

CLONING

Competent Cells

+ How do you choose the right competent cell?

INNOVATIVE
SPECIALTY CELLS

HIGH
EFFICIENCY

UNIQUE
SOLUTIONS


STRATAGENE

An Agilent Technologies Company

+ **We have your competent cells.**
 Finding the right competent cell for your application is easy with our extensive line of competent cells! We have innovative strains to match every cloning strategy. Our comprehensive collection of strains includes the highest transformation efficiencies available. Our specialty cells are ideal for your difficult or unusual cloning projects. Use the accompanying chart on this page to match your application to the perfect strain.

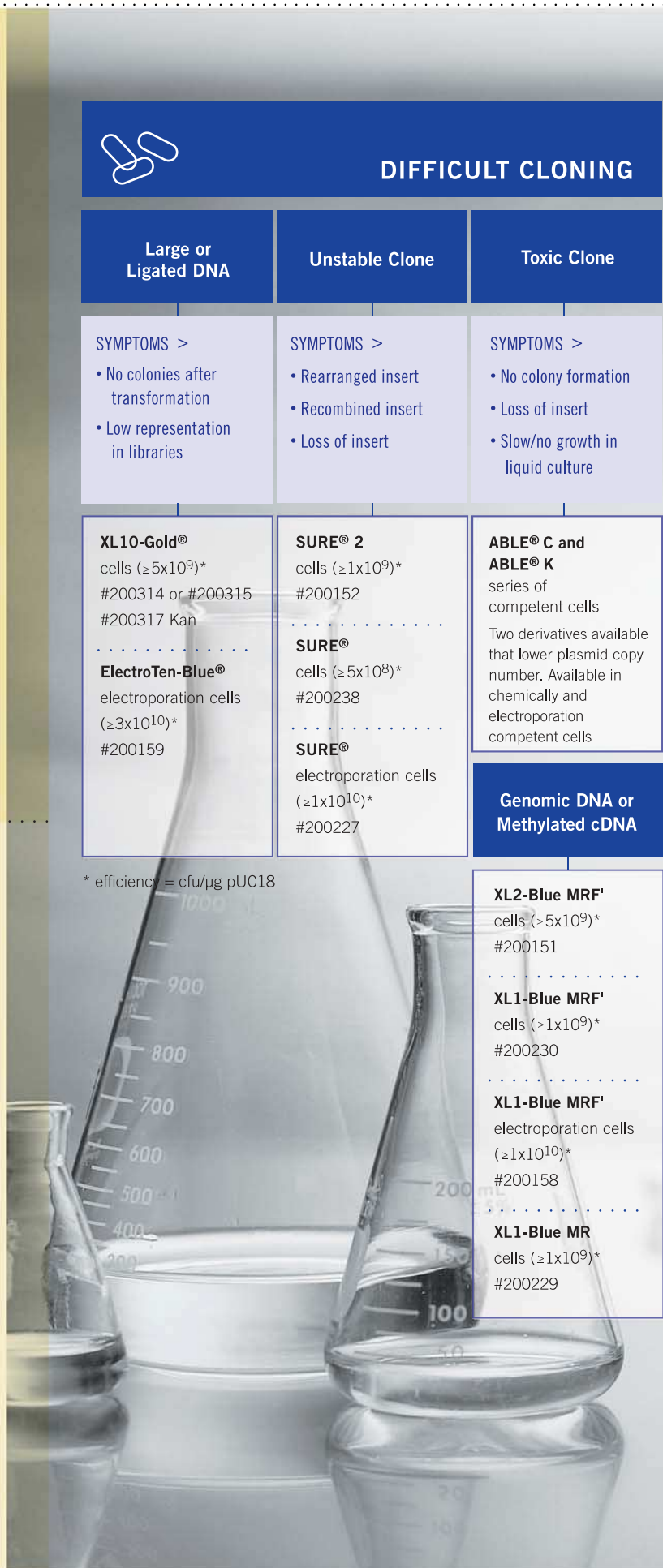
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 DIFFICULT CLONING		
Large or Ligated DNA	Unstable Clone	Toxic Clone
SYMPTOMS > <ul style="list-style-type: none"> • No colonies after transformation • Low representation in libraries 	SYMPTOMS > <ul style="list-style-type: none"> • Rearranged insert • Recombined insert • Loss of insert 	SYMPTOMS > <ul style="list-style-type: none"> • No colony formation • Loss of insert • Slow/no growth in liquid culture
XL10-Gold® cells ($\geq 5 \times 10^9$)* #200314 or #200315 #200317 Kan ElectroTen-Blue® electroperoration cells ($\geq 3 \times 10^{10}$)* #200159	SURE® 2 cells ($\geq 1 \times 10^9$)* #200152 SURE® cells ($\geq 5 \times 10^8$)* #200238 SURE® electroperoration cells ($\geq 1 \times 10^{10}$)* #200227	ABLE® C and ABLE® K series of competent cells Two derivatives available that lower plasmid copy number. Available in chemically and electroperoration competent cells
		Genomic DNA or Methylated cDNA

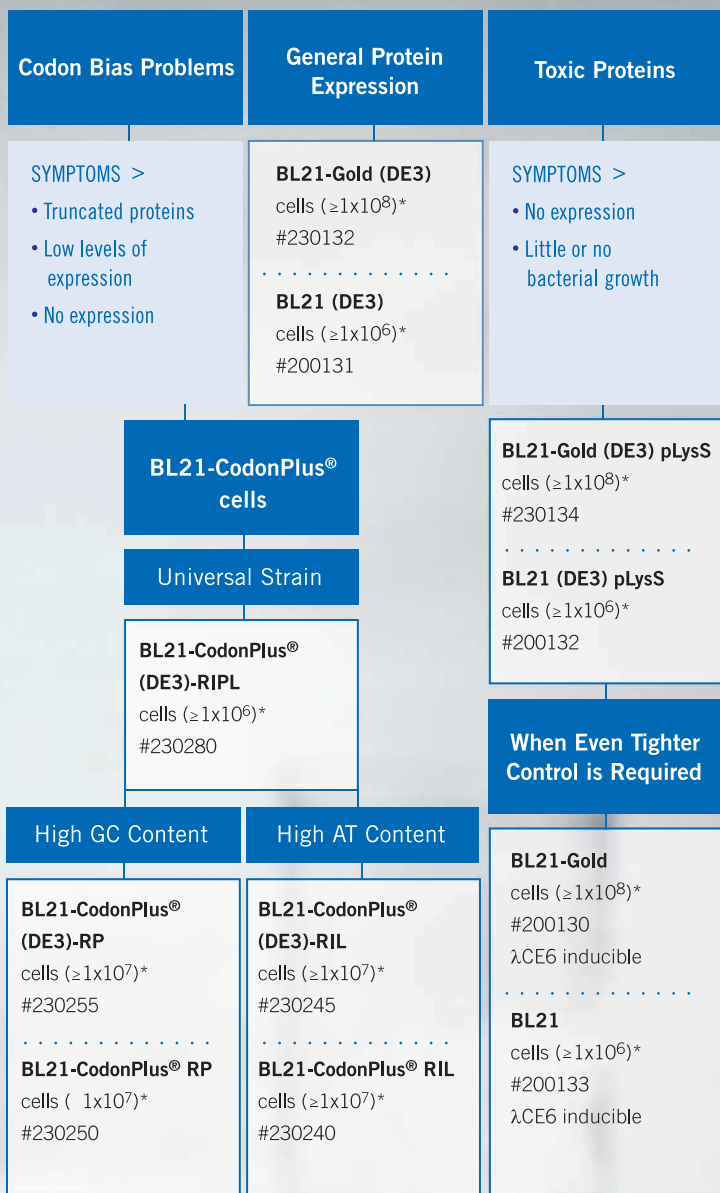
* efficiency = cfu/ μ g pUC18

XL2-Blue MRF® cells ($\geq 5 \times 10^9$)* #200151 XL1-Blue MRF® cells ($\geq 1 \times 10^9$)* #200230 XL1-Blue MRF® electroperoration cells ($\geq 1 \times 10^{10}$)* #200158 XL1-Blue MR cells ($\geq 1 \times 10^9$)* #200229





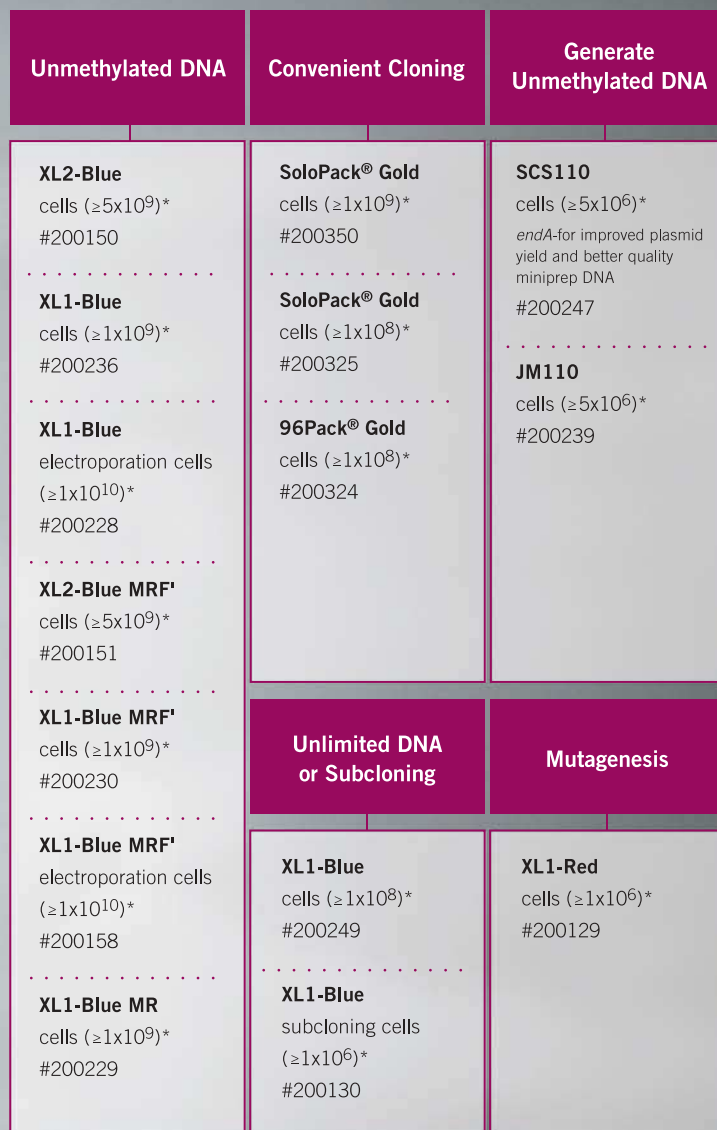
PROTEIN EXPRESSION



* efficiency = cfu/ μ g pUC18



GENERAL CLONING



* efficiency = cfu/ μ g pUC18

Get Your Clone with Our Competent Cells

From the high-efficiency ultracompetent and electroporation-competent cells to the reliable subcloning-grade competent cells, our competent cells feature the widest range of cloning efficiencies available. Whether you are cloning small amounts of DNA or doing routine day-to-day cloning, we have the right efficiency and genotype for every application.

Subcloning-Grade Cells

$\geq 1 \times 10^6$ transformants/ μg of supercoiled DNA

- + **SUBCLONING-GRADE COMPETENT CELLS** are perfect when you don't need high efficiency, but do need consistent results every day. They are the economical choice for routine subcloning procedures when DNA is not limited. The XL1-Blue strain is available as subcloning-grade competent cells.

