Invitrogen™
life technologies

Instruction Manual

ChargeSwitch® Plasmid Yeast Mini Kit

For purification of plasmid DNA from 1 ml *S. cerevisiae* culture

Catalog no. CS10203

Version A
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Table of Contents

Kit Contents and Storage

Shipping and Storage

All components of the ChargeSwitch® Plasmid Yeast Mini Kit are shipped at room temperature.

Upon receipt, store components as follows:

- Mix RNase A in Resuspension Buffer (R4) as described on page 6 and store RNase A in Resuspension Buffer (R4) at 4°C
- Store Precipitation Buffer (N5) at 4°C to improve performance.
- Store the remaining kit components at room temperature

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

Kit Contents

The components supplied in the ChargeSwitch® Plasmid Yeast Mini Kit are listed below. The reagents supplied are sufficient to perform 50 purifications.

Note: Some reagents in the kit maybe provided in excess in the amount needed.

Component	Amount
ChargeSwitch® Magnetic Beads (25 mg/ml in 10 mM MES, pH 5.0, 10 mM NaCl, 0.1% Tween 20)	2 ml
RNase A (5 mg/ml in 10 mM Tris-HCl, pH 8.5, 10 mM EDTA)	0.4 ml
ChargeSwitch® Resuspension Buffer (R4)	15 ml
ChargeSwitch® Lysis Buffer (L9)	15 ml
ChargeSwitch® Precipitation Buffer (N5)	15 ml
ChargeSwitch® Wash Buffer (W11)	50 ml
ChargeSwitch® Wash Buffer (W12)	50 ml
ChargeSwitch® Elution Buffer (E5; 10 mM Tris-HCl, pH 8.5)	10 ml

Accessory Products

Additional Products

The table below lists additional products available from Invitrogen that may be used with the ChargeSwitch® Plasmid Yeast Mini Kit.

In addition, the table lists ChargeSwitch® gDNA Kits that are available for purification of genomic DNA from other sources. For more information about these and other ChargeSwitch® gDNA Kits, refer to our website at www.invitrogen.com or call Technical Service (see page 14).

Product	Quantity	Catalog no.
MagnaRack™ Magnetic Rack	1 rack	CS15000
ChargeSwitch® gDNA 10-20 µl Blood Kit	96 purifications	CS11010
ChargeSwitch® gDNA 50-100 µl Blood Kit	50 purifications	CS11000
ChargeSwitch® gDNA 1 ml Blood Kit	20 purifications	CS11001
ChargeSwitch® gDNA 1 ml Serum Kit	50 purifications	CS11040
ChargeSwitch® gDNA Mini Bacteria Kit	50 purifications	CS11301
ChargeSwitch® gDNA Mini Tissue Kit	25 purifications	CS11204
ChargeSwitch® gDNA Micro Tissue Kit	50 purifications	CS11203
ChargeSwitch® gDNA Normalized Buccal Cell Kit	50 purifications	CS11020
ChargeSwitch® gDNA Buccal Cell Kit	50 purifications	CS11021
ChargeSwitch® Forensic DNA Purification Kit	100 purifications	CS11200
Quant-iT™ DNA Assay Kit, High Sensitivity	1000 assays	Q33120
Quant-iT™ DNA Assay Kit, Broad-Range	1000 assays	Q33130
Quant-iT™ PicoGreen® dsDNA Assay	1 kit, 1 ml	P7589

E-Gel® Agarose Gels and DNA Ladders

E-Gel® Agarose Gels are bufferless pre-cast agarose gels designed for fast, convenient electrophoresis of DNA samples. E-Gel® agarose gels are available in different agarose percentages and well formats for your convenience.

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

For more information about these products, visit www.invitrogen.com or call Technical Service (page 14).

Introduction

Overview

Introduction

The ChargeSwitch® Plasmid Yeast Mini Kit allows rapid and efficient purification of plasmid DNA from 1 ml of *Saccharomyces cerevisiae* grown in selective minimal media.

After preparing the lysates, you can purify DNA in less than 15 minutes using the ChargeSwitch® Technology. For more information about the Charge Switch® Technology, see below.

The purified plasmid DNA is suitable for bacterial transformation and PCR.

The ChargeSwitch® Technology

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

In low pH conditions, the ChargeSwitch® beads have a positive charge that binds the negatively charged nucleic acid backbone (see figure below). Proteins and other contaminants are not bound and are simply washed away in an aqueous wash buffer. To elute nucleic acids, the charge on the surface of the bead is neutralized by raising the pH to 8.5 using a low salt elution buffer (see figure below). Purified DNA elutes instantly into this elution buffer, and is ready for use in downstream applications.

