Maximize Your Success with Lucigen Competent Cells

Introduction

The ability of bacteria to take up foreign DNA has been known since the 1940s. This technique, known as transformation, has been exploited in the laboratory to become one of the fundamental tools of molecular biology.

Under normal conditions, transformation occurs at extremely low efficiency. However, bacterial cells can be made more permeable, thereby significantly increasing transformation efficiency. This process of making the cells "competent" can be mediated by chemical treatment and/or extensive washing. DNA transformation is then accomplished by exposing cells to a sudden increase in temperature (heat shock) or by applying high-voltage electrical fields (electroporation). The goal of both heat shock and electroporation methods is to alter the fluidity of—and create pores in—the cellular membrane, allowing DNA to enter the cell.

Commercially available *E. coli* competent cells offer a wide variety of strain and format choices for the molecular biologist. This document provides guidance on selecting the right cell types and provides an overview of ways to maximize success when working with competent cells.

Choosing a Strain

Selecting the right bacterial strain of competent cells is a critical factor in determining the success of an experiment. Lucigen provides many different types of competent cells for routine cloning to the most challenging applications. For detailed information, see the Competent Cell Selection Guide (*link to new Selection Guide on web*).

- *General cloning*: The E. cloni® 10G, E. cloni 5-alpha, and TransforMax™ EC100 competent cells are "workhorse" products, suitable for most general-purpose cloning experiments.
- *Large fragment cloning*: These specialized competent cell strains enable the cloning of DNA >10 kb, such as multigene expression constructs, bacterial artificial chromosomes (BACs), and fosmids. They include 10G BAC-Optimized and BAC Replicator cells. They are also ideal for lentiviral cloning in CRISPR-Cas workflows. E. cloni 10G chemically competent cells and TransforMax EPI300 electrocompetent cells are also suitable for Gibson Assembly cloning experiments.
- *Difficult cloning*: Some strains facilitate the cloning of unstable inserts—such as toxic coding sequences, AT-rich DNA, or repetitive sequences—by special modifications to bacterial genes, such as the Endura™ and CopyCutter™ EPI400™ lines of competent cells. These strains work with most commonly used cloning vectors, so subcloning is not required. Another approach uses a complete vector/host system to provide for cloning at low copy number and then inducing to high copy number for large-scale DNA preparation. Examples include the CopyControl™ vectors and TransforMax EPI300 competent cells, and the pJAZZ® vector and BigEasy®-TSA competent cells.
- *Protein expression*: For routine protein expression, most popular vectors are based on the bacteriophage T7 promoter. The E. cloni EXPRESS and HI-Control™ BL21(DE3) strains are ideal for these applications. HI-Control 10G cells are suitable for non-T7 lac-based systems. The OverExpress™ strains are widely used to express toxic or unstable proteins, and they are supported by over 500 publications. ClearColi® Bl21(DE3) cells are specially designed for expression systems where low endotoxin levels are desired.
- *Phage display*: Several strains are available for phage display experiments, including TG1, ER2738, and SS320.

Selecting a Format

When planning a transformation experiment, you should consider whether chemically competent or electrocompetent cells would be the best choice. Although the workflows are fairly similar (Table 1), each method has unique characteristics.

Chemically Competent Cells	Electrocompetent Cells
1. Remove cells from storage at –80°C and thaw on ice	1. Remove cells from storage at –80°C and thaw on ice
2. Add DNA to cells	2. Add DNA to cells
3. Incubate on ice 30 minutes	3. Transfer cells and DNA to prechilled electroporation cuvette
4. Heat shock at 42°C for 45 seconds	4. Perform electroporation using recommended parameters
5. Incubate on ice for 2 minutes	5. Immediately add recovery medium
6. Add Recovery Medium	6. Incubate at 37°C for 1 hour with shaking
7. Incubate at 37°C for 1 hour with shaking	

Table 1. Comparison of workflows for chemically competent and electrocompetent cells.

