IG® Recombineering Electrocompetent Cells

Manual

Catalog #	1266-12	1266-48
Package Size	6x50 µl	24x50 µl

Important!

-80°C Storage Required

* Immediately inspect packages
* Freeze upon receipt

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Intact Genomics, Inc.

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Description:

Intact Genomics (IG®) Recombineering Electrocompetent Cells are designed for high-efficiency transformation in a wide range of applications, including DNA, plasmid, and BAC engineering, as well as homologous recombination. This strain is a SW102 derivative containing a defective lambda prophage in a DH10B [λcI857 ind1 (cro-bioA<>tet)] background. In addition, it carries a fully functional gal operon except for a galK deletion, which enables efficient DNA or BAC modification through galK positive/negative selection. This strain is tetracycline resistant (5 µg/mL).

Specifications:

Competent cell type: Electrocompetent

Derivative of: SW102

Species: *E. coli*

Format: Tubes

Transformation efficiency: ≥ 1.0 x 10^{10} cfu/µg pUC19 DNA

Blue/white screening: Yes

Shipping condition: Dry ice

Reagents Needed for One Reaction:

- IG Recombineering Electrocompetent Cells: 25 µl
- DNA (or pUC19 Control, 10 pg/µl): 1 µl
- Recovery Medium: 1 ml

Product Components and Recommended Storage Condition:

- IG Recombineering Electrocompetent Cells: -80 °C
- pUC19 Control DNA: -20 °C
- Recovery Medium: 4 °C

Genomic Features and Benefits

IG Recombineering Electrocompetent cells have the following features:

- This strain carries an engineered, heat-inducible prophage in which red genes (exo, bet, gam) are placed under the control of the heat-inducible promoter pL, which is repressed at 32 °C and induced at 42 °C.
- It is a ΔgalK derivative. It supports galK positive/negative selection for precise, scarless engineering.
- TetR: The cro-bioA region has been replaced with a tetracycline resistance gene (tetra, 5 µg/ml).

Genotype:

DH10B [λcI857ind1 (cro-bioA<>tet)]

Quality Control:

Transformation efficiency is tested by using the pUC19 control DNA supplied with the kit and the high efficiency transformation protocol listed below. Transformation efficiency should be ≥1 x 10^{10} CFU/µg pUC19 DNA.

Untransformed cells are tested for appropriate antibiotic sensitivity.

General Guidelines:

Follow these guidelines when using IG Recombineering 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) pUC19 control plasmid into 25 µl of cells, add 975 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 = 2.0x10^{10}

Transformation Protocol:

Use this procedure to transform IG® Recombineering Electrocompetent Cells. Do not use these cells for chemical transformation.

1. DNA/Cell Mixture Preparation

1.1. Remove competent cells from the -80 °C freezer and thaw completely on wet ice (10–15 minutes). Place sterile electroporation cuvettes and microcentrifuge tubes on ice. Bring IG Recovery Medium to room temperature.

1.2. Aliquot 1–5 µl DNA (1 pg–100 ng) into chilled microcentrifuge tubes on ice. If using the pUC19 control, add 1 µl pUC19 DNA (10 pg/µl) to a chilled microcentrifuge tube.

1.3. Once the cells are thawed, gently add 25 µl of competent cells to each DNA tube while keeping the mixture on ice. Mix gently by tapping the tube 4–5 times.

⚠ *Do not pipette up and down or vortex, as this may damage the cells and reduce transformation efficiency.*

2. Electroporation

2.1. Pipette 26 µl of the cell/DNA mixture into a chilled electroporation cuvette, avoiding bubbles. Quickly flick the cuvette downward to ensure the mixture settles evenly across the bottom of the cuvette, then proceed with electroporation.

Standard electroporation settings for E. coli:
Voltage: 1.8 kV, Resistance: 200 Ω, Capacitance: 25 µF, Cuvette gap: 0.1 cm.

3. Cell Recovery

3.1. Immediately add 974 µl of room-temperature IG Recovery Medium (or another suitable medium) to the cuvette. Gently pipette up and down three times to resuspend the cells.

3.2. Transfer the entire mixture to a culture tube (17 mm × 100 mm) and incubate in a 30 °C shaking incubator at 210 rpm for 1 hour. *(Do NOT grow this cell and its transformants at > 32°C)*

4. Cell Plating

4.1. Prepare pre-warmed selection plates during the recovery period. Plates should be equilibrated to 30 °C and free of condensation to prevent contamination and mixed colonies.

4.2. Dilute the recovered cells 10–100X, if necessary, to obtain well-isolated colonies. Plate 20–200 µl of cells onto pre-warmed LB agar plates containing the appropriate antibiotic(s). For the pUC19 control, plate 50 µl of a 100X diluted transformants onto an LB plate containing 100 µg/ml ampicillin. Spread the cells evenly using a sterile spreader or autoclaved ColiRoller™ plating beads.

4.3. Incubate the plates overnight (12–16 hours) at 30 °C.

References on How Recombineering Works:

In addition to selection, successful transformation of plasmids or vectors into IG® Recombineering Electrocompetent Cells can be verified by plasmid/vector identification and/or PCR. The references below describe how to use a transformed and confirmed clone carrying your target plasmid or vector in IG® Recombineering Electrocompetent Cells.

- Warming S, Costantino N, Court DL, Jenkins NA, Copeland NG. Simple and highly efficient BAC recombineering using galK selection. Nucleic Acids Res. 2005 Feb 24;33(4):e36. doi: 10.1093/nar/gni035. PMID: 15731329; PMCID: PMC549575.
- Sawitzke JA, Thomason LC, Bubunenko M, Li X, Costantino N, Court DL. Recombineering: highly efficient in vivo genetic engineering using single-strand oligos. Methods Enzymol. 2013;533:157-77. doi: 10.1016/B978-0-12-420067-8.00010-6. PMID: 24182922; PMCID: PMC7518103.

Related Products:

- T4 DNA Ligase (Cat.# 3212)
- i7® High Fidelity DNA Polymerase (Cat.# 3254)
- igFusion™ Cloning Kit (Cat.# 4111)
- ig®Max™ DH10B Electrocompetent Cells (Cat.# Cat.# 1284-48)
- ig® 10B Electrocompetent Cells (Cat.# 1212-24)
- ig® 5-Alpha Chemically Comp. Cells (Cat.# 1031-12)

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  Email: **sales@intactgenomics.com**
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Our hours are Monday - Friday, 8AM to 5PM, U.S. Central Standard Time.

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