provided buffers in the Maxiprep kit are not sufficient due to the requirements of isolating low copy number plasmids, you may obtain additional buffers by ordering the PureLink® HiPure BAC Buffer kit (see **Additional Products**, page 33).



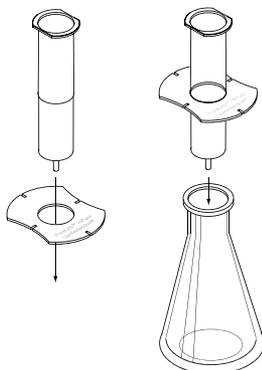
Follow the recommendations below to obtain the best results:

- Maintain a sterile workspace and equipment (including pipette tips and tubes) to avoid DNase contamination.
 - Ensure that no DNase is introduced into the sterile solutions supplied with the kit.
 - Use the PureLink® Nucleic Acid Purification Rack for column purification (see **Purification Rack**, page 7).
 - Perform all recommended wash steps for best results.
 - Use the TE Buffer (TE) provided or 10 mM Tris-HCl, pH 8.5 to resuspend the DNA pellet.
-

Using the Column Holder

The Column Holders in the kit allow Midi and Maxi Columns to be supported in an upright position when placed in the mouth of an Erlenmeyer (or similar) flask.

To use the Column Holder, slip the column through the hole in the center of the Column Holder. The column with Column Holder can then be placed in the mouth of a flask.



Continued on next page

Before Starting, Continued

Purification Rack

The PureLink® Nucleic Acid Purification Rack (see page 33) is designed specifically for use with PureLink® HiPure Plasmid DNA Miniprep, Midiprep, and Maxiprep Kits. The PureLink® Nucleic Acid Purification Rack consists of a Column Holder Rack (for processing 12 miniprep, 8 midiprep, and 4 maxiprep columns), a Collection Tube Rack (capable of accommodating various types and sizes of recovery tubes), and a large capacity Waste Tray for collecting waste.

Buffer Preparation

Resuspension Buffer (R3)

Add RNase A to the Resuspension Buffer (R3) according to instructions on the label of the bottle. Mix well. Mark the bottle label to indicate that it contains RNase A (**100 µg/mL** final concentration). Store the buffer with RNase A at 4°C.

Lysis Buffer (L7)

Check the Lysis Buffer (L7) for precipitates. If present, warm the solution briefly at 37°C to dissolve the precipitate.

Specific Protocols

Specific protocols for plasmid DNA purification using the various kits are described in this manual as shown in the table below.

Protocol	Page no.
Purifying plasmid DNA using:	
Miniprep kit	8
Midiprep kit	11
Maxiprep kit	15
Purifying BAC DNA	20
Purifying Bacmid DNA	24
Purifying Cosmid DNA	26
Purifying ssM13 DNA	28

Miniprep Procedure

Introduction

The PureLink® HiPure Plasmid DNA Miniprep Kit allows you to purify up to 30 µg of high quality plasmid DNA from 1–3 mL overnight *E. coli* cultures in ~1 hour when cloning high copy number plasmids.

Before Starting

Verify that RNase A is added to the Resuspension Buffer (R3). Warm Lysis Buffer (L7) briefly at 37°C to redissolve any particulate matter. See page 7 for details.

Materials Needed

- Overnight culture of transformed *E. coli* cells (page 5)
 - Isopropanol
 - 70% ethanol
 - Sterile, microcentrifuge tubes
 - PureLink® Nucleic Acid Purification Rack (page 33)
 - Microcentrifuge capable of centrifuging at >12,000 × g
-

Components Supplied with the Kit

- Resuspension Buffer (R3) with RNase A (page 7)
 - Lysis Buffer (L7)
 - Precipitation Buffer (N3)
 - Equilibration Buffer (EQ1)
 - Wash Buffer (W8)
 - Elution Buffer (E4)
 - TE Buffer (TE)
 - PureLink® HiPure Mini Columns
-

Equilibrating the Column

Place the PureLink® HiPure Mini column on the PureLink® Nucleic Acid Purification Rack (refer to the manual supplied with the rack for more details). Apply 2 mL Equilibration Buffer (EQ1) to the column. Allow the solution in the column to drain by gravity flow. Proceed to **Preparing Cell Lysate** (next page) while the column is equilibrating.

Continued on next page

Miniprep Procedure, Continued

Preparing Cell Lysate

1. For **high copy number plasmids**, use 1–3 mL of an overnight LB culture per sample in a microcentrifuge tube.
Note: When using 2–3 mL of culture, pellet 1–1.5 mL culture *twice* in the *same* microcentrifuge tube.
If you are using >5 mL of culture volume of high copy plasmids, add twice the amount of Resuspension Buffer (R3) with RNase A, Lysis Buffer (L7), and Precipitation Buffer (N3) as directed in steps 3, 4, and 5, below for best results.
For **low copy number plasmids**, use 10–15 mL of an overnight LB culture per sample in a 15-mL disposable tube.
2. Harvest the cells by centrifuging the overnight LB culture at $4000 \times g$ for 5–10 minutes.
Remove all medium.
3. Add 0.4 mL Resuspension Buffer (R3) with RNase A to the pellet and resuspend cells until homogeneous.
Note: If cells were resuspended in a 15-mL disposable tube, then transfer the cells in a microcentrifuge tube.
4. Add 0.4 mL Lysis Buffer (L7). Mix gently by inverting the capped tube until the lysate mixture is thoroughly homogenous. **Do not vortex.** Incubate at room temperature for 5 minutes.
Note: Do not allow lysis to proceed for more than 5 minutes.
5. Add 0.4 mL Precipitation Buffer (N3) and mix immediately by inverting the tube until the mixture is thoroughly homogeneous. **Do not vortex.**
6. Centrifuge the lysate at $>12,000 \times g$ for 10 minutes at room temperature.
Note: If the pellet does not adhere to the bottom of the tube, incubate the tube at room temperature for 5 minutes to allow the lysate and gelatinous pellet to separate. Pipet the clear lysate into another sterile tube and centrifuge at $>12,000 \times g$ for 5 minutes at room temperature to remove any remaining cellular debris.
7. Proceed to **Binding and Washing DNA** (next page).