Chemically competent cells are easier to work with, using well-established protocols, and don't require specialized equipment (electroporators and cuvettes). Consequently, they are comparatively inexpensive to use. However, the transformation efficiency for chemically competent cells is typically 1–3 orders of magnitude lower than for the equivalent electrocompetent cells.

Electrocompetent cells can achieve high transformation efficiencies, and therefore are recommended for the production of large, high-diversity libraries. However, they require larger amounts of DNA for each transformation, compared to chemically competent cells. Electroporation conditions also need to be optimized for each strain.

Purchase or prepare your own?

The decision whether to purchase competent cells or prepare your own is an individual one. It is certainly cheaper, in terms of the reagent costs, to prepare chemically competent cells in your laboratory. However, the process can be tedious, and many laboratories purchase competent cells to save time—and time is money. Purchased competent cells also offer higher transformation efficiencies than "homebrew" cells, and they come with established quality control measures that ensure reproducibility from batch to batch.

Optimizing transformation efficiency

Transformation efficiency is a measure of the amount of DNA that is successfully taken up by bacterial cells. It is typically measured by doing a control transformation with intact, supercoiled plasmid DNA, calculated as follows:

$$\frac{\text{number of colonies}}{\mu\text{g of DNA}} \times \frac{\text{final volume at recovery (mL)}}{\text{volume plated (mL)}} = \text{number of colonies per } \mu\text{g DNA}$$

For example, if you transformed 25 ng (0.025 µg) of plasmid DNA, plated 100 µL (0.1 mL) of a 1 mL final transformation volume, and obtained 100 colonies or colony-forming units (cfu), the transformation efficiency would be:

$$\frac{100 \text{ cfu}}{0.025 \text{ µg}} \times \frac{1 \text{ mL}}{0.1 \text{ mL}} = 4 \times 10^5 \text{ cfu/µg}$$

Cloning and ligation reactions

- Optimizing the ligation reaction by using high-quality DNA inserts and vector preparations will increase the chances of obtaining the desired clones and, in turn, yield better results from transformation. Using a high-quality DNA ligase is also important. If the ligation reaction contains polyethylene glycol (PEG), heat-inactivation should not be used. Instead, purify the ligation products by ethanol precipitation or using commercial DNA cleanup kits.
- Using too much DNA can also lower transformation efficiency. In general, use up to 25 ng of DNA per 50–100 µL of chemically competent cells, and up to 300 ng (in 1 µL of water) per 25 µL of electrocompetent cells.

Routine cloning

- In many cases, heat-inactivating the ligase and using an aliquot of the ligation reaction directly for transformation of chemically competent cells is sufficient.

Library generation

- To maximize the diversity of libraries, we recommend using electrocompetent cells. DNA purification is generally necessary before electroporation, especially if the mixture contains high salt concentrations, which could cause arcing in the electroporation cuvettes.

Heat shock/electroporation

Heat shock

- The temperature and time specified for heat shock when transforming chemically competent cells is critical. Follow the manufacturer's recommended heat-shock conditions. A shorter heat-shock time or a lower temperature will result in less DNA uptake, while a longer time or higher temperature will damage the cells.

Electroporation

- Make sure the electroporation cuvettes are chilled on ice.
- Competent cell suspensions for electroporation are very dense and can be challenging to pipette. Mix the cells and DNA gently but thoroughly before adding them to the cuvette.

- When adding the cells and DNA mixture to the cuvette, avoid introducing bubbles, which can cause arcing.
- For the highest post-transformation viability, transfer cells out of the cuvette immediately after electroporation and into the tube for recovery.

