SMOBIO®
Small Bio, Smart Tool

Champion™

E. coli Transformation Kit

Catalog number : CK1000

Flexible | High Efficiency | Fast and Easy

For research use only

Table of Contents

Product Information

(1) General Information

Champion™ *E. coli* Transformation Kit provides an easy method for rapid preparation of chemically competent cells with high transformation efficiency from fresh culture, overnight culture, or even directly from bacterial colonies on the plate. The competent cell preparation method eliminates the requirement of time-wasting wash step. In addition, preparation of competent cells from overnight culture or directly from bacterial colonies provides flexibility to cloning experiment. The resultant competent cells can be immediately used or stored at -70°C for one year.

This kit includes a specialized SMO-Broth™ medium and a unique Champion™ CC Buffer for culturing and preparing competent cells efficiently. Following the simple and quick competent cell preparation protocol from fresh culture, the transformation efficiency is typically ranged from 10^8–10^9 transformants/μg of pUC19 plasmid DNA, but varies depending on the *E. coli* strains.

The resultant competent cells can be further transformed using time-saving transformation protocol, eliminating the requirement of heat-shock and recovery steps.

(2) Features

- ✓ **Flexible –** fresh culture, overnight culture, 4°C stored liquid culture or even colonies on agar plate can be used for transformation.
- ✓ **Fast and Easy –** only few steps for preparation; suitable for time-saving transformation
- ✓ **High efficiency –** up to 10^9 cfu/µg
- ✓ **Personalization –** suitable for most *E. coli* strains

(3) Kit Contents

Champion™ CC Buffer	20 ml
SMO-Broth™	100 ml x 2
pUC19 Control Plasmid (10^{-4} µg/µl)	5 µl
Instruction Manual	1
Champion™ Competent Cell Preparation Card	1

(4) Storage and Expiration

Champion™ CC Buffer	4°C	≥ 12 months
SMO-Broth™	4°C	≥ 12 months
pUC19 Control Plasmid	4°C	≥ 12 months

Standard Protocol for Preparation of Competent Cells

For the best result, we recommend to freshly culture *E. coli* with SMO-Broth™ at 16~25°C.

1. Prepare *E. coli* overnight culture by inoculating a single colony in 2 ml growth medium.
2. Refresh *E. coli* culture by inoculating 0.5 ml overnight culture into 50 ml SMO-Broth™, SOB, SOC or LB medium in 250 ml flask. (Volume can be adjusted according to your requirements.)
3. Incubate fresh *E. coli* culture at suitable temperature with 150~250 rpm shaking until the OD_{600nm} reaches to 0.4~0.8. (The culture time is varied and depends on the temperature, shaking speed, and *E. coli* strains.)

The following steps should be performed on ice; also the Champion™ CC Buffer must be kept at 0~4°C.

4. Collect the *E. coli* culture by centrifuge at 4000 x *g* for 5 min at 4°C.
5. Remove the growth medium and gently resuspend the *E. coli* in pre-cooled Champion™ CC Buffer.

$$\text{Volume of Champion™ CC Buffer (ml)} = \frac{\text{Volume of medium (ml)} \times OD_{600}}{5}$$

6. Aliquot 100~200 µl *E. coli* suspension into sterile microcentrifuge tubes. The *E. coli* suspension is ready for transformation with DNA or can be stored at -70°C for 1 year. (**Do not store at temperature above -70°C.**)

Instant Protocol for Preparation of Competent Cells

(1) From Overnight Bacterial Culture (OD_{600nm}: 1~2.5)

Overnight incubation at 25°C with 250 rpm shaking in **SMO-Broth™** is recommended.

1. Harvest the *E. coli* with 4000 x *g* for 5 min at 4°C.
2. Remove the growth medium and gently resuspend the *E. coli* in suitable amount of pre-cooled Champion™ CC Buffer.

$$\text{Volume of Champion™ CC Buffer (ml)} = \frac{\text{Volume of medium (ml)} \times OD_{600}}{5}$$

3. Aliquot 100~200 µl *E. coli* suspension into sterile microcentrifuge tubes. The *E. coli* suspension is ready for transformation (efficiency is about 10^7~10^8).

(2) Directly from Bacterial Colonies (Fresh, or 4°C stored within 7 days)

1. Gently collect and mix five *E. coli* colonies (diameter ~1 mm^2) into 100 µl pre-cooled SMO-Broth™.
2. Harvest the *E. coli* with 4000 x *g* for 5 min at 4°C.
3. Remove the medium and gently resuspend the *E. coli* in 100 µl pre-cooled Champion™ CC Buffer. The *E. coli* suspension is ready for transformation (efficiency is about 10^5~10^6).

Tips for High Competence of *E. coli*

Culture Condition

E. coli cells present high competence when freshly cultured from 1/100~1/20 dilution of overnight culture and incubated at 16~25°C prior to preparation. Higher temperature (37°C) leads to competence decrease.