Continued on next page

Miniprep Procedure, Continued

Binding and Washing DNA

1. Load the supernatant from step 6 (**Preparing Cell Lysate**) onto the equilibrated column. Allow the solution in the column to drain by gravity flow.
 2. Wash the column **twice** with 2.5 mL Wash Buffer (W8). Allow the solution in the column to drain by gravity flow after each wash. Discard the flow-through.
 3. Proceed to **Eluting and Precipitating DNA**, below.
-

Eluting and Precipitating DNA

1. Place a sterile microcentrifuge tube (elution tube) under the column.
 2. Add 0.9 mL Elution Buffer (E4) to the column to elute the DNA. Allow the solution to drain by gravity flow. Do not force out any remaining solution.
The elution tube contains the purified DNA.
Discard the column.
 3. Add 0.63 mL isopropanol to the elution tube. Mix well.
 4. Centrifuge the elution tube at $>12,000 \times g$ for 30 minutes at 4°C. Carefully remove and discard the supernatant.
 5. Resuspend the DNA pellet in 1 mL 70% ethanol.
 6. Centrifuge the tube at $>12,000 \times g$ for 5 minutes at 4°C. Carefully remove and discard the supernatant.
 7. Air-dry the pellet for 10 minutes.
 8. Resuspend the DNA pellet in 50 μ L TE Buffer (TE).
Note: Occasionally, insoluble particles may be present. These particles do not influence the quality of the DNA and can be easily removed. To remove insoluble particles, centrifuge the DNA solution at high speed for 1 minute at room temperature. Transfer the supernatant (DNA sample) into a fresh tube.
-

Storing DNA

To avoid repeated freezing and thawing of DNA, store the purified DNA at 4°C for immediate use or aliquot the DNA and store at -20°C for long-term storage.

Midiprep Procedure

Introduction The PureLink® HiPure Plasmid Filter Midiprep Kit allows you to purify 100–350 µg of high-quality plasmid DNA from 15–25 mL overnight *E. coli* cultures in ~2 hours when cloning high copy number plasmids.

Before Starting Verify that RNase A is added to the Resuspension Buffer (R3). Warm Lysis Buffer (L7) briefly at 37°C to redissolve any particulate matter. See page 7 for details.

Materials Needed

- Overnight culture of transformed *E. coli* cells (page 5)
- Isopropanol
- 70% ethanol
- Sterile, microcentrifuge tubes
- PureLink® Nucleic Acid Purification Rack (page 33)
- Tubes or centrifuge bottles for harvesting cells
- Centrifuge and rotor appropriate for harvesting cells
- 15-mL centrifuge tubes (elution tubes) capable of withstanding centrifugation forces >12,000 × g
- Centrifuge capable of centrifuging at >12,000 × g at 4°C
- *Optional:* PureLink® HiPure Precipitator Module (page 33)

Components Supplied with the Kit

- Resuspension Buffer (R3) with RNase A (page 7)
- Lysis Buffer (L7)
- Precipitation Buffer (N3)
- Equilibration Buffer (EQ1)
- Wash Buffer (W8)
- Elution Buffer (E4)
- TE Buffer (TE)
- PureLink® HiPure Midi Columns
- Column Holder

Equilibrating the Column Use the Column Holder to support a HiPure Midi Column in a flask (see page 6), or place the Midi Column on the PureLink® Nucleic Acid Purification Rack (see the manual supplied with the rack for more details). Apply 10 mL Equilibration Buffer (EQ1) to the column. Allow the solution in the column to drain by gravity flow. Proceed to Preparing Cell Lysate (next page), while the column is equilibrating.

Continued on next page

Midiprep Procedure, Continued

Preparing Cell Lysate

1. For **high copy number plasmids**, use 15–25 mL of an overnight LB culture per sample in a disposable 50-mL conical tube.
Note: If you are using >25 mL of culture volume of high copy plasmids, add twice the amount of Resuspension Buffer (R3) with RNase A, Lysis Buffer (L7), and Precipitation Buffer (N3) as directed in steps 3, 4, and 5, below, for best results.
For **low copy number plasmids**, use 25–100 mL of an overnight LB culture per sample in a 50-mL tube.
2. Harvest the cells by centrifuging the overnight LB culture at $4000 \times g$ for 10 minutes. Remove all medium.
3. Add 4 mL Resuspension Buffer (R3) with RNase A to the cell pellet and resuspend the cells until homogeneous.
4. Add 4 mL Lysis Buffer (L7). Mix gently by inverting the capped tube until the lysate mixture is thoroughly homogenous. **Do not vortex.** Incubate at room temperature for 5 minutes.
Note: Do not allow lysis to proceed for more than 5 minutes.
5. Add 4 mL Precipitation Buffer (N3) and mix immediately by inverting the capped tube until the mixture is thoroughly homogeneous. **Do not vortex.**
6. Centrifuge the mixture at $>12,000 \times g$ for 10 minutes at room temperature.
Note: If the pellet does not adhere to the bottom of the tube, incubate the tube at room temperature for 5 minutes to allow the lysate and gelatinous pellet to separate. Pipet the clear lysate into another, sterile tube and centrifuge at $>12,000 \times g$ at room temperature for 5 minutes to remove any remaining cellular debris.
7. Proceed to **Binding and Washing DNA**, next page.

