Invitrogen™
life technologies

Instruction Manual

PureLink™ 384 Plasmid Purification System

Catalog no. 12263-026

Version B
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Introduction

Introduction

PureLink™ 384 Plasmid Purification Plates are part of a unique system for high-throughput plasmid DNA isolation. This solid-phase lysis system replaces the numerous reagents and mixing steps associated with traditional alkaline lysis. Since the lysis occurs within a solid-phase matrix, there is no mixing or transfer of lysate to create cross-contamination. Harvesting and resuspending of cells are avoided by growing cultures in rich media.

System Overview

To use PureLink™ 384 Plasmid Purification Plates as part of a high-throughput plasmid purification system, first you prepare cell cultures from rich media in 96- or 384-well growth blocks (see page 3). Since proper growth of bacterial cultures is a key element in this system, we strongly recommend that you grow and analyze test cultures before attempting purification.

Then, depending on the desired yield of plasmid DNA, proceed to Protocol A, Direct Load Method (2–3 µg DNA), or Protocol B, Harvested Cells Method (3–5 µg DNA). Protocol A is the simpler, less time-consuming method and should provide ample yield for most users.

In Protocol A (page 6), you stack the 384-well filter plate on top of a receiver plate containing isopropanol and load pre-lysis buffer containing lysozyme onto the filters. Then you load the cells directly into the wells of the filter plate, followed by lysis buffer. Centrifuge the stacked plates to recover the plasmid DNA and precipitate it in one step. Genomic DNA and other cell components will remain within the filter matrix.

In Protocol B (page 9), you stack the 384-well filter plate on top of a receiver plate containing isopropanol and load pre-lysis buffer containing lysozyme onto the filters. You then resuspend harvested cells to the desired concentration and load them into the wells of the filter plate, followed by lysis buffer. Centrifuge the stacked plates to recover the plasmid DNA and precipitate it in one step. Genomic DNA and other cell components will remain within the filter matrix.

System Features

This system has the following features:

- Protocols are compatible with a variety of automated devices.
- Approximately 66 plates (>25,000 samples) can be processed in one shift using a robot with a 96-well head, a 96/384 well dispenser, and one centrifuge with a 6-place rotor.
- Yields of 2–3 µg/well for Protocol A (Direct Load Method) and 3–5 µg/well for Protocol B (Harvested Cells Method) are typical for high copy number plasmids.
- Filter plates can be processed one at a time or in stacks of up to three filter plates per centrifuge plate carrier.

Plasmid DNA isolated using this method is suitable for use in automated fluorescent DNA sequencing, PCR, or restriction digestion.

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PureLink™ 384 Plasmid Purification Plates are sold in packages of 25 filter plates (Cat. no. 12263-026).

The plates are shipped at room temperature and should be stored at room temperature.

Reagents Needed

The following reagents are needed before starting the procedures.

Component	Composition	Amount	Cat. No.
Cell suspension buffer	50 mM Tris-HCl (pH 8.0), 10 mM EDTA	500 ml	12056-016
Pre-lysis buffer	Proprietary	125 ml	12263-034
Lysis buffer	Proprietary	1 liter	12057-022
RNase A solution	20 mg RNase A/ml in 50 mM Tris-HCl (pH 8.0), 10 mM EDTA	10 ml 25 ml	12091-021 12091-039
TE buffer	10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA	100 ml	12090-015
Media			
Terrific Broth	See page 3 for recipe	500 g	22711-022
MR2001	See page 3 for source		—
Antibiotic	See page 3 for concentrations		—
Lysozyme			—
Isoproponal			—
70% ethanol			—

Materials Needed

The following equipment and materials are needed before starting the procedures.

- PureLink™ Air-Porous Tape (Catalog no. 12262-010)
- PureLink™ Foil Tape (Catalog no. 12261-012)
- PureLink™ 96-well Growth Blocks (Catalog no. 12256-020) **or** 384-well growth blocks (required volume: 200 µl/well)
- 384-well polypropylene receiver plates (required volume: 200 µl/well)
  Contact technical service (see page 16) for assistance with obtaining appropriate receiver plates
- Shaking incubator at 37°C
- Refrigerated centrifuge with swinging bucket rotor capable of reaching 3000 × *g* and with a plate height clearance of 3.6 cm (see page 14 for additional information)

Preparing 96- or 384-Well Cell Cultures

Procedural Overview

In this procedure, you prepare cell cultures from rich media in 96- or 384-well growth blocks.