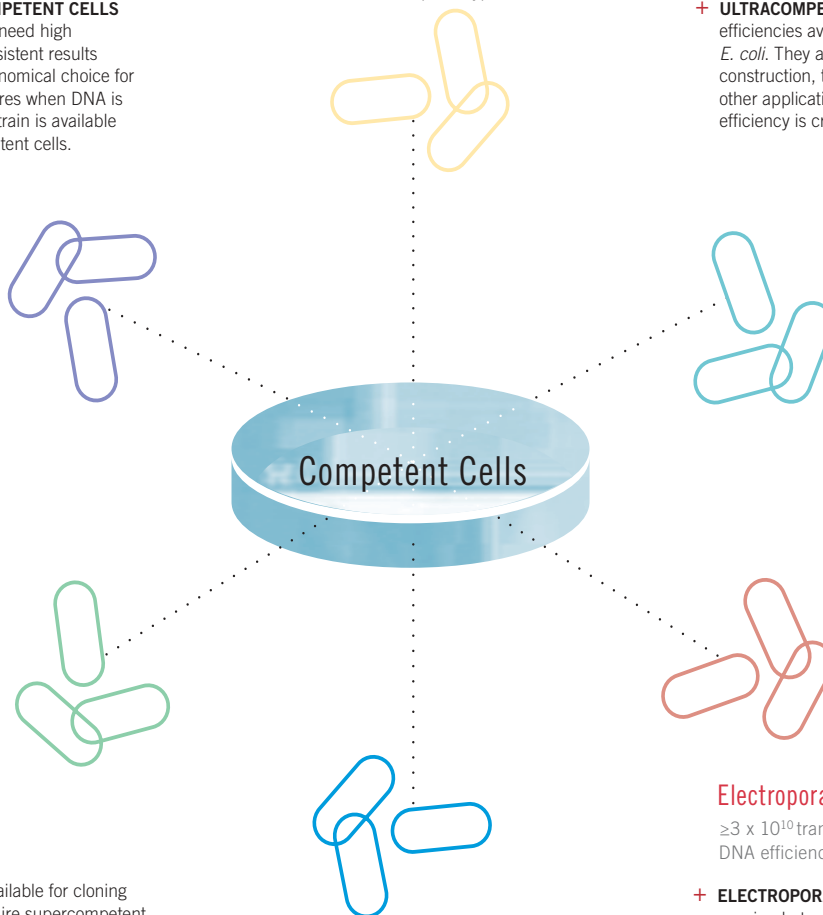
Hte Phenotype¹/Hee Phenotype

- + **HTE AND HEE PHENOTYPES** were developed to enhance competent cell performance. These phenotypes allow you to efficiently transform large plasmids and ligated DNA. XL10-Gold², BL21-Gold³, BL21-CodonPlus⁴, SoloPack⁵ Gold⁵ and 96Pack⁶ Gold⁶ cells each contain this novel phenotype. ElectroTen-Blue⁷ electrocompetent cells contain the Hee phenotype.

Ultracompetent Cells

$\geq 5 \times 10^9$ transformants/ μg of supercoiled DNA

- + **ULTRACOMPETENT CELLS** provide the highest efficiencies available for chemically competent *E. coli*. They are perfect for plasmid library construction, transforming large constructs or other applications where optimal transformation efficiency is critical.



Competent Cells

$\geq 1 \times 10^8$ transformants/ μg of supercoiled DNA

- + **COMPETENT CELLS** are available for cloning procedures that do not require supercompetent efficiencies. At 1×10^8 transformants/ μg , this group of competent cells is the economical alternative for routine cloning.

Supercompetent Cells

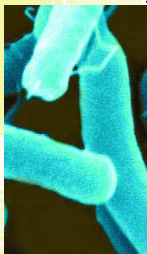
$\geq 1 \times 10^9$ transformants/ μg of supercoiled DNA

- + **SUPERCOMPETENT CELLS** are available in a wide variety of strains at efficiencies greater than 1×10^9 transformants/ μg . These superior-quality cells include the following: XL1-Blue, XL1-Blue MRF⁷, XL1-Blue MR, XL1-Blue MRF⁷ Kan, SoloPack⁸ Gold and SURE⁹2 supercompetent cells.

Electroporation - Competent Cells

$\geq 3 \times 10^{10}$ transformants/ μg of supercoiled DNA efficiency for ElectroTen-Blue⁷ cells

- + **ELECTROPORATION-COMPETENT CELLS** are simple-to-use. Ease-of-use and high efficiency make electroporation a popular method for library construction, cloning large inserts or cloning limited amounts of DNA. Our high-performance ElectroTen-Blue⁷ cells survive electroporation treatment better than other cells, giving them superior cloning efficiency for ligated DNA. XL1-Blue⁸, XL1-Blue MRF⁹, SURE¹⁰, ABLE¹¹ and TG1¹² cells are also available as high efficiency ($\geq 1 \times 10^{10}$ transformants/ μg of supercoiled DNA) electroporation-competent cells.



The Hte and Hee Phenotypes

Large and Ligated DNA

At Stratagene, we know it is more difficult to introduce large or ligated DNA constructs into competent cells than supercoiled DNA or small plasmids. We have developed the Hte (high transformation efficiency) and Hee (high electroporation efficiency) phenotypes to enhance competent cell performance for your chemical and electroporation transformations. Increased performance translates into increased success in obtaining representative primary and cDNA libraries.

High Transformation Efficiency

The XL10-Gold[®] ultracompetent cells were designed to transform large plasmids and ligated DNA with the highest transformation efficiency possible, while exhibiting faster growth and larger colonies. This strain was created by moving the Hte phenotype into our highest-efficiency strain, XL2-Blue MRF⁺.

We ran a series of assays to demonstrate the Hte phenotype's ability to improve competent cell performance. In the first assay, 500 ng of the pRK2013 plasmid (25kb) was transformed into XL10-Gold cells, XL2-Blue cells and DH10B cells. The XL10-Gold cells were 80-fold more efficient than the other cell lines with this large supercoiled plasmid (Figure 1). In the second assay, we tested for the ability of XL10-Gold cells to transform an 8-kb, non-supercoiled DNA molecule, generated by the ligation-independent cloning technique (LIC). The XL10-Gold strain proved 27-fold more efficient than the general cloning host DH5 α (data not shown).

High Electroporation Efficiency

The Hee (high electroporation efficiency) phenotype improves the survival rate of electroporated cells, resulting in a significant increase in transformation efficiencies. The theoretical efficiency with which *E. coli* cells become transformed is approximately 3×10^{11} colony-forming units (cfu) per microgram of supercoiled pUC plasmid DNA. To date, the actual values from the highest-efficiency hosts have ranged from 5×10^9 for chemical transformations to 1×10^{10} cfu for electroporation procedures. Data suggests this difference is partly due to harsh electroporation conditions that reduce the number of surviving cells taking up the plasmid DNA. Our ElectroTen-Blue[®] Electroporation-Competent cells, with an average efficiency of $\geq 3.0 \times 10^{10}$ (Figure 2) and the Hee phenotype significantly increase your ability to transform large or ligated DNA, obtain representative primary libraries, and ensure success in any cloning project.

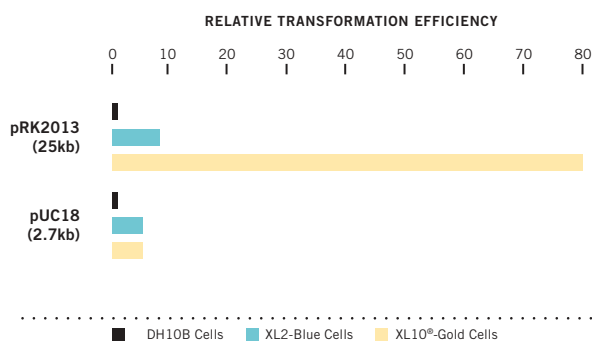


FIGURE 1
XL10-GOLD[®] CELLS TRANSFORM LARGE DNA AT HIGHER EFFICIENCIES 100 μ g of the pUC18 plasmid (2.7 kb) or 500 ng of the pRK2013 plasmid (25 kb) was transformed into 100 μ l of *E. coli* competent cells. 500 ng of the pRK2013 plasmid is used to compensate for the lower transformation efficiency. Aliquots of each transformation were selected on the appropriate antibiotic-agar plates and the lowest efficiency was set to one to calculate relative transformation efficiency.

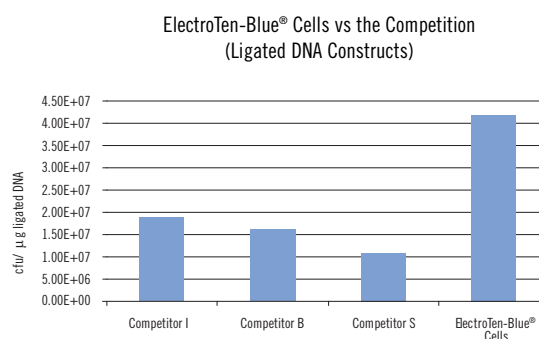


FIGURE 2
ELECTROTEN-BLUE[®] CELLS AND LIGATED DNA ElectroTen-Blue[®] Electroporation-Competent Cells consistently outperform "highest efficiency" electroporation-competent cells from other suppliers. The pBluescript[™] cloning vector was ligated to a 0.8-kb orange fluorescent protein (OFP) fragment and electroporated following manufacturing guidelines.

XL10-Gold[®] Ultracompetent Cells

Highest Efficiency Chemically Competent Cells

XL10-Gold[®] ultracompetent cells provide the highest chemical transformation efficiencies of large plasmids and ligated DNA. The XL10-Gold strain allows cloning of methylated DNA and produces high-quality miniprep DNA. Plasmid libraries constructed in this strain are more representative because XL10-Gold cells decrease the bias against large inserts.

Large DNA

XL10-Gold[®] cells are the only chemically competent cells that allow you to efficiently transform large DNA molecules, including expression vectors and genomic DNA. XL10-Gold ultracompetent cells are the host cells of choice when you need the highest transformation efficiencies for large constructs.

Optimal Plasmid Libraries

XL10-Gold ultracompetent cells are ideal for plasmid library construction. Ligated plasmid DNA generally transforms with significantly lower efficiency than supercoiled plasmids and larger plasmids will transform less efficiently than the smaller plasmids. The bias against large DNA molecules impacts the construction of plasmid libraries and reduces the probability of finding full-length cDNA clones. In addition, larger plasmid library vectors, such as two-hybrid vectors and eukaryotic expression vectors,

potentially increase this size bias. XL10-Gold cells decrease this size bias and produce more colonies for a more representative library.

To demonstrate the ability of XL10-Gold cells to produce the largest number of colonies, we transformed them with several plasmid cDNA libraries. The libraries were transformed into other cloning hosts and the resulting colonies were counted. Compared to the other hosts, XL10-Gold cells produced the most colonies, with 25-fold higher efficiency (Figure 3).

