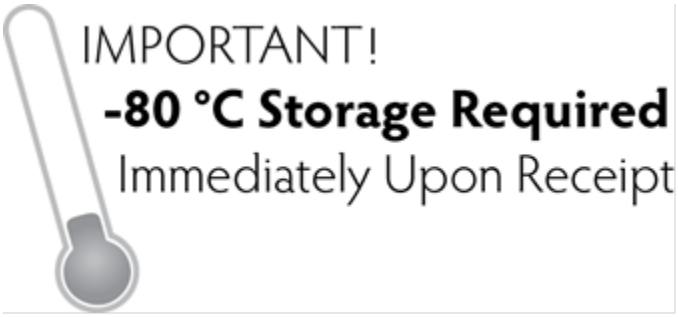
Lucigen®
Simplifying Genomics

E. cloni® 10G
Chemically Competent Cells



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Technical Support

Lucigen is dedicated to the success and satisfaction of our customers. Our products are tested to assure they perform as specified when used according to our recommendations. It is imperative that the reagents supplied by the user are of the highest quality. Please follow the instructions carefully and contact our technical service representatives if additional information is necessary. We encourage you to contact us with your comments regarding the performance of our products in your applications. Thank you.

Lucigen Technical Support

Email: techserv@lucigen.com

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Product Guarantee: Lucigen guarantees that this product will perform as specified for one year from the date of receipt. Please avoid using reagents for more than one year after receipt.

Components & Storage Conditions

Lucigen *E. cloni* 10G Chemically Competent Cells yield either $\geq$ 1 x 10^9 cfu/µg pUC19 ("standard" cells) or 1 x 10^6 cfu/µg pUC19 (Subcloning Grade). The cells are shipped on dry ice, with supercoiled control pUC19 DNA at 10 pg/µL, and Recovery Medium. *E. cloni* 10G Chemically Competent Cells are available in 40 µL aliquots (SOLOs), sufficient for one transformation per tube; 80 µL aliquots (DUOs), sufficient for two transformations per tube; 480 µL aliquots (Subcloning Grade), sufficient for 12 transformations per tube. Please refer to the table below for catalog numbers.

All competent cells require storage at -80 °C

E. cloni ® Chemically Competent Cells:

STRAIN	Efficiency (cfu/µg pUC19)	Transformations	Catalog #	Storage
E. cloni 10G Chemically Competent DUOs (Yellow cap)	$\geq$ 1 x 10^9	4 (2 x 80 µL) 12 (6 x 80 µL) 24 (12 x 80 µL) 48 (24 x 80 µL) 96 (48 x 80 µL)	60107-0 60107-1 60107-2 60107-3 60107-4	**-80 °C**
E. cloni 10G Chemically Competent SOLOs (Yellow cap)		12 (12 x 40 µL) 24 (24 X 40 µL) 48 (48 x 40 µL)	60106-1 60106-2 60106-3	
E. cloni 10G Chemically Competent Subcloning Grade (Yellow cap)	$\geq$ 1 x 10^6	48 (4 x 480 µL) 96 (8 x 480 µL)	60108-1 60108-2	**-80 °C**
Recovery Medium	----	4 (4 x 1 mL) 12 (1 x 12 mL) 24 (2 x 12 mL) 48 (4 x 12 mL) 96 (8 x 12 mL)	---- ---- ---- ---- 80026-1	**-20 to -80 °C**
Supercoiled pUC19 DNA (10 pg µL)	----	1 x 20 µL	----	**-20 to -80 °C**

E. cloni 10G & 10GF´ Chemically Competent Cells

E. cloni 10G Chemically Competent Cells are a derivative of *E. coli* that have been optimized for high efficiency transformation by heat shock. These cells are ideal for cloning and propagation of plasmid, cosmid, or fosmid clones. They can directly replace commonly used cloning strains, such as DH10B.

E. cloni 10G contain inactive *mcr* and *mrr* alleles, allowing methylated genomic DNA isolated directly from mammalian or plant cells to be cloned without deletions or rearrangements. They give high yield and high quality plasmid DNA due to the *end*A1 and *rec*A1 mutations. The *rps*L mutation confers resistance to streptomycin.

Genotypes:

***E. cloni* 10G:**

F⁻ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) *end*A1 *rec*A1 ϕ80d*lac*ZΔM15 Δ*lac*X74 *ara*D139 Δ(*ara,leu*)7697 *gal*U *gal*K *rps*L (Str^R) *nup*G λ– *ton*A

Cell Line	Supports cloning methylated DNA	Contains F- plasmid	Suitable for Blue/White Screening	IPTG Required for Blue/White Screening
E. cloni 10G	Yes	No	Yes	No

Transformation Control

As a control for transformation, *E. cloni* Chemically Competent Cells are provided with supercoiled pUC19 DNA at a concentration of 10 pg/µL. Use 1 µL of pUC19 for transformation. Plate transformants on plates containing ampicillin or carbenicillin.

Preparation for Transformation

To ensure successful transformation results, the following precautions must be taken:

- For best results, Lucigen CloneSmart® ligation reactions must be heat inactivated at 70 ºC for 15 minutes before transformation. Alternately, the reactions may be purified. For other ligation reactions, follow the manufacturer's recommendations.
- Prepare nutrient agar plus antibiotic for selection. We suggest using LB-Lennox agar to achieve optimal cell growth.
- All microcentrifuge tubes must be thoroughly pre-chilled on ice before use.
- The cells must be completely thawed **on ice** before use.
- For highest transformation efficiency, use the provided Recovery Medium to resuspend the cells after transformation.
- Perform the heat shock in a **15 mL disposable polypropylene culture tube** (17 x 100 mm). The use of other types of tubes may dramatically reduce transformation efficiency.

