



**Champion™**

# Competent Cell

For Research Use Only

*Mix then Spread*

High Efficiency  Easy to Use



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## General information

Champion™ Competent Cells are chemically competent cells, which were prepared by SMOBIO to make *E. coli* perform excellent transformation efficiency. Standard transformation protocol is recommended for large plasmids or non-ampicillin selection. Time-saving transformation protocol is recommended for simple and rapid transformation. Champion™ Competent Cells are one of the fastest and simplest ready-to-use competent cell products in the world.

## Kit contents

- (1) Champion™ Competent Cells
- (2) pUC19 Control Plasmid (5  $\mu$ l,  $10^{-4}$   $\mu$ g/ $\mu$ l)
- (3) Champion™ Transformation Protocol Card

## Shipping condition

Throughout the shipping process, the temperature is maintained under  $-70^{\circ}\text{C}$ .

## Storage and expiration

Champion™ Competent Cells must be stored between  $-70^{\circ}\text{C}$  to  $-80^{\circ}\text{C}$ . Subsequent freeze-thaw cycles will reduce transformation efficiency. If high efficiency is required for the experiment, do not use aliquots that have gone through several freeze-thaw cycles. The efficiency of Champion™ Competent Cells lasts for 1 year with proper storage.

## Items and ordering information

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Product Name	Compatible to <i>E. coli</i> strain	Efficiency (cfu/μg)	Quantity	Cat. No.
Champion™ 109 High	<i>E. coli</i> JM109	>1 x 10 <sup>8</sup>	100 μl x 80 vials	CC0202
			100 μl x 24 vials	CC0204
Champion™ 21	<i>E. coli</i> BL21 (DE3)	>1 x 10 <sup>7</sup>	100 μl x 80 vials	CC2102
			100 μl x 24 vials	CC2104
Champion™ DH5α High	<i>E. coli</i> DH5α	>3 x 10 <sup>8</sup>	100 μl x 80 vials	CC5202
			100 μl x 24 vials	CC5204

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## Genotypes and applications

Product Name	Genotype	Application
Champion™ 109 High	<i>e14(McrA<sup>-</sup>)recA1 endA1 gyrA96 hsdR17(r<sub>K</sub><sup>-</sup>, m<sub>K</sub><sup>+</sup>) phoA supE44 thi-1 relA1 Δ(lac-proAB) (F' traD36 proAB lacI<sup>q</sup>ΔM15</i>	Appropriate for blue-white color and robotic screening. It is a fast growing strain forming visible colonies within 8~10 hours.
Champion™ 21	<i>F' ompT hsdS<sub>β</sub>(r<sub>β</sub><sup>-</sup>m<sub>β</sub><sup>-</sup>) dcm gal λ (DE3)</i>	Appropriate host for recombinant protein expression using T7-based expression vectors.
Champion™ DH5α High	<i>recA1 endA1 gyrA96 hsdR17(r<sub>K</sub><sup>-</sup>, m<sub>K</sub><sup>+</sup>) phoA supE44 relA1 thi-1 Δ(lacZYA- argF)U 169 φ80 Δ(lacZ)M15 F'</i>	Suitable for cloning with large plasmid and cDNA library construction, and also for blue-white colony selection.

## Time-saving transformation protocol for Champion™ Competent Cells

1. Quickly thaw  $-70^{\circ}\text{C}$  stored competent cells with hand (body temperature) for 10~20 seconds, then keep thawed competent cells on ice.
2. Add DNA (ex. ligation products) to cells, keep volume of DNA less than 10% of competent cell volume.
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  $37^{\circ}\text{C}$ ) selective LB agar plate (LB + antibiotic).
6. Incubate the plates at  $37^{\circ}\text{C}$  until the colonies are suitable for analysis.

\* *Notice: For large plasmid (>6 Kb) or non-ampicillin selection, steps below are highly recommended to be added between step 4 and 5.*

- a. *Heat shock the competent cells at  $42^{\circ}\text{C}$  for 45~90 seconds.*
- b. *Incubate on ice for 1~5 mins.*
- c. *Add 900  $\mu\text{l}$  LB or SOC medium to the competent cells and incubate at  $37^{\circ}\text{C}$  for 30~60 mins with a 200 rpm shaking.*

## Time-saving transformation protocol for Champion™ Competent Cells



**Notice:** the antibiotics concentration for Champion™ Competent Cells

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



## Quality Control

### Efficiency assay

The transformation efficiency of each batch of Champion™ Competent Cells is greater than that described in “Items and ordering information” at the time of production. The transformation efficiency is determined following time-saving transformation protocol with incubation on ice for 5 mins.

Calculation of transformation efficiency is as follows:

- Equation for transformation efficiency= transformed colonies (transformants)/ $\mu\text{g}$  of plasmid.
- Example: 100  $\mu\text{l}$  of competent cells have been transformed with  $10^{-6}$   $\mu\text{g}$  of pUC19 plasmid. If 550 colonies are observed on the selective plate. The transformation efficiency is:  
 $550/10^{-6}=5.5 \times 10^8$  transformants/ $\mu\text{g}$  of pUC19 plasmid.

### Contamination assay

No colony on the LB agar plate with 20~50  $\mu\text{g}/\text{ml}$  of ampicillin when transformation is performed without plasmid.

### $\alpha$ -Complementation assay

The ratio of the white colonies/ white & blue colonies of the tested plate is less than 3% (for Champion™ 109 High, and Champion™ DH5 $\alpha$  high)



## 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.

### Methods of mixing DNA with competent cells

A one-second vortex provides more reliable transformation efficiency (1.1 times compared with mixing by a finger tap).

### Size of plasmid

Plasmid size is known to affect the efficiency greatly. For instance, the efficiency of a supercoiled 2.7 kb and a 10 Kb plasmid (using time-saving transformation protocol) has a difference of approximately 100 times. For large plasmids (> 6 kb), the heat shock method and the recovery procedure as shown in the standard transformation protocol will improve the efficiency.

### Heat shock treatment

For large plasmids (> 6kb), transformation efficiency with heat shock treatment will be nearly double to that without heat-shock treatment.

### 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.

#### (1) For ampicillin

The recommended concentration is 20  $\mu\text{g/ml}$  for fresh ampicillin (Ap) and 50  $\mu\text{g/ml}$  for old Ap. If a higher concentration (50~100  $\mu\text{g/ml}$ ) is used, the efficiency will reduce by 3~10 times.

#### (2) For other antibiotics

25  $\mu\text{g/ml}$  Kanamycin; 7.5  $\mu\text{g/ml}$  Tetracycline; 20  $\mu\text{g/ml}$  Chloramphenicol.

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.

## Fast and easy method for plasmid construction using SMOBIO smart cloning tools

### PCR amplification

Blunt-end PCR amplification of target gene with HiFi™ DNA polymerase. (TF1000, TF2000, TF3000)



Blunt end amplicon  
DNA fragment

### Ligation

Ligation with GetClone™ PCR Cloning Vector. (CV1000, CV1100)



Blunt end amplicon  
DNA fragment

pGet

pGet

### Colony PCR

Analyze colonies with ExcelTaq™ PCR Master Mix. (TP1100, TP1200)



### Transformation

Transformation to Champion™ Competent Cells.





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

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