Note

For direct loading of cultures, it may be easier to attain optimal concentrations of cells using 96-well growth blocks.

We strongly recommend that you grow and analyze some test cultures before attempting purification.

Recommended Cell Type

While this system is compatible with various strains of *E. coli,* DH10B cells are recommended for optimal performance and plasmid yield. This strain is compatible with direct cloning of genomic DNA and is available as both chemically competent and electrocompetent cells.

Media Recipes and Sources

The following table shows the recipe or source for recommended rich media:

Media	Recipe for 1 liter or Source
Terrific Broth	Available from Invitrogen (500 g, Cat. no. 22711-022) Recipe: 11.8 g peptone 23.6 g yeast extract 9.4 g dipotassium hydrogen phosphate (K_2HPO_4) 2.2 g potassium dihydrogen phosphate (KH_2PO_4) 4 ml glycerol Bring to 1 liter with deionized water and autoclave for 15 minutes at 121º C.
MR2001	MacConnell Research, San Diego, CA

Recommended Antibiotic Concentrations

The following table shows the recommended concentrations for different types of antibiotics:

Antibiotic	Concentration in culture medium
Ampicillin (Cat. no. 11593-019)	100 μg/ml
Chloramphenicol	30 μg/ml
Gentamicin (Cat. no. 15750-060)	10 μg/ml
Kanamycin (Cat. no. 15160-054)	50 μg/ml
Streptomycin (Cat. no. 11860-038)	100 μg/ml
Tetracycline	25 μg/ml

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Preparing 96- or 384-Well Cell Cultures, Continued

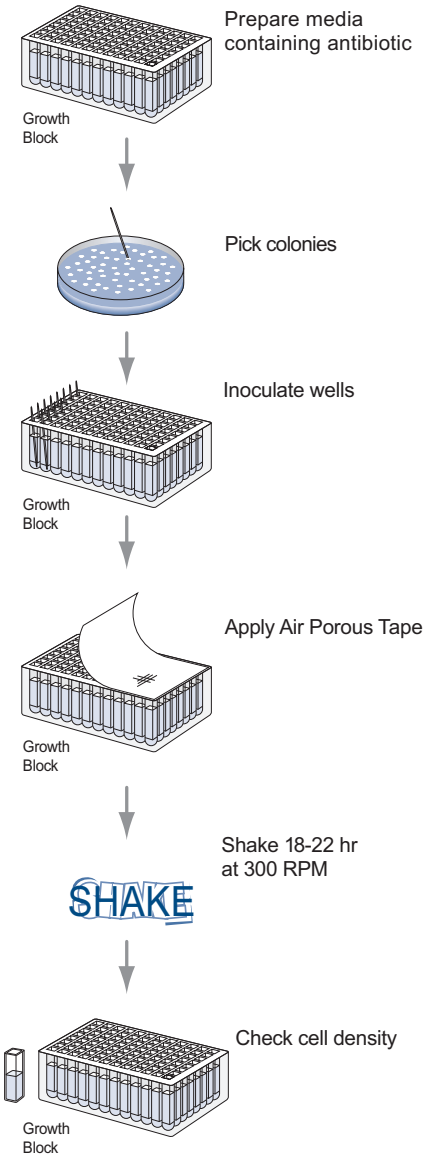
Critical Parameters

For the following procedure, the parameters listed below are critical:

- Antibiotic: Verify concentration and freshness (see previous page).
- Age of colonies: <1 week (the fresher the better).
- Recommended media: Terrific Broth or MR2001 (check for clarity).
- Incubation temperature: 37°C.
- Shaking speed: 300–320 RPM.
- Incubation time: 18–22 hours (20–24 hours for glycerol stock inoculation).

Preparing Cultures

Follow the steps below to prepare your 96- or 384-well cell cultures:



1. Add an appropriate concentration of antibiotic to rich media (Terrific Broth or MR2001 is recommended; see previous page for recipe/source).

2. Add 0.5 ml of antibiotic-containing media to each well of a 96-well growth block
OR
Add 0.15 ml of antibiotic-containing media to each well of a 384-well growth block (well capacity: 200 µl/well).

3. Inoculate all wells of the growth block with ***fresh*** colonies (<1 week old).

   Note: Glycerol stocks should be used with great care. Old glycerol stocks can produce low plasmid copy numbers, even though the cells grow adequately. Cell growth will be delayed out of glycerol stocks (see increased culture times in step 5).

