Lucigen®
Simplifying Genomics

E. cloni® 10G and 10GF' Electrocompetent Cells

FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE

IMPORTANT!
-80 °C Storage Required
Immediately Upon Receipt

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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

Phone: (888) 575-9695

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

Two strains of Lucigen's *E. cloni* Electrocompetent Cells are available: 10G and 10GF'. The 10G strain may be purchased at three transformation efficiency levels, designated SUPREME, ELITE, or CLASSIC. The 10GF' strain has the same chromosomal genotype as 10G, but harbors the F' plasmid. The 10GF' strain is available as ELITE only.

E. cloni cells are pre-dispensed in aliquots of several different sizes. SOLOs are aliquots of 25 µL for individual transformations; DUOs are 50-µL aliquots, sufficient for two transformation reactions of 25 µL each. SixPacks are 150-µL aliquots, sufficient for six transformations.

The cells are shipped on dry ice in one container, along with Recovery Medium and supercoiled control pUC19 DNA at 10 pg/µL. Please refer to Table 1 for a compete listing of *E. cloni* Electrocompetent Cells, efficiencies, and catalog numbers.

All competent cells require storage at -80 ˚C

Table 1: ***E. cloni*** **Electrocompetent Cells**

STRAIN	Efficiency (cfu/µg pUC19)	Transformations	Catalog #	Storage
E. cloni 10G SUPREME DUOs *(Red cap)*	> 4 x 10^{10}	12 (6 x 50 µL) 24 (12 x 50 µL)	60080-1 60080-2	**-80 °C**
E. cloni 10G SUPREME SOLOs *(Red cap)*	> 4 x 10^{10}	12 (12 x 25 µL) 24 (24 x 25 µL)	60081-1 60081-2	
E. cloni 10G ELITE SOLOs *(Red cap)*	> 2 x 10^{10}	12 (12 x 25 µL) 24 (24 x 25 µL)	60051-1 60051-2	**-80 °C**
E. cloni 10G ELITE DUOs *(Red cap)*		4 (2 x 50 µL) 12 (6 x 50 µL) 24 (12 x 50 µL)	60052-0 60052-1 60052-2	
E. cloni 10G ELITE SixPacks *(Red cap)*		24 (4 x 150 µL) 48 (8 x 150 µL)	60052-3 60052-4	
E. cloni 10G CLASSIC SixPacks *(Red cap)*	> 5 x 10^{9}	24 (4 x 150 µL) 48 (8 x 150 µL)	60117-1 60117-2	**-80 °C**
E. cloni 10GF' DUOs *(Blue cap)*	> 2 x 10^{10}	12 (6 x 50 µL) 24 (12 x 50 µL)	60061-1 60061-2	**-80 °C**
Recovery Medium		12 (1 x 12 mL) 24 (1 x 24 mL) 48 (2 x 24 mL) 96 (8 x 12 mL)	---- ---- ---- 80026-1	**-80 to +20 °C**
Supercoiled pUC19 DNA (10 pg/µL)		(1 x 20 µL)	----	**-80 to -20 °C**

E. cloni Electrocompetent Cells

E. cloni 10G and 10GF' Electrocompetent Cells are *E. coli* strains optimized for high efficiency transformation. They are ideal for cloning and propagation of BAC, cosmid, or plasmid clones. They give high yield and high quality plasmid DNA due to the *end*A1 mutation.

E. cloni ® 10G and 10GF´ Electrocompetent Cells

E. cloni 10G and10GF' contain the inactive *mcr* and *mrr* mutations, allowing methylated genomic DNA that has been isolated directly from mammalian or plant cells to be cloned without deletions or rearrangements. The *rps*L mutation confers resistance to streptomycin.

E. cloni 10GF' has the same chromosomal genotype as 10G, but it harbors the F' plasmid. This plasmid confers tetracycline resistance and allows the cells to be infected with bacteriophage M13 for ssDNA production. The F' plasmid also carries the *lac*Iq repressor allele; therefore, when using a blue/white screening vector (such as Lucigen's pEZSeq™ or pUC19) addition of IPTG is necessary to induce expression of the *lac*Zα peptide. In the absence of IPTG, blue/white screening will produce only faint color, and the associated transcription of insert DNA from the *lacZ* promoter may be minimal.

