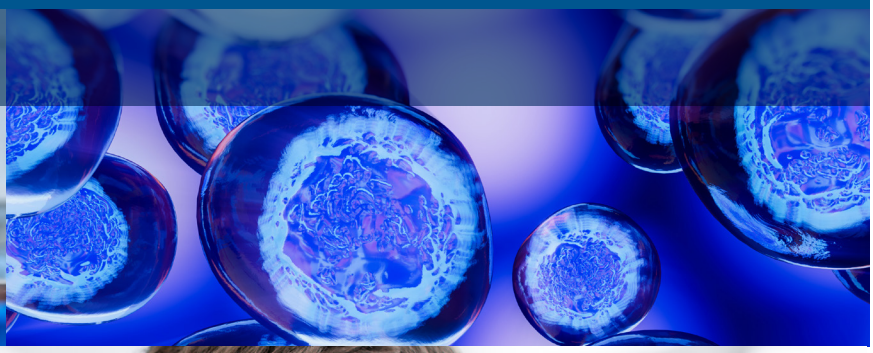
Agilent

Trusted Answers

Competent Cells

How do you choose the right cell line?



We Have Your Competent Cells

Finding the right competent cell for your application is easy with our extensive cell line portfolio. Our comprehensive collection includes innovative strains with high transformation efficiencies designed to match a variety of cloning strategies. Our specialty cells are also ideal for difficult or unusual cloning projects. Use our decision trees to match your application to the perfect strain.

Difficult Cloning

Large or Ligated DNA

Symptoms >
- No colonies after transformation
- Low representation in libraries

XL10-Gold
cells (≥ 5 x 10^9)*
#200314 or
#200315

ElectroTen-Blue
electroporation cells
(≥ 3 x 10^{10})*
#200159

Unstable Clone

Symptoms >
- Rearranged insert
- Recombined insert
- Loss of insert

SURE 2
cells (≥ 1 x 10^9)*
#200152

SURE
cells (≥ 5 x 10^8)*
#200238

SURE
electroporation cells
(≥ 1 x 10^{10})*
#200227

Toxic Clone

Symptoms >
- No colony formation
- Loss of insert
- Slow/no growth in liquid culture

ABLE K
series of competent cells
ABLE cells only available as chemically competent.

Genomic DNA or Methylated cDNA

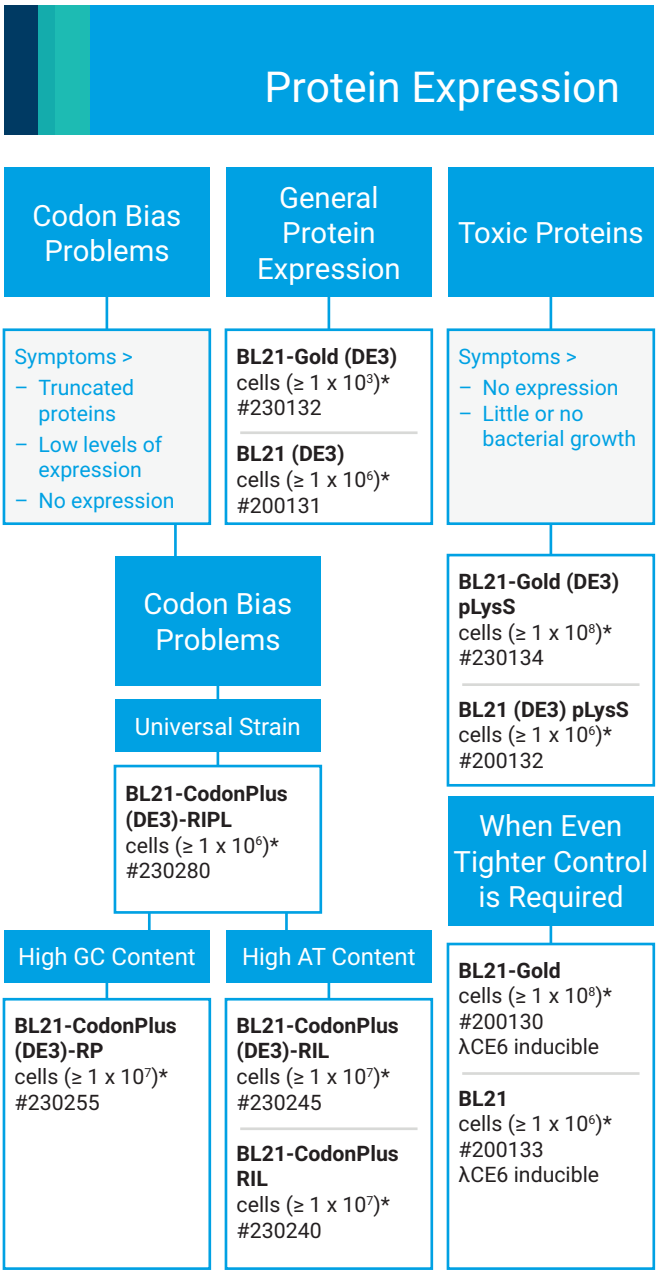
XL2-Blue MRF'
cells (≥ 5 x 10^9)*
#200151

