



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 μ l, 10^{-4} μ g/ μ l)
- (3) Champion™ Transformation Protocol Card

Shipping condition

Throughout the shipping process, the temperature is maintained under -70°C .

Storage and expiration

Champion™ Competent Cells must be stored between -70°C to -80°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

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 ⁸	100 μl x 80 vials	CC0202
			100 μl x 24 vials	CC0204
Champion™ 21	<i>E. coli</i> BL21 (DE3)	>1 x 10 ⁷	100 μl x 80 vials	CC2102
			100 μl x 24 vials	CC2104
Champion™ DH5α High	<i>E. coli</i> DH5α	>3 x 10 ⁸	100 μl x 80 vials	CC5202
			100 μl x 24 vials	CC5204



Genotypes and applications

Product Name	Genotype	Application
Champion™ 109 High	<i>e14(McrA⁻)recA1 endA1 gyrA96 hsdR17(r_k⁻, m_K⁺) phoA supE44 thi-1 relA1 Δ(lac-proAB) (F'traD36 proAB lacI^qΔM15</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_β(r_β⁻m_β⁻) 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_k⁻, m_K⁺) 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°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°C) selective LB agar plate (LB + antibiotic).
6. Incubate the plates at 37°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°C for 45~90 seconds.*
- b. *Incubate on ice for 1~5 mins.*
- c. *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.*



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)/ μg of plasmid.
- Example: 100 μl of competent cells have been transformed with 10^{-6} μg 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/ μg 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.

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

(2) For other antibiotics

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

P

P

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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