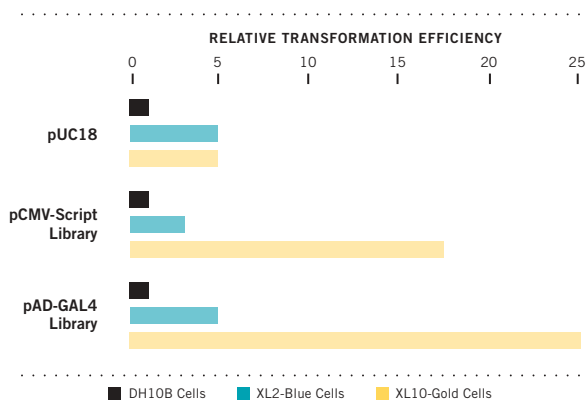
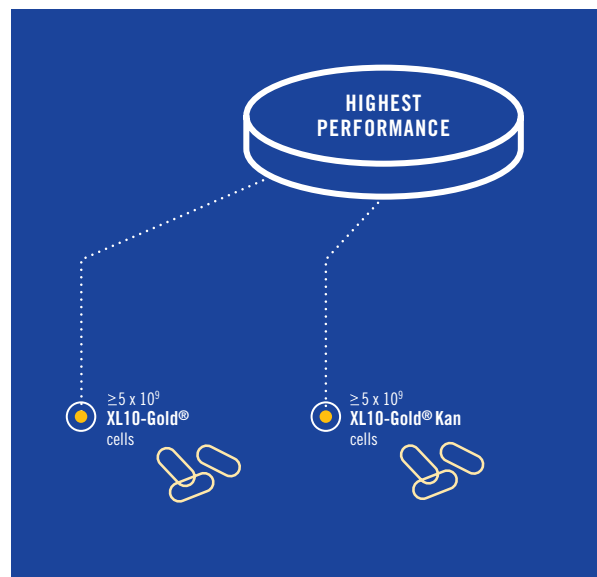


FIGURE 3
XL10-GOLD[®] CELLS DEMONSTRATE SUPERIOR TRANSFORMATION OF LARGE DNA Relative transformation efficiency comparison between XL10-Gold, XL2-Blue and DH10B cells. XL10-Gold cells reduce bias against transformation of large DNA compared to these other cells. 30 ng of the pCMV-Script vector (4.2 kb) or the pAD-GAL4 vector (7.2 kb) were ligated to 10 ng cDNA for construction of a plasmid library. Of the 15- μ l aliquots were used to transform 100 μ l of DH10B, XL2-Blue or XL10-Gold competent cells. Supercoiled pUC18 plasmid was used for the transformation control.



Newly Improved Electro-Ten Blue® Cells

Highest Efficiency Electroporation-Competent Cells

Stratagene's ElectroTen-Blue® electrocompetent cells offer the highest available transformation efficiencies of $\geq 3.0 \times 10^{10}$ cfu/ μg of supercoiled pUC DNA (Figure 4). High efficiency, ease-of-use, and the Hee phenotype make ElectroTen-Blue cells ideal for your most demanding cloning projects.

Easily Transform Large and Ligated DNA

ElectroTen-Blue® electrocompetent cells exhibit the high efficiency electroporation (Hee) phenotype. This phenotype improves the survival rate of cells, increasing cloning efficiency of large plasmids and ligated DNA. Derived from XL1-Blue cells, ElectroTen-Blue cells possess all of the same cloning features such as T1 phage-resistance, and RecA and EndA negative phenotypes, with the addition of 3-fold higher efficiency over our previous electrocompetent cell line (Figure 5). These cells are perfect when you have limited amounts of DNA or when generating cDNA, genomic, and subtractive libraries. Use this strain when your experiment must work the first time!

Spend Less Time Preparing Electroporation-Ready DNA

Before electroporation, ligated DNA to be transformed must be purified to remove DNA ligase, a potential inhibitor of electroporation. Our StrataClean™ resin dramatically simplifies this process. Because of its high affinity for proteins, StrataClean resin removes protein contamination with extraction complete in only 5 minutes. Use StrataClean resin for all of your electroporation experiments, a well-established alternative to phenol extractions and time-consuming ethanol precipitations. This resin is included in our ElectroTen-Blue® electroporation competent cell kit.

Your Favorite Competent Cells

Our most popular strains are available as electroporation-competent cells. These include our XL1-Blue, XL1-Blue MRF¹, SURE®, ABLE® and TG1 cells.

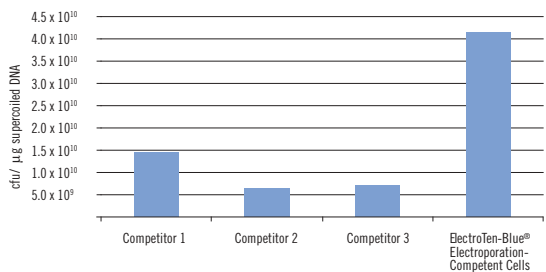
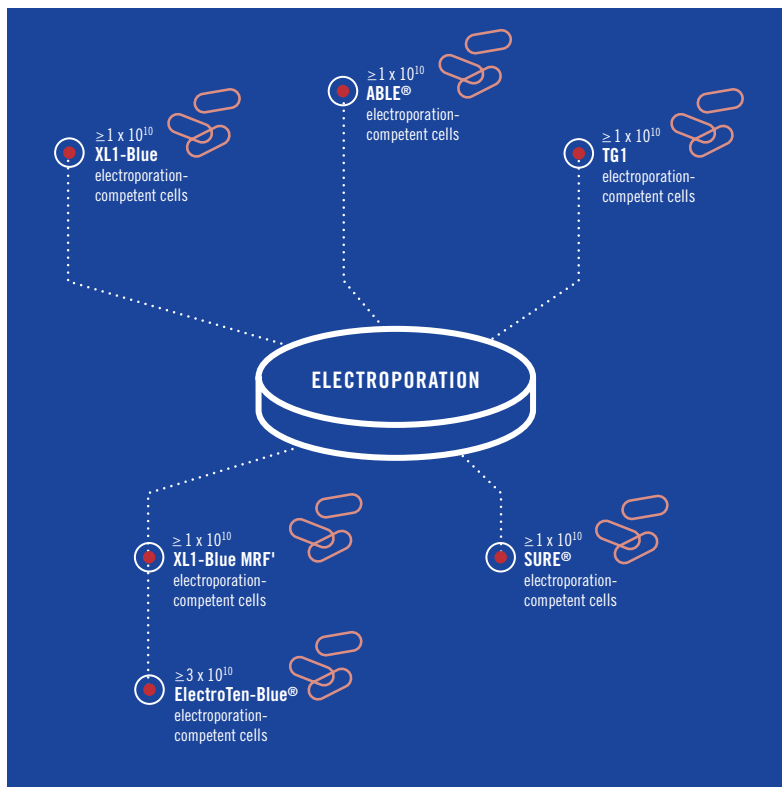


FIGURE 4
ELECTROTEN-BLUE® CELLS VS. THE COMPETITION USING SUPERCOILED pUC DNA

ElectroTen-Blue® Electrocompetent Cells consistently outperform "highest efficiency" electroporation-competent cells from other suppliers. Supercoiled pUC was electroporated following manufacturer's instructions.

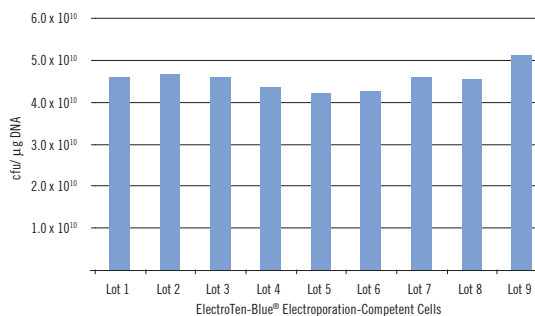
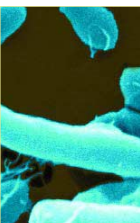


FIGURE 5
LOT-TO-LOT EFFICIENCY

We compared transformation efficiencies across several lots of ElectroTen-Blue® Electrocompetent Cells. Consistent lot-to-lot results ensure success in all of your cloning projects.



Cloning Difficult DNA

Unstable DNA / Toxic DNA / Methylated DNA

We have created strains that solve some of the toughest cloning challenges. Our SURE® series is engineered to improve cloning of unstable DNA. The ABLE® series offers a simplified approach for propagating toxic DNA. Our MR (Restriction Minus) series is deficient in all known *E. coli* K12 restriction systems to eliminate cleavage of eukaryotic DNA with methylation patterns that are different than the *E. coli* host methylation patterns.

Unstable DNA

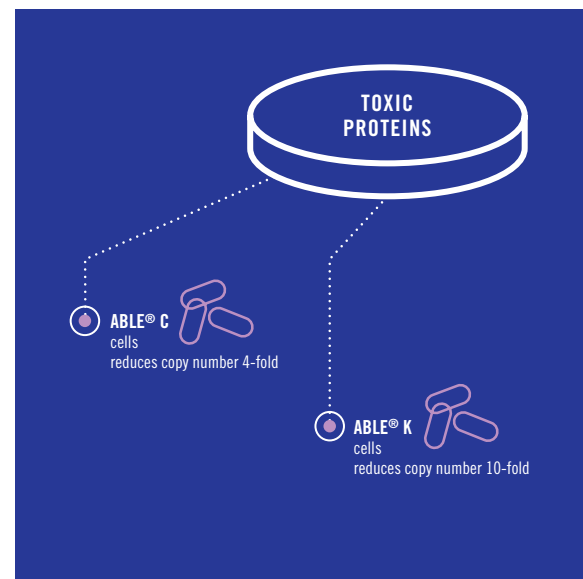
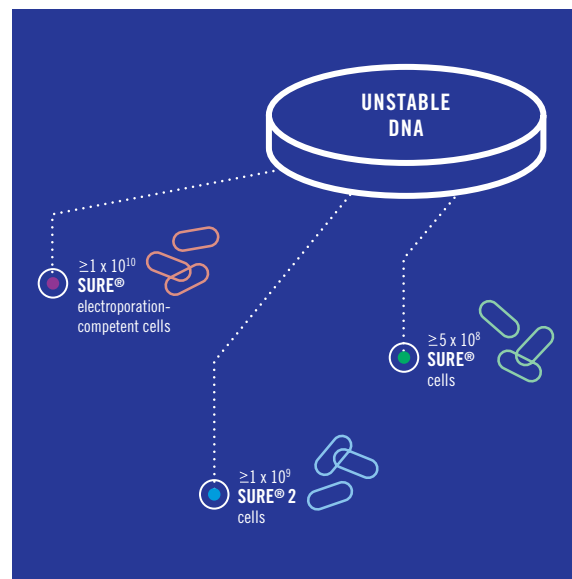
Replicating eukaryotic DNA in prokaryotic cells can be problematic. Particular eukaryotic genes may contain inverted repeats or secondary structures, such as Z-DNA, that can be rearranged or deleted by *E. coli* DNA repair systems. The SURE® competent cells¹³ were designed to easily clone DNA containing these irregular structures by removing *E. coli* genes involved in the rearrangement and deletion of DNA. The UV repair system (*uvrC*) and the SOS repair pathway (*umuC*) are both involved in repairing DNA lesions. Removal of these genes results in a 10- to 20-fold increase in the stability of DNA containing long inverted repeats. Another set of *E. coli* proteins, the SbcC and RecJ proteins, are involved in certain types of recombination. Mutations in these genes greatly increase stability of Z-DNA structures.

The combination of *recB* and *recJ* mutations confers a recombination deficient phenotype to the SURE cells, greatly reducing homologous recombination, similar to a mutation in the *recA* gene. These cells are also restriction negative, $\Delta(mcrCB-hsdSMR-mrr)$ 171, to allow cloning of methylated DNA. The *endA1* gene has been mutated so high-quality plasmid miniprep

DNA can be produced from these cells. SURE cells are available electroporation competent ($\geq 1 \times 10^{10}$ transformants/ μg DNA), as competent-grade ($\geq 5 \times 10^8$ transformants/ μg DNA) and as a highly efficient derivative SURE 2 supercompetent¹⁴ cells ($\geq 1 \times 10^9$ transformants/ μg DNA).

