Subcloning Efficiency™ DH5α™ Chemically Competent *E. coli*

Catalog no. 18265-017

General Information and Kit Contents

Shipping and Storage

The kit is shipped on dry ice. Upon receipt, store the kit at -70°C. Do not store in liquid nitrogen.

Kit Contents

The kit contains the following reagents.

Reagent	Composition	Amount
DH5α™cells	--	4 x 500 µl
pUC19	100 pg/µl in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8	20 µl (2 ng)

Genotype

F- φ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F)U169 *deo*R *rec*A1 *end*A1 *hsd*R17(r_k^-, m_k^+) *pho*A *sup*E44 *thi*-1 *gyr*A96 *rel*A1 λ^-

Quality Control Procedure

Competent cells (100 µl) are transformed with 500 pg of supercoiled pUC19 plasmid. Transformed cultures are plated on LB plates containing 100 µg/ml ampicillin and the transformation efficiency is calculated. Test transformations are performed in duplicate. Transformation efficiency should be > 1 x 10^6 cfu/µg DNA.

Untransformed cells are tested for:

- Absence of contamination by plating on five different LB plates containing either 100 µg/ml ampicillin, 50 µg/ml kanamycin, 15 µg/ml tetracycline, 20 µg/ml chloramphenicol, or 100 µg/ml streptomycin
- Inhibited growth on nitrofurantoin (*rec*A)
- Lac^- and Gal^+ phenotypes
- Absence of lambda phage contamination

Note: Saturating amounts of control pUC19 DNA (25 ng) generates > 1 × 10^4 transformants from a 50 µl reaction.

Patent Information

This product is covered by U.S. patent no. 4,981,797 and foreign equivalents.

Overview

Applications

Subcloning Efficiency™ DH5α™ Chemically Competent *E. coli* are suitable for:

- Routine subcloning into plasmid vectors
- Blue/white screening of transformants on selective plates containing Bluo-gal or X-gal
- High-quality plasmid preparation
- Transformation of large plasmids (up to 30 kb)
- Transiently hosting M13mp cloning vectors (see page 5)

Subcloning Efficiency™ DH5α™ are **not** suitable for generation of cDNA libraries. We recommend Max Efficiency™ DH5α™-T1^R Chemically Competent *E. coli* (Catalog no. 12034-013) or UltraMax™ DH5α™-ft Chemically Competent *E. coli* (Catalog no. 10643013).

Important

DH5α™ *E. coli* **does not require** IPTG to induce expression from the *lac* promoter even though the strain expresses the Lac repressor. The copy number of most plasmids exceeds the repressor number in the cells. If you are concerned about obtaining maximal levels of expression, add IPTG to a final concentration of 1 mM.

If blue/white screening is required to select for transformants spread 40 µl of 40 mg/ml X-Gal in dimethylformamide on top of the agar. Let the X-Gal diffuse into the agar for at least 1 hour.

General Handling

Be extremely gentle when working with competent cells. Competent cells are highly sensitive to changes in temperature or mechanical lysis caused by pipetting. Transformation should be started immediately following the thawing of the cells on ice. **Mix by swirling or tapping the tube gently, not by pipetting or vortexing.**

Transforming Chemically Competent Cells

Materials Supplied by the User

You will need the following items for transformation:

- 37°C shaking and non-shaking incubator
- 10 cm diameter LB agar plates with appropriate antibiotic (100 µg/ml ampicillin to select transformants containing pUC19 control DNA)
- LB, YT, or SOC Medium
- Dry ice and ethanol
- 37°C water bath

Before Starting

- Prepare a dry ice/ethanol bath and maintain at -70°C
- Equilibrate a water bath to 37°C
- Spread X-Gal onto LB agar plates with antibiotic, if desired
- Warm the medium and plates in a 37°C incubator for 30 minutes
- Obtain a test tube rack that will hold all transformation tubes so that they can all be put into a 37°C water bath at once.

Transformation Procedure

The instructions provided below are for general use. Plasmid DNA should be free of phenol, ethanol, protein, and detergents for maximum transformation efficiency.

1. Briefly centrifuge the ligation reaction and place on wet ice.
2. Remove one 500 µl tube of DH5α™ cells and thaw on wet ice.
3. Place the required number of sterile 1.5 ml microcentrifuge tubes on wet ice.
4. Gently mix cells with the pipette tip and aliquot 50 or 100 µl into each microcentrifuge tube.
5. Re-freeze any unused cells in the dry ice/ethanol bath for 5 minutes before returning the tube to the -70°C freezer. **Do not use liquid nitrogen.**
6. Pipet 1 to 5 µl (1-10 ng DNA) of each ligation reaction directly into the competent cells and mix by tapping gently. **Do not mix by pipetting up and down.** Store the remaining ligation reaction at -20°C.
7. (Optional) Pipet 5 µl (500 pg) pUC19 control DNA into 100 µl competent cells and mix as described in Step 6.
8. Incubate the vial on ice for 30 minutes.
9. Heat-shock for exactly 20 seconds in the 37°C water bath for 50 µl volume (45 seconds for 100 µl transformation). Do not mix or shake.
10. Remove vial from the 37°C bath and place on ice for 2 minutes.
11. Add 900 to 950 µl of pre-warmed medium of choice to each vial. Proceed to next page.