Lucigen's *E. cloni* Electrocompetent cells are available at three transformation efficiency levels to match any application:

> ***E. cloni* 10G SUPREME Electrocompetent Cells** deliver $\geq$ 4 x 10^{10} cfu/µg. Ideal for the most demanding applications requiring the greatest number of transformants, such as construction of large, high complexity libraries or cloning difficult targets.
>
> ***E. cloni* 10G or 10GF' ELITE Electrocompetent Cells** deliver $\geq$ 2 x 10^{10} cfu/µg. These preparations provide large numbers of transformants from hard-to-clone fragments or limited DNA at a lower price than SUPREME cells.
>
> ***E. cloni* 10G CLASSIC Electrocompetent cells**, deliver $\geq$ 5 x 10^{9} cfu/µg. The 10G CLASSIC cells are the most economical choice for standard subcloning and library construction. They are available in larger package sizes for use in higher volume cloning applications.

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

***E. cloni* 10GF':**
[F' *pro*A+B+ *lac*I^{q}ZΔM15::Tn10 (TetR)] / *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
E. cloni 10GF'	Yes	Yes	Yes	Yes

Preparation for Transformation

Transformation is carried out in a 0.1 cm gap cuvette using 25 µL of *E. cloni* 10G ELITE or SUPREME Electrocompetent Cells. Optimal settings for electroporation are listed in the table below. Typical time constants are 3.5 to 4.5 msec.

Optimal Setting		Alternate Settings (~ 20-50% lower efficiencies)

1.0 mm cuvette 10 µF 600 Ohms 1800 Volts		1.0 mm cuvette 25 µF 200 Ohms 1400 – 1600 Volts

Suggested Electroporation Systems:
Bio-Rad Micro Pulser (Cat. #165-2100); Bio-Rad E. coli Pulser (Cat. #165-2102); Bio-Rad Gene Pulser II (Cat. #165-2105); BTX ECM630 Electroporation System; Eppendorf Electroporator 2510.
Optional transformation control reactions include electroporation with 1 µL (10 pg) of supercoiled pUC19 DNA.

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

- Ligation reactions performed with Lucigen's CloneDirect™ Ligation Buffer (included with Lucigen's Cloning or Ligation Kits) must be heat killed at 70 ºC for 15 minutes before transformation. The ligation reaction can be used directly after heat inactivation, without purification of the ligation products.
- DNA samples in other buffers must be purified and dissolved in water or a buffer with low ionic strength, such as TE. The presence of salt in the electroporation sample leads to arcing at high voltage, resulting in loss of the cells and DNA.
- Microcentrifuge tubes and electroporation cuvettes must be thoroughly pre-chilled on ice before use. Successful results can be obtained with cuvettes from many sources including BTX (Model 610), Eppendorf (Cat. #940001005), and BioRad (Cat. #165-2089).
- The cells must be completely thawed **on ice** before use.
- For highest transformation efficiency, use the provided Recovery Medium to resuspend the cells after electroporation. Use of SOC or other media will result in lower transformation efficiencies.
- Prepare nutrient agar plus appropriate antibiotic.

Transformation Protocol for Cells

Use the following protocol for cells provided in microfuge tubes.

1. Have Recovery Medium and 17 mm x 100 mm sterile culture tubes readily available at room temperature (one tube for each transformation reaction). Transformation efficiency may decrease with the use of SOC or other media.
2. Place electroporation cuvettes (0.1 cm gap) and microcentrifuge tubes on ice (one cuvette and one microfuge tube for each transformation reaction).
3. Remove *E. cloni* cells from the -80 °C freezer and place on wet ice until they thaw **completely** (10-20 minutes).
4. When cells are thawed, mix them by tapping gently. Aliquot 25 µL of cells to the chilled microcentrifuge tubes on ice. (Omit this step if using *E. cloni* SOLOs, which contain 25 µL of cells per tube).
5. If using ligation buffer from any Lucigen Cloning Kit, add 1 µL of the heat-denatured ligation reaction to the 25 µL of cells on ice. Failure to heat-inactivate the ligation reaction will prevent transformation. Stir briefly with pipet tip; **do not** pipet up and down to mix, which can introduce air bubbles and warm the cells. Use of more than 2 µL of ligation mix may cause electrical arcing during electroporation.

   For ligation reactions using other commercial kits, please refer to the manufacturer's instructions.

6. Carefully pipet 25 µL of the cell/DNA mixture into a chilled electroporation cuvette without introducing bubbles. Quickly flick the cuvette downward with your wrist to deposit the cells across the bottom of the well. Electroporate according to the conditions recommended above.
7. Within 10 seconds of the pulse, add 975 µL of Recovery Medium to the cuvette and pipet up and down three times to resuspend the cells. Transfer the cells and Recovery Medium to a culture tube.
8. Place the tube in a shaking incubator at 250 rpm for 1 hour at 37 °C.
9. Spread up to 100 µL of transformed cells on nutrient agar plates containing the appropriate antibiotic.
10. Incubate the plates overnight at 37 °C.
11. Transformed clones can be further grown in any rich culture medium.

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. Autoclave and cool to 55 °C.