Toxic DNA

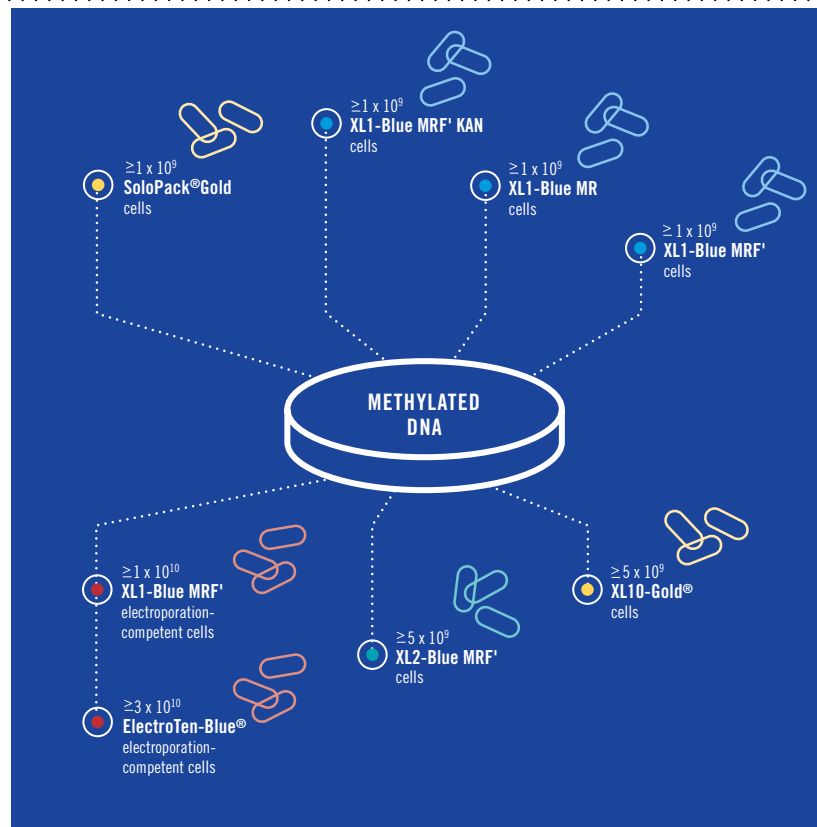
Many genes are difficult to clone in *E. coli*. Sometimes, the insert codes for a protein that is toxic to the *E. coli* host. Often, it is not known if the gene of interest is toxic or if it is just difficult to clone. The high-copy number of most commonly used cloning vectors amplifies this cloning problem. When this problem occurs, the gene of interest must be recloned into a low-copy-number plasmid or an inducible system with extremely tight control of gene expression. The ABLE® strains provide an easy alternative to these recloning projects. The ABLE C strain reduces the copy number of ColE1-derived plasmids (such as pUC and pBluescript® plasmids) four-fold. The ABLE K strain reduces the copy number of plasmids 10-fold. Reducing the plasmid copy number will usually decrease the level of cloned protein product. This results in increased cell viability and avoids



generating mutations within the gene of interest when your protein is toxic to the cells. Try both strains to obtain the highest copy number that still allows growth of your construct. The ABLE strains are available as both chemically and electroporation-competent cells.

Methylated DNA

Eukaryotic genomic DNA can be highly methylated; the methylation patterns can vary in different tissues and at different times during development. cDNA is often methylated during synthesis to protect internal restriction sites from cleavage during later processing. Cloning methylated DNA is more efficient when you use our restriction-minus competent cells. When DNA is methylated in a fashion unlike the bacterial host patterns, it is cleaved by the *E. coli* host restriction systems. Cleavage of DNA before host replication creates libraries that lack complete representation. The bacterial strains in our MR (Minus Restriction) series are deficient in all known *E. coli* K12 restriction systems to eliminate this problem. The *mcrA*, *mcrCB* and *mrr* mutations prevent cleavage of cloned DNA carrying cytosine and/or adenine methylation. Absence of these endogenous bacterial restriction



systems increases the efficiency of introducing eukaryotic DNA into *E. coli* and increases the size and representation of libraries constructed with methylated or hemi-methylated DNA. *E. coli* deficient in these restriction systems are optimal hosts for constructing cDNA and genomic libraries.

We carry eight different strains that lack methylation restriction pathways: XL10-Gold® ultracompetent cells for the highest efficiency cloning of large plasmids, ElectroTen-Blue® electroporation-competent cells for the highest electroporation efficiency cloning of ligated DNA, SoloPack® Gold supercompetent and competent cells in a convenient single-tube reaction format, XL2-Blue MRF' ultracompetent cells for the highest efficiency cloning of a variety of plasmids, XL1-Blue MRF' for electroporation, XL1-Blue MRF' Kan for use with tetracycline resistant plasmids, XL1-Blue MR for cloning without the F' episome and SURE cells for cloning DNA with secondary structures.

Protein Expression

Powerful T7 RNA Polymerase

The T7 RNA polymerase-based protein expression system¹⁵ is extremely popular because it provides the highest levels of recombinant protein expression in *E. coli*. We offer the BL21, BL21-Gold and BL21-CodonPlus® competent cell strains specifically for use with T7 promoter-driven vectors, such as the pET and pCAL protein expression vectors. All BL21 strains are deficient in the OmpT and Lon proteases, which may interfere with isolation of intact recombinant proteins.

The Problem of Codon Bias

Expression of heterologous recombinant genes in *E. coli* is difficult when the codon use in the recombinant gene differs from the codon use in the host cells. Forced high-level expression of a gene with codons that are rarely used by *E. coli* causes depletion of the internal tRNA pools. This is called codon bias. Translation of the recombinant RNA is delayed, resulting in degraded RNA or codon substitutions and misincorporations that destroy the functional characteristics of the protein. This problem has been most thoroughly documented for the arginine codons AGA and AGG, which are the rarest *E. coli* codons. However, codons for isoleucine (AUA), leucine (CUA) and proline (CCC) are also known to affect the amount and quality of protein produced in *E. coli* hosts (Table 1). BL21-CodonPlus® series of competent cells offer a novel solution to successfully expressing sequences with codon bias in *E. coli*.

Eliminate Codon Bias for High-Level Expression

The BL21-CodonPlus cells dramatically improve protein expression in *E. coli* by overcoming the problem of codon bias. We added extra copies of tRNA genes that are rare in *E. coli* but used more frequently in other organisms. This modification allows for high-level expression of many proteins that are difficult or impossible to express in conventional *E. coli* hosts due to the presence of rare codons. These cells eliminate the need to replace rare codons with more frequently used codons or move the gene of interest into an eukaryotic expression system to get expression.

BL21-CodonPlus(DE3)-RIPL strain contains extra copies of the *E. coli*, *argU*, *ileY*, *leuW* and *proL* tRNA genes. Use this strain to overcome expression problems due to codon bias from both AT- and GC-rich genomes. The original BL21-CodonPlus-RIL and RP

organism	AGG arginine	AGA arginine	CUA leucine	AUA isoleucine	CCC proline
<i>Escherichia coli</i>	1.2	2.1	3.9	4.4	5.5
<i>Homo sapiens</i>	11.4	11.5	6.5	6.9	20.0
<i>Drosophila melanogaster</i>	6.4	5.1	8.2	9.2	18.0
<i>Caenorhabditis elegans</i>	4.0	15.4	8.0	9.7	4.5
<i>Saccharomyces cerevisiae</i>	9.3	21.3	13.4	17.8	6.8
<i>Plasmodium falciparum</i>	4.1	20.2	15.2	33.2	8.5
<i>Clostridium pasteurianum</i>	2.4	29.4	6.2	50.0	0.9
<i>Pyrococcus horikoshii</i>	30.1	20.1	18.2	44.5	10.2
<i>Thermus aquaticus</i>	14.3	1.3	3.6	1.4	38.8
<i>Arabidopsis thaliana</i>	10.9	18.8	10.0	12.7	5.3

Table 1

CODON USAGE IN VARIOUS ORGANISMS Codon frequencies are expressed as codons used per 1000 codons encountered. The arginine codons AGG and AGA are recognized by the same tRNA and should therefore be combined. Codon frequencies of more than 15 codons/1000 codons are shown in bold to help identify a codon bias that may cause problems for high level expression in *E. coli*. * These frequencies are updated regularly. A complete compilation of codon usage of the sequences in the gene bank database can be found at www.kazusa.or.jp/codon/.

strains have been optimized for expression of AT- and GC-rich genomes respectively. Use these strains when the codon usage of your sequence is known. Use the genotypes table to determine the most appropriate strain for your gene of interest.

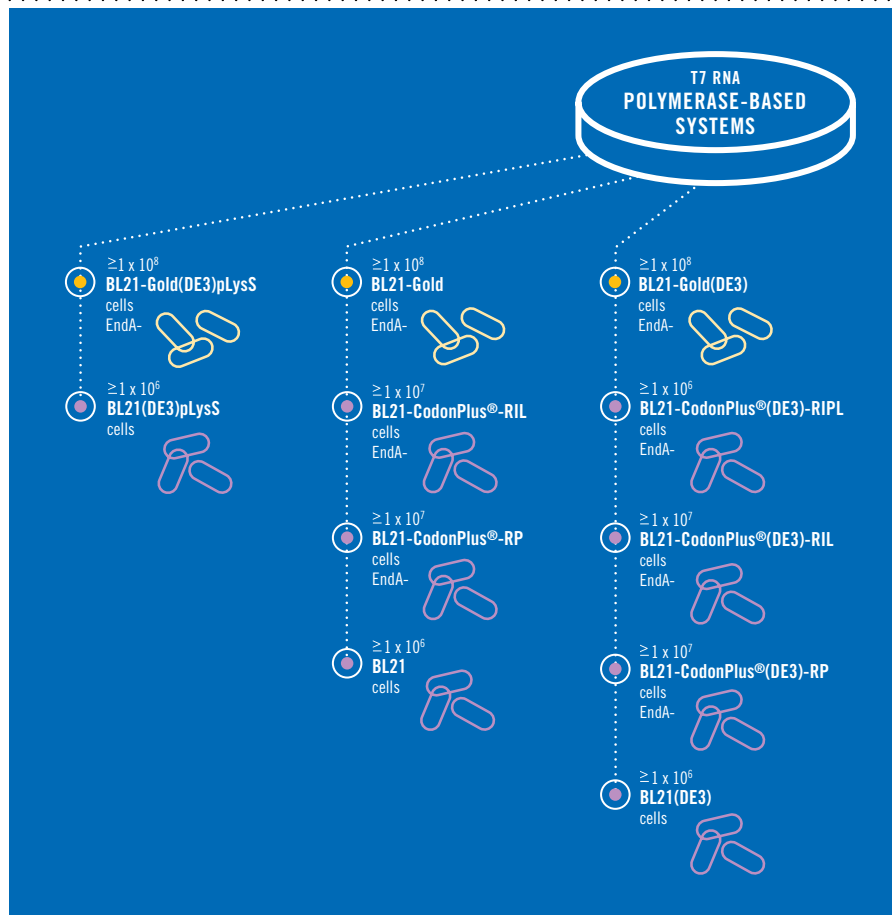
BL21-CodonPlus(DE3)-RIL-X and -RP-X are methionine auxotrophs for metabolic labeling of proteins for x-ray crystallography.

Save 2 Days with BL21-Gold Competent Cells

The BL21-Gold competent cells incorporate major improvements over the original BL21 series. The BL21-Gold cells feature the Hte phenotype. Presence of the Hte phenotype contributes to a 100-fold increase in transformation efficiency, to greater than 1×10^8 transformants/ μg of pUC18 DNA. In addition, the gene encoding endonuclease I (*endA*), which rapidly degrades plasmid DNA isolated by most miniprep procedures, is inactivated. These two improvements allow direct cloning for most protein expression constructs. By cloning directly in the strain you save 2 days of work normally spent on subcloning procedures.

Original BL21 Competent Cells

The original BL21-derived competent cells are an economical alternative when high efficiency is not a concern and plasmid DNA preparation is not necessary. The original BL21 cells provide the same high protein expression levels as BL21-Gold and are also deficient in the Lon and OmpT proteases. Use these cells for established expression constructs that have already been cloned and sequenced.



Controlling Expression Levels

BL21, BL21-Gold and BL21-CodonPlus®

The basic BL21 strain does not contain the T7 RNA polymerase gene and can be used with non-T7 RNA polymerase protein expression systems. To induce protein expression from T7 promoter-driven vectors, the host is infected with lambda CE6 bacteriophage, which provides the T7 RNA polymerase. Since induction cannot occur until infection, this strain provides the tightest control of protein expression for extremely toxic proteins.