XL1-Blue MRF'
electroporation cells
(≥ 1 x 10^{10})*
#200158

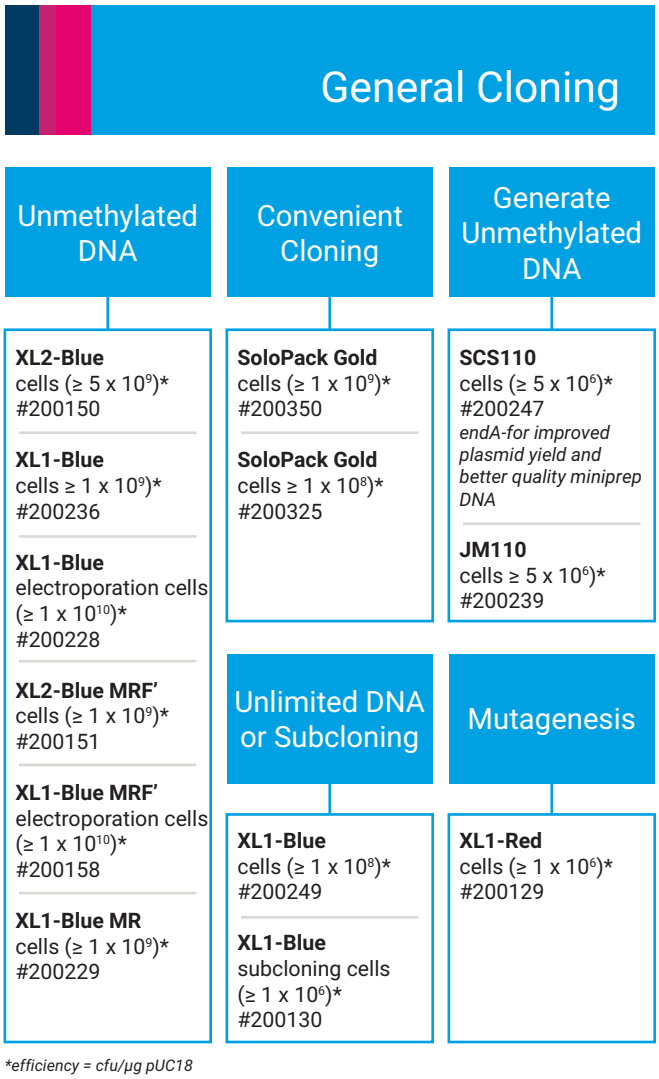
XL1-Blue MR
cells (≥ 1 x 10^9)*
#200229

**efficiency = cfu/µg pUC18*

Contents



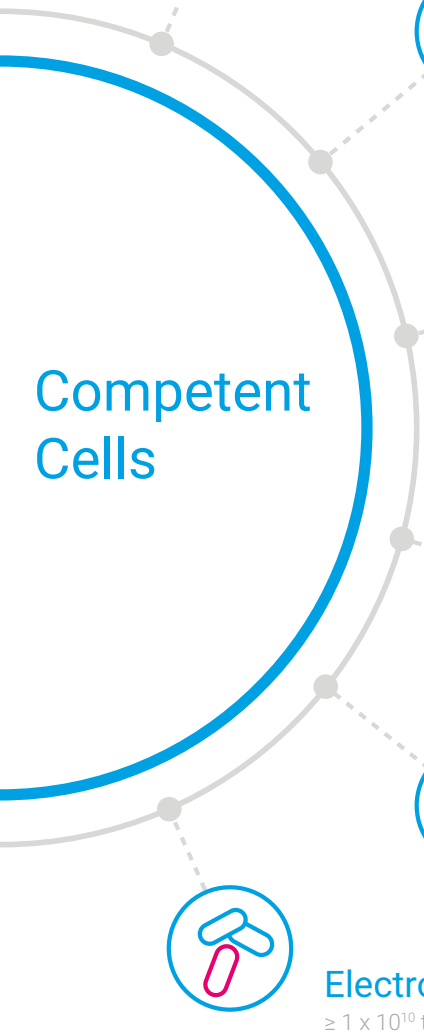
*efficiency = cfu/µg pUC18



*efficiency = cfu/µg 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 cells feature a wide range of cloning efficiencies. 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

≥ 1 x 10^6 transformants/µg of supercoiled DNA

Subcloning-grade competent cells are perfect when you don't need the highest efficiency, but do need consistent every day results. They are an economical choice for routine subcloning procedures when DNA is not limited. The Agilent XL1-Blue strain is available as a subloning-grade competent cell line.

Hte Phenotype/Hee Phenotype

Hte and Hee phenotypes were developed to enhance competent cell performance. These phenotypes allow you to efficiently transform large plasmid and ligated DNA. Agilent XL10-Gold, Agilent BL21-Gold, Agilent BL21-CodonPlus, and Agilent SoloPack Gold cells each contain this phenotype. Agilent ElectroTen-Blue electroporation-competent cells contain the Hee phenotype.

Ultracompetent Cells

≥ 5 x 10^9 transformants/µg of supercoiled DNA

Ultracompetent cells provide the highest efficiencies 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

≥ 1 x 10^8 transformants/µg of supercoiled DNA

Competent cells are available for cloning procedures that do not require supercompetent efficiencies. At 1 x 10^8 transformants/µg, this group of competent cells is the economical alternative for routine cloning.

Supercompetent Cells

≥ 1 x 10^9 transformants/µg of supercoiled DNA

Supercompetent cells are available in various strains at efficiencies greater than 1 x 10^9 transformants/µg. These superior-quality cells include the following: Agilent XL1-Blue, Agilent XL1-Blue MR, Agilent XL1-Blue MRF' Kan, Agilent SoloPack Gold, and Agilent SURE2 supercompetent cells.