Recovery

- Recovery, or outgrowth period, allows the cells to recover from the trauma of transformation and to begin expressing any antibiotic resistance genes present on the incoming DNA plasmid. Recovery is generally accomplished with gentle shaking at 37˚C.
- Efficient aeration of the culture is important for proper outgrowth. If performing the transformation in 96-well plates, it may be necessary to adjust the shaker speed or transfer the cells to a deep-well plate to achieve good aeration.
- Use recovery media optimal to the specific cell strain. SOC and LB media are popular for a wide range of bacterial cells. Lucigen supplies optimized Recovery Medium (Cat. No. 80026-1) for cloning strains and Expression Recovery Medium (Cat. No. 80030-1) for protein expression strains.
- Ensure that the cells are allowed to recover for at least 1 hour before plating.

Plating

- If performing a transformation for the first time, try plating different amounts of cells to determine the optimal amount of colonies per plate. For example, prepare serial 10-fold dilutions, and plate 100 µL of each dilution. This experiment should help you identify the best plating conditions to obtain even coverage with isolated colonies.
- Ensure that the plates contain the correct antibiotic for the cell strain and vector being used, and that the antibiotic concentration is correct.
- Always plate a control reaction with uncut, supercoiled vector to compare transformation efficiency.
- Use freshly prepared antibiotic solutions and agar plates (after drying overnight) for optimal results. Previously prepared, refrigerated plates can also be used. However, in either case, make sure the plates are at 37˚C at the time of plating.
- The salt concentration in the plating medium is critical for optimal bacterial growth. Lucigen recommends low-salt LB-Lennox media for all of its competent cell strains except ClearColi cells, which require high-salt LB-Miller media (Table 2).

LB-Lennox Media (per liter)	LB-Miller Media (per liter)
5 g NaCl	10 g NaCl
5 g Yeast extract	5 g Yeast extract
10 g Tryptone	10 g Tryptone
15 g Plating agar	15 g Plating agar

Table 2. Plating media for Lucigen competent cells.

Difficult cloning

Cloning unstable DNA

- For cloning toxic proteins, select CopyControl vectors, which lower the plasmid copy number. This method reduces the burden on the cell and helps to stabilize the clone.
- For library generation, select a vector/strain combination that allows cloning at low copy number and propagation at high copy number, e.g., CopyControl vectors and TransforMax EPI300 cells, or pJAZZ vectors and BigEasy-TSA cells.

Cloning repetitive regions

- Use Endura cells, which are optimized for stabilizing repeat sequences such as those found in lentiviral clones.

Protein expression

- For expression of toxic or membrane proteins, use OverExpress strains.
- To reduce background expression from T7 promoters, use the HI-Control 10G strain for cloning and the HI-Control BL21(DE3) strain for expression. Both contain a copy of the lac repressor (lacIq), which decreases background in uninduced cells.

Summary

Molecular cloning techniques remain one of the most common tools in many areas of biology. Choosing the appropriate strain of competent cells for your experiment and following the instructions provided will help to ensure optimal results for each transformation experiment.

BigEasy, Clear Coli, and E. cloni are registered trademarks, and CopyControl, CopyCutter, Endura, Hi-Control, OverExpress, and TransforMax are trademarks of Lucigen.

Do you need a specialized strain of competent cells? Are you performing transformation experiments in 96-well plates or other high-throughput formats?

Lucigen's Custom Competent Cell Preparation Services provide:

- **Support**—Personalized service from initial discussion through delivery
- **Flexibility**—Choose from the following options to match your specific needs:
  - » **Style**: Chemically competent or electrocompetent cells
  - » **Dispense volume**: bulk or small aliquots
  - » **Format**: 96-well plates or tubes of your choice
  - » **Strains**: Lucigen or any BSL1 *E. coli* strain of choice that meets your application needs
- **Transformation Efficiency**—High efficiencies that match your needs, up to an industry-leading 4×10^{10} cfu/µg DNA
- **Rapid Turnaround Time**—Average 2-3 weeks
- **Quality**—ISO 13485 Certified with 10+ years of manufacturing excellence, available for OEM and partnerships

Request a free, no-obligation quote today!

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