E. coli Competent Cells

Technical Bulletin No. 095

INSTRUCTIONS FOR USE OF PRODUCTS L1001, L1011, L1191, L1201, L2001 AND L2011.
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I. Description

Promega's *E. coli* Competent Cells are prepared according to a modified procedure of Hanahan (1). The competent cells can be used for many standard molecular biology applications. Competent cells of strains HB101 and JM109 are available for convenient transformation in two efficiencies: High Efficiency at greater than 10^8cfu/µg and Subcloning Efficiency at greater than 10^7cfu/µg. JM109 cells (2) are an ideal host for many molecular biology applications. HB101 cells (3) are useful for cloning in vectors that do not require α-complementation for blue/white screening. The BMH 71-18 *mut*S strain is suitable for use in in vitro mutagenesis procedures where a repair (–) strain is required. BL21(DE3)pLysS cells[(a)] can be used with protein expression vectors that are under the control of the T7 promoter, such as pET vectors. This strain is lysogenic for lambda-DE3 (4), which contains the T7 bacteriophage gene 1, encoding T7 RNA polymerase (5) under the control of the *lac* UV5 promoter. BL21(DE3)pLysS also contains the pLysS plasmid, which carries the gene encoding T7 lysozyme. T7 lysozyme lowers the background expression level of target genes under the control of the T7 promoter but does not interfere with the level of expression achieved following induction with IPTG. For genotypic information on Promega's *E. coli* Competent Cells, see Table 1.

Table 1. Genotypes of *E. coli* Competent Cells Offered by Promega.

Strain	Genotype
BL21(DE3)pLysS	F–, *omp*T, *hsd*S$_B$ (r$_B$–, m$_B$–), *dcm*, *gal*, λ(DE3), pLysS, Cm^r
BMH 71-18 *mut*S	*thi*, *sup*E, Δ(*lac*-*pro*AB), [*mut*S::Tn10], [F′, *pro*AB, *laq*I^{q}ZΔM15]
HB101	F–, *thi*-1, *hsd*S20 (r$_B$–, m$_B$–), *sup*E44, *rec*A13, *ara*-14, *leu*B6, *pro*A2, *lac*Y1, *gal*K2, *rpsL*20 (str^r), *xyl*-5, *mtl*-1
JM109	*end*A1, *rec*A1, *gyr*A96, *thi*, *hsd*R17 (r$_K$–, m$_K$+), *rel*A1, *sup*E44, Δ(*lac*-*pro*AB), [F′, *tra*D36, *pro*AB, *laq*I^{q}ZΔM15]

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II. Product Components

Product	Size	Cat.#
JM109 Competent Cells, High Efficiency ($>10^8$cfu/µg)	1ml (5 × 200µl)	L2001
JM109 Competent Cells, Subcloning Efficiency ($>10^7$cfu/µg)	1ml (5 × 200µl)	L1001
HB101 Competent Cells, High Efficiency ($>10^8$cfu/µg)	1ml (5 × 200µl)	L2011
HB101 Competent Cells, Subcloning Efficiency ($>10^7$cfu/µg)	1ml (5 × 200µl)	L1011
BL21(DE3)pLysS Competent Cells[a] ($>10^6$cfu/µg)	1ml (5 × 200µl)	L1191
BMH 71-18 *mut*S Competent Cells ($>10^7$cfu/µg)	1ml (5 × 200µl)	L1201

Do Not refreeze thawed, unused aliquots.

Storage Conditions: Always store Competent Cells at –70°C. Thaw on ice when ready for use. **Do not refreeze thawed, unused aliquots.**

All cells are supplied in 200µl aliquots and are provided with 3ng of Competent Cells Control DNA for use as a positive control. Typically, 100µl of Competent Cells are required for standard transformations.

III. Standard Transformation Protocol

Materials to Be Supplied by the User
(Solution compositions are provided in Section V.)