Continued on next page

Overview, Continued

Advantages

The ChargeSwitch® Plasmid Yeast Mini Kit provides the following advantages:

- Uses a magnetic bead-based technology to isolate DNA without the need for centrifugation or vacuum manifold
- Rapid and efficient purification of plasmid DNA from yeast culture in less than 15 minutes following sample preparation
- Simple lysis of cells without the need for any mechanical lysis
- Minimal contamination with RNA
- Purified DNA that demonstrates improved downstream performance in applications including PCR and bacterial transformations

System Specifications

Starting Material:	1 ml liquid culture (~2 x 10^7 cells/ml)
Bead Binding Capacity:	1 mg beads bind ~25 µg DNA
Bead Size:	<1 µm
Bead Concentration:	25 mg/ml
Bead Storage Buffer:	10 mM MES, pH 5.0, 10 mM NaCl, 0.1% Tween 20
Elution Volume:	100 µl
DNA Yield:	Up to 25 ng

Methods

General Information

Follow the recommendations below to obtain the best results:

- Maintain a sterile environment when handling DNA to avoid any contamination from DNases
- Ensure that no 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
- Perform the centrifugation step during cell lysis at 4°C to improve the efficiency of the precipitation reaction
- Perform the recommended wash steps during purification to obtain the best results

Safety Information

Follow the safety guidelines below when using the ChargeSwitch® gDNA Mini Bacteria Kit.

- Treat all reagents supplied in the kit as potential irritants.
- Always wear a suitable lab coat, disposable gloves, and protective goggles.
- Do not add bleach or oxidizing agents directly to the sample preparation waste.
- 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.

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General Information, Continued

MagnaRack™ Magnetic Rack

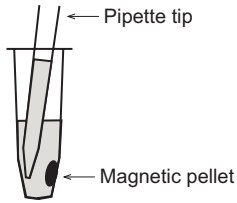
The MagnaRack™ Magnetic Rack available from Invitrogen (catalog no. CS15000) is a two-piece magnetic separation rack for use in protocols with magnetic beads. The MagnaRack™ Magnetic Rack consists of a magnetic base station and a removable tube rack. The tube rack can hold up to 24 microcentrifuge tubes. The tube rack fits onto the magnetic base station in two different positions associating the row of 12 neodymium magnets with a single row of 12 tubes for simple and easy "on the magnet" and "off the magnet" sample processing (see figure below). For more information, see www.invitrogen.com or call Technical Service (page 14).

Continued on next page

Handling Magnetic Beads

Follow the recommendations below for best results:

- During the mixing and washing steps of the ChargeSwitch® Magnetic Beads, mix beads by pipetting up and down gently to avoid forming bubbles as directed in the protocol.
- Do not allow the beads to dry as drying reduces the bead binding efficiency.
- To aspirate the supernatant after bead washing, place the pipette tip away from the beads by angling the pipette such that the tip is pointed away from the pellet and carefully remove the supernatant without disturbing or removing any beads (see figure below).



- **Do not freeze the magnetic beads** as freezing damages the beads and cannot be used for nucleic acid purifications.

Elution Buffer

The DNA is eluted with Elution Buffer (E5; 10 mM Tris-HCl, pH 8.5). To obtain the best results, always use Elution Buffer (E5) to elute the DNA. If you wish to elute the DNA in any other buffer, be sure to use a buffer of **pH 8.5-9.0**. If the pH of the buffer is <8.5, the DNA will not elute.

Do not use water for elution.

The volume of elution buffer can be changed to obtain DNA in the desired final concentration. To obtain the best results, always use a volume of elution buffer that is equal or greater than the volume of beads used in the protocol. If the volume of elution buffer is lower than the volume of beads, DNA elution is incomplete and you may need to perform a second elution to recover all DNA.

Using the Plasmid Yeast Mini Kit

Introduction

Instructions for isolating plasmid DNA from 1 ml culture are described in this section.

The procedure is designed for isolating plasmid DNA using the ChargeSwitch® Magnetic Beads procedure in a total time of **~15 minutes** after sample preparation.

Materials Needed

- MagnaRack™ Magnetic Rack (see page 4)
- β-mercaptoethanol (14 M)
- Lyticase (*e.g.* Sigma catalog no. L5263; 2,000 U/ml in water)
- Sterile 1.5 ml microcentrifuge tubes
- Microcentrifuge
- Vortex mixer
- Adjustable pipettes and sterile tips
- Light microscope and slides

Components supplied with the kit

- RNase A in Resuspension Buffer (R4), see below
- ChargeSwitch® Magnetic Beads
- ChargeSwitch® Precipitation Buffer (N5)
- ChargeSwitch® Lysis Buffer (L9)
- ChargeSwitch® Wash Buffer (W11)
- ChargeSwitch® Wash Buffer (W12)
- ChargeSwitch® Elution Buffer (E5)

Before Starting

- Add the entire content of supplied RNase A to the Resuspension Buffer (R4). Mix well. Mark the bottle label to indicate that RNase A is added. Store the buffer with RNase at 4°C.
- Prepare fresh Yeast Spheroplast Mix by adding 950 µl Resuspension Buffer (R4) with RNase A, 50 µl lyticase (2,000 U/ml), and 1 µl β-mercaptoethanol.
- Chill an aliquot of the Precipitation Buffer (N5) to 4°C.
- Check the Lysis Buffer (L9) for precipitates. If present, warm the solution briefly at 37°C to dissolve the precipitate.

