Lucigen®
Simplifying Genomics

BAC-Optimized Replicator™ v2.0 Electrocompetent Cells

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MA052 31OCT2016

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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, especially the DNA targets to be cloned, are of the highest quality. Please follow the manual 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: techsupport@lucigen.com

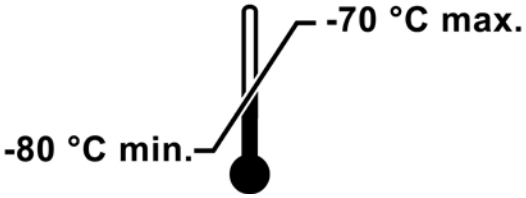
Phone: (888) 575-9695

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

BAC-Optimized Replicator v2.0 Electrocompetent Cells are shipped on dry ice with supercoiled control pKanR DNA (10 pg/µl), Arabinose Induction Solution, and Recovery Medium. BAC-Optimized Replicator v2.0 Cells are packaged in 50 µl aliquots, sufficient for two transformations per tube (DUOS).

BAC-Optimized Replicator v2.0 Electrocompetent Cells require storage at –80 ºC.



BAC-Optimized Replicator v2.0 Electrocompetent Cells

	Catalog #	Reactions
BAC-Optimized Replicator v2.0 Electrocompetent Cells (DUOS) (Green cap) Store at **-80 °C**	60210-1	12 (6 x 50 µl)
	60210-2	24 (12 x 50 µl)
Positive Control Plasmid (pKanR 10 pg/µl) Store at **-20 °C** to **-80 °C**	----	(1 x 20 µl)
Arabinose Induction Solution Store at **-20 °C** to **-80 °C**	----	1 ml
Recovery Medium Store at **-20 °C** to **-80 °C**	---- ----	12 (1 x 12 ml) 24 (2 x 12 ml)

Note: YT Agar packets are no longer provided by Lucigen.

BAC-Optimized Replicator™ v2.0 Electroporation Competent Cells

Lucigen's BAC-Optimized Replicator v2.0 Electrocompetent Cells are an *E. coli* strain that contains an inducible *trf*A gene, which is required for induction of replication from the *ori*V origin in BAC and fosmid clones. Most lab strains of *E. coli* do not contain a *trf*A gene, and thus will not support copy number amplification of such vectors.

BAC-Optimized Replicator v2.0 Cells are designed for high efficiency transformation by electroporation, yielding $\geq 1 \times 10^{10}$ cfu/µg pKanR DNA. These cells are ideal for cloning and propagating BAC, fosmid or plasmid clones. In addition to the *trf*A gene, they contain the *end*A1 mutation for high yield and high quality plasmid DNA. They also contain the *mcr* and *mrr* mutations, stable cloning of methylated genomic DNA isolated directly from mammalian or plant cells. They do not contain the F plasmid. The *rps*L mutation confers resistance to streptomycin.

BAC-Optimized Replicator v2.0 Genotype: F^- *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) *end*A1 *rec*A1 Φ80d*lac*Z ΔM15 Δ*lac*X74 *ara*D139 Δ(*ara,leu*)7697 *gal*U *gal*K *rps*L (Str^R) *nup*G (*att*L *ara*C-P_{BAD}-*trf*A250 *bla att*R) λ^-

Note that Replicator v2.0 cells are resistant to ampicillin (*bla* gene).

Preparation for Transformation

Large-insert DNA cloning and BAC library construction demand the highest transformation efficiency and recovery of the largest clones possible. Lucigen's BAC-Optimized Replicator v2.0 Electrocompetent Cells were developed exclusively for this purpose. These cells are provided in 50 µl aliquots, sufficient for two transformation reactions per tube. 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 BAC-Optimized Replicator 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 pKanR DNA. The plasmid pKanR is provided as a control for transformation of Lucigen's Replicator v2.0 cells. Kanamycin is required for selection of pKanR transformants.

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 (Catalog# 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. Colony size will be small or variable on LB agar plates. YT 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. 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.
2. Place electroporation cuvettes and microcentrifuge tubes on ice (one cuvette and one 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. Add 20 µl of *E. cloni* cells to the chilled microcentrifuge tube on ice.
5. 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.
6. Carefully pipet the cell/DNA mixture into a chilled electroporation cuvette without introducing bubbles. Electroporate according to the conditions recommended on p. 4.
7. After electroporation, quickly transfer the cells into the Recovery Medium in the culture tube at room temperature.
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 YT agar plates containing the appropriate antibiotic.
10. Incubate the plates overnight at 37 °C.
11. Transformed clones can be further grown in TB or in any other rich culture medium.

DNA Isolation

Transformants are grown in TB medium. Stable inserts of 10-40 kb can be grown overnight with shaking at 37 ºC in the presence of 1X Arabinose Induction Solution. DNA minipreps can be performed by standard methods.

For BACs and unstable smaller inserts, it may be necessary to grow the cultures without induction to an OD_{600} of 0.2-0.3. To reach this OD, it is convenient to grow the cultures overnight at 37 ºC without shaking. The following morning, dilute the cultures 2-10 fold, and grow at 37 ºC with shaking at 225 rpm for 30 minutes. For each ml of culture, add 1 µl of 1000 X Arabinose Induction Solution. Continue growth for 2-3 hours at 37 ºC with shaking at 225 rpm.

Prepare DNA minipreps according to standard protocols. BAC-Optimized Replicator v2.0 cells are *recA endA* deficient and will provide high quality plasmid DNA.

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 plus appropriate antibiotics.

YT Agar Plates

Prepare YT Agar. 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; 50 mg ampicillin or carbenicillin for ampicillin-resistant transformants). For blue/white screening, add 1.5 ml 100 mM IPTG and 5 ml 2% X-gal to the molten agar at 55 °C before pouring. Pour approximately 25 ml per petri plate.

Temperatures of >55 °C may destroy the antibiotics. Do NOT add antibiotics to hot media!

Per liter YT is:
- 5 g yeast extract
- 8 g tryptone
- 5 g NaCl
- 15 g agar

Add deionized water to 1 liter. Adjust pH to 7.0 with NaOH.

YT 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.

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