Temperature	OD_{600}	Culture time	Efficiency (cfu/μg)*
16°C	0.4~0.8	12~20 hr	~$1x10^9$
25°C	0.4~0.8	3~8 hr	~$5x10^8$
37°C	0.4~0.8	1~1.5 hr	~$1x10^8$
25°C	1~2.5	4~10 hr	~$3x10^8$

E. coli cells in liquid culture are higher competent than colonies on plate.

Culture Condition	Efficiency (cfu/μg)*
Liquid culture (fresh or overnight culture)	10^8~10^9
Fresh colonies (grown overnight at 37°C)	~$2x10^6$
Old colonies (4°C stored for 7 days)	~$5x10^5$

*Efficiency is measured by using JM109 strain and following efficiency assay protocol at page 11.

E. coli Strains

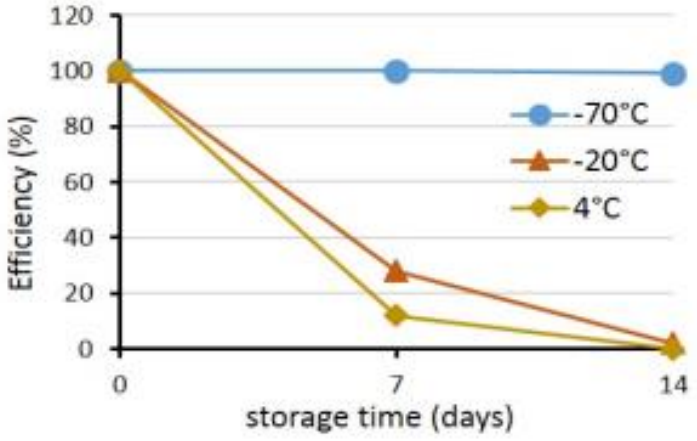
Different *E. coli* strains vary in their ability to be transformed with DNA. Efficiency of strains and their derivatives is listed below, when prepared with the Champion™ *E. coli* Transformation Kit.

E. coli strains	Efficiency (cfu/µg)**
JM109	~$5x10^8$
XL-1 blue	~$3x10^8$
DH5α	~$5x10^8$
stbl 3	~$2x10^8$
BL21	~$1x10^7$
Rosseta 2	~$1x10^7$

***E. coli* strains are liquid cultured following standard protocol for preparation of competent cells (**at 25˚C until OD_{600} reached ~0.5**). Efficiency is measured following efficiency assay protocol at page 11.

Storage Condition

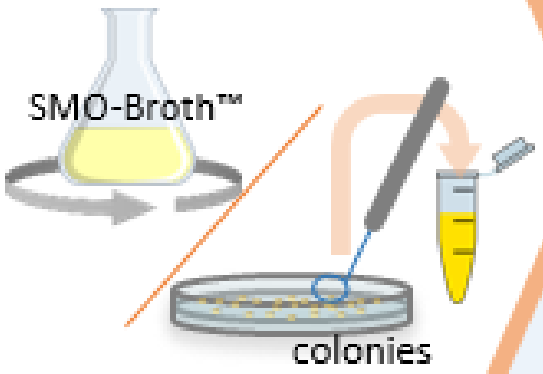
To maintain high competence, *E. coli* should be stored at -70°C. Higher temperature (above -50°C) leads to competence decrease.



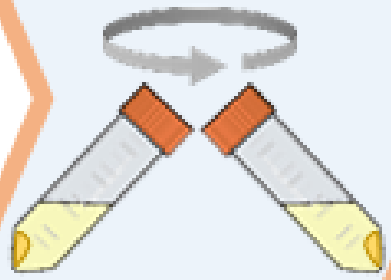
Champion™ *E. coli* Transformation Protocol