BL21(DE3), BL21-Gold(DE3) and BL21-CodonPlus®(DE3)

The DE3-derivatives contain the T7 RNA polymerase gene controlled by the *lacUV5* promoter. Expression is induced with IPTG. This all-purpose derivative yields high-level expression and provides easy induction. Use this derivative with nontoxic proteins.

BL21(DE3)pLysS and BL21-Gold(DE3)pLysS

The DE3 pLysS-derivatives contain the pLysS plasmid as well as the gene for T7 RNA polymerase. The pLysS plasmid codes for T7 lysozyme, a natural inhibitor of T7 RNA polymerase. The presence of this inhibitor prevents leaky expression in uninduced cells. When induced with IPTG, the inhibition by the T7 lysozyme is overcome by the stronger T7 promoter. This derivative provides tighter control for expression of toxic proteins.

XL1-Blue Strain

Versatile Cloning

We designed the XL1-Blue strain to provide a host for optimal propagation of both plasmid and lambda phage vectors. Over the years, we have introduced derivatives of this popular strain which enable higher transformation efficiency, transformation of methylated DNA, choice of antibiotic resistance and a derivative without an F' episome. XL1-Blue cells are available in a wide range of cloning efficiencies as well.

All-Purpose Cloning

The strain of choice for many cloning experiments is the XL1-Blue strain. The XL1-Blue strain allows blue-white color screening, single-strand rescue of phagemid DNA and preparation of high-quality plasmid DNA. This strain is available in a wide variety of transformation efficiencies (Figure 6). For the most colonies, use electroporation-competent XL1-Blue cells or the high-efficiency derivative, XL2-Blue ultracompetent cells. Electroporation-competent XL1-Blue cells are guaranteed to give you $\geq 1 \times 10^{10}$ transformants/ μg of DNA, and chemically competent XL2-Blue cells give you $\geq 5 \times 10^9$ transformants/ μg of DNA. When ultimate efficiency is not as critical, try the supercompetent-grade ($\geq 1 \times 10^9$ transformants/ μg DNA), competent-grade ($\geq 1 \times 10^8$ transformants/ μg DNA) or the subcloning-grade ($\geq 1 \times 10^6$ transformants/ μg DNA) competent cells.

Restriction-Minus

To allow high efficiency and representational cloning of methylated DNA, we created XL1-Blue MRF' cells, restriction-minus versions of XL1-Blue competent cells. All known *E. coli* K12 restriction systems have been deleted from these cells. Use XL1-Blue MRF' cells when cloning methylated cDNA or genomic DNA, or when cloning methylated PCR products. The XL1-Blue MRF' strain is also available as the high-efficiency chemically competent derivative XL2-Blue MRF' ($\geq 5 \times 10^9$ transformants/ μg DNA), or as supercompetent ($\geq 1 \times 10^9$ transformants/ μg DNA) cells. When the F' episome and blue-white screening are unnecessary, use XL1-Blue MR supercompetent cells ($\geq 1 \times 10^9$ transformants/ μg DNA). Finally, when cloning tetracycline-resistant plasmids, use XL1-Blue MRF' Kan supercompetent cells ($\geq 1 \times 10^9$ transformants/ μg DNA). The XL1-Blue MRF' Kan cells carry the kanamycin-resistance gene instead of the tetracycline gene to select for the F' episome and provide a more intense blue color for blue-white screening.

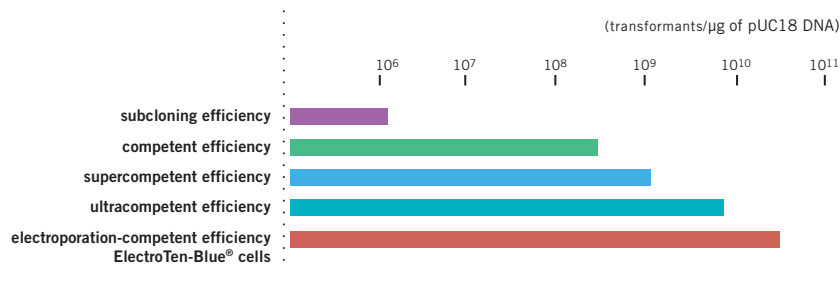
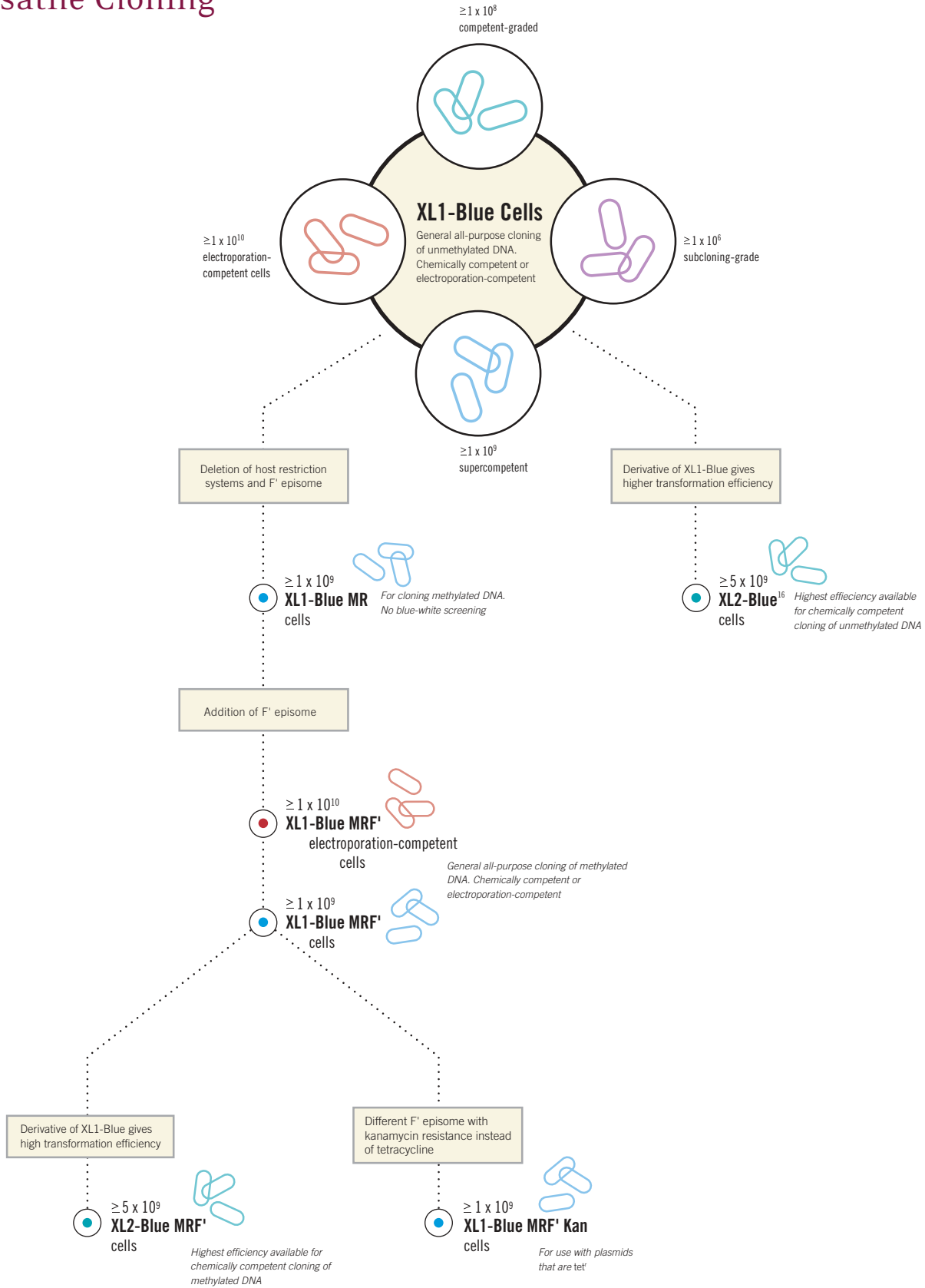


FIGURE 6

COMPETENT CELL EFFICIENCIES Our XL1-Blue series of competent cells are available in every efficiency so you can choose the derivative that matches the demands of your cloning experiment.

Versatile Cloning



Convenient Cloning

Packaging Simplifies Cloning

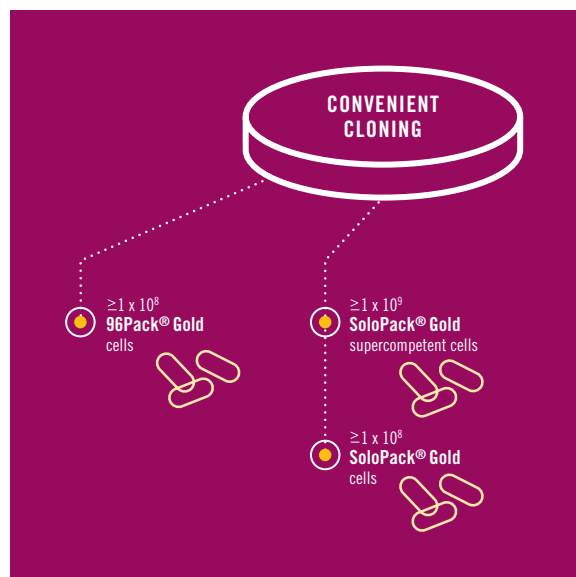
Our competent cells in convenient packaging simplify transformations without compromising performance. Choose the single-reaction format for routine cloning or the 96-well format when high-throughput is a necessity.

Single Transformation in the Tube

The SoloPack® Gold cells provide the high performance and convenience of single-tube transformation in efficiencies for everyday cloning. With the SoloPack single-reaction format, there is no more thawing, aliquotting and refreezing. Thaw only the cells you need. There are fewer pipetting steps because the entire transformation reaction occurs in the tube supplied.

High-Throughput Cloning

96Pack® Gold competent cells are in a convenient 96-well format for rapid transformation. Just add DNA, heat shock and add outgrowth media directly to the plate. This protocol saves you time and reagents, while providing the best possible results.



Generating Unmethylated DNA

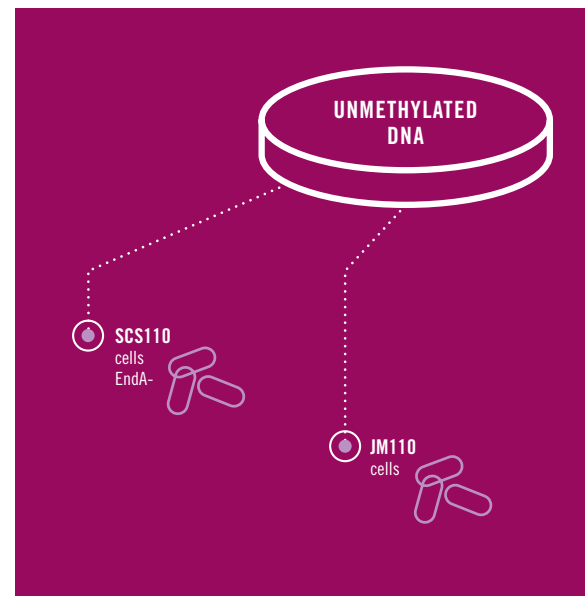
Using Methylation-Sensitive Restriction Enzymes

Sometimes the easiest cloning strategy for your experiment involves the use of methylation-sensitive restriction enzymes. These hosts generate unmethylated DNA so you can use methylation-sensitive restriction enzymes with no problem. We further created an *EndA*-variant (SCS110 cells) for improved mini-prep DNA.

