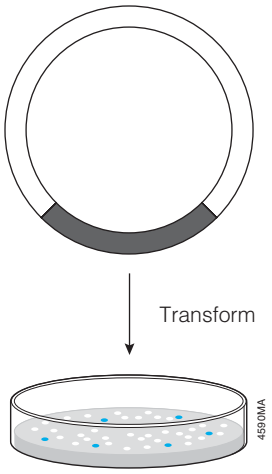
Transforming Bacteria

Properties of E. coli Strains for Subcloning

Common laboratory strains of *E. coli,* like JM109, DH5α™, and XL-1 Blue, are different from their wildtype counterparts. These strains carry some mutations designed to help you propagate plasmids. Typically laboratory strains have a mutation in the *rec*A gene (*rec*A1), a gene involved in recombination. The mutant gene limits recombination of the plasmid with the *E. coli* genome so that the plasmid inserts are more stable (the *rec*A1 mutation is more effective than the *rec*A13 mutation). Each of these strains also carries the *end*A1 mutation that inactivates a nuclease that might copurify with plasmids during purification. This mutation helps you to purify higher quality plasmids. Special treatments must be performed on plasmids from strains that do not have this mutation (e.g., RR1, HB101, etc.) to eliminate the nuclease from the plasmid prep (e.g., the Alkaline Protease digestion in the Wizard® *Plus* SV Miniprep protocol).

Common laboratory strains of *E. coli* are typically defined as K strains or B strains based on the presence of the restriction and modification system that functions around *Eco*K I or *Eco*B I, respectively. In a wildtype K strain, the *E. coli* will have both the *Eco*K I restriction enzyme to cleave foreign DNA and *Eco*K I methylase to protect and mask host DNA recognition sequences. In B strains, the *Eco*B I restriction enzyme and methylase serve the same purpose. Strains like JM109, DH5α™ and XL-1 Blue are K strains but carry the *hsd*R17 (r_{K^-}, m_{K^+}) mutation. This mutation knocks out the *Eco*K I restriction enzyme but leaves the methylase intact. Therefore, these strains will not degrade plasmid DNA isolated from a B or K strain but will methylate it. This is useful if the DNA must be transferred to a K strain with an intact K restriction and methylation system.

If you wish to incorporate blue/white selection into your subcloning scheme, you need to transform *E. coli* carrying a *lac*ZΔ. This mutation deletes a portion of the β-galactosidase gene leaving what is termed the ω-fragment. The plasmid vector supplies this deleted portion, or α-fragment. Once inside the bacterium, the plasmid produces the α-fragment and the *E. coli* produces the ω-fragment, which combine to make a



functional β-galactosidase. If grown on plate containing 5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside (X-gal), the colony will turn blue as a result of β-galactosidase activity and indicate full complementation of the bacterium by the plasmid. This is termed α-complementation. Blue/White cloning methods use plasmids with a multiple cloning region within the coding sequence of the α-fragment. Disruption of the reading frame due to the presence of the insert will produce a non-functional α-fragment incapable of α-complementation. These disrupted plasmids are differentiated from the plasmids without insert by the color of the colony (white versus blue), hence the term blue/white selection. Strains like JM109, DH5α™ and XL-1 Blue have the necessary deletion. One difference between these strains lies in how you get the bacterium to produce the ω-fragment. Both JM109 and XL-1 Blue have a second mutation call *lac I*^{q}. This mutation leads to increased production of the *lacI* repressor that stops transcription from the *lac* operon until substrate is present. To relieve this repression, these strains are grown on media containing the non-cleavable lactose analog, isopropyl-β-D-thiogalactopyranoside (IPTG). DH5α™ does not have the *lac I*^{q} mutation and constantly produces a low level of the ω-fragment through leaky transcription of the *lac* operon and therefore does not require IPTG for blue/white selection.

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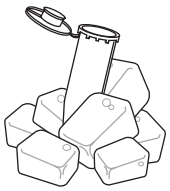
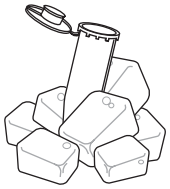
Ready-to-Use Competent Cells



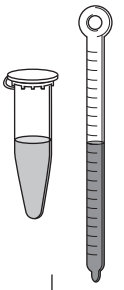
Thaw on crushed ice.