Prepare Competent Cells

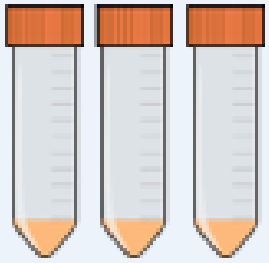
Culture
16~25˚C, OD_{600} 0.4~2.5



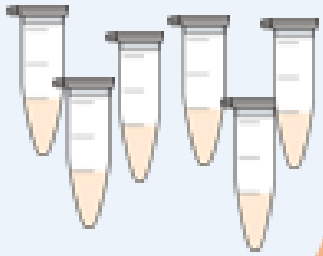
Harvest
4˚C, 4000 x *g*, 5 min



Resuspend
Operate on ice

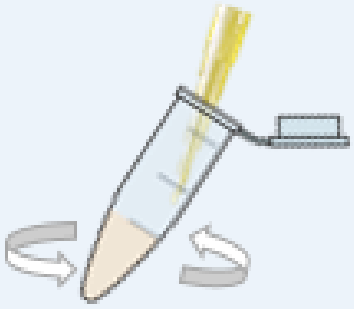


Aliquot
Operate on ice

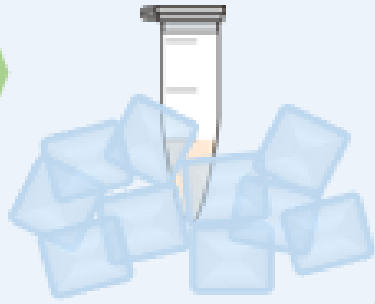


Time-Saving Transformation

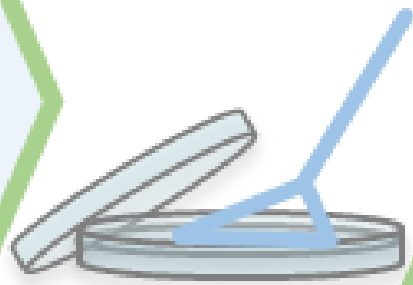
Mix DNA
Vortex 1 sec



Incubate on Ice
0~10min



Spread
Pre-warmed selective plate



Standard Transformation Protocol

For large plasmid (>6 Kb) or non-ampicillin antibiotic

1. Quickly thaw the competent cells with hand (body temperature), warm water bath, or running tap water for 10~20 seconds until 1/3~1/2 volume is thawed.
2. Add less than 10% of competent cell volume DNA (ex. ligation product) to cells.
3. Vortex 1 sec or tap on the tube with finger to mix well.
4. Incubate on ice for 5 mins.
5. Heat shock the competent cells at 42°C for 45~90 seconds.
6. Incubate on ice for 1~5 mins.
7. Add 900 µl LB or SOC medium to the competent cells and incubate at 37°C for 30~60 mins with a 200 rpm shaking (optional for optimized efficiency).
8. Plate the bacteria/DNA mixture onto a pre-warmed (room temperature to 37°C) and dried selective LB agar plate (LB + antibiotic).
9. Incubate the plates at 37°C until the colonies are suitable for analysis.

Time-Saving Transformation Protocol

1. Quickly thaw the competent cells with hand (body temperature), warm water bath, or running tap water for 10~20 seconds until 1/3~1/2 volume is thawed.
2. Add less than 10% of competent cell volume DNA (ex. ligation products) to cells.
3. Vortex 1 sec or tap on the tube with finger to mix well.
4. Incubate on ice for 0~10 mins. (The ice-incubation will slightly increase efficiency.)
5. Plate the bacteria/DNA mixture onto a pre-warmed (room temperature to 37°C) and dried selective LB agar plate (LB + antibiotic).
6. Incubate the plates at 37°C until the colonies are suitable for analysis.

Efficiency Assay

1. Dilute pUC19 Control Plasmid to 10^{-6} µg/µl
2. Transform 10^{-6} µg of pUC19 plasmid to 100 µl of competent cells following time- saving transformation protocol with incubation on ice for 5 mins.
3. Calculation of transformation efficiency is as follows:
   a. Equation for transformation efficiency= transformed colonies (transformants)/µg of plasmid.
   b. If 550 colonies are observed on the selective plate.
      The transformation efficiency is:
      $550/10^{-6} = 5.5x10^{8}$ transformants/µg of pUC19 plasmid.

Factors Affecting Transformation Efficiency

Thawing methods

Shorter thawing time is more efficient than a longer thawing time. Slow thawing caused by power shortages and unstable freezers will result in decreased efficiency.

Size of plasmid

Plasmid size affect the efficiency greatly. The efficiency of transforming a supercoiled 2.7 kb is approximately 100 times higher than that of a 10 Kb plasmid (using time-saving transformation protocol). For large plasmids (> 6 kb), standard transformation protocol is recommended.

Heat shock treatment

Heat shock treatment will enhance the efficiency about 1~2 folds versus non-heat shock method.

Plating methods

Bent glass rods show the greatest efficiency, while plating loop shows less efficiency than plating beads. When handling a large quantity of samples at the same time, plating beads are recommended.

Concentration of antibiotic

Antibiotic concentration is critical to use of the time-saving transformation protocol.

Antibiotic	concentration
Ampicillin (Ap)	20 μg/ml
Kanamycin (Km)	25 μg/ml
Tetracycline (Tc)	7.5 μg/ml
Chloramphenicol (Cm)	20 μg/ml

For plasmid size <6 Kb, the efficiency of kanamycin selection is usually 3~10 times less than the ampicillin selection. For plasmid size > 6 Kb, the efficiency of kanamycin selection is much lower than ampicillin. We suggest using the standard transformation protocol (with heat shock and recovery steps) to enhance the efficiency.

Quality Control

All components of the kit were tested in transformation of *E.coli* JM109 strains using 10^{-6} μg of pUC19 plasmid following standard preparation of competent cell (page 4) and time-saving transformation protocol (page 10). The transformation yields more than 5 x 10^{8} transformants per μg of pUC19 plasmid DNA.

Other Information

SMOBIO Technology, Inc. claims all warranties with respect to this document, expressed or implied, including but not limited to those of merchantability or fitness for a particular purpose. In no event shall SMOBIO Technology, Inc. be liable, whether in contract, tort, warranty, or under any statute or any other basis for special, incidental, indirect, punitive, multiple or consequential damages in connection with or arising from this document, including but not limited to the use thereof.

SMOBiO®

Small Bio, Smart Tool

More technical information can be found by visiting our website:
http://www.smobio.com/Support.html

save an hour