- LB or SOC medium
- LB plates with antibiotic
- 17 × 100mm polypropylene culture tubes, sterile (e.g., Falcon® 2059)
- IPTG (Cat.# V3955; optional, see Note 4)
- X-Gal (Cat.# V3941; optional, see Note 4)

1. Chill sterile 17 × 100mm polypropylene culture tubes on ice, one per transformation (e.g., Falcon® 2059). Use of a standard microcentrifuge tube reduces the transformation efficiency by approximately 50% due to inefficient heat-shock treatment of the cells.
2. Remove frozen Competent Cells from –70°C and place on ice for 5 minutes, or until just thawed. Once the cells have thawed, pipet quickly or use chilled (4°C) pipette tips to prevent the cells from warming above 4°C.
3. Gently mix the thawed Competent Cells by flicking the tube, and transfer 100µl to each of the chilled culture tubes.
4. Add 1–50ng of DNA (in a volume not greater than 10µl) per 100µl of Competent Cells. Move the pipette tip through the cells while dispensing. Quickly flick the tube several times.
5. Immediately return the tubes to ice for 10 minutes.
6. Heat-shock the cells for 45–50 seconds in a water bath at exactly 42°C. **Do not shake.**
7. Immediately place the tubes on ice for 2 minutes.

Note: To determine the transformation efficiency, we recommend using 1µl (0.1ng) of Competent Cells Control DNA at Step 4.

8. Add 900µl of cold (4°C) SOC medium to each transformation reaction, and incubate for 60 minutes at 37°C with shaking (approximately 225rpm).
9. For each transformation reaction, we recommend diluting the cells 1:10 and 1:100 and plating 100µl of the undiluted, 1:10 and 1:100 dilutions on antibiotic plates (see Notes 1–3). Incubate the plates at 37°C for 12–14 hours.

Notes:

1. For transformations using the Competent Cells Control DNA, we recommend diluting the cells 1:10 and then plating 100µl on LB/ampicillin plates.
2. Do not dilute BL21(DE3)pLysS Competent Cells; spread 100µl of these cells directly onto antibiotic plates.
3. If desired, pellet the cells by centrifugation at 1,000 × *g* for 10 minutes, then resuspend in 200µl of SOC or LB medium and plate (see margin note).
4. **Blue/white screening** can be used with a variety of vectors in conjunction with JM109 Competent Cells. To use blue/white color screening for recombinants, plate the transformed cells on LB plates containing 100µg/ml ampicillin, 0.5mM IPTG (Cat.# V3955) and 40µg/ml X-Gal (Cat.# V3941). Incubate overnight at 37°C.

   An alternative to preparing plates containing X-Gal and IPTG is to spread 20µl of 50mg/ml X-Gal and 100µl of 0.1M IPTG onto LB ampicillin plates and allow these components to absorb for 30 minutes at 37°C prior to plating cells.
5. Solutions and media containing **tetracycline** must be stored protected from light in order to maintain potency.

Note: Use high-quality deionized water (e.g., MilliQ® or NANOpure®) for SOC medium (see recipe in Section V). If LB or other media is used, transformation efficiencies will be reduced.

Note: HB101 and BL21(DE3)pLysS Competent Cells cannot be used for blue/white color screening.

IV. Calculation of Transformation Efficiency (Colony Forming Units [cfu])

Transformation efficiency is defined as the number of colony forming units (cfu) produced by 1µg of Competent Cells Control DNA (supercoiled plasmid DNA) and is measured by performing a control transformation reaction using a known quantity of DNA, typically 0.1ng, then calculating the number of cfu formed per microgram DNA.

Note: The Competent Cells Control DNA (pGEM®-3Z Vector[b]) is supplied at a concentration of 0.1ng/µl in TE buffer.

Equation for Transformation Efficiency (cfu/µg)

$$\frac{\text{cfu on control plate}}{\text{ng of Competent Cells Control DNA plated}} \times \frac{1 \times 10^3\text{ng}}{\mu\text{g}}$$

Example

After adding 900µl SOC medium to 100µl competent cells that have been transformed with 0.1ng Competent Cells Control DNA, transfer 100µl (equivalent to 0.01ngDNA) to 900µl SOC medium and plate 100µl (equivalent to 0.001ng DNA). If 100 colonies are observed on the plate, the transformation efficiency is:

$$\frac{100\text{cfu}}{0.001\text{ng}} \times \frac{1 \times 10^3\text{ng}}{\mu\text{g}} = 1 \times 10^8\text{cfu}/\mu\text{g}$$

Note: Transformation with ligated plasmid DNA will produce fewer colonies than transformation with supercoiled plasmid DNA.