Continued on next page

Midiprep Procedure, Continued

Binding and Washing DNA

1. Load the supernatant from step 6 (**Preparing Cell Lysate**) onto the equilibrated column. Allow the solution in the column to drain by gravity flow.
 2. Wash the column **twice** with 10 mL Wash Buffer (W8). Allow the solution in the column to drain by gravity flow after each wash. Discard the flow-through.
 3. Proceed to **Eluting and Precipitating DNA**.
-



Note

For DNA precipitation, you can use the PureLink® HiPure Precipitator Module (page 33) which allows you to precipitate DNA within 10 minutes without using a centrifuge. Alternatively, follow **Eluting and Precipitating DNA** on page 14 to perform traditional DNA precipitation using centrifugation.

Refer to the manual supplied with the PureLink® HiPure Precipitator Module for a detailed protocol.

Continued on next page

Midiprep Procedure, Continued

Eluting and Precipitating DNA

1. Place a sterile 15-mL centrifuge tube (elution tube) under the column.
2. Add 5 mL Elution Buffer (E4) to the column to elute the DNA. Allow the solution to drain by gravity flow. Do not force out any remaining solution.
The elution tube contains the purified DNA.
Discard the column.
3. Add 3.5 mL isopropanol to the elution tube. Mix well.
Note: Proceed to the protocol described in the PureLink® HiPure Precipitator manual after this step, if you are using the precipitator module.
4. Centrifuge the tube at $>12,000 \times g$ for 30 minutes at 4°C. Carefully remove and discard the supernatant.
5. Resuspend the pellet in 3 mL 70% ethanol.
6. Centrifuge the tube at $>12,000 \times g$ for 5 minutes at 4°C. Carefully remove and discard the supernatant.
7. Air-dry the pellet for 10 minutes.
8. Resuspend the DNA pellet in 200 μ L TE Buffer (TE). For low copy number plasmids, use 100 μ L of TE Buffer.
Note: Occasionally, insoluble particles may be present. These particles do not influence the quality of the DNA and can be easily removed. To remove insoluble particles, centrifuge the DNA solution at high speed for 1 minute at room temperature. Transfer the supernatant (DNA sample) into a fresh tube.

Storing DNA

To avoid repeated freezing and thawing of DNA, store the purified DNA at 4°C for immediate use or aliquot the DNA and store at -20°C for long-term storage.

Maxiprep Procedure

Introduction

The PureLink® HiPure Plasmid Filter Maxiprep Kit allows you to purify 500–850 µg of high-quality plasmid DNA from 100–200 mL overnight *E. coli* cultures in ~2 hours when cloning high copy number plasmids.

Before Starting

Verify that RNase A is added to the Resuspension Buffer (R3). Warm Lysis Buffer (L7) briefly at 37°C to redissolve any particulate matter. See page 7 for details.

Materials Needed

- Overnight culture of transformed *E. coli* cells (page 5)
 - Isopropanol
 - 70% ethanol
 - Sterile, microcentrifuge tubes
 - PureLink® Nucleic Acid Purification Rack (page 33)
 - Tubes or centrifuge bottles for harvesting cells
 - Centrifuge and rotor appropriate for harvesting cells
 - Sterile 50-mL centrifuge tube (elution tube) capable of withstanding centrifugation forces >12,000 × g
 - Centrifuge capable of centrifuging at >12,000 × g at 4°C
 - *Optional:* PureLink® HiPure Precipitator Module (page 33)
-

Components Supplied with the Kit

- Resuspension Buffer (R3) with RNase A (page 7)
 - Lysis Buffer (L7)
 - Precipitation Buffer (N3)
 - Equilibration Buffer (EQ1)
 - Wash Buffer (W8)
 - Elution Buffer (E4)
 - TE Buffer (TE)
 - PureLink® HiPure Maxi Columns
 - Column Holder
-

Equilibrating the Column

Use the Column Holder to support a HiPure Maxi Column in a flask (see page 6), or place the Maxi Column on the PureLink® Nucleic Acid Purification Rack (refer to the manual supplied with the rack for more details). Apply 30 mL Equilibration Buffer (EQ1) to the column. Allow the solution in the column to drain by gravity flow. Proceed to **Preparing Cell Lysate** while the column is equilibrating.

Continued on next page

Maxiprep Procedure, Continued

Preparing Cell Lysate

1. For **high copy number plasmids**, use 100–200 mL of an overnight LB culture per sample.
For **low copy number plasmids**, use 250–500 mL of an overnight LB culture per sample.
Note: For culture volumes >200 mL, add twice the amount of Resuspension Buffer (R3) with RNase A, Lysis Buffer (L7), and Precipitation Buffer (N3) as directed in steps 3, 4, and 5, below.
2. Harvest the cells by centrifuging the overnight LB culture at $4000 \times g$ for 10 minutes. Remove all medium.
3. Add 10 mL Resuspension Buffer (R3) with RNase A to the pellet and resuspend the cells until homogeneous.
4. Add 10 mL Lysis Buffer (L7). Mix gently by inverting the capped tube until the lysate mixture is thoroughly homogenous. **Do not vortex**. Incubate the tube at room temperature for 5 minutes.
Note: Do not allow lysis to proceed for more than 5 minutes.
5. Add 10 mL Precipitation Buffer (N3) and mix immediately by inverting the tube until the mixture is thoroughly homogeneous. **Do not vortex**.
6. Centrifuge the mixture at $>12,000 \times g$ for 10 minutes at room temperature.
Note: If the pellet does not adhere to the bottom of the tube, incubate the tube at room temperature for 5 minutes to allow the lysate and gelatinous pellet to separate. Pipet the clear lysate into another tube and centrifuge the tube at $>12,000 \times g$ for 5 minutes at room temperature to remove any remaining cellular debris.
7. Proceed to **Binding and Washing DNA**.