Add 1–50ng of DNA from ligation reacton.

Use in blue/white selection cloning procedures!

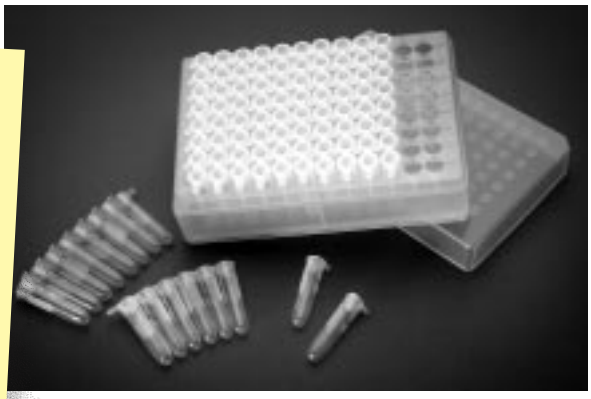


Incubate on crushed ice for 30 minutes.

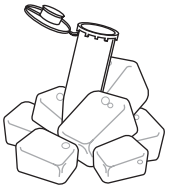


Heat shock at 42°C for 30 seconds.

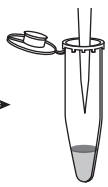
Single-use aliquots! Use one-at-a-time or all 96 at one time.



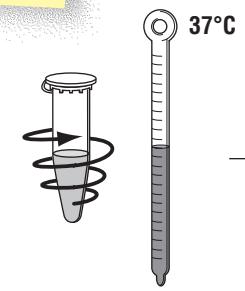
3722CA05_2A



Place on crushed ice for 2 minutes.



Add 250µl room temperature SOC Media.



Incubate at 37°C; with shaking (225–250rpm) for 60 minutes.

4591MA

Spread 100µl per plate. Incubate at 37°C overnight.

Select96™ Competent Cells

Select96™ Competent *E. coli* cells are single-use aliquots of high competency cells provided in 12 eight-strip tubes ready for use. Simply snip off the number of transformations you need, thaw on ice and proceed. Handle all steps in the tubes from beginning to end. No need to transfer to other tubes. Cells are guaranteed to provide at least 1 × 10^8 cfu/µg of supercoiled DNA. The cells are the *rec*A1, *end*A1, and *lac*ZΔM15. Blue/White selection does not require IPTG induction. For more information on genotype, see the table on page 64.

Select96™ Competent Cells
Cat.# L3300 1 x 96 reactions

Full protocol available at:
www.promega.com/tbs/tb301/tb301.html

Transforming Bacteria

Ready-to-Use Competent Cells

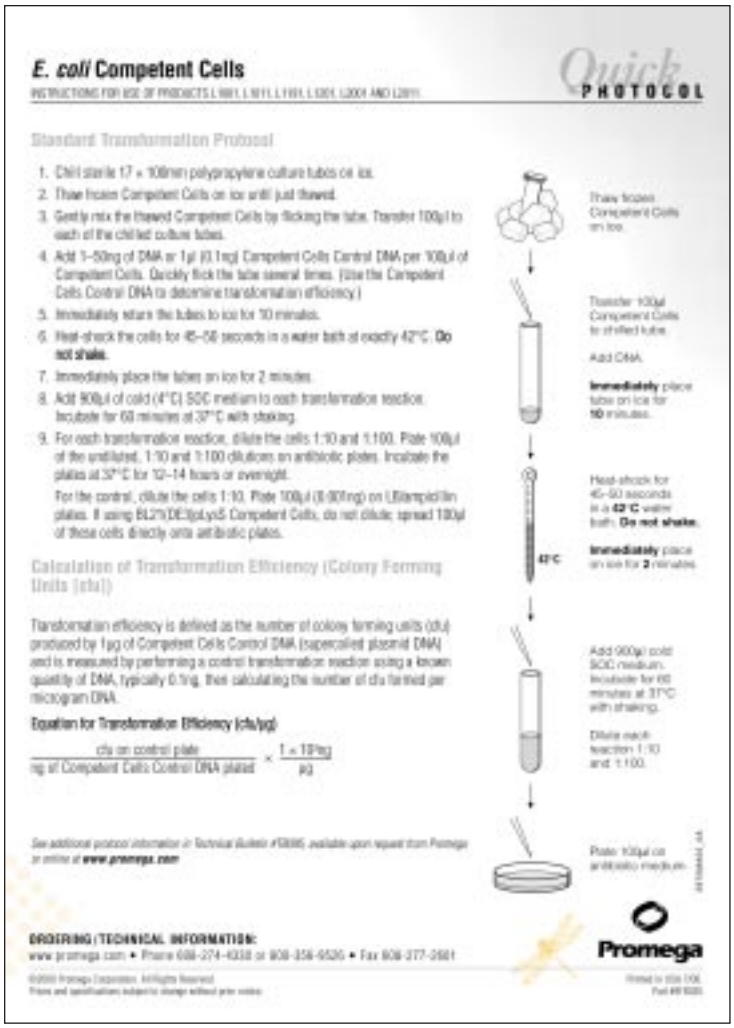
JM109 Competent Cells, >10^8 cfu/µg
Cat.# L2001 5 x 200µl