V. Composition of Buffers and Solutions

glucose, 2M

180.16g glucose

Add distilled water to 500ml, filter-sterilize through a 0.2µm filter unit and store in aliquots at –20°C. Stable for 1 year.

IPTG stock solution, 0.1M

1.2g IPTG (Cat.# V3955)

Add water to 50ml final volume. Filter-sterilize through a 0.2µm filter unit and store at 4°C.

LB medium with ampicillin

10g/L	Bacto®-Tryptone
5g/L	Bacto®-Yeast Extract
5g/L	NaCl

Adjust the pH to 7.5 with NaOH. Autoclave to sterilize. Allow the autoclaved medium to cool to 55°C and add ampicillin (final concentration 100µg/ml). For LB plates, include 15g agar prior to autoclaving.

X-Gal

Available from Promega (Cat.# V3941) at a concentration of 50mg/ml in dimethylformamide.

Mg^{2+} stock solution, 2M

101.5g	$MgCl_2 \bullet 6H_2O$
123.3g	$MgSO_4 \bullet 7H_2O$

Add distilled water to 500ml and filter-sterilize through a 0.2µm filter unit.

Note: Filter-sterilizing units should be prerinsed with distilled water before use to remove any toxic material.

SOC medium

2.0g	Bacto®-tryptone
0.5g	Bacto®-yeast extract
1ml	1M NaCl
0.25ml	1M KCl
1ml	Mg^{2+} stock ($1M\ MgCl_2 \bullet 6H_2O$, $1M\ MgSO_4 \bullet 7H_2O$), filter-sterilized
1ml	2M glucose, filter-sterilized

Bring to 100ml with distilled water.

Add Bacto®-tryptone, Bacto®-yeast extract, NaCl and KCl to 97ml distilled water. Stir to dissolve. Autoclave and cool to room temperature. Add 2M Mg^{2+} stock and 2M glucose stock, each to a final concentration 20mM. Filter the complete medium through a 0.2µm filter unit. The pH should be 7.0.

VI. References

1 Hanahan, D. (1985) In: *DNA Cloning*, Vol. 1, Glover, D., ed., IRL Press, Ltd., 109.

2. Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* **33**, 103.

3. Lacks, S. and Greenberg, B. (1977) Complementary specificity of restriction endonucleases of *Diplococcus pneumoniae* with respect to DNA methylation. *J. Mol. Biol.* **114**, 153.

4. Studier, F.W. and Moffatt, B.A. (1986) Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J. Mol. Biol.* **189**, 113.

5. Davanloo, P. *et al.* (1984) Cloning and expression of the gene for bacteriophage T7 RNA polymerase. *Proc. Natl. Acad. Sci. USA* **81**, 2035.

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[b]U.S. Pat. No. 4,766,072 has been issued to Promega Corporation for transcription vectors having two different bacteriophage RNA polymerase promoter sequences separated by a series of unique restriction sites into which foreign DNA can be inserted.

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E. coli Competent Cells: *Experienced User's Protocol*

This quick protocol is intended as an easy-to-follow reminder for experienced users. Please follow the complete protocol (Section III) the first time you use *E. coli* Competent Cells.

Standard Transformation Protocol (Section III)	1. Chill sterile 17 × 100mm polypropylene culture tubes on ice. 2. Thaw frozen Competent Cells on ice for 5 minutes. 3. Gently mix the thawed Competent Cells. Transfer 100µl to each of the chilled culture tubes. 4. Add 1–50ng of DNA or 0.1ng Competent Cells Control DNA per 100µl of Competent Cells. Quickly flick the tube several times. (Use Competent Cells Control DNA to determine transformation efficiency.) 5. Immediately return the tubes to ice for 10 minutes. 6. Heat-shock the cells for 45–50 seconds in a water bath at exactly 42°C. **Do not shake.** 7. Immediately place the tubes on ice for 2 minutes. 8. Add 900µl of cold (4°C) SOC medium to each transformation reaction Incubate for 60 minutes at 37°C with shaking. 9. For each transformation reaction, dilute the cells 1:10 and 1:100. Plate 100µl of the undiluted, 1:10 and 1:100 dilutions on antibiotic plates. Incubate the plates at 37°C for 12–14 hours. For the control, dilute the cells 1:10. Plate 100µl (0.001ng) on LB/ampicillin plates. If using BL21(DE3)pLysS Competent Cells, see Section III, Note 2.