Intact Genomics®

10B Copy-up BAC ElectroCompetent Cells

Catalog #	Package Size
1322-12	6x40 µl
1322-24	12x40 µl
1324-30	6x100 µl

Description

Intact Genomics 10B Copy-up BAC ElectroCompetent E. coli cells offer the highest transformation efficiencies of ≥5 x 10^{10} cfu/µg plasmid DNA which are ideal for applications requiring high transformation efficiencies, such as with cDNA or gDNA library construction. The cells contain a mutant trfA gene, whose protein product is required for initiation of replication from the oriV origin of replication. The trfA gene is under control of an inducible promoter. When grown in standard LB or Recovery Medium, expression of the trfA gene is repressed. Addition of L-arabinose induces expression of the trfA gene and subsequent utilization of the oriV origin of replication and high copy amplification of the Copy-Up BAC, fosmid and PCR clones.

Specifications

Competent cell type:	ElectroCompetent
Derivative of:	DH10B™
Species:	*E. coli*
Format:	Tubes
Transformation efficiency:	≥ 5 x 10^{10} cfu/µg plasmid DNA
Inducible Promotor:	Yes
Blue/white screening:	Yes
Shipping condition:	Dry ice

Reagents Needed for One Reaction

1). 10B Copy-up BAC ElectroCompetent Cells:	20 µl
2). Recovery Medium:	1 ml

Storage

10B Copy-up BAC ElectroCompetent Cells:	-80 ºC
Recovery medium:	4 ºC

Genomic Features

10B Copy-up BAC ElectroCompetent Cells have the following features:

- Mutant trfA gene under control of an inducible promoter for complete copy number control of Copy-up clones.
- Φ80lacZΔM15 marker provides α-complementation of the β-galactosidase gene with blue/white screening.
- mcrA genotypic marker and the mcrBC, mrr deletion allows for cloning DNA that contains methylcytosine and methyladenine.

Genotype

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 (*att*L *ara*C-PBAD-*trf*A250 *bla att*R) λ- galUgalK rpsL nupG

Quality Control

Transformation efficiency is tested by using the pUC19 control DNA supplied with the kit and using the protocol given below. Transformation efficiency should be ≥5 x 10^{10} CFU/µg pUC19 DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

General Guidelines

Follow these guidelines when using BAC 10B Copy-up ElectroCompetent *Cells:*

- Handle competent cells gently as they are highly sensitive to changes in temperature or mechanical lysis caused by pipetting.
- Thaw competent cells on ice, and transform cells immediately following thawing. After adding DNA, mix by tapping the tube gently. Do not mix cells by pipetting or vortexing.

Note: A high-voltage electroporation apparatus such as Bio-Rad Gene Pulser II #165-2105, capable of generating field strengths of 16 kV/cm is required.

Calculation of Transformation Efficiency

Transformation Efficiency (TE) is defined as the number of colony forming units (cfu) produced by transforming 1µg of plasmid into a given volume of competent cells.

TE = Colonies/µg/Dilution

Transform 1 µl of (10 pg/µl) any control plasmid into 50 µl of cells, add 950 µl of Recovery Medium. Dilute 10 µl of this in 990 µl of Recovery Medium and plate 50 µl. Count the colonies on the plate the next day. If you count 100 colonies, the TE is calculated as follows:

Colonies = 100
µg of DNA = 0.00001
Dilution = 50/1000 x 10/1000 = 0.0005
TE = 100/.00001/.0005 = 2.0 x 10^{10}

Transformation Protocol

Use this procedure to transform 10B copy-up BAC ElectroCompetent Cells. Do not use these cells for chemically transformation.

1) Place sterile cuvettes and microcentrifuge tubes on ice.
2) Remove competent cells from the -80 °C freezer and thaw completely on wet ice (10-15 minutes).
3) Aliquot 1 µl (1 pg-10 ng) of DNA to the chilled microcentrifuge tubes on ice.
4) When the cells are thawed, add 20 µl of cells to each DNA tube on ice and mix gently by tapping 4-5 times. Do not pipette up and down or vortex to mix, this can harm cells and decrease transformation efficiency.
5) Pipette 21 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 and then electroporate.

6) Immediately add 979 µl of Recovery Medium or any other medium of choice to the cuvette, pipette up and down three times to re-suspend the cells. Transfer the cells and Recovery Medium to a culture tube.
7) Incubate tubes at 37 °C for 1 hour at 210 rpm.
8) Dilute the cells as appropriate then spread 20-200 µl cells onto a pre-warmed selective plate. Use sterilized spreader or autoclaved ColiRoller™ plating beads to spread evenly.
9) Incubate the plates overnight at 37 °C.