TaKaRa *E.coli* JM109 Competent Cell

Cat.# 9052
v.0610

TaKaRa

Contents:

TaKaRa *E.coli* JM109 Competent Cells ....10 vials x 100 μ l
pBR322 plasmid (0.1 ng/ μ l) ....10 μ l
SOC Medium*................................ ....10 vials x ml

* ・ SOC medium:

2%	Bacto tryptone
0.5%	Bacto yeast extract
10 mM	NaCl
2.5 mM	KCl
10 mM	$MgSO_4$
10 mM	$MgCl_2$
20 mM	Glucose

Specification:

TaKaRa Competent Cells are prepared by Hanahan's method modified by TaKaRa and have a transformation efficiency of >1 x 10^8 cfu/ μ g when 100 μ l of the cells are transformed by 1 ng pBR322.
As TaKaRa *E.coli* JM109 Competent Cells contains F' plasmid, it can be used as a host of M13 vector DNA as well as for preparation of DNA library or subcloning. When transformation of pUC vectors or transduction of M13 phage vector DNAs, recombinants can be selected easily by adding X-Gal and IPTG to a media utilizing the α -complimentarity to β -galactosidase of the competent cell.
X-Gal : 5-Bromo-4-Chloro-3-Indolyl- β -D-Galactoside
IPTG : Isopropyl- β -D-thiogalactopyranoside

Protocols:

A. Transformation into a plasmid vector

1) Thaw TaKaRa *E.coli* JM109 Competent Cells in an ice bath just before use.
2) Gently mix cells and transfer 100 μ l into a polypropylene tube (BD Falcon 352059 or 352057).
   Important: Do not use a vortex to mix cells.
   It is important that BD Falcon 352059 or 352057 tubes are used for the transformation protocol, as the incubation period during the heat pulse step (step 5) is critical and has been calculated for the thickness and shape of the BD Falcon tubes.
3) Add DNA sample (≦ 10 ng is recommended.)
4) Keep in the ice bath for 30 min.
5) Incubte cells for 45 sec. at 42°C.
6) Return to the ice bath for 1-2 min.
7) Add SOC medium (pre-incubated at 37°C) up to a final volume of 1 ml.
8) Incubate by shaking (160-225 rpm) for 1 hour at 37°C.
9) Plate on selective media*.
   * ≦ 100 μ l is recommended for plating on dish with ∅ 9cm.
10) Incubate overnight at 37°C.

B. Transduction of a M13 phage vector

1) Follow the step 1)-8) mentioned in A.
2) Add 200 μ l of the host (*E.coli* JM109, A_{600}=0.8-1.0) into 3.5 ml of YT soft agar (pre-incubated at 46-48°C).
3) Add a proper amount of the solution prepared at 1) into the agar, mix, and immediately spread it onto a L-plate.
4) Incubate at room temperature for 10-15 min. and then, at 37°C over night.

Please read before proceeding:

1) Place a vial of competent cells in a dry ice / EtOH bath immediately upon removal from -80°C freezer. Keep cells in bath until you are ready to proceed.
2) When using 100 μl of competent cell, apply high-purified sample DNA in less than 10 ng. If not, transformation efficiency might decrease.
3) When changing an experiment scale, optimum condition should be considered.
4) L-broth or φ b-broth can be used instead of SOC medium. In this case, lower efficiency might be obtained.

・L-broth :

Ingredient	per liter water
Bacto tryptone	10 g
Bacto yeast extract	5 g
NaCl	5 g

Adjust to around pH7.5 with 1N NaOH and autoclave.

・φ b-broth:

Ingredient	per liter water
Bacto tryptone	20 g
Bacto yeast extract	5 g
$MgSO_4 \cdot 7H_2O$	5 g

Adjust to around pH7.5 with 1N KOH and autoclave.

5) When diluting, use SOC medium which has been added in the step 7) of A.
6) YT soft agar:

Ingredient	per 100 ml water
Bacto tryptone	0.8 g
Bacto yeast extract	0.5 g
NaCl	0.5 g

Adjust to around pH7.6 with NaOH, add agar to the concentration of 0.6%, and autoclave.

7) Host can be prepared by culturing competent cells.
8) L-plate

Ingredient	per liter
Bacto tryptone	10 g
Bacto yeast extract	5 g
NaCl	5 g

Adjust to pH7.5 with NaOH, add agar to the concentration of 1.5%, and autoclave.

9) When adding X-Gal or IPTG, follow the procedures described as below:
   - ・Add 100 ml IPTG into 100 μl/100 ml agar medium and 25 μl/3 ml soft agar.
   - ・Add 20 mg/ml dimethylhormeamide of X-Gal into 200 μl/100 ml medium and 50 μl/3 ml soft agar.
10) It is not recommended to freeze and store the thawed competent cells. However, if necessary, freeze in a dry ice/EtOH bath and return to -80°C. The transformation efficiency can be lowered by more than one magnitude.

Transformation efficiency:

1 ng of pBR322 was transformed and selected by Amp^+ selective media plating.
Transformation efficiency ＞ 1 x 10^8 cfu / μg pBR322v

Stability of F' plasmid:

Less than 1% of white colonies appeared when transformation with a plasmid vector followed by plating on a L-agar medium containg 100 μl/ml amplicilin, 0.2 mM IPTG, and 40 μg/ml X-Gal.

Genotype:

E.coli JM109: *recA1, endA1, gyrA96, thi-1, hsdR17, supE44, relA1, Δ (lac-proAB)/ F' [traD36, proAB$^+$, lacI^q, lacZ Δ M15]*

Cell density 1-2 x 10^9 cells/ml

Storage: -80° C

Note: If it is not stroed at -80° C, transformation efficiency may decrease. In this case, it is recommended to confirm the efficiency by using supplied pBR322 prior to use an application.

Reference:

1. Hanahan, D. (1983) *J.Mol.Biol.* **166**, 557.
2. Messing, J. (1985) *Gene* **33**, 103.

NOTE: **For research use only.**
Not for use in diagnostic or therapeutic procedures.