4. Cover the growth block with air porous tape. Seal cover securely.

5. Incubate in a 37°C shaker at 300–320 RPM for 18–22 hours (20–24 hours for cells from glycerol stocks).

6. Check growth of a few random wells in the block by measuring OD_{600} of a 1:33 dilution in TE. (Note that different dilutions will result in different calculated undiluted OD_{600}.) Be certain that cells are well suspended before removing aliquot. Use 30 µl media plus 970 µl TE as a blank. See recommended cell densities for the different protocols on the next page.

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Preparing 96- or 384-Well Cell Cultures, Continued

Recommended Cell Densities

For optimal performance, the OD_{600} of a 1:33 dilution should be **0.18–0.33** (undiluted OD_{600} 6–10) for Protocol A, Direct Load, or **>0.12** (undiluted OD_{600} >4) for Protocol B, Harvested Cells.

Storing Cultures

Cultures can be refrigerated or frozen, depending on how soon you want to use them:

- Cultures can be stored up to 3 days at 4°C before use. Resuspend cultures thoroughly just before using.
- Cultures can be stored up to one month frozen at -20°C. Air porous tape should be removed and replaced with foil tape before storing.

Thawing Cultures

To thaw frozen cultures:

1. Store block at 4°C for at least 18 hours.
2. Resuspend cells thoroughly before use.

Protocol A, Direct Load Method

Protocol Overview

In this protocol, you stack the 384-well filter plate on top of a receiver plate containing isopropanol and load pre-lysis buffer containing lysozyme onto the filters. Then you load the cells directly into the wells of the filter plate, followed by lysis buffer. Centrifuge the stacked plates to recover the plasmid DNA and precipitate it in one step. Genomic DNA and other cell components will remain within the filter matrix.

Required Cell Density

For optimal performance, this protocol requires an OD_{600} of **0.18–0.33** for a 1:33 dilution (undiluted OD_{600} 6–10). If the density of the 1:33 dilution is lower, optimize growth conditions as described in the previous section or use Protocol B, Harvested Cells (see page 9).

Preparing the Pre-lysis Buffer with Lysozyme

Immediately before using this protocol, follow the steps below to prepare the pre-lysis buffer with lysozyme:

1. Calculate the necessary volume to prepare using the following formula:

   (total vol pre-lysis buffer) = [(# plates) × (2 ml/plate)] + (waste vol)

   The waste volume is the volume required to fill the lines or troughs of the robot or pipetting device to be used. The waste volume should be a minimum of 2 ml.

2. Calculate the necessary amount of lysozyme to weigh using the following formula:

   (total mg lysozyme) = (total vol pre-lysis buffer) × (5 mg/ml)

3. Add the lysozyme to the pre-lysis buffer and invert 30 or more times to thoroughly dissolve.

   Note: This solution should be prepared fresh daily. Alternatively, a large batch can be prepared and stored aliquoted for single use at –20°C. Aliquots should be frozen and thawed only once.

Preparing the Lysis Buffer with RNase A

Immediately before using this protocol, prepare the lysis buffer/RNase A mixture according to the directions below:

1. Calculate the necessary volume of lysis buffer and RNase A using the following formulas:

   [(# plates) × (12 ml/plate)] + (waste volume) = (total vol lysis buffer)

   (total vol lysis buffer) × (0.05) = (total vol RNase A)

2. Mix the lysis buffer and RNase A by inverting 20 times to mix thoroughly. Use this solution within 24 hours.

Expected Amount

The expected amount of recovered DNA for Protocol A is **2–3 µg DNA per well**.