Dam-/Dcm- Strains

Most *E. coli* hosts contain both DNA adenine methylation (*dam*) and DNA cytosine methylation (*dcm*) genes. These genes code for proteins that methylate specific sequences when DNA is propagated, making subsequent digestion with methylation-sensitive restriction enzymes impossible. We offer the SCS110 and JM110 strains, which lack both *dam* and *dcm* activity. DNA propagated in these strains can be digested by methylation-sensitive enzymes such as *Xba* I, *Cla* I and *EcoR* II.

While both strains can be used to propagate unmethylated DNA, the JM110 strain is *EndA*+. The wild-type *endA* gene encodes for endonuclease I, which nonspecifically cleaves dsDNA approximately every 400 bp. The yield and quality of plasmid miniprep DNA are greatly improved when DNA is isolated from *EndA*- strains. The SCS110 is *EndA*- and was derived from the JM110 strain, greatly improving the quality of plasmid purified from this strain. Both strains are available at efficiencies greater than 5×10^6 transformants/ μg DNA.



Mutagenesis

Fast, Easy Random Mutagenesis

We created the XL1-Red mutator strain for simple, rapid and economical random mutagenesis. Use this strain for highly efficient and reproducible isolation of random mutations.

Easy Random Mutagenesis

Procedures developed to generate random mutations within a gene, such as chemical treatment of DNA and PCR, can be time-consuming, laborious and expensive. We constructed the XL1-Red mutator strain for the highly efficient and reproducible isolation of random mutations. The XL1-Red strain carries mutations in *mutS*, *mutD* and *mutT* and is deficient in three of the primary DNA repair pathways in *E. coli*. Its mutation rate is approximately 5,000-fold higher than that of its wild-type parent. The method is easy: simply transform your construct into the XL1-Red strain, propagate and purify the mutant plasmids. Then, retransform into provided XL1-Blue competent cells.



The Widest Selection

We are not content to just be competent! We have designed strains for protein expression, plasmid stability, large plasmids and ligated DNA as well as everyday cloning.

+ Our complete line of competent cells includes specialty strains for a wide variety of applications and a selection of useful packaging formats, each designed to increase your chances of getting your clone.

The Highest Efficiency

Our ultracompetent cells provide the highest transformation efficiency in the world. Our ultracompetent cells are your best insurance for successful cloning. + When you use XL10-Gold® ultracompetent cells, you will get more colonies than with any other commercially available cells. XL10-Gold cells have been engineered to transform large plasmids and ligated DNA more efficiently than other cell lines and are ideal for plasmid library construction.

Appendix

Key to Genotypes

Transformation Efficiency

- + **Hte** The Hte phenotype increases transformation efficiency and improves competent cell performance. In XL10-Gold® ultracompetent cells, it allows transformation of large plasmid DNA and provides 20- to 30-fold higher transformation efficiency of ligated DNA. In BL21-Gold competent cells, it increases transformation efficiency 100-fold, to greater than 1×10^8 transformants/ μ g.
- + **Hee** The Hee phenotype improves the survival rate of electroporated cells, resulting in a significant increase in transformation efficiencies.

Recombination

When foreign DNA is propagated in *E. coli*, there are always risks of recombination. The following genes in the *E. coli* chromosome are involved in these recombination events.

- + **recA** This gene is central to general recombination and DNA repair. Mutations in this gene reduce homologous recombination of DNA propagated in this strain and renders the bacteria sensitive to UV light. Most competent cells from Stratagene have this mutation.
- + **recB** The *recB* gene product is involved in general recombination. Strains containing a mutation in both *recB* and *recJ* confer a RecA phenotype. SURE® and SURE 2 cells contain this mutation.
- + **recJ** The RecJ exonuclease is involved in recombination pathways alternate to the RecA pathways. Mutation in conjunction with *sbcC* reduces Z-DNA rearrangements. Mutations in conjunction with *recB* confer a RecA-phenotype. SURE and SURE 2 cells contain this mutation.
- + **uvrC and umuC** These genes are components in UV repair and SOS repair pathways respectively. Mutations in these pathways reduce rearrangement of inverted repeats. SURE and SURE 2 cells contain these mutations.
- + **sbcC** Mutation in conjunction with *recJ* reduces rearrangements in Z-DNA structures. SURE and SURE 2 cells contain this mutation.

Restriction Systems

The following genes code for pathways in *E. coli* that restrict DNA methylated in a pattern unlike *E. coli* methylation. Most eukaryotic DNA is methylated and will be restricted by the *E. coli* as it enters the cell. This greatly reduces cloning efficiencies and changes the representation of methylated genes in the library. Elimination of these pathways increases cloning efficiencies of methylated DNA and increases representation of methylated sequences.

- + **hsdR** *E. coli* (or EcoK) restriction endonuclease. Absence of this activity permits the introduction of DNA propagated from non-*E. coli* sources. Most Stratagene strains carry this mutation.
- + **hsdS** Specificity determinant for *hsdM* and *hsdR*. Mutation of this gene eliminates both HsdM and HsdR activity. Most Stratagene strains carry this mutation.

- + **mcrA** *E. coli* restriction system that recognizes methylated DNA of sequence 5' C*CGG (*internal cytosine methylated). Mutation in this gene prevents cleavage of this sequence. Many Stratagene strains carry this mutation.
- + **mcrCB** *E. coli* restriction system that cleaves methylated DNA of sequence 5' G⁵*C, 5' G^{5h}*C or 5' G^{N4}*C (*methylated cytosine). Mutations in this gene prevent restriction of these sequences. Absence of McrCB activity is important when cloning genomic DNA or methylated cDNA. XL10-Gold, SoloPack Gold, 96Pack Gold, XL1-Blue MR, XL1-Blue MRF', XL2-Blue MRF', SURE and SURE 2 strains contain these mutations.
- + **mrr** *E. coli* restriction system that recognizes methylated DNA of sequence 5'-G*AC or C*AG (*methylated adenine). Mutation in this gene prevents cleavage of these sequences. Mutation also prevents McrF restriction of methylated cytosine sequences. XL10-Gold, SoloPack Gold, 96Pack Gold, XL1-Blue MR, XL1-Blue MRF', XL2-Blue MRF', SURE and SURE 2 strains contain these mutations.

DNA Preparation

The following genes are important for preparing high-quality plasmid DNA.

- + **endA** DNA specific endonuclease I. Mutation in the gene dramatically improves the yield and quality of plasmid miniprep DNA prepared from alkaline lysis and rapid boiling miniprep procedures. Most Stratagene strains have this mutation.
- + **dam** DNA adenine methylase. Mutation blocks methylation of adenine residues in the recognition sequence 5'-G-*ATC-3' (*methylated) allowing cleavage with methylation-sensitive restriction enzymes such as *Bcl* I.
- + **dcm** DNA cytosine methylase. Mutation blocks methylation of internal cytosine residues in the recognition sequences 5'-C*CAGG-3' or 5'-C*CTGG-3' (*methylated) allowing cleavage with methylation-sensitive restriction enzymes such as *EcoR* II.

Blue-white Color Screening

When using the appropriate vectors, blue-white screening is an important tool for selecting colonies that contain insert. The following genes are involved in this process.

- + **lacI** Repressor protein of *lac* operon. *Lac^H* is a mutant of *lacI* that overproduces the repressor protein. Repression is overcome by addition of IPTG to the cells.
- + **lacZ** This gene codes for β -D-galactosidase, a protein involved in lactose utilization. Cells with *lacZ* mutations produce white colonies in the presence of X-gal; wild type produces blue colonies.
- + **lacZ³M15** A specific N-terminal deletion which permits the α -complementation segment present on pUC-based plasmids, such as the pBluescript® phagemid or lambda vectors such as the Lambda ZAP® II vector, to make a functional *lacZ* protein.

