

**Please note:** The concentration of the included pUC19 has been changed from 1ng/μl to 10pg/μl. Do not dilute the plasmid before performing the transformation positive control. Please contact Lucigen if you have any questions.

# ***E. coli*<sup>®</sup> 10G & 10GF' Chemically Competent Cells**

**IMPORTANT!  
-80°C Storage Required  
Immediately Upon Receipt**

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# *E. cloni*<sup>®</sup> 10G & 10GF' Chemically Competent Cells

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# *E. coli*<sup>®</sup> 10G & 10GF' Chemically Competent Cells

## Components & Storage Conditions

Lucigen's *E. coli* 10G Chemically Competent Cells yield either  $\geq 1 \times 10^9$  cfu/ $\mu$ g ("standard" cells) or  $1 \times 10^6$  cfu/ $\mu$ g pUC19 (subcloning grade). *E. coli* 10GF' Chemically Competent Cells yield  $\geq 5 \times 10^8$  cfu/ $\mu$ g pUC19. The cells are shipped on dry ice, with supercoiled control pUC19 DNA at 10 pg/ $\mu$ l, and Recovery Medium. *E. coli* 10G Chemically Competent Cells are available in 40- $\mu$ l aliquots (SOLOs), sufficient for one transformation per tube; 80- $\mu$ l aliquots (DUOs), sufficient for two transformations per tube; 480- $\mu$ l aliquots (Subcloning Grade), sufficient for 12 transformations per tube. *E. coli* 10GF' Chemically Competent Cells are available in DUO packaging only. Please refer to the table below for catalog numbers.

### *E. coli*<sup>®</sup> Chemically Competent Cells: Store at **-80° C**

STRAIN	Efficiency (cfu/ $\mu$ g pUC19)	Transformations	Catalog #	Storage
<i>E. coli</i> 10G Chemically Competent DUOs	$\geq 1 \times 10^9$	4 (2 x 80 $\mu$ l)	60107-0	<b>-80°C</b>
		12 (6 x 80 $\mu$ l)	60107-1	
		24 (12 x 80 $\mu$ l)	60107-2	
		48 (24 x 80 $\mu$ l)	60107-3	
		96 (48 x 80 $\mu$ l)	60107-4	
		<i>E. coli</i> 10G Chemically Competent SOLOs	$\geq 1 \times 10^9$	
24 (24 X 40 $\mu$ l)	60106-2			
48 (48 x 40 $\mu$ l)	60106-3			
<i>E. coli</i> 10G Chemically Competent Subcloning Grade	$\geq 1 \times 10^6$	48 (4 x 480 $\mu$ l)	60108-1	<b>-80°C</b>
		96 (8 x 480 $\mu$ l)	60108-2	
<i>E. coli</i> 10GF' Chemically Competent DUOs	$\geq 5 \times 10^8$	12 (6 x 80 $\mu$ l)	60062-1	<b>-80°C</b>
		24 (12 x 80 $\mu$ l)	60062-2	
Recovery Medium		4 (4 x 1 mls)	----	<b>-20 to -80°C</b>
		12 (1 x 12 mls)	----	
		24 (2 x 12 mls)	----	
		48 (4 x 12 mls)	----	
		96 (8 x 12 mls)	80026-1	
Supercoiled pUC19 DNA (10 pg/ $\mu$ l)		1 x 20 $\mu$ l	----	<b>-20 to -80°C</b>

# *E. cloni*<sup>®</sup> 10G & 10GF' Chemically Competent Cells

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## *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 like DH10B.

The 10GF' strain has the same chromosomal genotype as 10G, but harbors the F' plasmid, which allows production of single-stranded DNA by infection with phage M13 and carries the *lacIq* allele to control expression of the *lacZ* promoter.

The strains *E. cloni* 10G and 10GF' 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 *endA1* and *recA1* mutations. The *rpsL* mutation confers resistance to streptomycin.