Binding and Washing DNA

1. Load the supernatant from step 6 (**Preparing Cell Lysate**) onto the equilibrated column. Allow the solution in the column to drain by gravity flow.
2. Wash the column with 60 mL Wash Buffer (W8). Allow the solution in the column to drain by gravity flow. Discard the flow-through.
3. Proceed to **Eluting and Precipitating DNA**.

Continued on next page

Maxiprep Procedure, Continued



Note

To precipitate DNA, you can use the PureLink® HiPure Precipitator Module (page 33) which allows you to precipitate DNA within 10 minutes without using a centrifuge. Alternatively, you can follow the protocol below to perform traditional DNA precipitation.

Refer to the manual supplied with the PureLink® HiPure Precipitator Module for a detailed protocol.

Eluting and Precipitating DNA

1. Place a sterile 30-mL centrifuge tube (elution tube) under the column.
 2. Add 15 mL Elution Buffer (E4) to the column to elute the DNA. Allow the solution to drain by gravity flow. Do not force out any remaining solution.
The elution tube contains the purified DNA.
Discard the column.
 3. Add 10.5 mL isopropanol to the elution tube. Mix well.
Note: Proceed to the protocol described in the PureLink® HiPure Precipitator manual after this step, if you are using the precipitator.
 4. Centrifuge the elution tube at $>12,000 \times g$ for 30 minutes at 4°C. Carefully remove and discard the supernatant.
 5. Resuspend the DNA pellet in 5 mL 70% ethanol.
 6. Centrifuge the elution tube at $>12,000 \times g$ for 5 minutes at 4°C. Carefully remove and discard the supernatant.
 7. Air-dry the pellet for 10 minutes.
 8. Resuspend the DNA pellet in 500 μ L TE Buffer (TE). For low copy number plasmids, use 200 μ L TE Buffer (TE).
Note: Occasionally, insoluble particles may be present. These particles do not influence the quality of the DNA and can be easily removed. To remove insoluble particles, centrifuge the DNA solution at high speed at room temperature for 1 minute. Transfer the supernatant (DNA sample) into a fresh tube.
-

Storing DNA

To avoid repeated freezing and thawing of DNA, store the purified DNA at 4°C for immediate use or aliquot the DNA and store at -20°C for long-term storage.

Estimating DNA Yield and Quality

Introduction

Once you have isolated DNA, you may determine the quantity and quality of the purified DNA as described below.

DNA Yield

Measure the DNA concentration using UV absorbance at 260 nm or Qubit® DNA Assay 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:

$$\text{DNA } (\mu\text{g/mL}) = A_{260} \times 50 \times \text{dilution factor}$$

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

Qubit® DNA Assay Kits

The Qubit® DNA Assay Kits (page 33) provide a rapid, sensitive, and specific method for measuring dsDNA concentration with minimal interference from RNA, protein, ssDNA (primers), or other common contaminants that affect UV absorbance.

The kits contain a state-of-the-art quantitation reagent, pre-diluted standards for standard curve, and a pre-made buffer. The assay is designed for reading in standard fluorescent readers/fluorometer or Qubit® 2.0 Fluorometer.

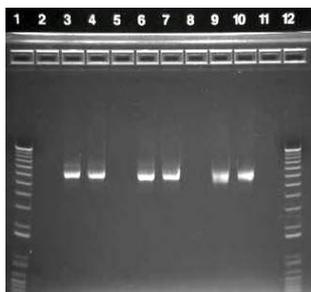
Estimating DNA Quality

Typically, DNA isolated using the PureLink® HiPure Plasmid Purification Kit has an A_{260}/A_{280} ratio >1.80 when samples are diluted in Tris-HCl pH 7.5, indicating that the DNA is substantially free of proteins that could interfere with downstream applications. The absence of contaminating RNA may be confirmed by agarose gel electrophoresis.

Expected Results

Results

Plasmid DNA was isolated in duplicates from *E. coli* (TOP10) transformed with pcDNA™ 3.1/His/LacZ using the PureLink® HiPure Plasmid DNA Purification Kits as described in this manual. The purified plasmid DNA was analyzed for yield, endotoxin levels, OD_{260/280} ratio, sequencing, restriction enzyme digestion and gel electrophoresis (100 ng) on a 0.8% E-Gel® agarose gel (see below).



Lanes 1, 12: TrackIt™ 1 Kb Plus DNA Ladder

Lanes 2, 5, 8, 11: Blank

Lanes 3, 4: Miniprep (3 mL culture)

Lanes 6, 8: Midiprep (25 mL culture)

Lanes 9, 10: Maxiprep (100 mL culture)

Summary of Expected Results

The summary of results using the PureLink® HiPure Plasmid DNA Purification Kits is listed in the table below.

Note: DNA yield depends on plasmid copy number and type, bacterial strain, and growth conditions.

Results for:	Miniprep	Midiprep	Maxiprep
Processing Time	~1 hour	~2 hours	~2 hours
Plasmid DNA Yield*	≤30 µg	100–350 µg	500–850 µg
Column Binding Capacity	30 µg	350 µg	850 µg
Endotoxin	0.1–1 EU/µg	0.1–1 EU/µg	0.1–1.5 EU/µg
OD _{260/280}	~1.87	~1.95	~1.98
Sequencing (Capillary)	Successful	Successful	Successful
Restriction Enzyme Digestion	Successful	Successful	Successful

* As determined by using Qubit® Kit or by measuring UV absorbance at 260 nm

Appendix

Procedure for BAC DNA

Introduction

The PureLink® HiPure Plasmid DNA Purification Kits allow you to purify high quality BAC (bacterial artificial chromosome) DNA from *E. coli* cultures.



