Yeast DNA Isolation System

INSTRUCTION MANUAL

Catalog #200052
Revision A

For In Vitro Use Only
200052-12

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Yeast DNA Isolation System

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MATERIALS PROVIDED

Materials provided[a]	Quantity
XL1-Blue supercompetent cells	8 × 240 µl
β-Mercaptoethanol (1.42 M)	2 × 25 µl
Lysis buffer[b]	400 µl
p53 control plasmid (100 ng/µl)	10 µl

[a] 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

Revision A

INTRODUCTION

The Yeast DNA Isolation System,[1] an innovative system for rapidly retrieving plasmid DNA from positive yeast clones identified from GAL4 two-hybrid system screenings,[2,3] 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 H_2O 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 H_2O to a final volume of 1 liter
Adjust to pH 7.5 using NaOH
Autoclave
Add the following filer-sterilized supplements prior to use:

12.5 ml of 1 M $MgCl_2$
12.5 ml of 1 M $MgSO_4$
20 ml of 20% (w/v) glucose (or 10 ml of 2 M glucose)

REFERENCES

1. Callahan, M. and Greener, A. (1996) *Strategies* 9(1):30–31.
2. Mullinax, R. L. and Sorge, J. A. (1995) *Strategies* 8(1):3-5.
3. Callahan, M., Jerpseth, B., Mullinax, R. L. and Greener, A. (1995) *Strategies* 8(2):45–46.

ENDNOTES

MSDS INFORMATION

The Material Safety Data Sheet (MSDS) information for Stratagene products is provided on the web at *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.

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QUICK-REFERENCE PROTOCOL

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