JM109 Competent Cells, >10^7 cfu/µg
Cat.# L1001 5 x 200µl

Protocol available at:
www.promega.com/tbs/tb095/tb095.html

JM109 Competent Cells

Available in two efficiencies: High Efficiency at greater than 10^8cfu/µg and Subcloning Efficiency at greater than 10^7cfu/µg. JM109 cells are an ideal host for many molecular biology applications including standard subcloning that requires blue/white screening, scale-up for large plasmid preparations and routine minipreps. The cells are *rec*A1, *end*A1, and *lac*Z∆*M*15.



***E. coli* Competent Cells**

Quick PROTOCOL

Standard Transformation Protocol

1. Chill sterile 17 x 100mm polypropylene culture tubes on ice.
2. Thaw frozen Competent Cells on ice until just thawed.
3. Gently mix the thawed Competent Cells by flicking the tube. Transfer 100µl to each of the chilled culture tubes.
4. Add 1–50ng of DNA or 1µl (0.1ng) Competent Cells Control DNA per 100µl of Competent Cells. Quickly flick the tube several times. (Use the 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 or overnight.

   For the control, dilute the cells 1:10. Plate 100µl (0.001ng) on LB/ampicillin plates. If using B121(DE3)pLysS Competent Cells, do not dilute; spread 100µl of these cells directly onto antibiotic plates.

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.

Equation for Transformation Efficiency (cfu/µg)

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

See additional protocol information in Technical Bulletin #TB095, available upon request from Promega or online at www.promega.com

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Transforming Bacteria

Making Your Own Competent Cells

Preparation of Competent Cells: Modified RbCl Method

This rubidium chloride protocol gives better transformation efficiencies than the $CaCl_2$ procedure for most strains. The procedure is an adaptation of one described in Hanahan, D. (1985) In: *DNA Cloning*, Volume 1, D. Glover, ed., IRL Press, Ltd., London, 109.

Materials to Be Supplied by the User
(Solution compositions are provided on page 48.)

- LB medium and plates
- LB + 20mM $MgSO_4$
- TFB1, ice-cold
- TFB2, ice-cold
- dry ice/isopropanol bath

1. Inoculate a single colony from an LB plate (for JM109, use M9 + B1 plate so that F′ episome is maintained) into 2.5ml of LB medium in a plating tube. Incubate overnight at 37°C with shaking (approximately 225rpm).
2. Subculture the overnight culture at a 1:100 dilution by inoculating 2.5ml into 250ml of LB supplemented with 20mM $MgSO_4$. Grow the cells in a 1L flask until the OD_{600} reaches 0.4–0.6 (usually 5–6 hours, but the time may vary).
3. Pellet the cells by centrifugation at 4,500 × *g* for 5 minutes at 4°C. For a 250ml culture, use two 250ml centrifuge bottles in a large rotor.
4. Gently resuspend the cell pellet in 0.4 original volume of ice-cold TFB1. For a 250ml subculture, use 100ml of TFB1 (50ml/bottle). Combine the resuspended cells in one bottle. For the remaining steps, keep the cells on ice and chill all pipettes, tubes and flasks.
5. Incubate the resuspended cells on ice for 5 minutes at 4°C.
6. Pellet the cells by centrifugation at 4,500 × *g* for 5 minutes at 4°C.
7. Gently resuspend the cells in 1/25 original volume of ice-cold TFB2. For a 250ml subculture, use 10ml of TFB2.
8. Incubate the cells on ice for 15–60 minutes, then dispense 100µl/tube for storage at –70°C. Quick-freeze the tubes in a dry ice/isopropanol bath. JM109 competent cells prepared by this method are stable for 1 year.