Note

Due to changes in the volumes of buffers used in this protocol, the amounts of buffers provided in the PureLink® HiPure Plasmid DNA Purification Kits may not be sufficient to utilize all of the columns provided in the kit. To obtain additional amounts of buffers, order the PureLink® HiPure BAC Buffer Kit (see page 33 for ordering information).

Before Starting

- Prepare a 20-hour culture of BAC containing bacteria in 2X YT medium and appropriate antibiotic. The absorbance at 600 nm of the final culture should be 5.0 ± 0.5 .
 - Warm an aliquot of Elution Buffer (E4) to 50°C.
 - Add 20 mg/mL RNase A to Resuspension Buffer (R3) to a final concentration of **400 µg/mL**.
 - Warm Lysis Buffer (L7) briefly at 37°C to redissolve any particulate matter (see page 7).
-

Continued on next page

Procedure for BAC DNA, Continued

Modified Column Wash Buffer

Isolating DNA from large amounts of bacterial culture increases the likelihood of residual RNA in the eluate. We recommend using a modified Column Wash Buffer (W8) with a higher salt concentration (850 mM NaCl) and lower pH (4.0) for more efficient removal of residual RNA (see **Binding and Washing DNA**, step 2, page 22).

Column Wash Buffer

100 mM sodium acetate, pH 4.0

850 mM NaCl

1. Prepare 100 mL of Modified Column Wash Buffer as follows:

Sodium acetate, anhydrous	0.82 g
Glacial acetic acid	1.43 mL
NaCl	4.97 g
Ultra pure water	80 mL
 2. Mix well and adjust with water to bring the final volume to 100 mL and check the pH. Slight variation for the final pH (± 0.1) is possible and can be tolerated. Do not adjust the pH value using salt or acid.
 3. Store the buffer at room temperature.
-

Equilibrating the Column

Support the column vertically using the PureLink[®] Nucleic Acid Purification Rack (refer to the manual supplied with the rack for more details), or the Column Holder (for PureLink[®] HiPure Midiprep and Maxiprep Columns, see page 6). Apply Equilibration Buffer (EQ1) to the column. Allow the solution in the column to drain by gravity flow.

Miniprep	Midiprep	Maxiprep
2 mL	10 mL	30 mL

Continued on next page

Procedure for BAC DNA, Continued

Preparing Cell Lysate

1. Harvest the bacterial cells by centrifuging the overnight culture at $9000 \times g$ for 15 minutes. Remove all medium.

Miniprep	Midiprep	Maxiprep
10–25 mL	100 mL	200–500 mL

2. Add Resuspension Buffer (R3) containing RNase A to the pellet and resuspend the cells until homogeneous.

Miniprep	Midiprep	Maxiprep
2 mL	8 mL	20 mL

3. Add Lysis Buffer (L7). Mix gently by inverting the capped tube until the mixture is thoroughly homogeneous. **Do not vortex.** Incubate at room temperature for 5 minutes.

Miniprep	Midiprep	Maxiprep
2 mL	8 mL	20 mL

4. Add Precipitation Buffer (N3) and mix immediately by inverting the capped tube until the mixture is thoroughly homogeneous. **Do not vortex.**

Miniprep	Midiprep	Maxiprep
2 mL	8 mL	20 mL

5. Centrifuge the mixture at $>12,000 \times g$ at room temperature for 10 minutes.

Note: If the pellet does not adhere to the bottom of the tube, incubate the tube at room temperature for 5 minutes to allow the lysate and gelatinous pellet to separate. Pipet the clear lysate into a sterile tube and centrifuge the tube at $>12,000 \times g$ for 5 minutes at room temperature to remove any remaining cellular debris.

Binding and Washing DNA

1. Load the supernatant from step 5 (**Preparing Cell Lysate**) onto the equilibrated column. Allow the solution in the column to drain by gravity flow.
2. Wash the column with Wash Buffer (W8). Allow the solution in the column to drain by gravity flow. Discard the flow-through.

Miniprep	Midiprep	Maxiprep
2×2.5 mL	2×10 mL	1×60 mL

Continued on next page

Procedure for BAC DNA, Continued

Eluting and Precipitating DNA

1. Place a sterile centrifuge tube (elution tube) under the column.
2. Add Elution Buffer (E4) **warmed to 50°C** onto the column to elute DNA. Allow the solution to drain by gravity flow. Do not force out any remaining solution.
The elution tube contains the purified DNA.
Discard the column.

Miniprep	Midiprep	Maxiprep
0.9 mL	5 mL	15 mL

3. Add 0.7 volumes of isopropanol to the elution tube. Mix well.

Miniprep	Midiprep	Maxiprep
0.63 mL	3.5 mL	10.5 mL

4. Centrifuge the mixture at $>12,000 \times g$ for 30 minutes at 4°C. Carefully remove and discard the supernatant.
5. Resuspend the DNA pellet in 70% ethanol.

Miniprep	Midiprep	Maxiprep
1 mL	3 mL	5 mL

6. Centrifuge at $>12,000 \times g$ for 5 minutes at 4°C. Carefully remove and discard the supernatant.
7. Air-dry the pellet for 10 minutes.
8. Resuspend the DNA pellet in TE Buffer (TE).

Miniprep	Midiprep	Maxiprep
10 μ L	50–100 μ L	200–400 μ L

Note: Occasionally, insoluble particles may be present. These particles do not influence the quality of the DNA and can be easily removed. To remove insoluble particles, centrifuge the DNA solution at high speed at room temperature for 1 minute. Transfer the supernatant (DNA sample) into a fresh tube.

Expected Results

The above procedure allows you to purify a ~100 kb BAC molecule with yields of approximately 40 μ g DNA per 100 mL culture.