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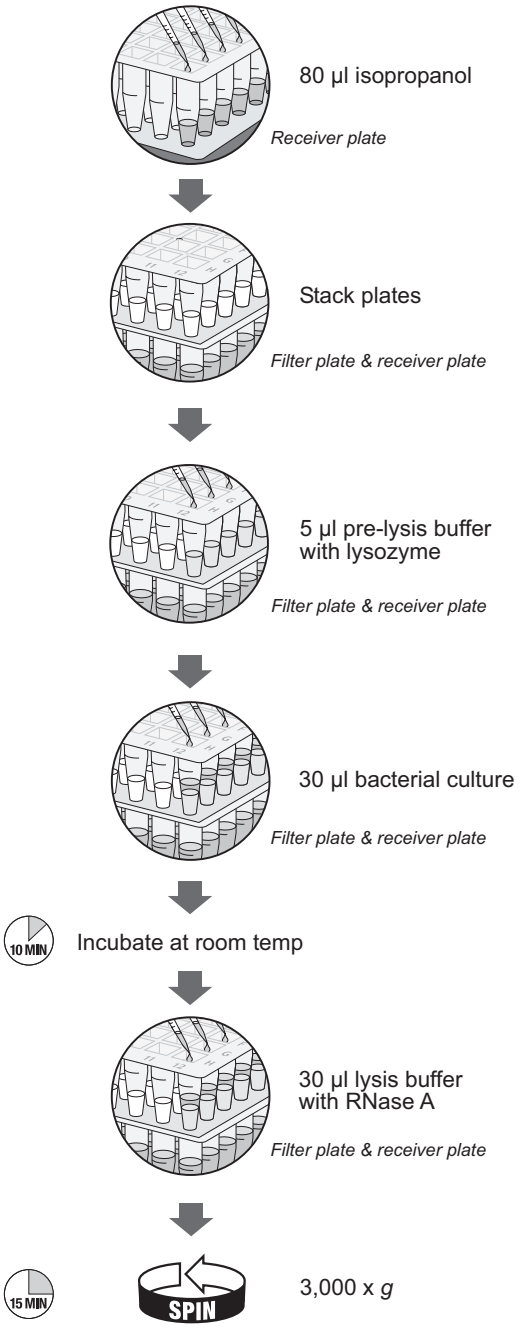
Protocol A, Direct Load Method, Continued

Protocol Procedure



1. Add 80 µl of isopropanol per well to a receiver plate.

2. Align the filter plate on top of the receiver plate. Keep the plates stacked through step 8. Do not place the filter plate on an absorbent surface for steps 3–8 or some sample may wick out.

3. Add 5 µl of the pre-lysis buffer containing lysozyme to each well of the filter plate. This can be done as much as 72 hours in advance. Store plates at room temperature after adding pre-lysis buffer.

4. If cells have settled in the growth block, mix cultures by pipetting up and down several times.

5. Transfer 30 µl of bacterial culture (OD_{600} 6–10) to each well of the filter plate.

6. Incubate 10 minutes at room temperature.

7. Add 30 µl lysis buffer containing RNase A to each well. Some of the liquid may not absorb into the filter. Do not mix.

8. Centrifuge the stacked plates at 3,000 × *g* for 15 min at 4°C. The formula for converting RCF (*g*) to RPM is on page 14. If you are stacking multiple sets of plates in the centrifuge, cushion the bottom plate as described in the note on page 14.

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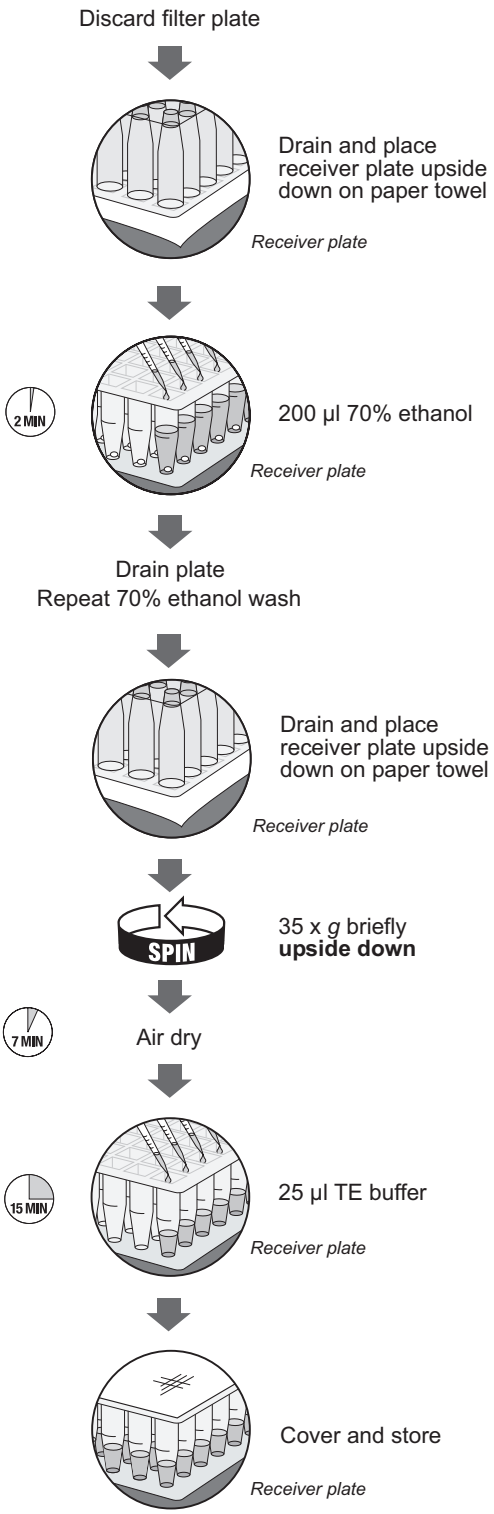
Protocol A, Direct Load Method, Continued



