Competent Cell Compendium:
Tools and Tips for Successful Transformations

- Competent Cell Genotypes and What They Mean
- Selecting the Right Cloning or Expression Strain
- Calculating Transformation Efficiencies
- Cloning and Expression Protocols
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We would like to thank GeneChoice, Inc. for their support and content in the Competent Cell Compendium: Tools and Tips for Successful Transformation.
Competent cells are *E. coli* cells that have been specially treated to transform efficiently. There are two types of competent cells: chemically competent and electrocompetent. If plasmid is simply added to *E. coli*, nothing happens! The cells must be competent!

**WHAT ARE COMPETENT CELLS?**

Chemically Competent
Chemically competent cells are treated with a buffer that contains CaCl2 and other salts that disrupt the cell membrane creating “holes” that allow the plasmids to pass into the cell. Most researchers use chemically competent cells because they are less expensive, can be made in the lab and do not require special equipment.

Electrocompetent
Electrocompetent transformations require a high density of cells and a non-ionic buffer. The cells are placed in an electroporation device that delivers a pulse of electricity to disrupt the membranes of the cells allowing the plasmids to enter the cells. Electrocompetent formats provide the highest transformation efficiencies, but do require an electroporation device.
Section 1: WHAT ARE COMPETENT CELLS?

How Do I Calculate Transformation Efficiency?

Competent cells have a range of transformation efficiencies. Transformation efficiency is a measure of how well the cells incorporate and duplicate DNA of interest. Transformation efficiency is measured in cfus, or Colony Forming Units, per input DNA. The unofficial standard is cfu/µg of pUC19 DNA.

Transformation efficiencies are based on the fraction of cells that can be transformed. No matter how much DNA is added, only a certain number of cells can be transformed. This fraction of the cells is called the “fraction competent.” The total number of transformants generated from a reaction is determined by the number of cells present and the fraction competent.

The following are typical transformation efficiencies:

- $10^6$ = value efficiency cloning strains and BL21 expression strains
- $10^7$ = general cloning purposes
- $10^8$ = high efficiency grade
- $10^9$ = highest efficiency (electrocompetent)

Value efficiency cells are suitable for most purposes. For instance, when transforming purified plasmid DNA or expressing after cloning has already been done in a cloning strain, value efficiency cells are sufficient. High efficiency cells are excellent for everything from general cloning to difficult cloning and libraries. Electrocompetent cells are suitable for most purposes with an efficiency of $10^{10}$.

Calculating Transformation Efficiency

Transformation efficiency is measured as the number of transformants per microgram of pUC19 DNA. Transformation efficiency is determined by dividing the number of transformants (A) by the amount of plasmid DNA (B).

$$ \frac{A}{B} = \frac{10^7}{0.001 \, \mu g} = 10^{10} $$

Example:

$$ \frac{A}{B} = \frac{10^7}{1 \, \mu g} = 10^7 $$

Note: Transformation efficiency is determined in the linear part of the curve. These are the fraction competent cells. In the non-linear portion of the curve are cells that cannot be transformed.
Section 2: STRAINS OF COMPETENT CELLS

There are many strains of competent cells that are grouped into cloning strains and expression strains. Each strain has beneficial properties. Selecting the strain most suitable for your application will provide optimal results.

Cloning Strains

Competent cells most often used in cloning and sequencing, but that are not well suited to gene expression work, are referred to as cloning strains. There are many different strains of *E. coli* available for cloning, nearly all of them derived from a single strain called K12 which was first isolated in 1922. Since 1922, many mutants have been made, resulting in the average strain containing a long list of genetic markers (the genotype).

The Ideal Cloning Host

Ideal traits of cloning strains are listed below. For a detailed explanation of these traits and others, please see page 20 Appendix 1: Genotypes.

- **hsdR** does not restrict unmethylated DNA
- **mcr-mrr** does not restrict methylated DNA
- **recA** does not recombine homologous DNA
- **endA** does not degrade DNA
- **lacZ** △ M15 blue/white screening
- **tonA, T1** resistant to T1 phage and its relatives

Cloning Strains from Sigma-Aldrich

### Cloning Strain Selection Guide

Sigma-Aldrich offers a variety of cloning strains. The table below features available cloning strains, their efficiencies, their traits, and the ideal uses for each strain, to aid in the selection of the optimal strain.

<table>
<thead>
<tr>
<th>Features</th>
<th>GCS™</th>
<th>GC10™</th>
<th>Thunderbolt™ GC10™</th>
<th>JM109</th>
<th>HB101</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain background</td>
<td>K12</td>
<td>K12</td>
<td>K12</td>
<td>K12</td>
<td>K12xB</td>
</tr>
<tr>
<td>Transformation efficiency</td>
<td>10⁹</td>
<td>10⁹</td>
<td>10¹⁰</td>
<td>10⁸</td>
<td>10⁸</td>
</tr>
<tr>
<td>Blue/White selection</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Recombination deficient (recA)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Endonuclease deficient (endA)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Restriction deficient (hsdR⁻)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Methyl restriction deficient (mcr-mrr)</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Phage resistant (tonA, T1*)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>F' episome for single strand rescue</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>lacI (higher level of lac repressor)</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Ideal for:</td>
<td>cloning larger plasmids and genomic DNA</td>
<td>cloning methylated and genomic DNA</td>
<td>cloning methylated and genomic DNA</td>
<td>generation of high-quality plasmid and single-stranded DNA</td>
<td>popular strain, cloning with pBR322 &amp; other vectors without α-complementation</td>
</tr>
</tbody>
</table>

Ideal for:

- cloning larger plasmids and genomic DNA
- cloning methylated and genomic DNA
- cloning methylated and genomic DNA
- generation of high-quality plasmid and single-stranded DNA
- popular strain, cloning with pBR322 & other vectors without α-complementation
Section 2: STRAINS OF COMPETENT CELLS

Cloning Strains from Sigma-Aldrich

**GC5™ Chemically Competent Cells**

Comparable to: DH5α™

Uses: plasmid propagation, cDNA library generation from plasmid based vectors, and general cloning protocols.

Ideal for: larger plasmids.

Transformation Efficiency: >1 X 10^9 cfu/µg when transformed with non-saturating amounts of pUC19 control DNA.

Beneficial Traits:
- recA increases plasmid stability
- endA improves the quality of plasmid preparations
- lacZΔM15 blue/white screening
- T1 phage resistance

Genotype of GC5: F ø80lacZΔM15ΔlacZYA-argF)U169 endA1 recA1 relA1 gyrA96 hsdR17 (r^-m^-) phoA supE44 thi-1 λ-T1R

Package Sizes: GC5 competent cells are available in convenient unipacks for one time use, standard aliquots and in a 96-well plate format.

**GC10™ Chemically Competent Cells**

Comparable to: DH10B™

Uses: cDNA library generation from plasmid-based vectors, construction of gene banks and general cloning protocols.

Ideal for: cloning methylated and genomic DNA. Elimination of host restriction systems allows construction of more representative genomic libraries (allows cloning of methylated DNA).

Transformation Efficiency: >1 X 10^9 cfu/µg when transformed with non-saturating amounts of pUC19 control DNA.