Procedure for Bacmid DNA

Introduction

The PureLink® HiPure Plasmid DNA Miniprep Kit allows you to purify high quality Bacmid DNA (DH10Bac™) from *E. coli*. The isolated bacmid DNA is suitable for use in insect cell transfections.

Before Starting

- Inoculate a single white bacterial colony into 2 mL LB medium with appropriate antibiotics. Incubate the culture at 37°C in a shaking water bath at 250 rpm for a minimum of 1 hour and up to overnight.
 - Verify that the Resuspension Buffer (R3) contains RNase A, and warm Lysis Buffer (L7) briefly at 37°C to redissolve any particulate matter (see page 7).
-

Equilibrating the Column

Place the PureLink® HiPure Mini column on the PureLink® Nucleic Acid Purification Rack (refer to the manual supplied with the rack for more details). Apply 2 mL Equilibration Buffer (EQ1) to the column. Allow the solution in the column to drain by gravity flow.

Preparing Cell Lysate

1. Harvest 1.5 mL bacterial cells by centrifuging the culture at 9000 × g for 15 minutes. Remove all medium.
2. Add 0.4 mL Resuspension Buffer (R3) containing RNase A to the pellet and resuspend the cells until homogeneous.
3. Add 0.4 mL Lysis Buffer (L7). Mix gently by inverting the capped tube until the mixture is thoroughly homogeneous. **Do not vortex.** Incubate at room temperature for 5 minutes.
4. Add 0.4 mL Precipitation Buffer (N3) and mix immediately by inverting the capped tube until the mixture is thoroughly homogeneous. **Do not vortex.**
5. Centrifuge the mixture at >12,000 × g at room temperature for 10 minutes.

Note: If the pellet does not adhere to the bottom of the tube, incubate the tube at room temperature for 5 minutes to allow the lysate and gelatinous pellet to separate. Pipet the clear lysate into a sterile tube and centrifuge at >12,000 × g for 5 minutes at room temperature to remove any remaining cellular debris.

Continued on next page

Procedure for Bacmid DNA, Continued

Binding and Washing DNA

1. Load the supernatant from step 5 (**Preparing Cell Lysate**) onto the equilibrated column. Allow the solution in the column to drain by gravity flow.
 2. Wash the column **twice** with 2.5 mL Wash Buffer (W8). Allow the solution in the column to drain by gravity flow after each wash. Discard the flow-through.
-

Eluting and Precipitating DNA

1. Place a sterile centrifuge tube (elution tube) under the column.
 2. Add 0.9 mL Elution Buffer (E4) to the column to elute DNA. Allow the solution to drain by gravity flow. Do not force out any remaining solution.
The elution tube contains the purified DNA.
Discard the column.
 3. Add 0.63 mL isopropanol to the elution tube. Mix and place on ice for 10 minutes.
 4. Centrifuge the mixture at $>12,000 \times g$ at 4°C for 20 minutes. Carefully remove and discard the supernatant.
 5. Resuspend the DNA pellet in 1 mL 70% ethanol.
 6. Centrifuge the tube at $>12,000 \times g$ at 4°C for 5 minutes. Carefully remove and discard the supernatant.
 7. Air-dry the pellet for 10 minutes.
 8. Resuspend the DNA pellet in 40 μL TE Buffer (TE). Allow the pellet to dissolve for at least 10 minutes on ice. To avoid shearing the DNA, pipet only 1–2 times to resuspend.
 9. Store the bacmid DNA at -20°C and avoid repeated freezing and thawing.
-

Procedure for Cosmid DNA

Introduction

The PureLink® HiPure Plasmid DNA Miniprep Kit allows you to purify high quality cosmid DNA from *E. coli*.

Before Starting

- Inoculate a bacterial culture containing your cosmid construct in LB medium with the appropriate selective antibiotic and grow the bacteria for 16 hours (or overnight) on a 225 rpm shaking incubator.
 - Verify that the Resuspension Buffer (R3) contains RNase A, and warm Lysis Buffer (L7) briefly at 37°C to redissolve any particulate matter (see page 7).
-

Equilibrating the Column

Place the PureLink® HiPure Mini column on the PureLink® Nucleic Acid Purification Rack (refer to the manual supplied with the rack for more details). Apply 2 mL Equilibration Buffer (EQ1) to the column. Allow the solution in the column to drain by gravity flow.

Preparing Cell Lysate

1. Harvest 3-mL cells by centrifuging the overnight culture at $9000 \times g$ for 15 minutes. **Thoroughly** remove all medium.
2. Add 0.4 mL Resuspension Buffer (R3) containing **100 µg/mL** RNase A to the pellet and resuspend the cells until homogeneous.
3. Add 0.4 mL Lysis Buffer (L7). Mix gently by inverting the capped tube until the mixture is thoroughly homogeneous. **Do not vortex**. Incubate at room temperature for 5 minutes.
4. Add 0.4 mL Precipitation Buffer (N3) and mix immediately by inverting the capped tube until the mixture is thoroughly homogeneous. **Do not vortex**.
5. Centrifuge the mixture at $>12,000 \times g$ at room temperature for 10 minutes.

Note: If the pellet does not adhere to the bottom of the tube, incubate the tube at room temperature for 5 minutes to allow the lysate and gelatinous pellet to separate. Pipet the clear lysate into a sterile tube and centrifuge at $>12,000 \times g$ at room temperature for 5 minutes to remove any remaining cellular debris.

