For Research Use

# TakaRa

# E. coli JM109 Competent Cell

Product Manual

v202001Da

#### Cat. #9052 v202001Da



I.	Components	3
II.	Storage	. 3
III.	Description	. 3
IV.	Protocol	.4
V.	Quality	. 6
VI.	Genotype	, 6
VII.	Cell density	. 6
VIII.	References	. 6



#### I. Components

<i>E. coli</i> JM109 Competent Cells pBR322 plasmid (0.1 ng/ μl) SOC Medium <sup>*</sup>		100 μl x 10 10 μl 1 ml x 10
* SOC Medium :	2% 0.5% 10 mM 2.5 mM 10 mM 10 mM 20 mM	Tryptone Yeast extract NaCl KCl MgSO4 MgCl <sub>2</sub> Glucose

#### II. 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. 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  $\alpha$ -complimentarity to  $\beta$ -galactosidase of the competent cells.

X-Gal : 5-Bromo-4-Chloro-3-Indolyl-  $\beta$ -D-Galactoside IPTG : Isopropyl- $\beta$ -D-thiogalactopyranoside



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#### 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  $\mu$ l into 14 ml round-bottom tube (BD Code: 352059 or 352057).
- Note: Do not use a vortex to mix cells.
- 3) Add DNA sample ( $\leq 10$  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  $37^{\circ}$ C) up to a final volume of 1 ml.
- 8) Incubate by shaking (160-225 rpm) for 1 hour at  $37^{\circ}$ C.
- 9) Plate on selective medium\*.
  - \* Plate no more than 100  $\mu$ l for a  $\phi$  9 cm plate. If necessary, dilute the culture with the same medium as used in step 7).
- 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<sub>600</sub>=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°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) 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  $\mu$  l 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  $\varphi$  b-broth can be used instead of SOC Medium. In this case, lower efficiency might be obtained.

• <u>L-broth :</u>	Ingredient	per liter water
	Tryptone	10 g
	Yeast extract	5 g
	NaCl	5 g
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Adjust to around pH 7.5 with 1N NaOH and autoclave.

• <u>φb-broth:</u>	Ingredient	per liter water
	Tryptone	20 g
	Yeast extract	5 g
	MgSO4·7H <sub>2</sub> O	5 g
Adjust to aro	und pH 7.5 with	1N KOH and autoclave.

6) <u>YT soft agar:</u> <u>Ingredient per 100 ml water</u> Tryptone 0.8 g Yeast extract 0.5 g NaCl 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
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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  $\mu$  l/100 ml agar medium and 25  $\mu$  l/3 ml soft agar.
    - Add 20 mg /ml X-Gal (dissolved in dimethylformamide) at a ratio of 200 300  $\mu$  l/100 ml agar medium, and 50  $\mu$  l/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°C.
  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<sup>+</sup> selective media plating. Transformation efficiency  $> 1 \times 10^8$  cfu /  $\mu$  g 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  $\mu$  l/ml amplicilin, 0.3 mM IPTG, and 60  $\mu$  g/ml X-Gal.

# VI. Genotype

*E*.coli JM109: recA1, endA1, gyrA96, thi-1, hsdR17 ( $r_k$ - $m_k$ +), e14- (mcrA-), supE44, relA1,  $\Delta$  (lac-proAB)/F'[traD36, proAB+, lacI9, lacZ  $\Delta$  M15]

# VII. Cell density

1 - 2 x 10<sup>9</sup> bacteria/ml

# **VIII. References**

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

**NOTE :** This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.

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