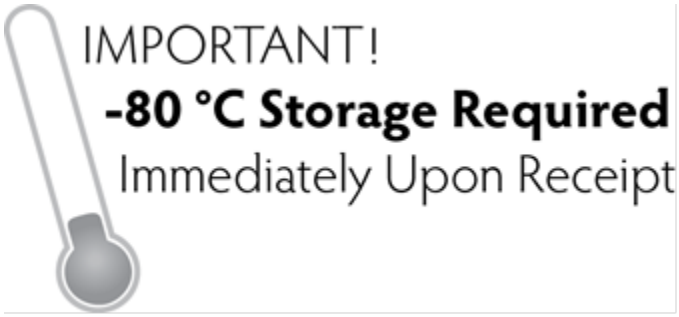
Lucigen®
Simplifying Genomics

E. cloni® 10G BAC-Optimized Electrocompetent 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 critical that the reagents supplied by the user, especially the DNA targets to be cloned, are of the highest quality. Please follow the manual carefully or contact our technical service representatives for information on preparation and testing of the target DNA. We encourage you to contact us with your comments regarding the performance of our products in your applications. Thank you.

Email: techsupport@lucigen.com

Phone: (888) 575-9695

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

Components & Storage Conditions

Lucigen E. cloni ® 10G BAC-Optimized Electrocompetent Cells are shipped on dry ice, with Recovery Medium and supercoiled control pUC19 DNA at 10 pg/µL. **Store Competent Cells at –80 °C**.

E. cloni ® 10G BAC-Optimized Electrocompetent Cells

	Efficiency (cfu/µg)		Transformations	Catalog #
	pUC19	150kb BAC		
E. cloni 10G BAC-Optimized *(Brown cap)*	≥1 X 10^{10}	≥1 X 10^{7}	25 (5 x 100 µL)	60215-2
Recovery Medium (Store at -20°C or -80°C)			36 (3 x 12 mL) 96 (8 x 12 mL)	---- 80026-1
Transformation Control DNA* Supercoiled pUC19 (10 pg/µL)			(1 x 20 µL)	----

* Provided as a control for transformation. Use 1 µL (10 pg) of the DNA for transformation.

E. cloni ® 10G BAC-Optimized Electrocompetent Cells

E. cloni ® 10G BAC-Optimized Electrocompetent Cells are *E. coli* strains optimized for the highest transformation efficiency of high molecular weight BAC DNA and BAC ligations. They also give the high yield and the high quality BAC/plasmid DNA due to the *end*A1 mutation.

These cells 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.

Genotypes

E. cloni *10G BAC-Optimized:* *F - mcrA Δ(mrr-hsdRMS-mcrBC) endA1 recA1 Φ80dlacZΔM15 ΔlacX74 araD139 Δ(ara, leu)7697 galU galK rpsL nupG λ- tonA (StrR)*

Cell Line	Suitable for Blue/White Screening	IPTG Required for Blue/White Screening
10G BAC-Optimized	Yes	No

Preparation for Transformation

Large-insert DNA cloning applications and BAC library construction demand the highest transformation efficiency and recovery of the largest clones possible. Lucigen's E. cloni ® 10G BAC-Optimized Electrocompetent Cells were developed exclusively for this purpose. These cells are provided in 100-µL aliquots, sufficient for five transformation reactions. We also recommend use of the Cell Porator and Voltage Booster System from Whatman Biometra. Optimal settings for electroporation are listed below:

Electroporation conditions for E. cloni ® 10G BAC-Optimized Electrocompetent Cells

Optimal Setting (Recommended)		Alternate Conditions (May give lower efficiencies)
Cuvette gap: 1.5 mm Voltage: 358 V Capacitance: 330 µF Impedance: Low ohms Charge rate: Fast Voltage Booster Resistance: 4000 ohms		Cuvette gap: 1.0 mm Voltage: 1800 V Capacitance: 25 µF Impedance: 200 ohms
Whatman Biometra: Cell Porator and Voltage Booster System, Cat. # 11609013		Eppendorf: Model 2510; Bio-Rad: Gene Pulser II #165-2105; BTX: ECM630
1.5 mm Cuvettes: Whatman Biometra Cat.#11608031		1 mm Cuvettes: Eppendorf Model 4307-000-569, BTX Model 610, or BioRad Cat. #165-2089. Users have reported difficulties using *E. cloni* cells with Invitrogen cuvettes Cat. # 65-0030.

Optional transformation control reactions include electroporation with 1 µL(10 pg) of supercoiled pUC19 DNA.

Take the following precautions to ensure successful transformation:

- For best results, the BAC ligation reaction must NOT be purified or heat treated.
- The DNA sample to be used for electroporation must be 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 the loss of the cells and DNA.
- Microcentrifuge tubes and electroporation cuvettes must be thoroughly pre-chilled on ice before use. Optimal results are obtained with the electroporator and cuvettes from Whatman Biometra (Cat.# 11609013 and #11608031, respectively). Successful transformation also can be achieved with alternate systems, although efficiency may be lower (see Table 2).
- 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 TB or SOC will result in lower transformation efficiencies.
- Use LB Lennox agar plus appropriate antibiotic for plating cells. LB Lennox Agar is used to maximize colony size. Cells may be plated on LB or other common media—colonies will be noticeably smaller upon comparison but adequate for the vast majority of intents and purposes.

Transformation Protocol

1. Prepare Agar plates.
2. Aliquot 1 mL of Recovery Medium into 17 mm x 100 mm sterile culture tubes at room temperature (one tube for each transformation reaction). Transformation efficiency may decrease with the use of other media.
3. Place electroporation cuvettes and microcentrifuge tubes on ice (one cuvette and one tube for each transformation reaction).
4. Remove E. cloni cells from the -80 °C freezer and place on wet ice until they thaw **completely** (10-20 minutes).
5. When cells are thawed, mix them by tapping gently. Add 20 µL of E. cloni cells to the chilled microcentrifuge tube on ice.
6. If using a Lucigen BAC Cloning Kit, add 1 µL of the BAC ligation reaction directly to the 20 µL of cells on ice. Do NOT heat inactivate the ligation reaction. (Heat-inactivating the ligation reaction will reduce the quality of BAC cloning.) Stir briefly with pipet tip; **do not** pipet up and down to mix, which can introduce air bubbles and warm the cells. Using more than 1 µL of ligation mix may cause electrical arcing during electroporation.

   For ligation reactions using other commercial kits, please refer to the manufacturer's instructions.
7. Carefully pipet the cell/DNA mixture into a chilled electroporation cuvette without introducing bubbles. Electroporate according to the conditions recommended on p.3.
8. After electroporation, quickly transfer the cells into the Recovery Medium in the culture tube at room temperature.
9. Place the tube in a shaking incubator at 250 rpm for 1 hour at 37 °C.
10. Spread up to 100 µL of transformed cells on agar plates containing the appropriate antibiotic.
11. Incubate the plates overnight at 37 °C.
12. Transformed clones can be further grown in TB or in any other rich culture medium.

Media Recipes

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.

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