Continued on next page

Procedure for Cosmid DNA, Continued

Binding and Washing DNA

1. Pipet the supernatant from step 5 (**Preparing Cell Lysate**) onto the equilibrated column. Allow the solution in the column to drain by gravity flow.
 2. Wash the column **twice** with 2.5 mL Wash Buffer (W8). Allow the solution in the column to drain by gravity flow after each wash. Discard the flow-through.
-

Eluting and Precipitating DNA

1. Place a sterile centrifuge tube (elution tube) under the column.
 2. Add 0.9 mL Elution Buffer (E4) to the column to elute DNA. Allow the solution to drain by gravity flow. Do not force out any remaining solution.
The elution tube contains the purified DNA.
Discard the column.
 3. Add 0.63 mL of isopropanol to the elution tube. Mix and place on ice for 10 minutes.
 4. Centrifuge the mixture at $>12,000 \times g$ at 4°C for 20 minutes. Carefully remove and discard the supernatant.
 5. Resuspend the DNA pellet in 1 mL 70% ethanol.
 6. Centrifuge at $>12,000 \times g$ at 4°C for 5 minutes. Carefully remove and discard the supernatant.
 7. Air-dry the pellet for 10 minutes.
 8. Resuspend the DNA pellet in 50 μ L TE Buffer (TE). Allow the pellet to dissolve for at least 10 minutes on ice. To avoid shearing the DNA, pipet only 1–2 times to resuspend.
 9. Store the cosmid DNA at –20°C and avoid repeated freezing and thawing.
-

Expected Results

This procedure allows you to purify a ~45 kb cosmid DNA with yields of ~4 μ g DNA per 3 mL culture.

Procedure for ssM13 DNA

Introduction

The PureLink® HiPure Plasmid DNA Purification Kits allow you to purify high quality ssM13 (single strand M13) DNA from bacteria.



Note

When using the PureLink® HiPure Plasmid DNA Purification Kits for this procedure, note that the number of reactions may vary from that stated on page 3 because of changes in the volumes used with the kit-supplied reagents.

Before Starting

- The Resuspension Buffer (R3) and Lysis Buffer (L7) provided in the kit are **not** used in this protocol.
- In addition to Precipitation Buffer (N3), Wash Buffer (W8), Elution Buffer (E4), and TE Buffer (TE) from the kit, the following solutions are required:
 - M1: 3 M NaCl, 30% (w/v) PEG 8000
 - M2: 100 mM Tris-HCl, pH 8.0, 25 mM EDTA
 - M3: 4% SDS

Read the protocol carefully to determine the volume of each solution you need to prepare.

Store solutions M1, M2, and M3, at room temperature.

- Inoculate an aliquot of YT medium with 1/150 volume of lawn cells (a confluent culture of the bacterial host strain). Infect the cells with an M13 colony or a phage stock. Shake vigorously for **no longer** than 5 hours (longer incubations may result in deletions).
 - Set a water bath or heat block to 70°C.
-

Continued on next page

Procedure for ssM13 DNA, Continued

Equilibrating the Column

Support the column vertically using the PureLink® Nucleic Acid Purification Rack (refer to the manual supplied with the rack for more details), or the Column Holder (for PureLink® HiPure Midiprep and Maxiprep Columns, see page 6). Apply Equilibration Buffer (EQ1) to the column. Allow the solution in the column to drain by gravity flow.

Miniprep	Midiprep	Maxiprep
2 mL	10 mL	30 mL

Preparing Cell Lysate

1. Spin the culture using a centrifuge to sediment the bacterial cells. **The ssM13 DNA is in the supernatant.** Transfer the supernatant to a sterile tube and centrifuge to remove any traces of bacterial cells. Transfer the supernatant to a new, sterile tube.

Miniprep	Midiprep	Maxiprep
1–10 mL	10–25 mL	25–100 mL

2. To each 10 mL of supernatant, add 2 mL solution M1. Mix thoroughly and incubate on ice for 15 minutes to precipitate the M13 phage particles.
3. Collect the phage particles by centrifuging the sample at $>10,000 \times g$ for 10 minutes. Discard the supernatant.
4. Resuspend the phage particles in solution M2 by pipetting up and down repeatedly.

Miniprep	Midiprep	Maxiprep
1 mL	3 mL	9 mL

5. Lyse the phage particles by adding solution M3. Mix thoroughly by inverting the tube until the lysate is thoroughly homogeneous. Incubate the tube at 70°C for the time indicated in the table.

Miniprep	Midiprep	Maxiprep
1 mL	3 mL	9 mL
10 min	20 min	20 min

6. Add solution N3 to the lysate. Mix thoroughly by inverting the tube until the solution is thoroughly homogeneous. Centrifuge the tube at $>12,000 \times g$ for 10 minutes at room temperature.

Miniprep	Midiprep	Maxiprep
1 mL	3 mL	9 mL

Continued on next page

Procedure for ssM13 DNA, Continued

Binding and Washing DNA

1. Load the supernatant from step 6 (**Preparing Cell Lysate**) onto the equilibrated column. Allow the solution in the column to drain by gravity flow.
2. Wash the column with Wash Buffer (W8). Allow the solution in the column to drain by gravity flow after each wash. Discard the flow-through.

Miniprep	Midiprep	Maxiprep
2 × 2.5 mL	2 × 10 mL	1 × 60 mL

Eluting and Precipitating DNA

1. Place a sterile centrifuge tube (elution tube) under the column.
2. Add Elution Buffer (E4) to the column to elute DNA. Allow the solution to drain by gravity flow. Do not force out any remaining solution. *The elution tube contains the purified DNA.* Discard the column.

Miniprep	Midiprep	Maxiprep
0.9 mL	5 mL	15 mL

3. Add 0.7 volumes of isopropanol to the elution tube. Mix well.

Miniprep	Midiprep	Maxiprep
0.63 mL	3.5 mL	10.5 mL

4. Centrifuge the mixture at $>12,000 \times g$ at 4°C for 30 minutes. Carefully remove and discard the supernatant.
5. Resuspend the DNA pellet in 70% ethanol.