Beneficial Traits:
- recA increases plasmid stability
- endA improves the quality of plasmid preparations
- lacZΔM15 blue/white screening
- mcrA, mrr does not restrict methylated DNA
- T1 phage resistance

Genotype of GC10: F mcrAΔ(mrr-hsdRMS-mcrBC) ø80lacZΔM15 ΔlacX74 endA1 recA1 Δ(ara, leu)7697 araD139 galU galK nupG rpsL λ-T1R

Package Sizes: GC10 chemically competent cells are available in convenient unipacks for one time use and in standard aliquots.

**Thunderbolt™ GC10™ Electrocompetent Cells**

Comparable to: DH10B™

Uses: cDNA library generation from plasmid-based vectors, construction of gene banks and general cloning protocols.

Ideal for: cloning methylated and genomic DNA.

Transformation Efficiency: >1 X 10^10 cfu/µg when transformed with non-saturating amounts of pUC19 control DNA.

Beneficial Traits:
- recA increases plasmid stability
- endA improves the quality of plasmid preparations
- lacZΔM15 blue/white screening
- mcrA, mrr does not restrict methylated DNA
- T1 phage resistance

Genotype of GC10: F mcrAΔ(mrr-hsdRMS-mcrBC) ø80lacZΔM15 ΔlacX74 endA1 recA1 Δ(ara, leu)7697 araD139 galU galK nupG rpsL λ-T1R

Package Sizes: Thunderbolt GC10 electrocompetent cells are only available in standard aliquots.
Section 2: STRAINS OF COMPETENT CELLS

Cloning Strains from Sigma-Aldrich

**JM109 Competent Cells**

**Uses:** generation of high-quality plasmid and single-stranded DNA.

**Ideal for:** generation of high quality plasmid and single-stranded DNA.

**Transformation Efficiency:** >1 x 10^8 cfu/µg when transformed with non-saturating amounts of pUC19 control DNA.

**Beneficial Traits:**
- **recA** increases plasmid stability
- **endA** improves quality of plasmid preparations
- **hsdR17** prevents the cleavage of heterologous DNA by an endogenous endonuclease
- **F’ episome** single strand stability
- **lacZΔM15** blue/white screening
- **T1** phage resistance

**Genotype of JM109:** F’ (traD36, proAB+ lacI, lacZΔM15) endA1 recA1 hsdR17(r−, m−) mcrA supE44 λ− gyrA96 relA1 Δ(lac-proAB) thi-1 lon

**Package Size:** JM109 competent cells are available in the convenient unipack format for single use.

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>J3895</td>
<td>JM109 Competent Cells, Unipack</td>
<td>10 x 50 µl</td>
</tr>
</tbody>
</table>

**HB101 Competent Cells**

**Uses:** routine subcloning and construction of cDNA libraries.

**Ideal for:** classic strain for general cloning purposes and also for cloning genomic DNA.

**Transformation Efficiency:** >1 x 10^8 cfu/µg when transformed with non-saturating amounts of pUC19 control DNA.

**Beneficial Traits:**
- **HB101** is a hybrid K12 x B strain.
- **recA13** minimizes recombination and aids in insert stability
- **hsdS20(r−, m−)** prevents cleavage of cloned DNA by endogenous restriction

**Genotype of HB101:** F−, hsdS20(r−, m−), xy/f5, λ−, recA13, galK2, ara14, supE44, lacY1, rpsL20(strr), leuB6, mtl-1

**Package Size:** HB101 competent cells are provided in the convenient unipack format for single use transformations.

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>H3788</td>
<td>HB101 Competent Cells, Unipack</td>
<td>10 x 50 µl</td>
</tr>
</tbody>
</table>
Section 2: STRAINS OF COMPETENT CELLS

Expression Strains

Expression strains are used to express a protein efficiently from a given construct. There are two points to consider when choosing an expression strain: the type of promoter system being used and the level of promoter control required.

T7 Promoter System

The first consideration is the type of promoter system being used. One of the most common systems is the T7 promoter system. The T7 promoter system works by utilizing T7 RNA polymerase to drive expression. Upon induction with IPTG, T7 RNA polymerase is made by the DE3 element in the chromosome. T7 RNA polymerase then recognizes the T7 promoter on the clone and leads to the production of RNA. Next, the RNA is translated into the protein. If a T7 promoter system is being used, BL21(DE3) strains are recommended. The DE3 designation indicates that the strain is lysogenic for a lambda prophage containing the inducible T7 RNA polymerase.

If the system does not require T7 RNA polymerase to drive expression, then the BL21 strain is a suitable choice. BL21 is an all-purpose expression strain directed by various expression vector systems such as, lac, trc, tac, λPL and araD. The strain naturally lacks two key proteases, lon and ompT. The absence of proteolytic activity from lon and ompT may reduce the degradation of some heterologous proteins expressed in the strain.

Promoter Control

Additional BL21(DE3) strains are available depending on the level of promoter control required. For instance, in the T7 system, T7 RNA polymerase expression is repressed by lacI, but a small amount of T7 RNA polymerase is still produced even without IPTG induction and with the presence of the lacIq allele. If the expressed protein is toxic, even the low level of protein expression is enough to make the cells sick. As a result, when producing a toxic protein, increased promoter control is vital. BL21(DE3) pLysS or pLysE competent cells are recommended for increased promoter control in T7 systems. BL21(DE3)pLysS competent cells express T7 lysozyme which is a natural inhibitor of T7 RNA polymerase. This allows for improved transcriptional control and reduction of “leaky” expression. BL21(DE3)pLysE competent cells also express the T7 lysozyme, but at higher levels than BL21(DE3)pLysS. This allows for a greater level of control over transcription and a greater reduction in “leaky” expression. This strain is usually required if the protein to be expressed is toxic to the cell.

Cloning in BL21

BL21 competent cells are traditionally referred to as “expression strains.” Cloning can be performed in BL21, but success depends on how efficient the cloning is. If using a cloning method with high yield and low background, then transformation can be done directly into BL21 to save time. If cutting, pasting, and screening, it is recommended to use the GC5 cloning strain to generate the construct and then put the constructed plasmid into BL21.

BL21 Geneotypes

The genotypes of BL21 and phage λDE3 are listed below. Note that the genotype of BL21(DE3) is just BL21 with the phage DE3 added.

BL21

\[ \text{F}, \text{ompT}, \text{hsdS}^{r,m}, \text{gal}, \text{dcm}, \text{lon} \]

λDE3

\[ \text{lacI}, \text{lacUV5-T7 gene 1}, \text{ind1}, \text{sam7}, \text{nin5} \]

BL21(DE3)

\[ \text{F}, \text{ompT}, \text{hsdS}^{r,m}, \text{gal}, \text{dcm}, \lambda\text{DE3 (lacI, lacUV5-T7 gene 1, ind1, sam7, nin5)}} \]

The Ideal Expression Strain

The ideal expression strain has the following traits:

- endA: does not degrade DNA
- hsd: does not restrict unmethylated DNA
- lacIq: better control over the lac promoter
- lon: lacks intracellular protease
- ompT: lacks extracellular protease
- pLysS: inhibits T7 RNA polymerase
- tonA: resistant to T1 phage
- trfA: replicated oriV plasmids

T7 RNA Polymerase

To learn more about the traits listed here and about other traits of expression strains, please see page 20 Appendix 1: Genotypes.
Section 2: STRAINS OF COMPETENT CELLS

Expression Strains from Sigma-Aldrich

Sigma-Aldrich offers BL21 strains with or without T7 RNA Polymerase and with varying levels of control over the lac promoter (pLysS and pLysE). The guide below will aid in the selection of the ideal BL21 strain for your expression needs.