Ordering Information

Cloning Large or Ligated DNA				
XL10-GOLD® ULTRACOMPETENT CELLS	5 x 0.1-ml aliquots	Highest cloning efficiency. Use with large plasmids, ligated DNA and plasmid libraries. $\geq 5 \times 10^9$ transformants/ μ g	Tetracycline resistant Chloramphenicol resistant*	#200314
	10 x 0.1-ml aliquots			#200315
XL10-GOLD® KAN^r ULTRACOMPETENT CELLS	10 x 0.1-ml aliquots	Highest cloning efficiency. Use with large plasmids, ligated DNA and plasmid libraries. Use with chloramphenicol-resistant plasmids. $\geq 5 \times 10^9$ transformants/ μ g	Tetracycline resistant Kanamycin resistant	#200317
ELECTROTEN-BLUE® ELECTROPORATION-COMPETENT CELLS	5 x 0.1-ml aliquots	Highest electroporation cloning efficiency. Use for cloning ligated DNA and generating libraries. StrataClean™ Resin included. $\geq 3 \times 10^{10}$ transformants/ μ g	Tetracycline resistant Kanamycin resistant	#200159
Convenient Cloning				
SOLOPACK® GOLD SUPERCOMPETENT CELLS	15 single-tube transformations	For high-efficiency cloning. Convenient single-reaction format. $\geq 1 \times 10^9$ transformants/ μ g	Tetracycline resistant Chloramphenicol resistant*	#230350
SOLOPACK® GOLD COMPETENT CELLS	15 single-tube transformations	For routine cloning. Convenient single-reaction format. $\geq 1 \times 10^8$ transformants/ μ g	Tetracycline resistant Chloramphenicol resistant*	#230325
96PACK® GOLD COMPETENT CELLS	Four 96-well plates	For routine cloning. High-throughput format. $\geq 1 \times 10^8$ transformants/ μ g	Tetracycline resistant Chloramphenicol resistant*	#200324
Routine Cloning				
XL1-BLUE ELECTROPORATION-COMPETENT CELLS	5 x 0.1-ml aliquots	For electroporation. $\geq 1 \times 10^{10}$ transformants/ μ g	Tetracycline resistant	#200228
XL1-BLUE MRF^r ELECTROPORATION-COMPETENT CELLS	5 x 0.1-ml aliquots	For electroporation. Restriction minus for cloning methylated DNA. $\geq 1 \times 10^{10}$ transformants/ μ g	Tetracycline resistant	#200158
XL2-BLUE ULTRACOMPETENT CELLS	10 x 0.1-ml aliquots	Highest cloning efficiency. $\geq 5 \times 10^9$ transformants/ μ g	Tetracycline resistant Chloramphenicol resistant*	#200150
XL2-BLUE MRF^r ULTRACOMPETENT CELLS	10 x 0.1-ml aliquots	Restriction minus for cloning methylated DNA. Highest cloning efficiency. $\geq 5 \times 10^9$ transformants/ μ g	Tetracycline resistant Chloramphenicol resistant*	#200151
XL1-BLUE SUPERCOMPETENT CELLS	5 x 0.2-ml aliquots	For high-efficiency cloning. $\geq 1 \times 10^9$ transformants/ μ g	Tetracycline resistant	#200236
XL1-BLUE MRF^r SUPERCOMPETENT CELLS	5 x 0.2-ml aliquots	Restriction minus for cloning methylated DNA. $\geq 1 \times 10^9$ transformants/ μ g	Tetracycline resistant	#200230
XL1-BLUE MRF^r KAN SUPERCOMPETENT CELLS	5 x 0.2-ml aliquots	Use with tetracycline-resistant plasmids. Restriction minus for cloning methylated DNA. $\geq 1 \times 10^9$ transformants/ μ g	Kanamycin resistant	#200248
XL1-BLUE MR SUPERCOMPETENT CELLS	5 x 0.2-ml aliquots	Use for cloning without the F' episome. $\geq 1 \times 10^9$ transformants/ μ g		#200229
XL1-BLUE COMPETENT CELLS	5 x 0.2-ml aliquots	For routine cloning. $\geq 1 \times 10^8$ transformants/ μ g	Tetracycline resistant	#200249
XL1-BLUE SUBCLONING-GRADE COMPETENT CELLS	8 x 0.5-ml aliquots	For cloning where DNA is not limited. $\geq 1 \times 10^6$ transformants/ μ g	Tetracycline resistant	#200130
Cloning Unstable DNA				
SURE® 2 SUPERCOMPETENT CELLS	10 x 0.1-ml aliquots	High-efficiency derivative. $\geq 1 \times 10^9$ transformants/ μ g	Tetracycline resistant Chloramphenicol resistant* Kanamycin resistant	#200152
SURE® COMPETENT CELLS	5 x 0.2-ml aliquots	$\geq 5 \times 10^8$ transformants/ μ g	Tetracycline resistant Kanamycin resistant	#200238
SURE® ELECTROPORATION-COMPETENT CELLS	5 x 0.1-ml aliquots	For electroporation. $\geq 1 \times 10^{10}$ transformants/ μ g	Tetracycline resistant Kanamycin resistant	#200227
Protein Expression				
BL21-CODONPLUS® (DE3)-RIPL COMPETENT CELLS	10 x 0.1-ml aliquots	Use to eliminate codon bias. Use with pET or pCAL vectors. Encodes T7 RNA polymerase under the control of the <i>lacUV5</i> promoter for easy induction of protein expression. $\geq 1 \times 10^6$ transformants/ μ g	Chloramphenicol resistant* Streptomycin/Spectinomycin resistant	#230280
BL21-CODONPLUS® RIL COMPETENT CELLS	10 x 0.1-ml aliquots	Use to eliminate codon bias. Use for non-T7 polymerase systems. Use with λ CE6 for extremely tight control of expression. $\geq 1 \times 10^7$ transformants/ μ g	Tetracycline resistant Chloramphenicol resistant*	#230240
BL21-CODONPLUS® RP COMPETENT CELLS	10 x 0.1-ml aliquots	Use to eliminate codon bias. Use for non-T7 polymerase systems. Use with λ CE6 for extremely tight control of expression. $\geq 1 \times 10^7$ transformants/ μ g	Tetracycline resistant Chloramphenicol resistant*	#230250
BL21-CODONPLUS® (DE3)-RIL COMPETENT CELLS	10 x 0.1-ml aliquots	Use to eliminate codon bias. Use with pET or pCAL vectors. Encodes T7 RNA polymerase under the control of the <i>lacUV5</i> promoter for easy induction of protein expression. $\geq 1 \times 10^7$ transformants/ μ g	Tetracycline resistant Chloramphenicol resistant*	#230245
BL21-CODONPLUS® (DE3)-RP COMPETENT CELLS	10 x 0.1-ml aliquots	Use to eliminate codon bias. Use with pET or pCAL vectors. Encodes T7 RNA polymerase under the control of the <i>lacUV5</i> promoter for easy induction of protein expression. $\geq 1 \times 10^7$ transformants/ μ g	Tetracycline resistant Chloramphenicol resistant*	#230255
BL21-CODONPLUS® (DE3)-RIL-X COMPETENT CELLS	10 x 0.1-ml aliquots	Methionine auxotroph for use in X-Ray crystallography. $\geq 1 \times 10^7$ transformants/ μ g	Tetracycline resistant Chloramphenicol resistant* Kanamycin resistant	#230265
BL21-CODONPLUS® (DE3)-RP-X COMPETENT CELLS	10 x 0.1-ml aliquots	Methionine auxotroph for use X-Ray crystallography. $\geq 1 \times 10^7$ transformants/ μ g	Tetracycline resistant Chloramphenicol resistant* Kanamycin resistant	#230275
BL21-GOLD CELLS	10 x 0.1-ml aliquots	Increased efficiency and EndA- for cloning many expression constructs. Use with non-T7 RNA polymerase-based systems or extremely toxic proteins. $\geq 1 \times 10^8$ transformants/ μ g	Tetracycline resistant	#230130
BL21-GOLD(DE3) CELLS	10 x 0.1-ml aliquots	Increased efficiency and EndA- for cloning many expression constructs. Use with nontoxic proteins. $\geq 1 \times 10^8$ transformants/ μ g	Tetracycline resistant	#230132
BL21-GOLD(DE3)pLysS CELLS	10 x 0.1-ml aliquots	Increased efficiency and EndA- for cloning many expression constructs. Use with both toxic and nontoxic proteins. $\geq 1 \times 10^8$ transformants/ μ g	Tetracycline resistant Chloramphenicol resistant*	#230134

BL21 CELLS	5 x 0.2-ml aliquots	Use with non-T7 polymerase-based systems or with λ CE6 for extremely toxic proteins. $\geq 1 \times 10^6$ transformants/ μ g		#200133
BL21(DE3) CELLS	5 x 0.2-ml aliquots	Use with nontoxic proteins. $\geq 1 \times 10^6$ transformants/ μ g		#200131
BL21(DE3)pLysS CELLS	5 x 0.2-ml aliquots	Use with both toxic and nontoxic proteins. $\geq 1 \times 10^6$ transformants/ μ g	Chloramphenicol resistant*	#200132
Cloning Sequences that Encode Toxic Proteins				
ABLE[®] COMPETENT CELL KIT	5 x 0.2-ml aliquot ABLE C 5 x 0.2-ml aliquot ABLE K	Chemically competent cells. Includes both ABLE C and K strains. $\geq 5 \times 10^6$ transformants/ μ g	Tetracycline resistant Kanamycin resistant	#200170
ABLE[®] C CELLS	5 x 0.2-ml aliquot ABLE C	$\geq 5 \times 10^6$ transformants/ μ g	Tetracycline resistant Kanamycin resistant	#200171
ABLE[®] K CELLS	5 x 0.2-ml aliquot ABLE K	$\geq 5 \times 10^6$ transformants/ μ g	Tetracycline resistant. Kanamycin resistant	#200172
ABLE[®] ELECTROPORATION-COMPETENT CELL KIT	5 x 0.1-ml aliquot ABLE C 5 x 0.1-ml aliquot ABLE K	Use for electroporation. Includes both ABLE C and K strains. $\geq 1 \times 10^{10}$ transformants/ μ g	Kanamycin resistant	#200160
ABLE[®] C ELECTROPORATION-COMPETENT CELLS	5 x 0.1-ml aliquots	$\geq 1 \times 10^{10}$ transformants/ μ g	Tetracycline resistant Kanamycin resistant	#200161
ABLE[®] K ELECTROPORATION-COMPETENT CELLS	5 x 0.1-ml aliquots	$\geq 1 \times 10^{10}$ transformants/ μ g	Tetracycline resistant Kanamycin resistant	#200162
Mutagenesis				
XL1-RED CELLS	5 x 0.2-ml aliquots	For random mutagenesis. Provided with XL1-Blue competent cells.	Tetracycline resistant	#200129
Generate Unmethylated DNA				
SCS110 CELLS	5 x 0.2-ml aliquots	EndA- for improved yield and quality of miniprep DNA. $\geq 5 \times 10^6$ transformants/ μ g	Streptomycin resistant	#200247
JM110 CELLS	5 x 0.2-ml aliquots	$\geq 5 \times 10^6$ transformants/ μ g	Streptomycin resistant	#200239
Electroporation-Competent Cells				
ELECTROTEN-BLUE[®] ELECTROPORATION-COMPETENT CELLS	5 x 0.1-ml aliquots	Highest electroporation cloning efficiency. Use for cloning ligated DNA and generating libraries. $\geq 3 \times 10^{10}$ transformants/ μ g	Tetracycline resistant Kanamycin resistant	#200159
XL1-BLUE ELECTROPORATION-COMPETENT CELLS	5 x 0.1-ml aliquots	For all-purpose cloning. $\geq 1 \times 10^{10}$ transformants/ μ g	Tetracycline resistant	#200228
XL1-BLUE MRF⁺ ELECTROPORATION-COMPETENT CELLS	5 x 0.1-ml aliquots	Restriction minus for cloning methylated DNA. $\geq 1 \times 10^{10}$ transformants/ μ g	Tetracycline resistant	#200158
SURE[®] ELECTROPORATION-COMPETENT CELLS	5 x 0.1-ml aliquots	For cloning unstable DNA. $\geq 1 \times 10^{10}$ transformants/ μ g	Tetracycline resistant Kanamycin resistant	#200227
ABLE[®] ELECTROPORATION-COMPETENT CELL KIT	5 x 0.1-ml aliquot ABLE C 5 x 0.1-ml aliquot ABLE K	Includes both ABLE C and K strains. For genes containing toxic proteins.	Tetracycline resistant Kanamycin resistant	#200160
ABLE[®] C ELECTROPORATION-COMPETENT CELLS	5 x 0.1-ml aliquots	$\geq 1 \times 10^{10}$ transformants/ μ g	Tetracycline resistant Kanamycin resistant	#200161
ABLE[®] K ELECTROPORATION-COMPETENT CELLS	5 x 0.1-ml aliquots	$\geq 1 \times 10^{10}$ transformants/ μ g	Tetracycline resistant Kanamycin resistant	#200162
TG1 ELECTROPORATION-COMPETENT CELLS	5 x 0.1-ml aliquots	For phage display. $\geq 1 \times 10^{10}$ transformants/ μ g		#200123
Classic Cells				
SCS1 SUPERCOMPETENT CELLS	5 x 0.2-ml aliquots	$\geq 1 \times 10^9$ transformants/ μ g		#200231
AG1 COMPETENT CELLS	5 x 0.2-ml aliquots	$\geq 1 \times 10^8$ transformants/ μ g		#200232
JM101 COMPETENT CELLS	5 x 0.2-ml aliquots	$\geq 1 \times 10^8$ transformants/ μ g		#200234
JM109 COMPETENT CELLS	5 x 0.2-ml aliquots	$\geq 1 \times 10^8$ transformants/ μ g		#200235
NM522 COMPETENT CELLS	5 x 0.2-ml aliquots	$\geq 1 \times 10^8$ transformants/ μ g		#200233
Competent Cell Reagents				
TURBO AMP[®] ANTIBIOTIC	10 grams, powder			#300024
AMP TABS[™]	200 x 2.5-mg tablets			#300020
	200 x 25-mg tablets	Ampicillin in premeasured tablets.		#300021
IPTG	1 gram			#300127
X-GAL	250 mg	For induction and blue-white color screening.		#300200
	1 gram	For blue-white color screening.		#300201
	10 grams			#300204

1 U.S. Patent No. 6,706,525 and patent pending

2 U.S. Patent Nos. 6,706,525, 5,512,468 and 5,707,841 and patents pending and equivalent foreign patents

3 U.S. Patent No. 6,706,525 and patents pending

4 U.S. Patent No. 6,706,525 and patents pending

5 U.S. Patent Nos. 6,706,525, 5,512,468 and 5,707,841 and patents pending and equivalent foreign patents

6 U.S. Patent Nos. 6,706,525, 5,512,468 and 5,707,841 and patents pending

7 U.S. Patent Nos. 6,635,457, 6,586,249, 6,338,965, 6,040,184 and patents pending

8 U.S. Patent Nos. 6,586,249, 6,338,965 and 6,040,184 and patents pending

9 U.S. Patent Nos. 6,586,249, 6,338,965 and 6,040,184

10 U.S. Patent Nos. 6,586,249, 6,338,965, 6,040,184, 6,017,748 and 5,552,314 and equivalent foreign patents

11 U.S. Patent Nos. 6,568,249, 6,338,965 and 6,040,184 and patents pending

12 U.S. Patent Nos. 6,586,249, 6,338,965 and 6,040,184

13 U.S. Patent Nos. 6,017,748 and 5,552,314 and patents pending and equivalent foreign patents

14 U.S. Patent Nos. 6,017,748, 5,707,841, 5,552,314 and 5,512,468 and patents pending and equivalent foreign patents

15 U.S. Patent No. 4,952,496. For academic and non-profit laboratories, and assurance letter accompanies the sale of the products. For commercial laboratories, a research use license agreement must be entered into prior to purchase of the products.