Continued on next page

Procedure, continued

12. Place the vial in a microcentrifuge rack on its side and secure with tape to avoid loss of the vial. Shake the vial at 37°C for exactly 1 hour at 225 rpm in a shaking incubator.
13. Spread 20 µl to 200 µl from each transformation vial on separate, labeled LB agar plates. We recommend that you plate two different volumes.

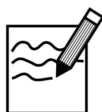
    Note: You may have to dilute cells 1:10 to obtain well-spaced colonies. Generally ligations are at least 10-fold lower efficiency.
14. (Optional) For cells transformed with pUC19 control DNA, plate 100 µl of the transformation reaction, then serially dilute the transformation reaction 1:10 and 1:100 and plate 100 µl of each dilution on plates containing 100 µg/ml ampicillin.
15. Store the remaining transformation reaction at +4°C and plate out the next day, if desired. If necessary, cells may be concentrated by centrifuging in a microcentrifuge (5 seconds) and resuspending them in 100 µl. Plate 1 to 90 µl.
16. Invert the plates and incubate at 37°C overnight.
17. Select colonies and analyze by plasmid isolation, PCR, or sequencing.

Calculation

Calculate the transformation efficiency as transformants per 1 µg of pUC19 plasmid DNA. Be sure to account for dilution or concentration of cells (DF = dilution factor).

$$\frac{\text{\# of colonies}}{\text{500 pg transformed pUC19 DNA}} \times \frac{10^6 \text{ pg}}{\mu\text{g}} \times \frac{\text{1000 } \mu\text{l total transformation volume}}{\text{100 } \mu\text{l plated}} \times \text{DF} = \frac{\text{\# transformants}}{\mu\text{g plasmid DNA}}$$

Expected transformation efficiency: >1 x 10^6 cfu/µg supercoiled plasmid.



Note

Transformation efficiencies for cDNA and ligation of inserts to vectors will be lower than for a supercoiled control plasmid such as pUC19.

- For cDNA, transformation efficiencies may be 10- to 100-fold lower.
- For ligation of inserts to vectors, transformation efficiencies may be 10-fold lower.

Using DH5α™ as a Transient Host

Introduction

DH5α™ competent *E. coli* support replication of M13mp vectors, but it does not support plaque formation. Plating on a lawn of *E. coli* containing the F episome will allow plaque formation.

Before Starting

You will need the following reagents:

- Dry ice/ethanol bath
- **Log-phase** *E. coli* containing the F episome (*e.g.* DH5α-FT™, DH5αF′™, DH5αF′IQ™, JM101, or JM107)
- Liquid top agar containing 50 µg/ml Bluo-gal or X-gal and 1 mM IPTG
- LB plates (no antibiotic)

Procedure

A general procedure is provided below for your convenience; you may have to optimize the protocol for your particular circumstances.

1. Remove one 500 µl tube of DH5α™ cells and thaw on wet ice.
2. Place the required number of sterile 1.5 ml microcentrifuge tubes on ice.
3. Gently mix cells with the pipette tip and aliquot 50 µl into each microcentrifuge tube.
4. Re-freeze any unused cells in the dry ice/ethanol bath for 5 minutes before returning the tube to the -70°C freezer. **Do not use liquid nitrogen.**
5. Transform 10 pg (or 1-10 ng of a RF ligation) of replicative form (RF) M13mp into 50 µl of competent cells.
6. Incubate the vial on ice for 30 minutes.
7. Heat-shock for exactly 20 seconds in the 37°C water bath. Do not mix or shake.
8. Remove vial from the 37°C bath and place on ice. **Note**: Since selection by antibiotic resistance is not necessary for plaque formation, recovery in medium is not necessary.
9. Take the log-phase *E. coli* containing the F episome and add to the liquid top agar.
10. Add 30-50 µl of the transformation reaction from Step 8 to the top agar.
11. Mix and pour the top agar onto LB plates (no antibiotic).
12. After the plate has solidified, invert and incubate at 37°C overnight or until plaques form.

Technical Service

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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!

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2. Follow instructions on the page and fill out all the required fields.
3. To request additional MSDSs, click the 'Add Another' button.
4. All requests will be faxed unless another method is selected.
5. When you are finished entering information, click the 'Submit' button. Your MSDS will be sent within 24 hours.

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3E Company
Voice: 1-760-602-8700

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