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Cell Lysis

Grow *Saccharomyces cerevisiae* in selective minimal media.

1. Transfer 1 ml overnight culture (~2 x 10^7 cells/ml) to a sterile microcentrifuge tube.
2. Centrifuge at room temperature for 10 seconds at 13,000 rpm. Discard the supernatant.

   The length of time required may vary for different species.
3. Resuspend the cell pellet in 300 µl Yeast Spheroplast Mix (see previous page for recipe) by pipetting up and down gently several times or by vortexing. Ensure that the cells are evenly distributed.
4. Incubate at room temperature for 30 minutes.

   Note: Visually check the progress of spheroplast formation using a microscope by adding 5 µl of resuspended cells to an equal volume of 1% SDS. Intact yeast cells have a refractile appearance, whereas cells lacking a cell wall appear as ghost-like spheroplasts. Proceed to Step 5 when at least 90% of cells appear like spheroplasts.
5. Add 300 µl Lysis Buffer (L9) and invert the capped tube 5 times to mix.
6. Incubate at room temperature for 3 minutes.

   Incubate until solution clears. Over-incubation at this stage may reduce the quality of your purified plasmid DNA.
7. Add 300 µl chilled Precipitation Buffer (N5) and invert the capped tube 5 times or until a white, free-flowing precipitate has formed.
8. Centrifuge at 4°C for 10 minutes at ~12,000 x g.
9. Carefully transfer the supernatant to a sterile labeled tube.
10. Proceed immediately to **Binding DNA**, next page.

Continued on next page

Binding DNA

Follow the procedure below to bind DNA to the ChargeSwitch® Magnetic Beads.

1. Vortex the tube containing the ChargeSwitch® Magnetic Beads to fully resuspend and evenly distribute the beads in the storage buffer.
2. Add 40 µl ChargeSwitch® Magnetic Beads to the lysate from Step 9, previous page.
3. Pipet up and down gently to mix without forming bubbles.
4. Incubate at room temperature for 1 minute.
5. Place the sample on the MagnaRack™ Magnetic Rack for 1 minute or until the beads have formed a tight pellet.
6. Without removing the tube from the MagnaRack™, carefully remove and discard the supernatant without disturbing the pellet of beads by angling the pipette such that the tip is pointed away from the pellet (see figure on page 5).
7. Proceed immediately to **Washing DNA**, next page.

Continued on next page

Washing DNA

1. Remove the tube containing the pelleted magnetic beads from the MagnaRack™.
2. Add 1 ml Wash Buffer (W11) to the tube and pipet up and down gently using a 1 ml pipette tip to mix the sample without forming bubbles.
3. Place the sample on the MagnaRack™ Magnetic Rack for 1 minute or until the beads have formed a tight pellet.
4. Without removing the tube from the MagnaRack™, carefully remove and discard the supernatant without disturbing the pellet of beads by angling the pipette such that the tip is pointed away from the pellet.
5. Remove the tube containing the pelleted magnetic beads from the MagnaRack™.
6. Add 1 ml **Wash Buffer (W12)** to the tube and pipet up and down gently using a 1 ml pipette tip to mix the sample without forming bubbles.
7. Place the sample on the MagnaRack™ Magnetic Rack for 1 minute or until the beads have formed a tight pellet.
8. Without removing the tube from the MagnaRack™, carefully remove and discard the supernatant without disturbing the pellet of beads by angling the pipette such that the tip is pointed away from the pellet.
9. Proceed immediately to **Eluting DNA**, next page.

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Eluting DNA

1. Remove the tube containing the pelleted magnetic beads from the MagnaRack™.
2. Add 100 µl Elution Buffer (E5; 10 mM Tris-HCl, pH 8.5) to the tube and pipet up and down gently 5-10 times to mix the sample without forming bubbles.

   Note: See page 5 for more information on elution buffer volume.
3. Incubate at room temperature for 1 minute.

   Tip: For maximum yield, mix the suspension of beads (by pipetting up and down gently) half way through the incubation.
4. Place the sample in the MagnaRack™ for 1 minute or until the beads have formed a tight pellet.
5. Without removing the tube from the MagnaRack™, carefully remove the **supernatant containing the DNA** to a sterile microcentrifuge tube. Take care not to disturb the pellet of beads by angling the pipette such that the tip is pointed away from the pellet.

