

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. cloni*[®]**

10G BAC-Optimized Electrocompetent Cells

IMPORTANT!
-80°C Storage Required
Immediately Upon Receipt

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E. cloni[®] 10G BAC-Optimized Electrocompetent Cells

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E. cloni[®] 10G BAC-Optimized Electrocompetent Cells

Components & Storage Conditions

Lucigen's *E. cloni*[®] 10G BAC-Optimized Electrocompetent Cells are shipped on dry ice, along with Recovery Medium and supercoiled control pUC19 DNA at 10 pg/μl. Please refer to Table 1 for a listing of *E. cloni*[®] 10G BAC-Optimized Electrocompetent Cells, efficiencies, and catalog numbers.

All *E. cloni*[®] 10G BAC-Optimized Electrocompetent Cells require storage at -80°C.

Table 1: *E. cloni*[®] 10G BAC-Optimized Electrocompetent Cells

| | Efficiency (cfu/μg) | | Transformations | Catalog # | Storage |
|---|-----------------------|----------------------|--|-------------------------|---------------------|
| | pUC19 | 150kb BAC | | | |
| <i>E. cloni</i> 10G BAC-Optimized | ≥1 X 10 ¹⁰ | ≥1 X 10 ⁷ | 10 (2 x 100 μl) 25 (5 x 100 μl) | 60215-1 60215-2 | -80°C |
| Recovery Medium | | | 12 (1 x 12 ml) 36 (3 x 12 ml) 96 (8 x 12 ml) | ---- ---- 80026-1 | -20 to -80°C |
| Transformation Control DNA* Supercoiled pUC19 (10 pg/μl) | | | (1 x 20 μl) | ---- | -20 to -80°C |
| YT Agar (powder) | | | | ---- | |

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

E. cloni[®] 10G BAC-Optimized Electrocompetent Cells

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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 *endA1* 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:

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

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:

Table 2. 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 | Cuvette gap: 1.0 mm Voltage: 1800 V Capacitance: 25 μF Impedance: 200 ohms |
| Voltage Booster Resistance: 4000 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 <i>E. cloni</i> cells with Invitrogen cuvettes Cat. # 65-0030. |

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

E. coli[®] 10G BAC-Optimized Electrocompetent Cells

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

- 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 YT agar plus appropriate antibiotic for plating cells. YT Agar is provided 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 YT Agar from powder included with the cells.
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. coli* 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. coli* 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.4.
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 YT 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.

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Media Recipes

TB Culture Medium for Growth of Transformants

Per liter: 11.8 g Bacto-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 except glycerol to deionized water. Autoclave and cool to 55°C. Immediately before use, add 8 ml of filter-sterilized 50% glycerol.

YT Agar Plates

Add the YT Agar powder* provided with the kit to 500 ml of deionized water. Autoclave and cool to 55°C. Add the appropriate filter-sterilized antibiotic to the cooled medium (e.g., 15 mg kanamycin for kanamycin-resistant transformants or 50 mg of chloramphenicol for chloramphenicol-resistance).

Temperatures of >55°C may destroy the antibiotics. Do NOT add antibiotics to hot media! Pour approximately 20-25 ml per petri plate.

YT Agar is per liter: 8 g Bacto-tryptone, 5 g yeast extract, 5 g NaCl, 15 g agar, plus antibiotic. Prepare YT+kan30 agar medium by adding kanamycin to a final concentration of 30 mg/l (equal to 30 µg/ml).

YT Agar is available to purchase separately as 5 packets with catalog number 60025-1.

For blue/white screening, add 3 ml per Liter of 100mM IPTG and 10 ml per Liter of 2% X-gal to the molten agar at 55°C before pouring.

*YT Agar is provided 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.

Related Lucigen Products

- CopyRight™ 2.0 BAC Cloning Kit
- CopyRight™ 2.0 FOS Cloning Kit
- BigEasy™ Linear Cloning Kit
- CloneSmart® Blunt Cloning Kits
- DNATerminator® End Repair Kit
- PCRTerminator® End Repair Kit
- UltraClone™ DNA Ligation & Transformation Kits
- CloneDirect™ Rapid Ligation Kit
- ClonePlex® Library Construction Kit
- pEZSeq™ Blunt Cloning Kits
- cSMART™ cDNA Cloning Kits
- *E. cloni*[®] EXPRESS Electrocompetent Cells
- OverExpress™ Competent Cells