Yeast DNA Isolation System

INSTRUCTION MANUAL

Catalog #200052
Revision A

For In Vitro Use Only
200052-12
LIMITED PRODUCT WARRANTY

This warranty limits our liability to replacement of this product. No other warranties of any kind, express or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Agilent. Agilent shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

ORDERING INFORMATION AND TECHNICAL SERVICES

United States and Canada
Agilent Technologies
Stratagene Products Division
11011 North Torrey Pines Road
La Jolla, CA 92037
Telephone  (858) 373-6300
Order Toll Free  (800) 424-5444
Technical Services  (800) 894-1304
Internet  techservices@agilent.com
World Wide Web  www.stratagene.com

Europe

<table>
<thead>
<tr>
<th>Location</th>
<th>Telephone</th>
<th>Fax</th>
<th>Technical Services</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria</td>
<td>0800 292 499</td>
<td>0800 292 496</td>
<td>0800 292 498</td>
</tr>
<tr>
<td>Belgium</td>
<td>00800 7000 7000</td>
<td>00800 7001 7001</td>
<td>08000 7400 7400</td>
</tr>
<tr>
<td></td>
<td>0800 15775</td>
<td>0800 15740</td>
<td>0800 15720</td>
</tr>
<tr>
<td>France</td>
<td>00800 7000 7000</td>
<td>00800 7001 7001</td>
<td>08000 7400 7400</td>
</tr>
<tr>
<td></td>
<td>0800 919 288</td>
<td>0800 919 287</td>
<td>0800 919 289</td>
</tr>
<tr>
<td>Germany</td>
<td>00800 7000 7000</td>
<td>00800 7001 7001</td>
<td>08000 7400 7400</td>
</tr>
<tr>
<td></td>
<td>0800 182 8232</td>
<td>0800 182 8231</td>
<td>0800 182 8234</td>
</tr>
<tr>
<td>Netherlands</td>
<td>00800 7000 7000</td>
<td>00800 7001 7001</td>
<td>08000 7400 7400</td>
</tr>
<tr>
<td></td>
<td>0800 023 0446</td>
<td>+31 (0)20 312 5700</td>
<td>08000 243 0448</td>
</tr>
<tr>
<td>Switzerland</td>
<td>00800 7000 7000</td>
<td>00800 7001 7001</td>
<td>08000 7400 7400</td>
</tr>
<tr>
<td></td>
<td>0800 563 080</td>
<td>0800 563 082</td>
<td>0800 563 081</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>00800 7000 7000</td>
<td>00800 7001 7001</td>
<td>08000 7400 7400</td>
</tr>
<tr>
<td></td>
<td>0800 917 3282</td>
<td>0800 917 3283</td>
<td>0800 917 3281</td>
</tr>
</tbody>
</table>

All Other Countries
Please contact your local distributor. A complete list of distributors is available at www.stratagene.com.
# Yeast DNA Isolation System

## CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Materials Provided</td>
<td>1</td>
</tr>
<tr>
<td>Storage Conditions</td>
<td>1</td>
</tr>
<tr>
<td>Additional Materials Required</td>
<td>1</td>
</tr>
<tr>
<td>Introduction</td>
<td>2</td>
</tr>
<tr>
<td>Protocol</td>
<td>2</td>
</tr>
<tr>
<td>Retrieving Plasmid DNA from a Single Yeast Colony</td>
<td>2</td>
</tr>
<tr>
<td>Transformation Guidelines</td>
<td>3</td>
</tr>
<tr>
<td>Transforming XL1-Blue Supercompetent Cells Using Plasmid DNA</td>
<td>3</td>
</tr>
<tr>
<td>Preparation of Media and Reagents</td>
<td>5</td>
</tr>
<tr>
<td>References</td>
<td>5</td>
</tr>
<tr>
<td>Endnotes</td>
<td>5</td>
</tr>
<tr>
<td>MSDS Information</td>
<td>5</td>
</tr>
<tr>
<td>Quick-Reference Protocol</td>
<td>7</td>
</tr>
</tbody>
</table>
Yeast DNA Isolation System

MATERIALS PROVIDED

<table>
<thead>
<tr>
<th>Materials provided</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>XL1-Blue supercompetent cells</td>
<td>8 × 240 μl</td>
</tr>
<tr>
<td>β-Mercaptoethanol (1.42 M)</td>
<td>2 × 25 μl</td>
</tr>
<tr>
<td>Lysis buffer</td>
<td>400 μl</td>
</tr>
<tr>
<td>p53 control plasmid (100 ng/μl)</td>
<td>10 μl</td>
</tr>
</tbody>
</table>

* The Yeast DNA Isolation System contains enough reagents for 20 transformation reactions and 4 control reactions.

b Immediately on receipt, thaw and dispense the lysis buffer into usable aliquots to avoid multiple freeze–thaw cycles that affect the buffer adversely. Store the aliquots at −80°C.

