Promega

Technical Bulletin

E. coli Competent Cells

INSTRUCTIONS FOR USE OF PRODUCTS L1001, L1191, L1195, L2001, L2005, L2011, L2015 AND L1221.

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E. coli Competent Cells

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1. Description

The *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. JM109 competent cells 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. HB101 competent cells are available in high efficiency at greater than 10^8cfu/ug. Pro 5-alpha competent cells are available in high efficiency at greater than 10^9cfu/µg. JM109 and Pro 5-alpha cells (2) are ideal hosts for many molecular biology applications, including blue/white screening. HB101 cells (3) are useful for cloning in vectors that do not require α-complementation for blue/white screening. 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 the *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
HB101	F-, *thi*-1, *hsd*S20 (r_B–, m_B–), *sup*E44, *rec*A13, *ara*-14, *leu*B6, *pro*A2, *lac*Y1, *gal*K2, *rps*L20 (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^qZΔM15]
Pro 5-alpha	*fhu*A2, Δ(*arg*F-*lac*Z), U169, *pho*A, *gln*V44, ϕ80, Δ(*lac*Z)M15, *gyr*A96, *rec*A1, *rel*A1, *end*A1, *thi*-1, *hsd*R17

2. Product Components and Storage Conditions

Product	Size	Cat.#
JM109 Competent Cells, >10^8cfu/µg	1ml (5 × 200µl)	L2001
Single-Use JM109 Competent Cells, >10^8cfu/µg	1ml (20 × 50µl)	L2005
JM109 Competent Cells, >10^7cfu/µg	1ml (5 × 200µl)	L1001
HB101 Competent Cells, >10^8cfu/µg	1ml (5 × 200µl)	L2011
Single-Use HB101 CompetentCells, >10^8cfu/µg	1ml (20 × 50µl)	L2015
BL21(DE3)pLysS Competent Cells, >10^6cfu/µg	1ml (5 × 200µl)	L1191
Single-Use BL21(DE3)pLysS Competent Cells, >10^6cfu/µg	1ml (20 × 50µl)	L1195
Single-Use Pro 5-alpha Competent Cells, >10^9cfu/µg	1ml (20 × 50µl)	L1221

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

Competent cells, supplied in 200µl aliquots, are provided with 3ng of competent cells control DNA for use as a positive control. Typically, 100µl of competent cells are required for a standard transformation. Competent cells supplied in 50µl aliquots do not include the control DNA. For the competent cells supplied in 50µl aliquots, transformation can be completed in the tube in which the cells are supplied.

3. Standard Transformation Protocol

Materials to Be Supplied by the User
(Solution compositions are provided in Section 6.)

- 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 chilled culture tube.

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.

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

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, and incubate for 60 minutes at 37°C with shaking (approximately 225rpm).

   Note: Use high-quality deionized water (e.g., Milli-Q® or NANOpure®) for SOC medium (see recipe in Section 6). If LB or other medium is used, transformation efficiencies will be reduced.

9. For each transformation reaction, we recommend diluting the cells 1:10 and 1:100 and plating 100µl of undiluted cells and 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, 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. Standard Transformation Protocol (continued)

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 note at Step 8).

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.

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

5. Solutions and media containing **tetracycline** must be stored protected from light to maintain potency.

4. Single-Use Competent Cells Standard Transformation Protocol

Materials to Be Supplied by the User
(Solution compositions are provided in Section 6.)

- LB or SOC medium
- LB plates with antibiotic appropriate for the plasmid
- IPTG (Cat.# V3955; optional, see Note 4)
- X-Gal (Cat.# 3941; optional, see Note 4)

1. Remove competent cells from –70˚C and place on ice for 5 minutes or until just thawed.

2. Add 1–50ng of DNA (in a volume not greater than 5µl) to the Competent Cells. Move the pipette tip through the cells while dispensing. Quickly flick the tube several times. Do not vortex!

   Note: To determine transformation efficiency, we recommend using 2µl of supercoiled plasmid DNA (e.g., pGEM®-3Z Vector, Cat.# P2151) diluted to 5pg/µl in TE buffer. See Section 5 for more information.