16 U.S. Patent Nos. 5,512,468 and 5,707,841 and patents pending and equivalent foreign patents

* Chloramphenicol resistant at concentrations of <40 μ g/ml, but sensitive at concentrations of 100 μ g/ml.

** The F' episome in ElectroTen-Blue cells is not functional for infection with M13 bacteriophage.

^a This strain, a derivative of *E. coli* B, is a general protein expression strain that lacks both the Lon protease and the OmpT protease, which can degrade proteins during purification. The Dcm methylase, naturally lacking in *E. coli* B, is inserted into the genome.

96Pack[®], ABLE[®], ElectroTen-Blue[®], BL21-CodonPlus[®], Lambda ZAP[®], pBluescript[®], SoloPack[®], SURE[®], TurboAmp[®] and XL10-Gold[®] are registered trademarks of Stratagene in the United States. Amp Tabs and StrataClean are trademarks of Stratagene.

Genotypes

Host Strain	Genotype
96PACK® GOLD STRAIN	Tet ^r Δ(<i>mcrA</i>)183 Δ(<i>mcrCB</i> - <i>hsdSMR</i> - <i>mrr</i>)173 <i>endA1 supE44 thi-1 recA1 gyrA96 relA1 lac Hte</i> [F' <i>proAB lac⁺ΔM15 Tn10</i> (Tet ^r) Amy Cam ^r]*
ABLE® C STRAIN	<i>E. coli</i> C <i>lac</i> (LacZω ⁻) [Kan ^r McrA ⁻ McrCB ⁻ McrF ⁻ Mrr ⁻ HsdR (r _K ⁻ m _K ⁻)] [F' <i>proAB lac⁺ΔM15 Tn10</i> (Tet ^r)]
ABLE® K STRAIN	<i>E. coli</i> C <i>lac</i> (LacZω ⁻) [Kan ^r McrA ⁻ McrCB ⁻ McrF ⁻ Mrr ⁻ HsdR (r _K ⁻ m _K ⁻)] [F' <i>proAB lac⁺ΔM15 Tn10</i> (Tet ^r)]
AG1 STRAIN	<i>recA1 endA1 gyrA96 thi-1</i> (r _K ⁻ m _K ⁻) <i>supE44 relA1</i>
BL21-GOLD STRAIN	<i>E. coli</i> B F ⁻ <i>dcm+</i> Hte <i>ompT hsdS</i> (r _B ⁻ m _B ⁻) <i>gal endA Tet^r</i> ^a
BL21-GOLD(DE3) STRAIN	<i>E. coli</i> B F ⁻ <i>dcm+</i> Hte <i>ompT hsdS</i> (r _B ⁻ m _B ⁻) <i>gal λ</i> (DE3) <i>endA Tet^r</i> ^a
BL21-GOLD(DE3)pLysS STRAIN	<i>E. coli</i> B F ⁻ <i>dcm+</i> Hte <i>ompT hsdS</i> (r _B ⁻ m _B ⁻) <i>gal λ</i> (DE3) [pLysS Cam ^r]* <i>endA Tet^r</i> ^a
BL21 STRAIN	<i>E. coli</i> B F ⁻ <i>dcm ompT hsdS</i> (r _B ⁻ m _B ⁻) <i>gal</i>
BL21(DE3) STRAIN	<i>E. coli</i> B F ⁻ <i>dcm ompT hsdS</i> (r _B ⁻ m _B ⁻) <i>gal λ</i> (DE3)
BL21(DE3)pLysS STRAIN	<i>E. coli</i> B F ⁻ <i>dcm ompT hsdS</i> (r _B ⁻ m _B ⁻) <i>gal λ</i> (DE3) [pLysS Cam ^r]*
BL21-CODONPLUS® (DE3)-RIPL STRAIN	<i>E. coli</i> B F ⁻ <i>ompT hsdS</i> (r _B ⁻ m _B ⁻) <i>dcm+</i> Tet ^r <i>gal λ</i> (DE3) <i>endA Hte</i> [<i>argU proL</i> Cam ^r] [<i>argU ileY leuW</i> Strep/Spec ^r]
BL21-CODONPLUS® RIL STRAIN	<i>E. coli</i> B F ⁻ <i>ompT hsdS</i> (r _B ⁻ m _B ⁻) <i>dcm+</i> Tet ^r <i>gal endA Hte</i> [<i>argU ileY leuW</i> Cam ^r]*, ^a
BL21-CODONPLUS®(DE3)-RIL STRAIN	<i>E. coli</i> B F ⁻ <i>ompT hsdS</i> (r _B ⁻ m _B ⁻) <i>dcm+</i> Tet ^r <i>gal λ</i> (DE3) <i>endA Hte</i> [<i>argU ileY leuW</i> Cam ^r]*, ^a
BL21-CODONPLUS® RP STRAIN	<i>E. coli</i> B F ⁻ <i>ompT hsdS</i> (r _B ⁻ m _B ⁻) <i>dcm+</i> Tet ^r <i>gal endA Hte</i> [<i>argU proL</i> Cam ^r]*, ^a
BL21-CODONPLUS® (DE3)-RP STRAIN	<i>E. coli</i> B F ⁻ <i>ompT hsdS</i> (r _B ⁻ m _B ⁻) <i>dcm+</i> Tet ^r <i>gal λ</i> (DE3) <i>endA Hte</i> [<i>argU proL</i> Cam ^r]*, ^a
BL21-CODONPLUS® (DE3)-RIL-X STRAIN	<i>E. coli</i> B F ⁻ <i>ompT hsdS</i> (r _B ⁻ m _B ⁻) <i>dcm+</i> Tet ^r <i>gal λ</i> (DE3) <i>endA Hte metA::Tn5</i> (Kan ^r) [<i>argU ileY leuW</i> Cam ^r]*, ^a
BL21-CODONPLUS® (DE3)-RP-X STRAIN	<i>E. coli</i> B F ⁻ <i>ompT hsdS</i> (r _B ⁻ m _B ⁻) <i>dcm+</i> Tet ^r <i>gal λ</i> (DE3) <i>endA Hte metA::Tn5</i> (Kan ^r) [<i>argU proL</i> Cam ^r]*, ^a
ELECTROTEN-BLUE® STRAIN	Δ(<i>mcrA</i>)183 Δ(<i>mcrB</i> - <i>hsdSMR</i> - <i>mrr</i>)173 <i>endA1 supE44 thi-1 recA1 gyrA96 relA1 lac Kan^r Hee</i> [F' <i>proAB lac⁺ΔM15Tn10</i> (Tet ^r)]**
JM101 STRAIN	<i>supE thi-1 Δ(lac-proAB)</i> [F' <i>traD36 proAB lac⁺ΔM15</i>]
JM109 STRAIN	e14 ⁻ (McrA ⁻) <i>recA1 endA1 gyrA96 thi-1 hsdR17</i> (r _K ⁻ m _K ⁺) <i>supE44 relA1 Δ(lac-proAB)</i> [F' <i>traD36 proAB lac⁺ΔM15</i>]
JM110 STRAIN	<i>rpsL</i> (Str ^r) <i>thr leu thi-1 lacY galK galT ara tonA tsx dam dcm supE44 Δ(lac-proAB)</i> [F' <i>traD36 proAB lac⁺ΔM15</i>]
NM522 STRAIN	<i>supE thi-1 Δ(lac-proAB) Δ(mcrB-hsdSM)5</i> (r _K ⁻ m _K ⁻) [F' <i>proAB lac⁺ΔM15</i>]
SCS1 STRAIN	<i>recA1 endA1 gyrA96 thi-1 hsdR17</i> (r _K ⁻ m _K ⁺) <i>supE44 relA1</i>
SCS110 STRAIN	<i>rpsL</i> (Str ^r) <i>thr leu endA thi-1 lacY galK galT ara tonA tsx dam dcm supE44 Δ(lac-proAB)</i> [F' <i>traD36 proAB lac⁺ΔM15</i>]
SURE® STRAIN	e14 ⁻ (McrA ⁻) Δ(<i>mcrCB</i> - <i>hsdSMR</i> - <i>mrr</i>)171 <i>endA1 supE44 thi-1 gyrA96 relA1 lac recB recJ sbcC umuC::Tn5</i> (Kan ^r) <i>uvrC</i> [F' <i>proAB lac⁺ΔM15 Tn10</i> (Tet ^r)]
SURE® 2 STRAIN	e14 ⁻ (McrA ⁻) Δ(<i>mcrCB</i> - <i>hsdSMR</i> - <i>mrr</i>)171 <i>endA1 supE44 thi-1 gyrA96 relA1 lac recB recJ sbcC umuC::Tn5</i> (Kan ^r) <i>uvrC</i> [F' <i>proAB lac⁺ΔM15 Tn10</i> (Tet ^r) Amy Cam ^r]*
TG1 STRAIN	<i>supE thi-1 Δ(lac-proAB) Δ(mcrB-hsdSM)5</i> (r _K ⁻ m _K ⁻) [F' <i>traD36 proAB lac⁺ΔM15</i>]
XL1-BLUE STRAIN	<i>recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac</i> [F' <i>proAB lac⁺ΔM15 Tn10</i> (Tet ^r)]
XL1-BLUE MR STRAIN	Δ(<i>mcrA</i>)183 Δ(<i>mcrCB</i> - <i>hsdSMR</i> - <i>mrr</i>)173 <i>endA1 supE44 thi-1 recA1 gyrA96 relA1 lac</i>
XL1-BLUE MRF' STRAIN	Δ(<i>mcrA</i>)183 Δ(<i>mcrCB</i> - <i>hsdSMR</i> - <i>mrr</i>)173 <i>endA1 supE44 thi-1 recA1 gyrA96 relA1 lac</i> [F' <i>proAB lac⁺ΔM15 Tn10</i> (Tet ^r)]
XL1-BLUE MRF' KAN STRAIN	Δ(<i>mcrA</i>)183 Δ(<i>mcrCB</i> - <i>hsdSMR</i> - <i>mrr</i>)173 <i>endA1 supE44 thi-1 recA1 gyrA96 relA1 lac</i> [F' <i>proAB lac⁺ΔM15 Tn5</i> (Kan ^r)]
XL2-BLUE STRAIN	<i>recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac</i> [F' <i>proAB lac⁺ΔM15 Tn10</i> (Tet ^r) Amy Cam ^r]*
XL2-BLUE MRF' STRAIN	Δ(<i>mcrA</i>)183 Δ(<i>mcrCB</i> - <i>hsdSMR</i> - <i>mrr</i>)173 <i>endA1 supE44 thi-1 recA1 gyrA96 relA1 lac</i> [F' <i>proAB lac⁺ΔM15 Tn10</i> (Tet ^r) Amy Cam ^r]*
XL10-GOLD® STRAIN	Tet ^r Δ(<i>mcrA</i>)183 Δ(<i>mcrCB</i> - <i>hsdSMR</i> - <i>mrr</i>)173 <i>endA1 supE44 thi-1 recA1 gyrA96 relA1 lac Hte</i> [F' <i>proAB lac⁺ΔM15 Tn10</i> (Tet ^r) Amy Cam ^r]*
XL10-GOLD® KAN STRAIN	Tet ^r Δ(<i>mcrA</i>)183 Δ(<i>mcrCB</i> - <i>hsdSMR</i> - <i>mrr</i>)173 <i>endA1 supE44 thi-1 recA1 gyrA96 relA1 lac Hte</i> [F' <i>proAB lac⁺ΔM15 Tn10</i> (Tet ^r) Tn5 (Kan ^r) Amy]
XL1-RED STRAIN	<i>endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac mutD5 mutS mutT Tn10</i> (Tet ^r)

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