<table>
<thead>
<tr>
<th>Features</th>
<th>BL21</th>
<th>BL21(DE3)</th>
<th>BL21(DE3) pLysS</th>
<th>BL21(DE3) pLysE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain background</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>Transformation efficiency</td>
<td>$10^6$</td>
<td>$10^7$</td>
<td>$10^6$</td>
<td>$10^6$</td>
</tr>
<tr>
<td>Restriction deficient (hsdSB)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>lon and/or ompT protease deficient</td>
<td>ompT, lon</td>
<td>ompT, lon</td>
<td>ompT, lon</td>
<td>ompT, lon</td>
</tr>
<tr>
<td>T7 Polymerase</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Deficient in cytosine methylation (dcm)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Deficient in galactose metabolism (gal)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>T1 phage resistant</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

BL21-T1

**Ideal for:** high level production of heterologous proteins directed by various expression vector systems (promoters such as lac, trc, tac, λPL and araD).

**Transformation Efficiency:** $>1 \times 10^6$ cfu/µg when transformed with non-saturating amounts of pUC19 control DNA.

**Beneficial Traits:**
- lon reduces degradation of some heterologous proteins expressed in the strain
- ompT reduces degradation of some heterologous proteins expressed in the strain
- tonA confers resistance to the lytic bacteriophages T1 and T5

**Genotype of BL21-T1**: FompT hsdSB($r_s^-$, $m_s^-$) gal dcm tonA lon

**Package Size:** BL21 competent cells are available in the convenient unipack format for one time use.

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>B2685</td>
<td>BL21-T1 Competent Cells, Unipack</td>
<td>10 x 50 µl</td>
</tr>
</tbody>
</table>

BL21(DE3)-T1

**Ideal for:** high level induction and expression of genes from any T7 promoter-based expression vector.

**Transformation Efficiency:** $>1 \times 10^7$ cfu/µg when transformed with non-saturating amounts of pUC19 control DNA.

**Beneficial Traits:**
- DE(3) indicates that the strain is lysogenic for a lambda prophage containing an inducible T7 RNA polymerase under control of the lacUV5 promoter
- lon reduces degradation of some heterologous proteins expressed in the strain
- ompT reduces degradation of some heterologous proteins expressed in the strain
- tonA confers resistance to the lytic bacteriophages T1 and T5

**Genotype of BL21(DE3)-T1**: FompT hsdSB($r_s^-$, $m_s^-$) gal dcm λ(DE3) tonA lon

**Package Size:** BL21(DE3)-T1 competent cells are available in the convenient unipack format for one time use.

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>B2935</td>
<td>BL21(DE3)-T1 Competent Cells, Unipack</td>
<td>10 x 50 µl</td>
</tr>
</tbody>
</table>
Section 2: STRAINS OF COMPETENT CELLS

Expression Strains from Sigma-Aldrich

**BL21(DE3)pLysS-T1**

**Ideal for:** high level induction and expression of genes from any T7 promoter-based expression vector.

**Transformation Efficiency:** >5 x 10^6 cfu/µg when transformed with non-saturating amounts of pUC19 control DNA.

**Beneficial Traits:**
- DE(3) indicates that the strain is lysogenic for a lambda prophage containing an inducible T7 RNA polymerase under control of the lacUV5 promoter.
- lon reduces degradation of some heterologous proteins expressed in the strain.
- ompT reduces degradation of some heterologous proteins expressed in the strain.
- tonA confers resistance to the lytic bacteriophages T1 and T5.
- pLysS The pLysS plasmid expresses T7 lysozyme, a natural inhibitor of T7 polymerase allowing for improved transcriptional control and reduction of “leaky” expression. The pLysS plasmid also renders the cell resistant to chloramphenicol (CmR) and contains the p15A origin. This allows pLysS to be compatible with plasmids containing the ColE1 or pMB1 origin.

**Genotype of BL21(DE3)pLysS-T1**: FompT hsdS(I−,mR−) gal dcm λ(DE3) tonA pLysS (CmR) lon

**Package Size:** BL21(DE3)pLysS-T1 competent cells are available in the convenient unipack format for one time use.

**BL21(DE3)pLysE-T1**

**Ideal for:** high level induction and expression of genes from any T7 promoter-based expression vector.

**Transformation Efficiency:** >1 x 10^6 cfu/µg when transformed with non-saturating amounts of pUC19 control DNA.

**Beneficial Traits:**
- DE(3) indicates that the strain is lysogenic for a lambda prophage containing an inducible T7 RNA polymerase under control of the lacUV5 promoter.
- lon reduces degradation of some heterologous proteins expressed in the strain.
- ompT reduces degradation of some heterologous proteins expressed in the strain.
- tonA confers resistance to the lytic bacteriophages T1 and T5.
- pLysE The pLysE plasmid expresses T7 lysozyme, a natural inhibitor of T7 polymerase allowing for improved transcriptional control and reduction of “leaky” expression. The pLysE plasmid expresses T7 lysozyme at higher levels than the pLysS plasmid conferring a greater level of control over the T7 polymerase. This is usually only required when the recombinant protein to be expressed may be toxic to the cell. The pLysE plasmid also renders the cell resistant to chloramphenicol (CmR) and contains the p15A origin. This allows pLysE to be compatible with plasmids containing the ColE1 or pMB1 origin.

**Genotype of BL21(DE3)pLysE-T1**: FompT hsdS(I−,mR−) gal dcm λ(DE3) tonA pLysE (CmR) lon

**Package Size:** BL21(DE3)pLysE-T1 competent cells are available in the convenient unipack format for one time use.

Visit sigma-aldrich.com/competentcells to view the presentation, “Everthing You Need to Know About Competent Cells,” by Michael Smith, Ph.D.
Section 3: TRANSFORMATION

What Is Important in a Transformation?

After selecting the ideal competent cell strain, transformation is the next step. There are two ways to accomplish transformation successfully: by chemical transformation or by electroporation. Regardless of whether the transformation is being done for cloning or expression, the transformation procedures are the same.

Before beginning a transformation, it is important to know what is required to achieve the best results. There are many factors that affect transformation including the following:

1. the form of DNA
2. the amount of DNA
3. the source of DNA
4. impurities in the DNA
5. storage and handling of the competent cells

Forms of DNA

Relaxed plasmids transform *E. coli* with the same efficiency as supercoiled plasmids. Linear plasmids and single-stranded plasmids transform very poorly (<1% as efficiently as double strand circles). A special host is needed to achieve chromosomal transformation, which is very inefficient. Usually, a transformation involves a mixture of linear (non-transforming) and circular (transforming) DNA.

Amount of DNA

Adding more DNA to a transformation does not necessarily lead to more transformants. For chemically competent cells, adding more than 10 ng of pUC19 DNA does not result in significantly more transformants. The point of diminishing returns is about 100 ng of pUC19 for electrocompetent cells.

With ligations, a ligation will have insert DNA, linear vector, re-circularized vector, and vector with insert (both circular and linear). The concentration of all components is usually about 50 ng/µL. Typically, the non-transforming DNA will be in the majority, but it will not usually out-compete the transforming DNA. With 20 ng of total DNA per reaction, the non-transforming DNA will decrease the efficiency of the transforming DNA approximately two-fold for chemically competent cells and will not affect the efficiency of electrocompetent cells.

If the ligation reaction is concentrated by precipitation and 500 ng of the ligation is added to a single reaction, the competition effects can drop the transformation efficiency ten-fold for chemically competent cells, but will not affect electroporation.