3. Immediately return the tubes to ice for 5–30 minutes.

4. Heat-shock cells for 15–20 seconds in a water bath at exactly 42˚C. Do not shake.

5. Immediately place the tubes on ice for 2 minutes.

6. Add 450µl of room-temperature SOC medium to each transformation reaction, and incubate for 60 minutes at 37˚C with shaking (approximately 225rpm). For best transformation efficiency, lay the tubes on their sides and tape them to the platform.

7. For each transformation reaction, we recommend plating 100µl of undiluted cells and 1:10 and 1:100 cell dilutions on antibiotic plates (see Notes 1–3). Incubate the plates at 37˚C overnight.

Notes:

1. For control transformations using supercoiled plasmid DNA (e.g., pGEM®-3Z Vector, Cat.# P2151), we recommend diluting transformed cells 1:4 before plating 100µl on LB/ampicillin plates.

2. If more colonies are desired, pellet the cells by centrifugation at 1,000 × *g* for 10 minutes, then resuspend in 200µl of SOC or LB medium and plate.

3. Use high-quality deionized water (e.g., Milli-Q® or NANOpure® water) for SOC medium (see Section 6). If LB or other medium is used, transformation efficiency will be reduced.

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.

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

5. Solutions and media containing **tetracycline** must be stored protected from light to maintain potency.

5. 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.

Notes:

1. The Competent Cells Control DNA (pGEM®-3Z Vector) is supplied at a concentration of 0.1ng/µl in TE buffer.
2. Transformation with ligated plasmid DNA will produce fewer colonies than transformation with supercoiled plasmid DNA.

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

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

6. 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 $MgCl_2 \cdot 6H_2O$
123.3g $MgSO_4 \cdot 7H_2O$

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 $MgCl_2 \cdot 6H_2O$, 1M $MgSO_4 \cdot 7H_2O$), 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 of 20mM. Filter the complete medium through a 0.2µm filter unit. The pH should be 7.0.

7. 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**, 103–19.
3. Lacks, S. and Greenberg, B. (1977) Complementary specificity of restriction endonucleases of *Diplococcus pneumoniae* with respect to DNA methylation. *J. Mol. Biol.* **114**, 153–68.
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**, 113–30.
5. Davanloo, P. *et al.* (1984) Cloning and expression of the gene for bacteriophage T7 RNA polymerase. *Proc. Natl. Acad. Sci. USA* **81**, 2035–9.

8. Related Products

Competent Cells

Product	Size	Cat.#
Single Step (KRX) Competent Cells	20 × 50µl	L3002

Bacterial Strains (not competent cells)

Product	Size	Cat.#
Bacterial Strain JM109, Glycerol Stock	500µl	P9751
Bacterial Strain JM109(DE3), Glycerol Stock	500µl	P9801

[a]**Usage Restrictions for the T7 Expression System**

The T7 expression system is based on technology developed at Brookhaven National Laboratory under contract with the U.S. Department of Energy and is the subject of patents assigned to Brookhaven Science Associates, LLC (BSA). This technology, including bacteria, phage and plasmids that carry the gene for T7 RNA polymerase, is to be used for academic or nonprofit laboratory or licensed commercial research purposes only. By accepting or using the T7 expression technology you agree to be bound by the following conditions set forth by BSA. The initial purchaser may refuse to accept the conditions of this notice by returning this product and the enclosed materials to Promega unused.

Academic and NonProfit Laboratories

No materials that contain the cloned gene for T7 RNA polymerase may be distributed further to third parties outside of your laboratory unless the recipient receives a copy of this assurance notice and agrees to be bound by its terms. This limitation applies to Bacterial Strains JM109(DE3), BL21(DE3)pLysS and KRX and to any derivatives thereof.

Commercial Laboratories

A license is required for any commercial use of the T7 expression system, including use of the T7 system for research purposes or for production purposes by any commercial entity. Information about commercial licenses may be obtained from the Licensing Office, Brookhaven National Laboratory, Upton, NY 11973, Telephone: 631-344-7134, FAX: 631-344-3729.