9. Remove the filter plate and discard.

10. Discard the liquid from the receiver plate by inverting the plate over a waste container and shaking briskly. Invert the plate on a stack of paper towels and tap to remove as much liquid as possible.

11. Add 200 µl 70% ethanol to each well of the receiver plate, and incubate at room temperature for 2 minutes.

12. Drain liquid from the receiver plate as in step 10.

13. Repeat steps 11 and 12 once.

14. It is important to remove any remaining alcohol. Invert the receiver plate onto a paper towel. Briefly centrifuge the inverted plate by bringing the centrifuge to 35 × *g*, then immediately turning it off.

15. Air dry the plate for 7 minutes at room temperature.

16. Dissolve the pellets by adding 25 µl TE Buffer to each well. Allow plate to stand at room temperature or 4°C for at least 15 minutes.

17. Cover the plate with foil tape and store at 4°C. Small quantities of insoluble material are sometimes present in the samples, but these do not affect performance. See page 13 for recommended sequencing reaction conditions. Expect 2–3 µg DNA/well.

Protocol B, Harvested Cells Method

Protocol B Overview

In this protocol, you stack the 384-well filter plate on top of a receiver plate containing isopropanol and load pre-lysis buffer containing lysozyme onto the filters. You then resuspend harvested cells to the desired concentration and load them into the wells of the filter plate, followed by lysis buffer. Centrifuge the stacked plates to recover the plasmid DNA and precipitate it in one step. Genomic DNA and other cell components will remain within the filter matrix.

Required Cell Density

For optimal performance, this protocol requires an OD_{600} of **>0.12** for a 1:33 dilution (undiluted OD_{600} >4).

Preparing the Pre-lysis Buffer with Lysozyme

Immediately before using this protocol, follow the steps below to prepare the pre-lysis buffer with lysozyme:

1. Calculate the necessary volume to prepare using the following formula:

   (total vol pre-lysis buffer) = [(# plates) × (2 ml/plate)] + (waste vol)

   The waste volume is the volume required to fill the lines or troughs of the robot or pipetting device to be used. The waste volume should be a minimum of 2 ml.

2. Calculate the necessary amount of lysozyme to weigh using the formula below:

   (total mg lysozyme) = (total vol pre-lysis buffer) × (5 mg/ml)

3. Add the lysozyme to the pre-lysis buffer and invert 30 or more times to thoroughly dissolve.

   Note: This solution should be prepared fresh daily. Alternatively, a large batch can be prepared and stored aliquoted for single use at –20°C. Aliquots should be frozen and thawed only once.

Preparing the Lysis Buffer with RNase A

Immediately before using this protocol, prepare the lysis buffer/RNase A mixture according to the directions below:

1. Calculate the necessary volume of lysis buffer and RNase A using the following formulas:

   [(# plates) × (10 ml/plate)] + (waste volume) = (total vol lysis buffer)

   (total vol lysis buffer) × (0.05) = (total vol RNase A)

2. Mix the lysis buffer and RNase A by inverting 20 times to mix thoroughly. Use this solution within 24 hours.

Expected Amount

The expected amount of recovered DNA for Protocol B is **3–5 µg DNA per well**.