Source of DNA

DNA from eukaryotes is heavily methylated and *E. coli* has restriction systems that restrict these types of methylation. As a result, when cloning genomic DNA, it is recommended to use a *mcr* mutant like GC10. DNA generated by PCR is unmethylated, so cloning a PCR fragment from genomic DNA does not require a *mcr* mutant.

Impurities in DNA

Donor DNA should not have detergent, phenol, alcohol, PEG, or DNA binding protein in it. For electroporation, donor DNA cannot have salt in it. Ligase and PEG strongly inhibit transformation. A central problem in molecular biology is that both ligase and PEG are components of most ligation reactions. The best way to resolve this problem is to precipitate the ligation mixture, dilute the mixture three-fold and transform with 1 µL.
Section 3: TRANSFORMATION

What is Important in a Transformation?

Storage and Handling of Competent Cells
Proper storage and handling of competent cells is one of the most important factors affecting transformation. Simply handling the cells with care will prevent loss of efficiency.

Arrival
When the cells arrive in dry ice, they must be kept at –70 °C to retain maximum efficiency. This can be achieved by taking a few precautions. First, make sure the space is ready in the –70 °C for the cells before unpacking them. Once the space is ready, unpack the box at the freezer, place the cells in the pre-selected space in the –70 °C, and shut the door. There is no need to rush, but try to get the cells into the –70 °C with as little delay as possible. Even though the cells are frozen, they lose efficiency when they do warm up even a little.

Storage
Although the cells are stored at –70 °C, they will be subjected to temperature fluctuations constantly. When the –70 °C is opened, warm air goes in and cold air goes out. This is unavoidable, but the cells can be protected if they are stored in a spot where they are not in the way and are stored on a shelf that is not accessed very often.

Thawing the Cells
To thaw the cells, remove the vial from the –70 °C and thaw on ice for 5 – 10 minutes. The cells must be directly on ice! If time is an issue, the cells can be thawed by rolling the vial between your fingers. DO NOT thaw the cells in a water bath. By doing so, warming cannot be stopped when the cells reach 0 °C. If the cells stay in an ice bucket for one hour, that is acceptable. Beyond one hour, cells start losing efficiency approximately two-fold each hour. If the cells are left overnight, DO NOT use them! Use a new vial of cells.

Handling
Treat the cells gently. DO NOT pipet or vortex the cells. Mix the cells by gently tapping on the tube.

Refreezing Cells
If a tube of cells is thawed and all of the cells are not used, the remainder can be frozen. To refreeze, place the tube in crushed dry ice, in a dry ice-ethanol bath (the best option), buried in a bed of dry ice (the second best option) or by itself on a metal shelf in the –70 °C for one hour before placing it in the box. Efficiency will drop about two-fold. If the tube is simply placed back into the box and stored in the freezer, efficiency will drop five- to ten-fold.

Tips for Handling Competent Cells

1. Prepare space in the –70 °C freezer before unpacking the cells.
2. Keep the freezer door open for the shortest amount of time possible.
3. Thaw the cells directly on ice.
4. Be gentle when mixing.
5. Refreeze properly.
Section 4: TRANSFORMATION PROTOCOLS — CLONING

Chemical Transformation

Chemical transformation is achieved by suspending the cells in an ice-cold buffer that contains calcium chloride and other salts. Transformation occurs then the cells are warmed briefly. After transformation, the cells are diluted into media to recover. Finally, the cells are plated onto media that selects for transformants. Although the process is simple, there are important factors to remember when performing a chemical transformation.

What is Important in Chemical Transformation?

Purity of the DNA
Transformation frequency is affected by the purity of the DNA used. For example, too much salt in the DNA when performing electroporation can cause cells to explode. Whether the DNA comes from a PCR reaction, ligation, endonuclease digestion or other treatment, procedures to further purify the DNA can be performed if needed.

The following procedures are recommended to remove the impurities listed:

<table>
<thead>
<tr>
<th>Impurity</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td>Column purify the DNA or perform a phenol extraction.</td>
</tr>
<tr>
<td>Detergents</td>
<td>Perform ethanol precipitation to purify the DNA.</td>
</tr>
<tr>
<td>PEG</td>
<td>Column purify of perform either precipitation or ethanol purification.</td>
</tr>
</tbody>
</table>

Amount of Ligation Mix
The most common mistake when performing a transformation is to put too much ligation mix into the transformation. The protocols suggest using less than 1 µl of ligation. This amount is sufficient for any type of transformation. Adding more lowers the number of transformants. For chemically competent cells, the ligase and PEG in the mix inhibits transformation. To get the most transformants out of a ligation, there are two options:

1. Precipitate the ligation and resuspend it in TE.
2. Dilute the reaction three-fold in TE and use 1 µl per 50 µl competent cells.

Incubating the DNA with the Cells on Ice
Incubating on ice for 30 minutes is required for chemically competent cells. If the ice step is omitted and heat shock is performed immediately, efficiency will drop ten-fold. If incubated for only 15 minutes, efficiency will decrease three-fold. Occasionally, this is a corner to cut if pressed for time and maximum efficiency is not an issue.

Heat Shock
The heat shock works best in a thin-walled tube with a 42 °C water bath. A 45-second heat shock at 42 °C produces the best results, but one minute at 37 °C works almost as well (down two-fold). With Sigma-Aldrich Unipack cells, a 30-second pulse at 37 °C in the tube provides the best results.

Recovery Time
The effect of the expression time depends on the plasmid and strain. With pUC19 and GC5™, the efficiency is down ten-fold if plated without any expression time at all. It is down seven-fold if plated after 15 minutes and down three-fold if plated after 30 minutes. SOC medium provides two-fold better results than LB medium for chemically competent cells.

Plates
Some plates provide better results than others, but there are no magic plates. Plates less than six months old that are not too dry will provide good results. Be especially cautious with tetracycline plates. Tetracycline breaks down in light and produces toxic products that kill everything but contaminants. For best results, add the tetracycline to the plates when the agar has cooled and is ready to pour. Discard the plates after three months.
Section 4: TRANSFORMATION PROTOCOLS — CLONING

Chemical Transformation

Chemical Transformation Protocol

**Preparation:**
1. Equilibrate a non-shaking water bath to 42 °C.
2. Prepare LB agar plates with the appropriate antibiotic. If blue/white screening is desired, the plates should include 40 µg/ml X-Gal and 1 mM IPTG.
3. Agar plates should be placed in a 37 °C incubator for 30 minutes prior to plating.
4. Warm SOC to room temperature (20 – 25 °C).

**Standard Procedure:**
1. Remove the required tubes of cells from the -70 °C freezer, including one extra for the control DNA if desired. Place tubes immediately on wet ice so that only the cap is visible above the ice. Allow the cells to thaw on ice for approximately 5 minutes.
2. Visually examine the cells to ensure that they are thawed. Gently tap the vial several times to resuspend cells.
3. (Optional) Add 1 µl pUC19 control DNA to one tube of cells. Mix gently by tapping the tube. Return the cells to the ice.
4. Add 1 – 50 ng of the ligation reaction or purified plasmid DNA directly to cells. Mix as in step 3.
5. Incubate the cells on wet ice for 30 minutes.
6. Heat shock the cells by incubating the tubes in a 42 °C water bath for exactly 45 seconds.
7. Place the cells on ice immediately for at least 2 minutes.
8. Add 450 µl of room temperature SOC medium into each tube containing the cell/DNA mixture.
9. Incubate the cells at 37 °C with shaking (225 – 250 rpm) for 1 hour.
10. Pipette 10 – 100 µl of each transformed cell suspension onto LB agar plates containing selection antibiotic and evenly distribute using a sterile spreader. Plates should be pre-warmed to 37 °C for optimal colony growth. When inoculating less than 25 µl of cell suspension, first pipette a drop of SOC onto the plate and then add the cell suspension to the SOC.

