Cat. # 9052

For Research Use

TaKaRa

E. coli JM109 Competent Cell

Product Manual

v202001Da

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I. Components

E. coli JM109 Competent Cells		100 µl x 10
pBR322 plasmid (0.1 ng/µl)		10 µl
SOC Medium*		1 ml x 10
* SOC Medium :	2%	Tryptone
	0.5%	Yeast extract
	10 mM	NaCl
	2.5 mM	KCl
	10 mM	$MgSO_4$
	10 mM	$MgCl_2$
	20 mM	Glucose

II. Storage

-80℃

Note: If it is not stroed at -80℃, transformation efficiency may decrease. In this case, it is recommended to confirm the efficiency by using supplied pBR322 prior to use an application.
Do not store in liquid nitrogen.

III. Description

E .coli JM109 Competent Cells are prepared by Hanahan's method modified by Takara Bio. Because of containing F' plasmid, it can be used as a host of M13 phage 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 cells.

X-Gal : 5-Bromo-4-Chloro-3-Indolyl-β-D-Galactoside
IPTG : Isopropyl-β-D-thiogalactopyranoside

IV. Protocol

A. Transformation into a plasmid vector

1) Thaw *E. coli* JM109 Competent Cells in an ice bath just before use.
2) Gently mix cells and transfer 100 μl into 14 ml round-bottom tube (BD Code: 352059 or 352057).
   Note: Do not use a vortex to mix cells.
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℃.
6) Return to the ice bath for 1-2 min.
7) Add SOC Medium (pre-incubated at 37℃) up to a final volume of 1 ml.
8) Incubate by shaking (160-225 rpm) for 1 hour at 37℃.
9) Plate on selective medium＊.
   ＊ Plate no more than 100 μl for a φ 9 cm plate. If necessary, dilute the culture with the same medium as used in step 7).
10) Incubate overnight at 37℃.

B. Transduction of a M13 phage vector

1) Follow the step 1) - 8) mentioned in A.
2) Add 200 μl of the host (*E. coli* JM109, A_{600}=0.8-1.0) into 3 ml of YT soft agar (pre-incubated at 46-48℃).
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℃ over night.

【Please read before proceeding】

1) Place a vial of competent cells in a dry ice / EtOH bath immediately upon removal from -80℃ freezer. Keep cells in bath until you are ready to proceed.
2) You may use 1.5 ml microcentrifuge tubes instead of 14 ml round bottom tubes (BD Code: 352059 or 352057, etc.) for transformation, but it may reduce efficiency.
3) When using 100 μl of competent cells, apply high-purified sample DNA in less than 10 ng. If not, transformation efficiency might decrease.
4) If you change the quantity of competent cells, or type of tubes used, it might be necessary to reevaluate the conditions. For example, when using 1.5 ml microcentrifuge tubes, heat shock for 60 sec at 42℃ rather than 45 sec.
5) L-broth or φb-broth can be used instead of SOC Medium. In this case, lower efficiency might be obtained.

・L-broth :

Ingredient	per liter water
Tryptone	10 g
Yeast extract	5 g
NaCl	5 g

Adjust to around pH 7.5 with 1N NaOH and autoclave.

・φb-broth:

Ingredient	per liter water
Tryptone	20 g
Yeast extract	5 g
$MgSO_4 \cdot 7H_2O$	5 g

Adjust to around pH 7.5 with 1N KOH and autoclave.

6) YT soft agar:

Ingredient	per 100 ml water
Tryptone	0.8 g
Yeast extract	0.5 g
NaCl	0.5 g

Adjust to around pH 7.6 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 water
Tryptone	10 g
Yeast extract	5 g
NaCl	5 g

Adjust to pH 7.5 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 mM IPTG into 100-300 μl/100 ml agar medium and 25 μl/3 ml soft agar.
   - Add 20 mg /ml X-Gal (dissolved in dimethylformamide) at a ratio of 200 - 300 μl/100 ml agar medium, and 50 μl/3 ml soft agar.
10) It is not recommended to refreeze and store the thawed competent cells. However, if necessary, freeze in a dry ice/EtOH bath and return to -80℃. The transformation efficiency can be lowered by more than one magnitude.

V. Quality

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

2. 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, 0.3 mM IPTG, and 60 μg/ml X-Gal.

VI. Genotype

E .coli JM109:
recA1, endA1, gyrA96, thi-1, hsdR17 ($r_k^- m_k^+$), *e14*$^-$ (*mcrA*$^-$), *supE44, relA1,*
Δ (*lac-proAB*)/F'[*traD36, proAB*$^+$, *lacI*^{q}, *lacZ* Δ M15]

VII. Cell density

1 - 2 x 10^9 bacteria/ml

VIII. References

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