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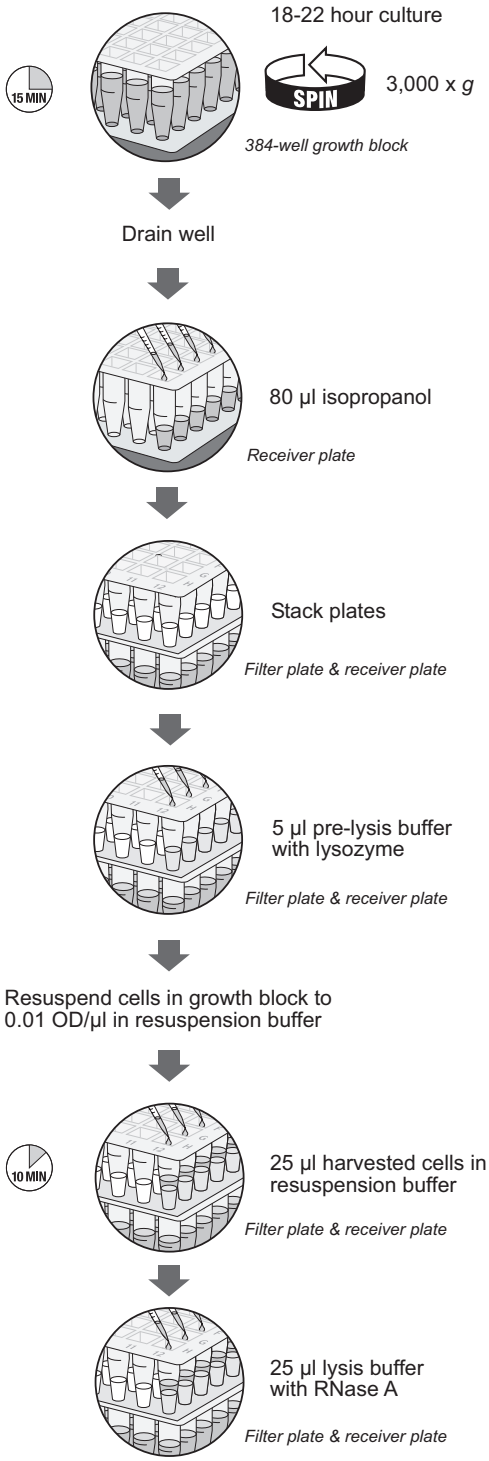
Protocol B, Harvested Cells Method, Continued

Protocol Procedure



1. Harvest cells from overnight growth by centrifuging growth block at 3,000 × *g* for 15 minutes at 4–25°C. Drain well.

2. While cells are pelleting, add 80 µl of isopropanol to each well of a receiver plate.

3. Align filter plate on top of receiver plate. Keep the plates stacked through step 8. Do not place the filter plate on absorbent surface for steps 3–8 or sample may wick out.

4. Pipet 5 µl of the pre-lysis buffer with lysozyme onto each filter in the plate. This can be done as much as 72 hours in advance. Store plates at room temperature after adding pre-lysis buffer.

5. Thoroughly resuspend cells in each well of the growth block in cell suspension buffer so that the final cell density will be 0.01 OD/µl. Complete resuspension is critical, as clumps of cells will lead to reduced yield. Pipet cells up and down repeatedly or vortex on a plate vortexer to resuspend thoroughly. (**Example:** If an OD_{600} reading of a 1:33 dilution of bacterial culture is 0.21 (7 OD/ml) and there is ~100 µl of culture in each well (150 µl media in a 384-well growth block will evaporate to ~100–110 µl overnight), then there are 7 × 0.100 = 0.7 OD of cells in each well. To obtain a cell suspension containing 0.01 OD/µl, resuspend cells in 70 µl of cell suspension buffer.)

6. Transfer 25 µl of cells into each well of the filter plate (0.25 OD/well). Incubate 10 minutes at room temperature.

7. Add 25 µl lysis buffer containing RNase A to each well of the filter plate. Some of the liquid may not absorb into the filter. Do not mix.