**Note:** The amount of transformation mixture to plate varies with the efficiency of both the ligation and the competent cells. When using the control DNA, add no more than 10 µl into a drop of SOC on an LB agar plate containing 100 µg/ml ampicillin or carbenicillin.

11. Incubate the plates at 37 °C overnight (12 – 16 hours).
Section 4: TRANSFORMATION PROTOCOLS — CLONING

Electroporation

Electroporation of *E. coli* requires a high cell density and a non-ionic buffer. The same rules for storing and handling chemically competent cells apply to electrocompetent cells. In electroporation, the competent cells are thawed, mixed with donor DNA and placed in an electroporation chamber attached to an electroporation device. The apparatus delivers a 5-millisecond pulse of about 1,900 volts. Efficiencies of $10^{10}$ transformants per µg pUC19 DNA are expected from commercially prepared cells and efficiencies of $10^9$ for home-made cells.

What's Important in Electroporation?

Conductivity of the Sample
This is the most important factor. The conductivity of the sample should be as close to zero as possible. The number one cause of exploding electrocompetent cells is putting too much ligation mix into the transformation reaction.

Thawing the Cells
To thaw the cells, remove the vial from the ~70 °C freezer and thaw on ice for 5 – 10 minutes. The cells must be directly on ice. If time is an issue, the cells can be thawed by rolling the vial between your fingers. DO NOT thaw cells in a water bath. By doing so, warming cannot be stopped when the cells reach 0 °C. If the cells stay in an ice bucket for one hour, that is acceptable. Beyond one hour, cells start losing efficiency approximately two-fold each hour. If the cells are left overnight, DO NOT use them! Use a new vial of cells.

Incubating the Cells with DNA on Ice
It is not necessary to incubate the cells with DNA on ice. The cells can be left on ice for one hour, after that, efficiency decreases.

The Pulse
Instead of a heat shock, the cells are exposed to a very short, intense electric field. The pulse has to be 4 – 5 milliseconds at a minimum field strength of 20 kV per cm for Thunderbolt™ GC10™ cells. The field strength is usually achieved with a voltage of 2.0 kV and a 0.1 cm cuvette. With a 0.2 cm cuvette, it is impossible to reach this field strength because most machines can only deliver 2.5 kV. The length of the pulse is often achieved with a 25 µF capacitor and a 200 Ω shunt resistor.

Note that there are two combinations of voltage and pulse length that have proven to be efficient and practical: a higher voltage with a shorter pulse length and a slightly lower voltage with a longer pulse length. Both have been used effectively, but one combination may prove to be better under certain circumstances.

Sigma-Aldrich offers two elecroporators: The Electroporator, EC100 (catalog number Z375942) with AC input 120V and the Electroporator, EC100 (catalog number Z375950) with AC input 240V.

Recovery Time
The effect of the expression time depends on the plasmid and the strain. Electroporation of Thunderbolt GC10 cells with pUC19 only decreases two-fold in efficiency if plated without any expression time at all.

Plates
Plates are not an issue. Since electrocompetent cells are so concentrated, there can be an increased tendency to form satellites when high cell densities are plated. Satellites are untransformed cells that form small colonies in clusters around real transformants. Satellites do not grow when streaked on selective agar or when inoculated into selective media.

Optimizing a New Strain
The best transformation uses pulses of 4 – 5 milliseconds, although some protocols call for as much as a 10-millisecond pulse or as little as 2. To optimize for a new strain, start with a 4 – 5 millisecond pulse and vary the voltage so that the field strength varies from 15 kV/cm to as high as possible without exploding the cells (20 – 25 kV/cm). The pulse can be varied from 2 ms to 10 ms by changing the value of the shunt resistor. Try combinations. If the cells always explode, the problem may not be the pulse and voltage combination used, but may be due to too much salt in the cells. Try washing them again before electroporating.

Salts and Buffers Inhibit Electroporation
Experimental DNA should be in a low ionic strength buffer such as TE. Samples containing too much salt will result in arcing at high voltage that could harm the sample and/or the equipment. Ligation reactions should be diluted 5-fold in TE buffer prior to transformation. Use 1 µL of the diluted ligation reaction per 40 µL of electrocompetent cells.
Section 4: TRANSFORMATION PROTOCOLS — CLONING

Electroporation

Electroporation Protocol
(Using Thunderbolt™ GC10™ cells)

Preparation
1. Place the electroporation chamber on ice.
2. Prepare LB agar plates with the appropriate antibiotic.
   If blue/white screening is desired, the plates should include 40 µg/mL X-gal and 1 mM IPTG.
3. Agar plates should be placed in a 37 °C incubator 30 minutes prior to plating.
4. Warm SOC medium to room temperature (20 – 25 ºC).

Procedure
1. Remove the cells from the -70 ºC freezer and place directly in ice.
2. Place 1 mm standard cuvettes and autoclaved microcentrifuge tubes on ice, one per transformation.
3. Place 960 µL SOC medium in culture tubes, one per transformation.
4. Add the DNA (or 1 µl of control DNA diluted 5-fold) to the microcentrifuge tubes on ice.
5. Gently mix cells by tapping the tube.
6. Transfer 40 µl of the cells into chilled tubes containing DNA.
7. Pipet 40 µl of the DNA/cells mixture into a chilled 1 mm cuvette.
8. Electroporate at a field strength of 20 kV/cm for 6 ms.
9. Remove cells from the cuvette and place in culture tubes containing SOC medium.
10. Incubate at 37 °C for one hour with shaking (225 – 250 rpm).
11. Spread on LB agar plates containing appropriate antibiotic (e.g., 100 µg/mL ampicillin for control pUC19).
12. Incubate the plates at 37 °C overnight (12 – 16 hrs).
Section 4: TRANSFORMATION PROTOCOLS — CLONING

FAQs on Cloning

What are the optimal settings for electroporation?
There are two combinations of voltage and pulse length that have proven to be efficient and practical: a higher voltage with a shorter pulse length and a slightly lower voltage with a longer pulse length. Both have been used effectively, but one combination may prove to be better in your laboratory.

Shorter pulse: 2.5 kV, 100 $\Omega$, 25 mF
Longer pulse: 2.0 kV, 200 $\Omega$, 25 mF

Why didn’t I get any transformants (chemical transformation)?
There are multiple reasons that this could occur:
- The cells might not have been competent. Try the control DNA to make sure the cells are competent.
- The ligation might have failed. Does this donor work on any cells?
- Was the correct selection used for the plasmid?
- Were the cells concentrated by centrifugation? If so, were the cells handled gently? Try plating 0.1 mL before concentrating the rest of the cells.

Why didn’t I get any transformants (electroporation)?
Try the suggestions listed for chemical transformation. If the control did not work either, make sure that everything is hooked up correctly. If it is, do a test pulse:
1. Add 40 $\mu$L of LB or SOC to a used cuvette. It should arc.
2. If it does not arc, there is no connection or the machine is dead.
   - Have the cuvette chamber and the cables tested to make sure they conduct electricity.
   - If they are OK, the electroporation machine may be dead. Call the manufacturer.