Miniprep	Midiprep	Maxiprep
1 mL	3 mL	5 mL

6. Centrifuge at $>12,000 \times g$ at 4°C for 5–10 minutes. Carefully remove and discard the supernatant.
7. Air-dry the pellet for 10 minutes.
8. Resuspend the DNA pellet in TE Buffer (TE).

Miniprep	Midiprep	Maxiprep
10–60 μ L	60–100 μ L	100–400 μ L

Troubleshooting

Observation	Cause	Solution
Pellet/debris is transferred onto column	Pellet is viscous and does not adhere to tube	After centrifuging the lysate, allow the tube sit for 5 minutes to separate the clear lysate from the pellet (the pellet may be floating). Remove the clear lysate to a fresh tube and centrifuge again to remove any remaining debris.
	Using a high volume of culture	Use the recommended culture volumes. If you are using higher culture volumes than the recommended volume, double the volumes of the Resuspension Buffer (R3), Lysis Buffer (L7), and Precipitation Buffer (N3) as designated in the protocol.
Low plasmid DNA yield	Buffers not stored correctly	Store Lysis Buffer (L7) and Equilibration Buffer (EQ1) at room temperature.
	Lysate centrifuged at 4°C	Make sure that the rotor and the centrifuge are at room temperature for the lysate centrifugation step. If centrifugation at 4°C is unavoidable, transfer the cleared lysate to a new tube and warm it to room temperature in a water bath before loading it onto the column.
	Low copy number plasmid	Increase the volume of starting culture. Carefully remove all medium before resuspending cells. Doubling the volumes of the Resuspension Buffer (R3), Lysis Buffer (L7) and Precipitation Buffer (N3) may increase plasmid yield and quality.
	Lysate at improper pH or salt concentration to bind column	Make sure that the correct volume of Precipitation Buffer (N3) is added when neutralizing the lysate.
	Plasmid DNA pellet over-dried	Do not dry the DNA pellet with a vacuum system.

Continued on next page

Troubleshooting, Continued

Observation	Cause	Solution
Slow column flow	Column clogged	Pipet the lysate supernatant onto the column. Do not pour the lysate onto the column, as some of the precipitate could enter the column.
Genomic DNA contamination	Genomic DNA sheared during handling	Gently invert tubes to mix after adding buffers. Do not vortex as it can shear genomic DNA.
Additional plasmid forms present	Plasmid DNA permanently denatured (band migrating faster than supercoiled DNA)	Incubate the lysate at room temperature for no longer than 5 minutes.
RNA contamination	Lysate at improper pH, salt concentration, or temperature	Carefully remove all medium before resuspending cells. Make sure not to add an excess of Precipitation Buffer (N3) when neutralizing the lysate. Do not warm the lysate above room temperature during while centrifuging.
	Lysate left on column too long	Once the lysate is loaded onto the column, avoid delays in processing.
	Lysate droplets remained on walls of column at elution	Wash droplets of lysate from the walls of the column with the Wash Buffer.
	RNase A digestion incomplete	Make sure RNase A is added to Resuspension Buffer (R3). Use recommended volume of buffer R3. Make sure that buffer with RNase A is stored at 4°C.

Accessory Products

Additional Products

The following products are also available from Invitrogen. For more details on these products, visit our website at www.invitrogen.com or contact **Technical Support** (page 34).

Product	Quantity	Catalog No.
Qubit® dsDNA Assay Kit, High Sensitivity	500 assays	Q32854
Qubit® dsDNA Assay Kit, Broad-Range	500 assays	Q32853
Qubit® 2.0 Fluorometer	1 each	Q32866
PureLink® Nucleic Acid Purification Rack	1 each	K2100-13
PureLink® HiPure Plasmid DNA Megaprep	4 preps	K2100-08
PureLink® HiPure Plasmid DNA Gigaprep	2 preps	K2100-09
PureLink® HiPure Plasmid Filter Midiprep Kits	25 preps	K2100-14
	50 preps	K2100-15
PureLink® HiPure Plasmid Filter Maxiprep Kits	10 preps	K2100-16
	25 preps	K2100-17
PureLink® HiPure BAC Buffer Kit	1 kit	K2100-18
PureLink® HiPure Precipitator Module	10 preps	K2100-21
	25 preps	K2100-22
Luria Broth Base (Miller's LB Broth Base), powder	500 g	12795-027
	2.5 kg	12795-084
Ampicillin Sodium Salt, irradiated	200 mg	11593-027
Carbenicillin, Disodium Salt	5 g	10177-012

E-Gel® Agarose Gels and DNA Ladders

E-Gel® Agarose Gels are bufferless pre-cast agarose gels with a variety of different agarose percentages and well formats designed for fast, convenient electrophoresis of DNA samples.

A large variety of DNA ladders is available from Invitrogen for sizing DNA.

Visit www.invitrogen.com or contact **Technical Support** (page 34) for more details on these products.

Technical Support

Obtaining Support

For the latest services and support information for all locations, go to www.invitrogen.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@invitrogen.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
-

SDS

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

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.invitrogen.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

Continued on next page

Technical Support, Continued

Limited Warranty

Invitrogen (a part of Life Technologies Corporation) 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.

All Invitrogen products are warranted to 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 the Company's liability to only the price 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. The Company reserves the right to select the method(s) used to analyze a product unless the Company 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 the Company makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, report it to our Technical Support Representatives.

Life Technologies Corporation shall have 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:
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 services of any kind, including, without limitation, 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.

©2011 Life Technologies Corporation. All rights reserved.

The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners.

Triton is a registered trademark of Union Carbide Chemicals & Plastics Technology Corp.

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.invitrogen.com/support or email techsupport@invitrogen.com
www.lifetechnologies.com