STORAGE CONDITIONS

All Components: −80°C

ADDITIONAL MATERIALS REQUIRED

- Sterile toothpicks
- Dry ice–methanol bath
- Water bath (37°C)
- Falcon® 2059 polypropylene tubes
INTRODUCTION

The Yeast DNA Isolation System, an innovative system for rapidly retrieving plasmid DNA from positive yeast clones identified from GAL4 two-hybrid system screenings, is designed specifically for the yeast host strains, YRG-2 and HF7c. The method uses a single lysis buffer combined with a rapid freeze–thaw cycle, followed by a heat step. The amount of plasmid DNA retrieved from a single yeast colony is sufficient to transform XL1-Blue supercompetent cells. The entire process, from picking the colony of interest to transformation, takes less than 15 minutes. Yields of 10–200 transformant colonies are typically achieved when using the Yeast DNA Isolation System.

PROTOCOL

Retrieving Plasmid DNA from a Single Yeast Colony

1. Aliquot 20 μl of lysis buffer into a 0.5-ml microcentrifuge tube.

   Warning The lysis buffer is a potential skin irritant. Wear gloves and avoid contact with skin and clothing.

2. Pick a positive yeast colony with a sterile toothpick. Place the tip of the sterile toothpick directly into the lysis buffer, stirring gently to disperse the yeast cells into the buffer.

3. Freeze the lysis reaction by immersing the microcentrifuge tube in a dry ice–methanol bath for 30 seconds. Thaw the lysis reaction in a 37°C water bath.

4. Heat the lysis reaction at 95°C for 5 minutes.

5. Spin the lysis reaction in a microcentrifuge at 14,000 × g for 30 seconds.

6. Transfer the lysate (i.e., the supernatant) to a fresh microcentrifuge tube. Use 2 μl of the lysate for transformation of XL1-Blue supercompetent cells (see Transforming XL1-Blue Supercompetent Cells Using Plasmid DNA).
Transformation Guidelines

Supercompetent Cells
XL1-Blue supercompetent cells are very sensitive to small variations in temperature and must be stored at the bottom of a –80°C freezer. Transferring tubes from one freezer to another may result in a loss of efficiency. The supercompetent cells should be placed at –80°C directly from the dry ice shipping container.

Use of Falcon® 2059 Polypropylene Tubes
It is important to use Falcon® 2059 polypropylene tubes for the transformation reactions, because other tubes may be degraded by β-mercaptoethanol. Additionally, the critical incubation period during heat pulsing is calculated for the thickness and shape of the Falcon 2059 polypropylene tubes.

Use of β-Mercaptoethanol
β-Mercaptoethanol has been shown to increase transformation efficiency. This kit includes prediluted, ready-to-use β-mercaptoethanol.

Transforming XL1-Blue Supercompetent Cells Using Plasmid DNA

Note  Keep the XL1-Blue supercompetent cells on ice at all times while aliquoting. It is essential that the Falcon 2059 polypropylene tubes are placed on ice before the supercompetent cells are thawed and that the supercompetent cells are aliquoted directly into the prechilled polypropylene tubes. Pipet the remaining supercompetent cells into 75-μl aliquots and freeze the aliquots at –80°C. Do not pass the frozen supercompetent cells through more than one freeze–thaw cycle.

1. Thaw the XL1-Blue supercompetent cells on ice.
2. Gently mix the XL1-Blue supercompetent cells.
3. To prepare the control and experimental transformation reactions, aliquot 75 μl of the XL1-Blue supercompetent cells into two separate prechilled 15-ml Falcon 2059 polypropylene tubes.
4. Add 1.3 μl of the β-mercaptoethanol to each Falcon 2059 polypropylene tube, yielding a final concentration of 25 mM β-mercaptoethanol/tube, and swirl the reactions gently.
5. Incubate the transformation reaction tubes on ice for 10 minutes, swirling each reaction gently every 2 minutes.
6. For the control transformation, dilute the p53 control plasmid 1:1000 in TE buffer to a final concentration of 0.1 ng/μl and mix gently. Add 1 μl of the diluted p53 control plasmid to a transformation reaction tube and swirl the control transformation reaction gently.