Why did my cells explode when I pulsed them (electroporation)?
- Too much DNA was added to the reaction. Try adding less DNA or ethanol precipitating the DNA.
- Check the settings. Use 200 $\Omega$, 2.0 kV, 25 $\mu$F capacitor. The lower voltage is less likely to pop the cells.
- The cells may have too much salt in them. If you made them yourself, wash them one more time.

Why do I have a lot of little colonies around the big colonies?
These are satellite colonies. They are not transformants. Incubate the plates for less time, use more antibiotic, or use fresh plates to get rid of them. If 0.1 mL of an electroporation was plated, there will most likely be satellites. Ignore them, they will not grow overnight if using selection.

Why does my plate have colonies of all sizes?
The selection is off. There is either too much or too little antibiotic. To determine the problem, streak the cells that should grow on the selection and cells that should not. If the cells that should grow are struggling, there is too much antibiotic and transformants are being lost. Try half as much. If the cells that should not grow are growing where the streak is heaviest, there is not enough antibiotic. If there is not enough antibiotic, “breakthrough” of non-transformed cells that are mutants to a low level of resistance will appear. Try using twice as much antibiotic.

Why do my plates look like one giant colony?
The first thing to determine is if there is antibiotic on the plate. If not, don’t forget it next time. Another possibility is that the plate was wet. If so, these cells are swimmers.

When I use the control and calculate transformation efficiency, I get a number that is 2 – 4 times as high as the specification. Is this OK?
The specification is a minimum. This is good!

I left my cells in the ice bucket overnight. Are they still OK?
No. Do not use them unless you are desperate.

My freezer died, but the temperature only went to –50 °C before I was able to transfer the cells to a different –70 °C freezer. Can I still use them?
Yes, but expect a two- to five-fold loss in efficiency.
Section 5: TRANSFORMATION PROTOCOLS — EXPRESSION

The procedures to transform are the same whether cloning or expressing. The important points to consider before beginning a transformation should still be followed. Additionally, there are steps to take when working with BL21 for successful transformation.

Making a Stock Culture

The first thing to do when working with BL21s is to make a stock culture. This ensures that the clone does not change and that each expression run gives optimal performance.

1. Transform the BL21 strain to be used with the plasmid.
2. Pick a single transformant colony from a fresh plate into 30 mL of LB + ampicillin (+ chloramphenicol as well for pLysS or pLysE). A small amount will do.
3. Grow overnight. Room temperature is best. Turn the heater off on the shaker. Don’t worry, the cells will grow. Many people cannot just shut off the heat on a shared lab shaker. In that case, grow the cells at 30 ºC. If this is not possible and they have to grow at 37 ºC, make three cultures: one at full strength, the second a 10-fold dilution of the first flask, the third a 10-fold dilution of that. Use the flask that grew from the most diluted inoculum (this is the one that spent the least amount of time at stationary phase).
4. In the morning, dilute 10 mL of overnight culture with 10 mL of LB-20% glycerol.
5. Distribute 1mL each into 1.2 mL cryotubes (5 – 20 tubes). Freeze and store at -70 ºC. As long as they stay at -70 ºC, they will be unchanged.
6. Each time an expression is done, thaw out a stock culture and use that to start the culture. When down to the last tube, make a new stock culture. Using the original plasmid, make it the same way. Just subculture from the stock culture. Do a new test expression first to make sure that the strain has not lost viability. DO NOT use a culture that has been thawed more than once.

To subculture from a master tube without thawing it, remove the tube and place it on dry ice. Open it and scrape some material from the top with an inoculation loop or a toothpick. Inoculate in 1 ml of culture media with the scraping. Replace the tube in the freezer. DO NOT store the cultures as stabs, on plates, or in a tube in the refrigerator. When this is done, most of the cells die. Often, the cells that do not die are the cells that will not make the protein of interest anymore.

DO NOT make stock cultures from cells that have been induced.

Important: When making a stock culture, remember that in log growth, the T7 system is repressed and all cells are more or less competitive. In stationary phase, cells are stressed and many will die. Cells with the least amount of stress will survive. Therefore, never make a stock culture from cells that have been induced.

Tips for Transformation:

What is Important

1. Purity of the DNA
2. Amount of Ligation Mix
3. Incubating the DNA with Cells on Ice
4. Heat Shock or the Pulse
5. Recovery Time
6. Plates

Please see p. 12 and p. 14 for detailed explanations.
Section 5: TRANSFORMATION PROTOCOLS — EXPRESSION

General Expression Protocol

Test Expression
The key to a successful gene expression is to do a test expression first to determine the following:

- Does the clone really make the desired protein?
- How much protein is made?
- Is the protein soluble or insoluble?

Growth and Sampling
The following is a generic protocol that lacks some detail as many different types of media and methods are used. Cells in LB grow to a maximum of about 3 OD with good aeration. In rich media such as Terrific Broth, culture OD can reach 20 with excellent aeration. Minimal media can give results as low as 0.5 OD or as high as 20 OD, depending on conditions.

1. Dilute 1 mL of stock culture into 100 mL of media + ampicillin (+ chloramphenicol as well for pLysS or pLysE) in a 500 mL baffled flask.
   [Amp = 100 µL/mL, Cam = 25 µL/mL]
2. Grow the cells to 0.5 OD at 37 °C. This takes 2 – 3 hours. During this time, label five 15 mL conical centrifuge tubes as follows: 0, 1, 2, 3, O/N.
3. Harvest 10 mL of the uninduced (0 hours) sample. Spin the tube at 4,000 rpm for 20 minutes. Pour off the supernatant. Freeze the pellets.
4. Add 1 mL of 100 mM IPTG to the culture [final concentration will be 10 mM].
5. Measure the OD of the cells for each of the next 3 hours. Harvest 10 mL samples of the culture at each time: 1 hour, 2 hours and 3 hours after induction. Store the pellets at –20 °C.
6. Continue to express the cells overnight.
7. The next morning, harvest 10 mL of the cells. Note the time.

Separation of Insoluble and Soluble Protein
1. Dilute the samples to 2 mg/mL protein.
2. Sonicate the samples to disrupt the cells.
3. Remove 10 mL and electrophorese. This is the total protein.
4. Spin at 13,000 rpm for 5 minutes.
5. Remove 10 mL and electrophorese. This is the soluble fraction.

Protein Determination
The approximate amount of total protein (in mg) that can be expected in each sample depends on the OD of the culture when it was sampled, as follows:

OD of culture when sampled:

| 0.5 | 1  | 2  | 3  | 4  | 5  | 7.5 | 10 | 15 | 20 |

Approximate concentration of protein in the 1 mL resuspension of the sample pellet (mg/mL):

| 0.25 | 0.5 | 1  | 1.5 | 2  | 2.5 | 3.8 | 5  | 7.5 | 10 |

Resuspend the pellets in 1 mL TE. Measure the total protein in each of the 1 mL samples using Bradford (Catalog Number B6916), BCA (Catalog Number BCA1), or equivalent with a standard curve. It is important to do this, as the gel analysis is much easier if each lane has the same amount of protein.