Many *E. coli* strains carry episomes (e.g., F′ and P2) expanding the capabilities of the bacterium for use in subcloning applications. For example, the XL1-Blue and JM109 strains carry the *lacI*^{q}ΔM15 mutation on the F′ episome. The episomes are extrachromosomal, replicating plasmids with a selectable marker (page 64). When making competent cells from strains with episomes, the bacteria must first be plated on selective media. For XL1-Blue, colonies are selected on tetracycline plate since the episome contains the Tet^R gene. Due to this, however, the strain cannot be used with subcloning plasmids containing the Tet^R gene for selection. JM109 cells should be selected first on M9 minimal media containing thiamine (vitamin B1). The bacterial chromosome lacks the biosynthetic genes for proline synthesis (*proAB*) but the episome carries those genes. Colonies grown on the M9 + B1 plates (recipe on page 48) can then be processed into competent cells ready for blue/white selection.

Note: Competent cells may be conveniently quick-frozen using ice bath racks, which have an ice compartment bottom and a removable rack (American Scientific Products, Cat.# S9233-1). Set up an ice bath in one rack and an ethanol bath in another. Place the top-labeled tubes in the rack with ice, dispense 100µl cells per tube, then close the tubes. Add the dry ice to the ethanol bath, wait for it to stop bubbling, then transfer the rack and tubes to the dry ice bath for about 15 seconds. Drain the ethanol, wipe with a tissue, and transfer to an empty bottom compartment and place in a –70°C freezer. Do not get alcohol on the lips of the tubes. Liquid nitrogen also can be used for quick-freezing, but not with these racks. Use only plasticware designed for liquid nitrogen.

Note: Be careful not to get alcohol on the labels because it will remove them.

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Determining Transformation Efficiency of Competent Cells

This is a general protocol for use with the procedure for producing competent cells that is provided on page 46. Please follow manufacturers' instructions when using purchased competent cells.

1. Thaw a 100µl aliquot of competent cells on ice.
2. Transfer 100µl of the cells to a 17 × 100mm polypropylene tube prechilled on ice.
3. Add 0.1ng of a supercoiled plasmid [e.g., pGEM®-3Zf(+) Vector] in a 10µl volume to the competent cells and gently mix by swirling the pipet tip (do not mix by pipetting).
4. Transfer the tubes from ice to a 42°C water bath and heat shock for 45–60 seconds. Place on ice immediately to cool for 2 minutes.
5. Add 890µl of SOC medium (giving a concentration of 0.1ng DNA/ml) and incubate for 45 minutes at 37°C with shaking (~150rpm).
6. Transfer 100µl of cells to 900µl of SOC medium (0.01ng DNA/ml) and plate 100µl of this (0.001ng/100µl) onto LB plates with the appropriate antibiotic. You may wish to plate 100µl of undiluted cells for determining efficiency as well. The 100µl aliquot will contain 0.01ng DNA.
7. Incubate the plates overnight in a 37°C incubator and count the number of colonies obtained. For example, if 200 colonies were obtained:

$$\frac{200\text{cfu}}{0.001\text{ng}} = 2 \times 10^5\text{cfu/ng} = 2 \times 10^8\text{cfu/µg DNA}$$

Competencies below 10^6 may not be useful for subcloning applications.

Transforming Ligation Reactions

This is a general protocol for use with the procedure for producing competent cells that is provided on page 46. Please follow manufacturers' instructions when using purchased competent cells.