### Genotypes:

#### *E. cloni* 10G Genotype:

F<sup>-</sup> *mcrA* Δ(*mrr-hsdRMS-mcrBC*) *endA1 recA1* φ80d*lacZ*ΔM15 Δ*lacX74 araD139* Δ(*ara,leu*)7697 *galJ galK rpsL nupGλ tonA*

#### *E. cloni* 10GF' Genotype:

[F' *proA+B+ lacI<sup>q</sup>Z*ΔM15::Tn10 (TetR)] / *mcrA* Δ(*mrr-hsdRMS-mcrBC*) *endA1 recA1* φ80d*lacZ*ΔM15 Δ*lacX74 araD139* Δ(*ara,leu*)7697 *galJ galK rpsL nupGλ tonA*

### 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 for transformation. Plate pUC19 transformants on plates containing ampicillin or carbenicillin.

## Preparation for Transformation

*E. cloni* Chemically Competent Cells are provided in aliquots of 40 μl (one transformation), 80 μl (two transformations), or 480 μl (12 transformations). Transformation is performed by heat shock at 42°C, followed by incubation on ice.

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

- For best results, Lucigen CloneSmart<sup>®</sup> ligation reactions must be heat killed at 70°C for 15 minutes before transformation. Alternately, the reactions may be purified, if desired. For other ligation reactions, follow the manufacturer's recommendations.
- Prepare nutrient agar plus antibiotic for selection.
- 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.

# *E. cloni*<sup>®</sup> 10G & 10GF' Chemically Competent Cells

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## Transformation Protocol for High Efficiency (>1 x 10<sup>9</sup> cfu/μg) Cells

1. Prepare nutrient agar (e.g., LB) plates with antibiotic for selection.
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 (10-20 minutes).
4. Add 40 μl of *E. cloni* cells to the chilled culture tube.
5. Add 1-4 μl of heat-inactivated ligation reaction or DNA sample to the 40 μl of cells on ice. (Failure to heat-inactivate—70°C for 15 minutes—or otherwise purify, the ligation reaction may prevent transformation.) Stir briefly with pipet tip; **do not** pipet up and down to mix, which can introduce air bubbles and warm the cells.
6. Incubate on ice for 30 minutes.
7. Heat shock cells by placing them in a 42°C water bath for 45 seconds.
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. 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 100 μl of transformed cells on nutrient agar plates containing the appropriate antibiotic. Note: the quality of LB plates varies widely. Transformants plated on LB 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 Subcloning Grade (1 x 10<sup>6</sup> cfu/μg) Cells

1. Prepare nutrient agar (e.g., LB) plates with antibiotic for selection.
2. Chill sterile microcentrifuge tubes on ice (one tube for each transformation reaction).
3. Remove *E. cloni* cells from the -80°C freezer and thaw completely on wet ice (10-20 minutes).
4. Add 40 μl of *E. cloni* cells to the chilled culture tube.
5. Add 1-4 μl of heat-inactivated ligation reaction or DNA sample to the 40 μl of cells on ice. (Failure to heat-inactivate—70°C for 15 minutes, or otherwise purify, the ligation reaction may prevent transformation.) Stir briefly with pipet tip; **do not** pipet up and down to mix, which can introduce air bubbles and warm the cells.
6. Incubate on ice for 30 minutes.
7. Heat shock cells by placing them in a 42°C water bath for 45 seconds.
8. Return the cells to ice for 2 minutes.
9. Add 160 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 100 μl of the resuspended cells on nutrient agar plates containing the appropriate antibiotic.
12. Incubate the plates overnight at 37°C.
13. Transformed clones can be further grown in rich culture medium (e.g., LB, or TB).

# *E. coli*<sup>®</sup> 10G & 10GF' Chemically Competent Cells

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## **Related Lucigen Products**

- CloneSmart<sup>®</sup> Blunt Cloning Kits
- DNATerminator<sup>®</sup> End Repair Kits
- PCRTerminator<sup>®</sup> End Repair Kits
- UltraClone<sup>™</sup> DNA Ligation & Transformation Kits
- CloneDirect<sup>™</sup> Rapid Ligation Kits
- PCR-SMART<sup>™</sup> Cloning Kits
- ClonePlex<sup>®</sup> Library Construction Kits
- pEZSeq<sup>™</sup> Blunt Cloning Kits
- cSMART<sup>™</sup> cDNA Cloning Kits
- *E. coli*<sup>®</sup> Electrocompetent Cells
- OverExpress<sup>™</sup> Competent Cells
- YT Agar Powder