Gel Electrophoresis
Load the same amount of protein in each lane of the gel. Analyze the samples by gel electrophoresis, looking at all of the samples: 0 (uninduced), 1, 2, 3, and O/N hours of induction. It is recommended to compare soluble protein and total protein. Insoluble protein is the total minus the soluble. The amount of protein in each sample depends on the amount of cells. The amount of cells in each sample depends on the clone and the media being used. Measure the amount of protein and load the same amount of protein in each lane of the gel.
Section 5: TRANSFORMATION PROTOCOLS — EXPRESSION

General Expression Protocol

The test expression experiment can be recorded in any way you like. A form such as this, or a derivative can be used:

<table>
<thead>
<tr>
<th>Time</th>
<th>Time post-inoc.</th>
<th>OD</th>
<th>Vol. Assayed</th>
<th>Reading</th>
<th>Protein/mL</th>
<th>Total Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>1</td>
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<td>3</td>
<td></td>
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</tr>
<tr>
<td>O/N</td>
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</tbody>
</table>

**Analysis of Test Expression Results**

There are many possible outcomes to the test expression experiment. Potential results include:

- Cells are not inhibited by induction and produce a lot of soluble protein. This is ideal. Scaling up is recommended.

- Cells are not inhibited by induction, but do not make very much protein. This is not the ideal result; however, an amino terminal fusion can be made with a protein that *E. coli* does express well, such as thioredoxin. If the fusion protein is made, initiation of translation of the protein is inefficient. If using *E. coli*, stay with the amino fusion.

- Cells are inhibited by induction and make a lot of protein. This is good. Induce at the highest OD possible, which will depend on the media being used and aeration. Induce at 1/3 of the final OD obtained when not inducing.

- Cells are inhibited by induction and do not make very much protein. This is not good. Induce at the highest OD possible, which will depend on the media being used and aeration. Induce at 1/3 the final OD obtained when not inducing. Whatever protein is being produced is killing off the cells. This is the best that can be achieved in this system.
FAQs on Expression

I store my cultures on plates that I keep in my refrigerator. When I inoculate media with these cultures, the cells are stringy and take a long time to grow. Why?

The stringy material is dead cells. The survivors will not make as much of the protein. Retransform BL21 with the original plasmid and make stock cultures. Never store BL21 on a plate as this discriminates against cells that are making the protein. Storage on a plate “favors” the wrong cells.

I’m working with BL21(DE3)pLysS and I’m getting a lot of background expression. Why?

Is selection done for pLysS as well as the plasmid? This should be done by placing chloramphenicol (Cm) in selective plates and growth media. If selecting with Cm and still getting a lot of background, then the construct is super hot. If this is a problem, switch to a promoter system that has better control, such as PL. Some researchers use BL21(DE3)pLysE because it produces even more inhibitor of background T7 expression. This strain can be tricky to use and sometimes grows poorly, which could be due to increased background expression of T7 lysozyme from the tet promoter.

When I induce my cells, they stop growing. After three hours, there is stringy stuff in the flask. After incubating overnight, there are not a lot of cells, but I can hardly see my protein. Why?

If the OD level is off when the cells are induced, this will happen. If the OD drops, that means that the induced protein is lethal. Overnight, something grows and it is probably something that has killed the protein gene. What can be done now is to grow to 1 OD before induction, and harvest in 1 – 3 hours.

I’m working with BL21(DE3)pLysS and I’m getting different sized colonies. Why?

Is there chloramphenicol in the plates? If not, big colonies may be ones in which the pLysS plasmid has been lost, because it inhibits growth. On the other hand, the big colonies might be the one WITH pLysS because the background expression of your gene is killing the cells in the absence of pLysS. Streak the big colonies and the little colonies on chloramphenicol to see which is occurring.

When do the control and calculate transformation efficiency, I get a number that is 2 – 4 times as high as the specification. Is this OK?

The specification is a minimum. We are getting BL21s at 10^8 quite often.

I left my cells in the ice bucket overnight. Can I still use them?

Not unless it is a desperate situation. Get a new vial of cells.

Can I clone in BL21?

Yes. Success depends on how efficient the cloning is. If using a fancy cloning method with high yield and low background, then transformation straight into BL21 can be done to save time. If cutting, pasting and screening, it is better to use a cloning strain (like GC5™) until the desired result is achieved before putting the plasmid into BL21.

I checked the plasmid in my BL21 transformant and the digest looks like it is degraded. What could be wrong?

BL21 derivatives have an endonuclease that degrades all DNA. Extract the plasmid prep with an equal volume of phenol, and then ethanol precipitate. The yield should be more than 50% and the resulting plasmid prep should be fine.

My freezer died, but the temperature only went to –50 °C before I was able to transfer the cells to a different –70 °C freezer. Can I still use them?

Yes, but expect a two- to five-fold decrease in efficiency.
Appendix 1: GENOTYPES

Genotypes

Blue/white screening: pUC19 and similar plasmids code for β-galactosidase (lacZ), which cleaves X-gal and turns colonies blue on X-gal plates. Inserts cloned into the plasmid disrupt the β-galactosidase gene resulting in white colonies. The plasmids only code for a small part of the β-galactosidase gene (the α peptide) and the chromosome codes for the rest. Both parts are required for activity. Since the plasmid is complementing the chromosomal mutation, this effect is called “α complementation.”

Cytosine Methylation deficient (dcm)
It is unknown why E. coli K12 methylates certain sites (CCAGG and CCTGG). E. coli B does not do this, therefore BL21 does not do this.

Endonuclease Deficient (endA)
E. coli has a powerful endonuclease on the outside of the cell that degrades any type of DNA. EndA mutants do not have this capability. The endA endonuclease has little or no effect on transformation efficiencies, but can have a great effect on the quality of plasmid DNA preparation. If plasmid DNA preps degrade when placed in magnesium-containing bugs, it is usually because the DNA was made from an endA bug. To prevent DNA degradation in plasmid preps, choose an endA– host.

Galactose Metabolism deficient (gal)
This mutation prevents BL21 from using galactose as a carbon source.

lac Promoter Control (lacI)
It is important when making plasmid constructs to keep the expression promoter off until ready to turn it on. High-level expression of many genes is bad for the host. When this happens, mutants that do not express the gene at a high level grow faster and take over the culture. Some expression systems use the lac, tac, or trc promoters to express cloned genes on high-copy plasmids, but do not have the cognate repressors on the expression plasmid. Under these circumstances, there is not enough lac repressor to keep the promoters off. A mutant that produces more lac repressor (lacI) can repress lac, tac, and trc promoters until IPTG is added to induce them.

Ion protease deficient (lon)
The parental E.coli B strain is a lon mutant, and therefore BL21s are lon mutants. The lon protein serves two functions in E. coli: it degrades misfolded (abnormal) proteins and it degrades normal proteins. This is an intracellular process, which includes degradation of the protein that you want to express. The lon protease degrades the protein before the cells are even lysed. To avoid this, expression strains are lon–.

Methyl Restriction Deficient (mcrA, mcrB, mrr)
E. coli has a system of enzymes that degrade DNA if it is methylated at the “wrong” sites. Genomic DNA from eukaryotic sources is methylated at all of the wrong sites as viewed by E. coli. When cloning genomic DNA from eukaryotic cells, it is essential to use a host that is deficient in all three of these methyl restriction systems. When cloning PCR fragments, cDNA, or fragments from previously made clones, there is no methyl restriction and it is not necessary to use a methyl restriction deficient host. When cloning genomic DNA, the cloning hosts should be mcr–.