1. Thaw a 100µl aliquot of competent cells on ice.
2. Transfer 100µl aliquot of the competent cells to a 17 × 100mm polypropylene tube prechilled on ice.
3. Add no more than 10ng of DNA in a maximum of 10µl from a ligation reaction to the cells and gently swirling the pipet tip (do not mix by pipetting). Incubate on ice for 30 minutes.
4. Transfer the tubes from ice to a 42°C water bath and heat-shock for 45–60 seconds. Place on ice immediately to cool for 2 minutes.
5. Add 1ml of LB or SOC medium and incubate for 45 minutes at 37°C with shaking (~150rpm).
6. Plate 100–200µl of the transformation mix, or an appropriate dilution onto selection plates. If you suspect low ligation efficiency, take the remaining cells and pellet by a quick 10–20 second spin in a microcentrifuge. Pour off media and resuspend pellet in about 200µl of SOC and plate.

Transformation Controls

Controls help you figure out where things may have gone wrong with the subcloning procedure. When transforming bacteria with your subcloning reaction DNA, also determine transformation and efficiency.

Transforming a ligation control (see page 26) of cut, dephosphorylated vector without insert can tell you how many background colonies you can expect in your actual vector + insert ligation.

Transforming more than 10ng of DNA from a ligation reaction may actually decrease transformation efficiency.

Transforming Bacteria

Media and Solutions

LB (Luria-Bertani) medium (1 liter)

10g tryptone
5g yeast extract
5g NaCl

Adjust pH to 7.5 with NaOH and autoclave.

LB plates (1 liter)

Add 15g agar to 1 liter of LB medium. Adjust to pH 7.5 with NaOH. Autoclave. Pour 30–35ml of medium into 85mm petri dishes. If necessary, flame the surface of the medium with a Bunsen burner to remove bubbles.

LB Medium

Allow the medium to cool to 55°C before adding antibiotic (either ampicillin, 125µg/ml final concentration; tetracycline, 12.5µg/ml final concentration; or chloramphenicol, 20µg/ml final concentration).

LB plates plus antibiotic (1 liter)

Add 15g agar to 1 liter of LB medium. Adjust to pH 7.5 with NaOH. Autoclave. Pour 30–35ml of medium into 85mm petri dishes. If necessary, flame the surface of the medium with a Bunsen burner to remove bubbles.

SOC medium

2.0g tryptone
0.5g 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

Add tryptone, 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. Adjust to pH 7.0. Filter the complete medium through a 0.2µm filter unit.

M9 + B1 plates

6g Na_2HPO_4
3g KH_2PO_4
0.5g NaCl
1g NH_4Cl
15g Agar

Add deionized water to approximately 1L. Adjust to pH 7.4 with 10N NaOH. Autoclave and cool to 50°C. Then add the following sterile solutions:

2.0ml 1M $MgSO_4$
0.1ml 1M $CaCl_2$
10.0ml 20% Glucose (Filter Sterilized)
1.0ml 1M Thiamine-KCl (Filter Sterilized)

Pour 30–35ml of medium into 85mm petri dishes.

Bacterial Plates for Blue/White Selection.

Ampicillin Stock Solution

Dissolve at 50mg/ml in water, filter sterilize, store in aliquots at –20°C.

IPTG stock solution (0.1M)

1.2g IPTG (Cat.# V3951)

Add water to 50ml final volume. Filter-sterilize and store at 4°C.

X-Gal (2ml)

100mg X-gal (Cat.# V3941)

Dissolved at 50mg/ml in N,N′-dimethyl-formamide.
Cover with aluminum foil and store at –20°C.

LB plates with ampicillin/IPTG/X-Gal

Add 15g agar to 1 liter of LB medium. Autoclave. Allow the medium to cool to 50°C before adding ampicillin to a final concentration of 100µg/ml then supplement with 0.5mM IPTG and 80µg/ml X-Gal and pour the plates. Pour 30–35ml of medium into 85mm petri dishes. Let the agar harden. Store at 4°C for up to 1 month or at room temperature for up to 1 week.

TFB1

30mM potassium acetate
10mM $CaCl_2$
50mM $MnCl_2$
100mM RbCl
15% glycerol

Adjust pH to 5.8 with 1M acetic acid. Be very careful as you approach 5.8; if the pH drops lower than 5.8, a black precipitate may form. Filter sterilize (0.2µM) and store at room temperature.

TFB2

100mM MOPS or PIPES (pH 6.5)
75mM $CaCl_2$
10mM RbCl
15% glycerol

Adjust the pH to 6.5 with 1M KOH. Filter sterilize (0.2µM) and store at room temperature.