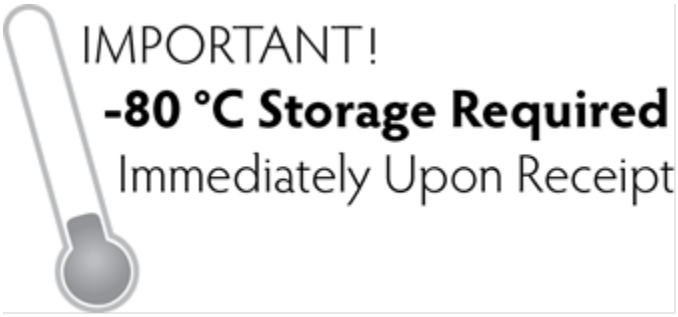
Lucigen®
Simplifying Genomics

E. cloni® EXPRESS Electrocompetent Cells



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Lucigen Corporation 2905 Parmenter St, Middleton, WI 53562 USA
Toll Free: (888) 575-9695 | (608) 831-9011 | FAX: (608) 831-9012
lucigen@lucigen.com www.lucigen.com

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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 shipment. Please avoid using reagents for greater than one year from receipt.

Components & Storage Conditions

The cells are shipped on dry ice in one container, along with supercoiled control pUC19 DNA at 10 pg/ µL , and Expression Recovery Medium (lactose minus). BL21(DE3) DUOs are packaged in 50 µL aliquots, sufficient for two transformations per tube.

***E. cloni* EXPRESS Competent Cells require storage at –80 °C**.

***E. cloni*® EXPRESS Electrocompetent Cells**

Description	Efficiency (cfu/µg pUC19)	Transformations	Catalog #	Storage
E. cloni EXPRESS BL21(DE3) DUOs (Orange caps)	≥ 5 x 10^9	12 (6 x 50 µL) 24 (12 x 50 µL)	60300-1 60300-2	**-80 °C**
Expression Recovery Medium (lactose minus)	----	12 (1 x 12 mL) 24 (1 x 24 mL)	----	**-20 to -80 °C**
Supercoiled pUC19 DNA (10 pg/ µL)	----	(1 x 20 µL)	----	**-20 to -80 °C**

As a control for transformation, *E. cloni* EXPRESS Electrocompetent Cells are provided with supercoiled pUC19 DNA at a concentration of 10 pg/ µL. Use 1 µL for transformation.

* Additional Expression Recovery Medium (lactose minus) can be ordered separately as catalog # 80030-1, 96 mL (8 x 12 mL).

E. cloni EXPRESS Electrocompetent Cells

E. cloni EXPRESS BL21(DE3) Electrocompetent Cells are an *E. coli* strain that is ideal for routine protein expression applications. NOTE: for expressing toxic proteins, we recommend Lucigen's OverExpress™ C41(DE3) and C43(DE3) Competent Cells.

BL21(DE3) is a lysogen of λDE3. This strain carries a chromosomal copy of the T7 RNA Polymerase gene under the control of the *lac*UV5 promoter. This strain is suitable for production of protein from target genes cloned into T7 driven expression vectors. E*. cloni* Express BL21(DE3) is also deficient in the *lon and ompT* proteases.

Strain and Safety Information

Genotype

F⁻ *ompT hsdS*$_B$ (*r*$_B^-$ *m*$_B^-$) *gal dcm* (DE3)

BL21(DE3) cells have a transformation efficiency yield of ≥ 5 x 10^9 cfu/µg pUC19.

Preparation for Transformation

E. cloni EXPRESS Electrocompetent Cells are provided in 50 µL aliquots (DUOs), sufficient for two transformation reactions of 25 µL each. Transformation is carried out in a 0.1 cm gap cuvette. Optimal settings for electroporation are listed in the table below. Typical time constants are 3.5 to 4.5 msec.

Electroporation Settings

Optimal Setting	Alternate Settings (~ 20-50% lower efficiencies)	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	2.0 mm cuvette 25 µF 750 Ohms 2400 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

Suggested Electroporation Cuvettes:
Successful results are obtained with cuvettes from BTX (Model 610), BioRad (Cat. #165-2089), or Eppendorf (Cat. # 4307-000-569). Users have reported difficulties using Invitrogen cuvettes (Cat.# 65-0030).

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:

- For best results, the ligation reaction must be purified or heat killed at 70 ºC for 15 minutes before transformation.
- 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.

  NOTE: Ligation reactions performed with Lucigen's CloneDirect™ Ligation Buffer (included with Lucigen's Cloning or Ligation Kits) can be used immediately after heat inactivation, without purification of the ligation products.
- Microcentrifuge tubes and electroporation cuvettes must be thoroughly pre-chilled on ice before use.
- The cells must be completely thawed **on ice** before use.
- Recovery Media must be at **Room Temperature** prior to use
- For highest transformation efficiency, use the provided Expression Recovery Medium to resuspend the cells after electroporation. Use of TB or other media will result in lower transformation efficiencies.

Transformation Protocol

1. Have Expression Recovery Medium and 17 mm x 100 mm sterile culture tubes readily available at room temperature (one tube for each transformation reaction).
   Note: 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 tube for each transformation reaction).
3. Remove *E. cloni* EXPRESS 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 25 µL of *E. cloni* EXPRESS cells to the chilled microcentrifuge tube on ice.
5. Add 1 µL of the heat-denatured CloneSmart Ligation reaction to the 25 µL of cells on ice. Stir briefly with pipet tip; **do not** pipet up and down to mix, which can introduce air bubbles and warm the cells.
   Note: Failure to heat-inactivate the ligation reaction will prevent transformation.
   Note: Do not use of more than 2 µL of ligation mix may cause electrical arcing during electroporation.
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 in the *Electroporation Settings* table on page 3.
7. Within 10 seconds of the pulse, add 975 µL of Expression 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 culture tube in a shaking incubator at 250 rpm for 1 hour at 37 °C.
9. Spread up to 100 µL of transformed cells on LB agar plates containing the appropriate antibiotic.
10. Incubate the plates overnight at 37 °C.
11. Transformed clones can be further grown in LB or in any other lactose-free medium.

Sample Induction Protocol

1. Inoculate a single colony from a freshly streaked plate into 5 mL of LB medium containing the appropriate antibiotic for the plasmid and host strain.
2. Incubate with shaking at 37 °C overnight. To minimize the amount of expression of the target protein prior to induction, add glucose to the growth medium at a concentration of 0.2% (w/v).
3. Inoculate 50 mL of LB medium containing the appropriate antibiotic with 0.5 mL of the overnight culture prepared in step 2.
4. Incubate with shaking at 37 °C until the OD_{600} reaches 0.6 - 0.8.
5. Add IPTG to a final concentration of 1 mM (Prepare a 1 M solution of IPTG by dissolving 2.38 g of IPTG in water and adjust the final volume to 10 mL. Filter sterilize before use). To determine the optimal concentration of IPTG for maximum expression of the target protein, test a range of IPTG concentrations from 0.25 - 2 mM.

6. Incubate at 37 °C for 3-4 hours. To determine the optimal time for induction of the target protein, it is recommended that a time course experiment be performed varying the induction from 2-16 hours.

7. Place the culture on ice for 10 minutes. Harvest cells by centrifugation at 5,000 x g for 10 minutes at 4 °C.

8. Remove the supernatant and store the cell pellet at -20 °C (storage at lower temperatures is also acceptable).

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

Pour approximately 25 mL per petri plate.

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