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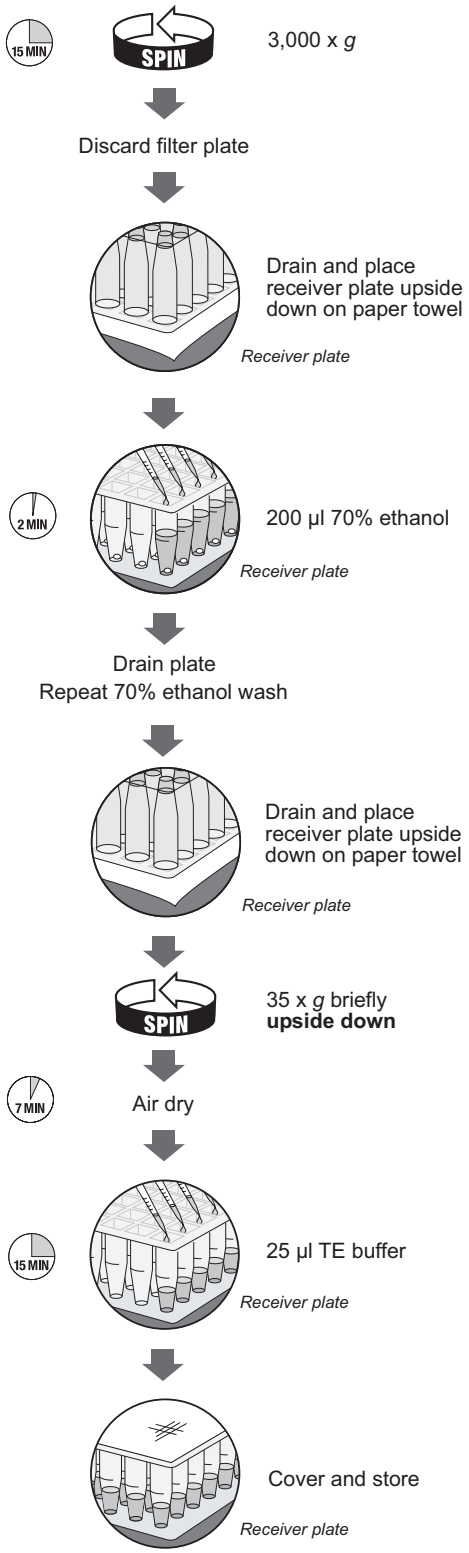
Protocol B, Harvested Cells Method, Continued



8. Centrifuge the stacked plates at 3,000 × *g* for 15 min at 4°C. The formula for converting RCF (*g*) to RPM is on page 14. If you are stacking multiple sets of plates in the centrifuge, cushion the bottom plate as described in the note on page 14.

9. Remove the filter plate and discard.

10. Discard the liquid from the receiver plate by inverting the plate over a waste container and shaking briskly. Invert the plate on a stack of paper towels and tap to remove as much liquid as possible.

11. Add 200 µl 70% ethanol to each well of the receiver plate, and incubate at room temperature for 2 minutes.

12. Discard the liquid from the receiver plate as in step 10.

13. Repeat steps 11 and 12 once.

14. It is important to remove any remaining alcohol. Invert the receiver plate onto a paper towel. Briefly centrifuge the inverted plate by bringing the centrifuge to 35 × *g*, then immediately turning it off.

15. Air dry the plate for 7 minutes at room temperature.

16. Dissolve the pellets by adding 25 µl TE Buffer to each well. Allow plate to stand at room temperature or 4°C for at least 15 minutes.

17. Cover the plate with foil tape and store at 4°C. Small quantities of insoluble material are sometimes present in the samples, but these do not affect performance. See page 13 for recommended sequencing reaction conditions. Expect 3–5 µg DNA/well.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solutions
Low yield of plasmid DNA	Culture not dense enough	Use fresh colonies, NOT glycerol stocks. Use fresh Terrific Broth or MR2001 media. Use fresh antibiotic at the concentration recommended on page 3. Ensure that the incubator is at 37°C. Ensure that the incubator is shaking at 300–320 RPM. Increase incubation time to as much as 22 hours.
	High copy number plasmid behaves like low copy number plasmid	Use fresh antibiotic at the concentration recommended on page 3 Use DH10B cells to ensure high plasmid DNA production. Ensure that the incubator is at 37°C. Low incubation temperature can reduce plasmid copy number. Ensure that all residual detergent has been rinsed from vessels.
	Insufficient centrifugation	Centrifuge at 3000 × *g* to recover and precipitate DNA. If your centrifuge cannot reach 3000 × *g*, longer centrifugation times can be used, as specified on page 14. Be sure to calculate *g*-force using the radius of the rotor with the microplate carrier in place and fully extended.
	Loss of pellet	Centrifuge at 4°C for best adherence of pellet to wall.
Excessive insoluble material	Too many cells used	For Protocol A, Direct Load Method, culture should be grown to an OD_{600} of 6–10. If cultures are growing to a higher density, reduce the incubation time or shaking speed. For Protocol B, Harvested Cells Method, check the density of a few random samples to confirm that the correct number of cells is being used (OD_{600} of 0.25 per well).
	Temperature too high during centrifugation	Ensure that the centrifuge is operating at 4°C during the run, or place the centrifuge in a cold room.
	Excessive centrifugation	Centrifuge at 3,000 × *g* to recover and precipitate DNA. Do not use higher *g*-force or longer times.
Weak fluorescence signal in sequencing	DNA pellet insufficiently washed	Two 200 µl washes with 70% ethanol are recommended in the protocol. Be sure to thoroughly remove the supernatant before each wash to minimize the salt carryover.
	Suboptimal sequencing reaction conditions	See the recommended sequencing reaction conditions on page 13.
Weak PCR Amplification	DNA pellet insufficiently washed	Two 200 µl washes with 70% ethanol are recommended in the protocol. Be sure to thoroughly remove the supernatant before each wash to minimize the salt carryover.

