Intact Genomics

Higher Quality, Lower Price, Better Result

Enzymes & Cloning Kits

Cas9 Nuclease • Hot Start Taq DNA Polymerase • Hot Start Taq 2x Master Mix • Taq DNA Polymerase • Taq DNA Polymerase with Dye • Taq DNA Polymerase 2x Premix with Dye • Hot Start Pfu DNA Polymerase • Pfu 2x Master Mix • Pfu DNA Polymerase • igScript™ Reverse Transcriptase • ig™ SYBR Green qPCR 2x Master Mix • Taq DNA Ligase • Klenow • Klenow Fragment, exo- • Pol I • T5 Exonuclease • ig-Fusion™ Cloning Kit

T4 Enzymes

T4 DNA Ligase • T4 DNA Polymerase • T4 Polynucleotide Kinase (PNK) • T4 gp46 Protein • T4 gp32 Protein • T4 gp59 Protein • T4 gp47 Protein • T4 UvsY Protein • T4 UsvX DNA Recombinase • T4 DNA Helicase (gp41)

RT-PCR & RT-qPCR Kits

igScript™ One Step RT-qPCR Kit • igScript™ First Strand cDNA Synthesis Kit • igScript™ RT-PCR Kit • igScript™ RT-qPCR Kit

Chemically & Electroporation Competent Cells

ig™ 5α • ig™ 10B • BL21 • BL21(DE3) • Phage Display Cells (TG1, SS320) • BAC Cells (10B, 10B Copy-Up) • Custom Cells

Custom Services

DNA Preparation: High HMW DNA • BAC DNA • High-Throughput DNA | **DNA Library Construction:** Random Shear BAC Library • Partial Digestion BAC Library • Fosmid Library | **Library Screening:** Colony Picking • Colony Duplication • 3D DNA Pools • Gridding & High Density Colony Filters • BAC/Fosmid end Sequencing | **Next Gen Sequencing:** Whole Genome Sequencing • *de novo* Sequencing • Exome Sequencing • RNA Sequencing • Small RNA Sequencing • Targeted Re-Sequencing • Metagenome Sequencing | **Other Services:** Large DNA Fragment Cloning and Manipulation • BAC Engineering • Custom Vector Construction

Intact Genomics, Inc.
1100 Corporate Square Drive, Suite 257
St. Louis, MO 63132-2933, USA
Phone 314.942.3655 **Fax** 314.942.3656
Email sales@intactgenomics.com
www.intactgenomics.com

Intact Genomics®

DL39 (DE3)
Chemically Competent Cells

Catalog #	Package Size
1061-12	12x50 µl
1061-24	24x50 µl

Intact Genomics

1100 Corporate Square Drive, Suite 257
Saint Louis, Missouri 63132, USA

DL39 (DE3) Chemically Competent Cells

Description

Intact Genomics chemically competent DL39 (DE3) E. coli cells are specific for transformation and protein expression in order to uniformly and specifically label :e.g. phenylalanine or leucine residues. DL39 (DE3) can also be used to reduce NMR cross-labeling via transaminase activity for valine, leucine, isoleucine, aspartate, phenylalanine, tyrosine and tryptophan residues.

Specifications

Competent cell type:	Chemically competent
Derivative of:	DL39
Species:	*E. coli*
Format:	Tubes
Transformation efficiency:	≥1 x 10^7 cfu/µg pUC19 DNA
Blue/white screening:	Yes
Shipping condition:	Dry ice

Reagents Needed for One Reaction

DL39 (DE3) chemically competent cells: 50 µl
DNA (or pUC19 Control, 10 pg/µl): 1 µl
Recovery medium: 1 ml

Storage

DL39 (DE3) competent cells:	-80 ºC
pUC19 control DNA:	-20 ºC
Recovery medium:	4 ºC

Genomic Features

DL39 (DE3) chemically competent cells have the following features:

- Deficient in the aromatic (TyrB), branched-chain (JlvE), and aspartate (AspC) transaminases.
- Modified to contain the T7 expression system

Genotype

F-, λ-, aspC13, fnr-25, rph-1, ilvE12, tyrB507,λDE3

Quality Control

Transformation efficiency is tested by using the pUC19 control DNA supplied with the kit and using the high efficiency transformation protocol listed below. Transformation efficiency should be ≥1x10^8 CFU/µg pUC19 DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

General Guidelines

Follow these guidelines when using DL39 (DE3) chemically competent *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.

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 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 = 2.0x10^{10}

High Efficiency Transformation Protocol

Use this procedure to transform DL39 (DE3) chemically competent cells. We recommend verifying the transformation efficiency of the cells using the pUC19 control DNA supplied with the kit. Do not use these cells for electroporation.

1) Remove competent cells from the -80 °C freezer and thaw completely on wet ice (10-15 minutes).
2) Aliquot 1-5 µl (1 pg-100 ng) of DNA to the chilled microcentrifuge tubes on ice.
3) When the cells are thawed, add 50 µl of cells to each DNA tube on ice and mix gently by tapping 4-5 times. For the pUC19 control, add 1 µl of (10 pg/µl) DNA to a chilled microcentrifuge tube, prior to adding 50 µl of cells. Mix well by tapping. Do not pipette up and down or vortex to mix, this can harm cells and decrease transformation efficiency.
4) Incubate the cells with DNA on ice for 30 minutes.
5) After 30 minute ice incubation, heat shock the cells at 42 °C for 45 seconds.
6) Transfer the tubes to ice for 2 minutes.
7) Add 950 µl of Recovery Medium or any other medium of choice to each tube.
8) Incubate tubes at 37 °C for 1 hour at 210 rpm.
9) Spread 50 µl to 200 µl from each transformation on Pre-warmed selection plates. We recommend plating two different volumes to ensure that at least one plate will have well-spaced colonies. For the pUC19 control, plate 50 µl on an LB plate containing 100 µg/ml ampicillin. Use sterilized spreader or autoclaved ColiRoller™ plating beads to spread evenly.
10) Incubate the plates overnight at 37 °C.

5 Minute Transformation Protocol

The following procedure results in only ~10% of the transformation efficiency as the protocol listed above.

1) Remove competent cells from the -80 °C freezer and thaw in your hand.
2) Aliquot 1-5 µl (1 pg-100 ng) of DNA to the microcentrifuge tubes. ***Do not*** pipette up and down or vortex to mix, this can harm cells and decrease transformation efficiency.
3) Incubate the cells with DNA on ice for 2 minutes.
4) After 2 minute ice incubation, heat shock the cells at 42 °C for 45 seconds.
5) Transfer the tubes to ice for 2 minutes.
6) Add 950 µl of Recovery Medium at room temperature or any other medium of choice to each tube. Immediately spread 50 µl to 200 µl from each transformation on pre-warmed selection plates. We recommend plating two different volumes to ensure that at least one plate will have well-spaced colonies. For the pUC19 control, plate 50 µl on an LB plate containing 100 µg/ml ampicillin. Use sterilized spreader or autoclaved ColiRoller™ plating beads to spread evenly.
7) Incubate the plates overnight at 37 °C.