Transformation Protocol for *E. cloni* 10G Cells

1. Prepare nutrient agar plates (LB-Lennox) with antibiotic for selection. Ensure that Recovery Medium is readily available at room temperature.

2. Chill sterile culture tubes on ice (17 mm x 100 mm tubes, one tube for each transformation reaction).

3. Remove *E. cloni* cells from the -80 °C freezer and thaw completely on wet ice (5-15 minutes).

4. Add 40 µL of *E. cloni* cells to the chilled culture tube.
5. Add 1-4 µL of DNA sample to the 40 µL of cells. Stir briefly with a pipet tip; **do not** pipet up and down to mix, which can introduce air bubbles and warm the cells. For the pUC19 control, add 1 µL (10 pg) of DNA to another chilled culture tube containing 40 µL of cells.

   Note: Lucigen CloneSmart® ligation reactions must be heat-inactivated or purified. For other ligation reactions, follow the manufacturer's recommendations.
6. Incubate the cell/DNA mixture on ice for 30 minutes.
7. Heat shock cells by placing the culture tubes in a 42 °C water bath for 45 seconds.

   Performing the heat shock in the 1.7 mL tube in which the cells are provided will significantly reduce the transformation efficiency.
8. Return the culture tubes to ice for 2 minutes.
9. Add 960 µL of room temperature Recovery Medium to the cells in the culture tube. When using these cells with a cloning kit, follow the Recovery Medium volume given in that kit manual.
10. Place the tubes in a shaking incubator at 250 rpm for 1 hour at 37 °C.
11. Plate up to 200 µL of the transformation on LB-Lennox or plates containing the appropriate antibiotic. The plating volume may need to be optimized depending on your DNA. For the pUC19 control, plate 25 µL of 10G cells LB-Lennox agar plates containing 100 µg/mL carbenicillin or ampicillin. Transformants plated on LB-Miller may grow slowly.
12. Incubate the plates overnight at 37 °C.
13. Transformed clones can be further grown in any rich culture medium (e.g. LB or TB).

Transformation Protocol for *E. cloni* 10G Subcloning Grade Cells

1. Prepare nutrient agar plates (LB-Lennox) with antibiotic for selection. Ensure that Recovery Medium is readily available at room temperature.
2. Chill sterile culture tubes on ice (one tube for each transformation reaction).
3. Remove *E. cloni* Subcloning Grade cells from the -80 °C freezer and thaw completely on wet ice (5-15 minutes).
4. Add 40 µL of cells to each chilled culture tube.
5. Add 1-4 µL of DNA to 40 µL of cells. Stir briefly with a pipet tip; do not pipet up and down to mix, which can introduce air bubbles and warm the cells. For the pUC19 control, add 1 µL (10 pg) of DNA to another chilled culture tube containing 40 µL of cells.

   Note: Lucigen CloneSmart®ligation reactions must be heat-inactivated or purified. For other ligation reactions, follow the manufacturer's recommendations.
6. Incubate the cell/DNA mixture on ice for 30 minutes.
7. Heat shock cells by placing the culture tubes in a 42 °C water bath for 45 seconds.

   Performing the heat shock in the 1.7 mL tube in which the cells are provided will significantly reduce the transformation efficiency.
8. Return the cells to ice for 2 minutes.
9. Add 960 µL of room temperature Recovery Medium to the cells in the culture tube.

10. Place the tubes in a shaking incubator at 250 rpm for 1 hour at 37 °C.
11. Plate up to 200 µL of the transformation on LB-Lennox agar plates containing the appropriate antibiotic. The plating volume may need to be optimized depending on your DNA. For the pUC19 control, plate 100 µL of cells on LB-Lennox or plates containing 100 µg/mL carbenicillin or ampicillin. Transformants plated on LB-Miller may grow slowly.
12. Incubate the plates overnight at 37 °C.
13. Transformed clones can be further grown in rich culture medium (e.g. LB or TB).

Calculating Transformation Efficiency

Use the following formula to calculate the transformation efficiency as the number of transformants (in cfu) per µg of plasmid DNA.

$$\frac{\text{\# cfu}}{\text{pg pUC 19 DNA}} \times \frac{10^6 \text{ pg}}{\text{µg}} \times \frac{\text{volume of transformants}}{\text{X µL plated}} \times \text{dilution factor} = \text{\# cfu/ µg pUC19}$$

For example, if 10 pg pUC19 yields 10 colonies when 100 µL of a 1mL transformation is plated, then:

$$\frac{10 \text{ cfu}}{10 \text{ pg pUC19}} \times \frac{1 \times 10^6 \text{ pg}}{\text{µg}} \times \frac{1000 \text{ µL}}{100 \text{ µL}} = 1.0 \times 10^7 \text{ cfu / µg pUC19}$$

Media Recipes

LB Lennox Agar Plates
Per liter:
- 10 g tryptone
- 5 g yeast extract
- 5 g NaCl
- 15 g agar

Medium for Growth of Transformants

LB Miller
Per liter:
- 10 g tryptone
- 5 g yeast extract
- 10 g NaCl

Add all components to deionized water. Adjust pH to 7.0 with NaOH. Autoclave and cool to 55 °C.

TB
Per liter:
- 11.8 g tryptone
- 23.6 g yeast extract
- 9.4 g dipotassium hydrogen phosphate (anhydrous)
- 2.2 g potassium dihydrogen phosphate (anhydrous)
- 0.4% glycerol

Add all components to deionized water. Adjust pH to 7.0 with NaOH. Autoclave and cool to 55 °C.