SMOBiO®
Small Bio, Smart Tool
コスモ・バイオ株式会社

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 µl, 10^{-4} µg/µl)
(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.

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Items and ordering information

Product Name	Compatible to *E. coli* strain	Efficiency (cfu/µg)	Quantity	Cat. No.
Champion™ 109 High	*E. coli* JM109	>1 x 10^8	100 µl x 80 vials	CC0202
			100 µl x 24 vials	CC0204
Champion™ 21	*E. coli* BL21 (DE3)	>1 x 10^7	100 µl x 80 vials	CC2102
			100 µl x 24 vials	CC2104
Champion™ DH5α High	*E. coli* DH5α	>3 x 10^8	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	*e*14(*McrA*$^-$)*rec*A1 *end*A1 *gyr*A96 *hsd*R17(r_K^-, m_K^+) *pho*A *sup*E44 *thi*-1 *rel*A1 Δ(*lac-pro*AB) (F'traD36 proAB *lac*I^{q}ZΔM15	Appropriate for blue-white color and robotic screening. It is a fast growing strain forming visible colonies within 8~10 hours.
Champion™ 21	F' *omp*T *hsd*S$_\beta$($r_\beta^-m_\beta^-$) *dcm gal* λ (DE3)	Appropriate host for recombinant protein expression using T7-based expression vectors.
Champion™ DH5α High	*rec*A1 *end*A1 *gyr*A96 *hsd*R17(r_K^-, m_K^+) *pho*A *sup*E44 *rel*A1 *thi*-1 Δ(*lac*ZYA-*arg*F)U 169 ϕ80 Δ(*lac*Z)M15 F'	Suitable for cloning with large plasmid and cDNA library construction, and also for blue-white colony selection.

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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.

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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:

a. Equation for transformation efficiency= transformed colonies (transformants)/μg of plasmid.
b. 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: $550/10^{-6}=5.5x10^{8}$ transformants/μg of pUC19 plasmid.

Contamination assay

No colony on the LB agar plate with 20~50 μg/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™ DH5α high)

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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 µg/ml for fresh ampicillin (Ap) and 50 µg/ml for old Ap. If a higher concentration (50~100 µg/ml) is used, the efficiency will reduce by 3~10 times.

(2) For other antibiotics

25 µg/ml Kanamycin; 7.5 µg/ml Tetracycline; 20 µg/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.

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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)

Ligation

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

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