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Troubleshooting Guide, Continued

Problem	Possible Cause	Suggested Solutions
Chromosomal DNA contamination	Too many cells used	For Protocol A, Direct Load Method, culture should be grown to OD_{600} of 6-10. If cultures are growing to a higher density, reduce the incubation time or shaking speed. For Protocol B, Harvested Cells Method, check the density of a few random samples to confirm that the correct number of cells are being used (OD_{600} of 0.25 per well).
RNA contamination	Excessive centrifugation	Centrifuge at 3,000 × *g* to recover and precipitate DNA. Do not use a higher *g*-force or longer times.
	Insufficient RNase A	Add RNase A to the lysis buffer immediately before use. Do not store reagent mix for more than one week.

Recommended Sequencing Reaction Conditions

The conditions below are provided as an example; other conditions may be suitable.

1/4 × sequencing reaction:

2.5 µl plasmid DNA from PureLink™ 384 Plasmid Purification System

2.0 µl BigDye Terminator Ready Reaction Mix (ABI)

0.8 pmole primer

1 µl sequencing buffer (see below)

Water sufficient to bring volume to 10.5 µl

Sequencing Buffer:

400 mM Tris-HCl, pH 9.0

10 mM $MgCl_2$

Note: Omission of the sequencing buffer can result in low fluorescent signal strength.

Centrifuge Information

Compatible Centrifuges and Rotors

The protocols described in this manual are compatible with a wide variety of centrifuges and rotors. Centrifuge and rotor combinations must be able to accommodate a 3.6-cm microtiter plate stack and provide refrigeration.

The protocols also specify a centrifugation speed of 3000 × *g;* however, if your centrifuge is not capable of reaching 3000 × *g*, longer centrifugation times can be used. See Centrifugation at Lower *g*-Force below for details.

Note

If two or three sets of filter and receiver plates are stacked in one plate carrier in the rotor, a cushion should be placed under the bottom receiver plate to prevent breakage of the outer plate walls. The cushion should be a firm silicon or rubber mat, fit inside the bottom of the receiver plate, and be about 0.5 cm thick.

Conversion Formula

The formula for converting RCF (*g*) to RPM is:

$$RCF\ (g) = 1.12 \times \text{radius in mm} \times (RPM/1000)^2$$

Where:

RPM is the centrifuge speed in revolutions per minute

RCF is the relative centrifugal force (*g*)

Radius is the distance (in mm) from the center of the centrifuge spindle to the bottom of the plate in the rotor when the microplate carrier is fully extended as during centrifugation.

Centrifugation at Lower *g*-Force

If your centrifuge is not capable of reaching 3000 × *g*, longer centrifugation times can be used. See the following table for guidelines:

***g*-Force**	**Centrifugation time**
1500	30 min
2000	25 min
2500	20 min
3000	15 min

Related Products

Related Products

The following table lists products that support PureLink™ 384 Plasmid Purification Plates:

Product	Size	Cat. No.
PureLink™ 96 Plasmid Purification System	4 plates	12263-018
PureLink™ 96 Plasmid Components:		
PureLink™ 96 Filter Plates	Pkg. of 50	12192-035
PureLink™ 96 Receiver Plates	Pkg. of 50	12193-025
Media:		
Terrific Broth	500 g	22711-022
Glycerol	500 ml	15514-011
Transformation and Testing:		
ElectroMAX™ DH10B™ Cells	5 × 0.1 ml	18290-015
ElectroMAX™ DH10B™ T1 Phage Resistant Competent Cells	5 × 0.1 ml	12033-015
MAX Efficiency® DH5α™ Competent Cells	5 × 0.2 ml	18258-012
MAX Efficiency® DH5α™ T1 Phage Resistant Competent Cells	5 × 0.2 ml	12034-013
CloneChecker System	100 reactions	11666-013
Sequencing:		
Custom Primers (see www.invitrogen.com for information)		

Technical Service

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

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MSDSs

MSDSs are available from our web site: www.invitrogen.com.

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