For the experimental transformation, add 2 μl of the lysate generated from Retrieving Plasmid DNA from a Single Yeast Colony to the remaining transformation reaction tube and swirl the experimental transformation reaction gently.

7. Incubate the transformation reactions on ice for 20–30 minutes.

8. Heat pulse the transformation reactions in a 42°C water bath for 40 seconds. **The duration of the heat pulse is critical for optimal transformation efficiencies.**

9. Incubate the transformation reactions on ice for 2 minutes.

10. Add 0.4 ml of NZY* broth to each transformation reaction tube and incubate the reactions at 37°C for 1 hour with shaking at 225–250 rpm.

11. Use a sterile spreader to plate the transformation reactions as outlined below:

   a. Plate 5 μl of the control transformation reaction on an LB–ampicillin agar plate.

   b. Plate the entire volume of the experimental transformation reaction onto an LB–ampicillin or LB–chloramphenicol agar plate for selection of the target or bait plasmid, respectively.

   **Note** If both the target and bait plasmids are ampicillin resistant, plate the entire volume of the experimental transformation reaction on an LB–ampicillin agar plate. For discrimination between the target and bait plasmids, additional screening must be performed.

12. Incubate the plates at 37°C overnight (16–18 hours).

**Expected Results**

The expected colony number of the control transformation reaction ranges from 100 to 500 cfu, and the expected colony number of the experimental transformation reaction ranges from 10 to 200 cfu.

---

* See Preparation of Media and Reagents.

* When spreading the transformation reactions onto the plate, tilt and tap the spreader to remove the last drop of cells. If plating <100 μl of the transformation reaction, plate into a 200-μl pool of NZY* broth. If plating ≥100 μl, the cells can be spread directly onto the plates.
## Preparation of Media and Reagents

### LB Agar (per Liter)

- 10 g of NaCl
- 10 g of tryptone
- 5 g of yeast extract
- 20 g of agar
- Add deionized H₂O to a final volume of 1 liter
- Adjust pH to 7.0 with 5 N NaOH
- Autoclave
- Pour into petri dishes (~25 ml/100-mm plate)

### LB–Ampicillin Agar (per Liter)

- 1 liter of LB agar, autoclaved
- Cool to 55°C
- Add 10 ml of 10-mg/ml filter-sterilized ampicillin
- Pour into petri dishes (~25 ml/100-mm plate)

### LB–Chloramphenicol Agar (per Liter)

- 1 liter of LB agar, autoclaved
- Cool to 55°C
- Add 30 mg of filter-sterilized chloramphenicol
- Pour into petri dishes (~25 ml/100-mm plate)

### TE Buffer

- 10 mM Tris-HCl (pH 7.5)
- 1 mM EDTA

### NZY+ Broth (per Liter)

- 10 g of NZ amine (casein hydrolysate)
- 5 g of yeast extract
- 5 g of NaCl
- Add deionized H₂O to a final volume of 1 liter
- Adjust to pH 7.5 using NaOH
- Autoclave
- Add the following filter-sterilized supplements prior to use:
  - 12.5 ml of 1 M MgCl₂
  - 12.5 ml of 1 M MgSO₄
  - 20 ml of 20% (w/v) glucose (or 10 ml of 2 M glucose)

## References


## Endnotes

Falcon® is a registered trademark of Becton Dickinson and Company.

## MSDS Information

The Material Safety Data Sheet (MSDS) information for Stratagene products is provided on the web at [http://www.stratagene.com/MSDS/](http://www.stratagene.com/MSDS/). Simply enter the catalog number to retrieve any associated MSDS’s in a print-ready format. MSDS documents are not included with product shipments.
QUICK-REFERENCE PROTOCOL

- Aliquot 20 μl of lysis buffer into a 0.5-ml microcentrifuge tube
- Pick a positive yeast colony with a sterile toothpick
- Place the tip of the sterile toothpick directly into the lysis buffer, stirring gently to disperse the yeast cells into the buffer
- Freeze the lysis reaction by immersing the microcentrifuge tube in a dry ice–methanol bath for 30 seconds
- Thaw the lysis reaction in a 37°C water bath
- Heat the lysis reaction at 95°C for 5 minutes
- Spin the lysis reaction in a microcentrifuge at 14,000 × g for 30 seconds
- Transfer the lysate (i.e., the supernatant) to a fresh microcentrifuge tube
- Use 2 μl of the lysate for transformation of XL1-Blue supercompetent cells (see Transforming XL1-Blue Supercompetent Cells Using Plasmid DNA in the instruction manual)