   Note: If the supernatant containing the DNA is discolored, repeat Steps 4-5.
6. Discard the used magnetic beads. **Do not** re-use the magnetic beads.

Storing DNA

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

Downstream Application

Use the recommended amount of purified DNA for the listed downstream applications:

- Use 2 µl of purified plasmid DNA (eluate) for PCR
- Use 5 µl of purified plasmid DNA (eluate) to transform 50 µl of chemically competent cells with a transformation efficiency of at least 1 x 10^7 cfu/µg of plasmid DNA

DNA Quantitation

DNA Yield

After using the ChargeSwitch® Plasmid Yeast Mini Kit, you may estimate the yield of purified plasmid DNA using the Quant-iT™ Kits.

Quant-iT™ DNA Assay Kits

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

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 DNA yield	Incomplete lysis	• Decrease the amount of starting material used. • Be sure to add lyticase and β-mercaptoethanol during lysis.
	Pellet of beads disturbed or lost during binding or washing steps	• Keep the sample in the MagnaRack™ when removing supernatant during the binding or washing steps. • Remove the supernatant without disturbing the pellet of beads by angling the pipette tip away from the pellet while the tube is on the magnetic rack.
	Incorrect elution conditions	• Do not use water to elute DNA. Use Elution Buffer (E5) or if you are using a buffer for elution, ensure the pH of the buffer is 8.5-9.0. • After adding ChargeSwitch® Elution Buffer to the sample, pipet up and down to resuspend the magnetic beads before incubation.

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Troubleshooting, Continued

Problem	Cause	Solution
No DNA recovered	Water used for elution	Do not use water for elution. The elution buffer **must** have a pH of 8.5-9.0 or the DNA will remain bound to the ChargeSwitch® Magnetic Beads. Use ChargeSwitch® Elution Buffer (E5).
	ChargeSwitch® Magnetic Beads stored or handled improperly	• Store beads at room temperature. **Do not freeze the beads** as they will become irreparably damaged. • Make sure that the beads are in solution at all times and do not allow the beads to dry. Dried beads are non-functional.
Eluate containing DNA is discolored	Magnetic pellet disturbed during elution	Place the sample on the MagnaRack™ until the beads form a tight pellet. Remove the eluate to a sterile microcentrifuge tube, taking care not to disturb the bead pellet.
RNA contamination	Forgot to add RNase A	Be sure to add RNase A to the Resuspension Buffer (R4) to perform RNase A digestion prior to binding the DNA to the magnetic beads.
DNA degraded	DNA contaminated with DNases	Maintain a sterile environment while working (*i.e.* wear gloves) and use DNase-free reagents.

Appendix

Technical Service

World Wide Web

Visit the Invitrogen Web Resource using your World Wide Web browser. At the site, you can:

- Get the scoop on our hot new products and special product offers
- View and download vector maps and sequences
- Download manuals in Adobe® Acrobat® (PDF) format
- Explore our catalog with full color graphics
- Obtain citations for Invitrogen products
- Request catalog and product literature

Once connected to the Internet, launch your Web browser (Internet Explorer 5.0 or newer or Netscape 4.0 or newer), then enter the following location (or URL):

http://www.invitrogen.com

...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!

Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our Web page (www.invitrogen.com).

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MSDS Requests

To request an MSDS, visit our Web site at **www.invitrogen.com.** On the home page, go to 'Technical Resources', select 'MSDS', and follow instructions on the page.

Product Qualification

Functional Qualification

Each kit is functionally tested to ensure conformance with the most current approved product specifications.

Current specifications consist of tests for:

- Bead size, charge, and binding capacity
- Nucleic acid quality and quantity
- Buffer turbidity, volume, and absence of RNases/DNases
- Kit packaging and labeling accuracy

For individual lot test results and more information, visit www.invitrogen.com to download the Certificate of Analysis.

Purchaser Notification

License

Limited Use Label License No. 265: ChargeSwitch® Technology

The use of this product may be covered by European Patent No. EP1036082B1 and foreign equivalents.

Limited Warranty

Invitrogen is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about an Invitrogen product or service, contact our Technical Service Representatives. Invitrogen warrants that all of its products will perform according to specifications stated on the certificate of analysis. The company will replace, free of charge, any product that does not meet those specifications. This warranty limits Invitrogen Corporation's liability only to the cost of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. Invitrogen reserves the right to select the method(s) used to analyze a product unless Invitrogen agrees to a specified method in writing prior to acceptance of the order. Invitrogen makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore Invitrogen makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, please report it to our Technical Service Representatives. **Invitrogen assumes no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose.**

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