Outer Membrane Protease deficient (ompT)
ompT is a protease that E. coli makes which sits on the outer surface to degrade extracellular proteins. It degrades the protein after the cells are lysed. Those that prefer intact protein demand that the E. coli proteases be eliminated, but this is not entirely possible. By eliminating ompT and lon, BL21s are able to maintain an optimal balance.

pLysS and pLysE
The pLysS and pLysE plasmids express T7 lysozyme, a natural inhibitor of T7 polymerase, allowing for improved transcriptional control and reduction of “leaky” expression. The pLysE plasmid expresses T7 lysozyme at higher levels than the pLysS plasmid conferring a greater level of control over the T7 polymerase. This is usually required only when the recombinant protein to be expressed may be toxic to the cell. Both the pLysS plasmid and the pLysE plasmid render the cells resistant to chloramphenicol (CmR) and also contain the p15A origin, which allows compatibility with plasmids containing CoIE1 or pMB1 (derivative of pBR322) origin.
Appendix 1: GENOTYPES

Genotypes

Phage Resistance (*fhuA*, *tonA*, T1*)
T1 phage and related phages kill *E. coli*. It is possible to have T1 phage infection. Unlike other phage, T1 is resistant to drying and is almost impossible to eliminate. The T1 resistance marker protects clones and cells with T1 resistance are fast becoming standards in the lab.

Recombination Deficient (*recA*)
*E. coli* has a repair system that will recombine homologous sequences. There is concern that recombination can cause plasmids to rearrange or to delete insertions. As a result, cloning strains are generally *recA* mutants. *recA* strains have the advantage of having a simple plasmid profile. *recA*+ strains have dimers, trimers and their nicked relatives. To prevent almost all homologous recombination, choose a *recA*– host.

Genomic clones often have duplicated regions, but they are generally short, tandem duplications. These duplications are unstable, but this is not due to *recA* function. If the length of the duplicated sequence is less than 200 bp, *recA* has no effect.

The *recA* repair system is also useful to *E. coli*. Disabling it will cause the cells to grow slower and they are generally less healthy. For this reason, some expression strains are not *recA* mutants.

Restriction Deficient (*hsdR*)
Most lab strains are *E. coli* K12 derived. K12 strains methylate their DNA at K12 sites (AAC(N6)GTGC and GCAC(N6)GTT). In K12 strains, DNA that is not methylated at these sites is degraded by a restriction enzyme. Many, but not all, cloning strains of *E. coli* are mutated in the gene that codes for this restriction enzyme. BL21 (expression strain) does not methylate, nor does it restrict unmethylated DNA. To avoid restriction problems, all hosts should be *hsdR*–.

Single Strand Ability (*F*+ or *F*)
*F* is a huge plasmid (99 kb) that is naturally found in *E. coli* K12. There are derivatives of the *F* plasmid that also contain chromosomal DNA. These *F* derivatives with a bit of chromosomal DNA are called *F*’ plasmid. *E. coli* with the *F* (or *F*’) plasmid make special surface features that allow them to be infected with M12 and similar phage. This property is useful if one wants to make single-stranded DNA or generate phage display libraries.

T7 polymerase (*λDE3* with *lacUV5*-T7 gene 1)
When people say BL21, they usually mean BL21(DE3), which is a derivative of BL21 that has the T7 RNA polymerase gene under the control of the *lacUV5* promoter. The *lacUV5* promoter is a mutant *lac* promoter that is stronger than a wild type *lac* promoter. The whole arrangement is on a *λ* phage genome, and this particular version of the *λ* phage is called *λDE3*. *λDE3* is inserted into the chromosome of BL21 to make BL21(DE3). There are other markers on this *λDE3* genome (*lac*, *ind1*, *sam7*, *nin9*), but none will affect expression.

For more information on genotypes, visit sigma-aldrich.com/competentcells
## Appendix 2: SUPPORTING PRODUCTS

### Supporting Products

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
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<tbody>
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### Ampicillin

<table>
<thead>
<tr>
<th>Description</th>
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<tbody>
<tr>
<td>Ampicillin Sodium Salt-powder</td>
</tr>
<tr>
<td>Ampicillin Sodium Salt-powder, cell culture tested</td>
</tr>
<tr>
<td>Ampicillin Sodium Salt-powder, cell culture tested, minimum 98% (titration)</td>
</tr>
<tr>
<td>Ampicillin Ready-Made Solution</td>
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</table>

### Carbenicillin

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<tr>
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<tbody>
<tr>
<td>Carbenicillin Disodium Salt</td>
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<tr>
<td>Carbenicillin Ready-Made Solution</td>
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### Electroporator, EC100

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<td>AC Input 120V</td>
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<td>AC Input 240V</td>
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### IPTG (Isopropyl β-D-1-thiogalactopyranoside)

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<tbody>
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<td>IPTG</td>
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<tr>
<td>IPTG Ready-Made</td>
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### LB Agar

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<tr>
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<tbody>
<tr>
<td>LB Agar Powder</td>
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<tr>
<td>LB Agar EZMix Powder</td>
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<tr>
<td>S-Gal™/LB Agar Blend</td>
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### LB Mediums

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<tr>
<th>Description</th>
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<tbody>
<tr>
<td>LB Broth Powder</td>
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<tr>
<td>LB Broth EZMix™ Powder</td>
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<tr>
<td>LB Broth Liquid</td>
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</tbody>
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### Pre-Poured Agar Plates

<table>
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</thead>
<tbody>
<tr>
<td>LB Agar Ampicillin - 100 (10 plates)</td>
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<tr>
<td>LB Agar (10 plates)</td>
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### Protein Determination Reagents

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<tbody>
<tr>
<td>Bicinchoninic Acid (BCA) Kit</td>
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<tr>
<td>Bradford Reagent</td>
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### SOC Medium

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<tr>
<td>SOC Medium</td>
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### Terrific Broth

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<tbody>
<tr>
<td>Terrific Broth, Modified Powder</td>
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<tr>
<td>Terrific Broth, Modified EZMix Powder</td>
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<tr>
<td>Terrific Broth Liquid</td>
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### X-gal

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<td>X-gal, minimum 98%</td>
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<td>X-gal, tablet</td>
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*EZMix™ and S-Gal™ are trademarks of Sigma-Aldrich.*
ACKNOWLEDGEMENTS AND GENERAL REFERENCES

Acknowledgements

We would like to thank GeneChoice, Inc. for their support and content in the Competent Cell Compendium: Tools and Tips for Successful Transformation.

General Reference

Original Papers on Chemical Transformation of Escherichia coli

High Efficiency Transformation of Escherichia coli Grown at 37 °C

General Review and Methods of Preparing and Using Competent Cells

General Review of Transformation of Bacteria

High Efficiency Transformation of Escherichia coli Grown at 18 °C

Electroporation of Escherichia coli

Practical Aspects of Electroporation

Everything about Molecular Cloning — Transformations, too

Original Description of Escherichia coli

Derivation of BL21

Original Description of the T7 System

pLysS and pLysE

Excellent Reviews on Gene Expression in Escherichia coli

Rare Codons

Fusion Proteins
To a modified DsbA

To a phage protein

To maltose binding protein
Kapust, R.B. and D.S. Waugh (1999). Escherichia coli maltose-binding protein is uncommonly effective at promoting the solubility of polypeptides to which it is fused. Protein Sci. 8: 1668-1674.

To anything, but the order of fusion matters

Proteases

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