Electroporation-Competent Cells

≥ 1 x 10^{10} transformants/µg of supercoiled DNA efficiency for Agilent ElectroTen-Blue cells

Electroporation-competent cells are simple to use. Ease-of-use and high efficiency make electroporation a popular method for library construction, cloning larger inserts, or cloning limited amounts of DNA. Our high-performance ElectroTen-Blue cells aptly survive electroporation treatment, giving them excellent cloning efficiency for ligated DNA.

The Hte and Hee Phenotypes

Large and ligated DNA

At Agilent, we know it's more difficult to introduce large or ligated DNA constructs into competent cells than supercoiled DNA or small plasmids. That's why we've 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 Agilent 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, Agilent 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 (25 kb) 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 DH5α (data not shown).

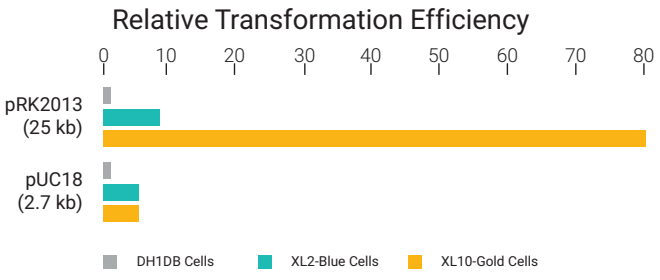


Figure 1. Agilent XL10-Gold ultracompetent cells transform large DNA at higher efficiencies. 100 pg of the pUC18 plasmid (2.7 kb) or 500 ng of the pRK2013 plasmid (25 kb) was transformed into 100 µL of *E. coli* competent cells. 500 ng of the pRK2013 plasmid is used to compensate for the lower transformation 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.

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 x 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 x 10^{9} for chemical transformations to 1 x 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. Agilent ElectroTen-Blue electroporation-competent cells, with an average efficiency of ≥ 3.0 x 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.

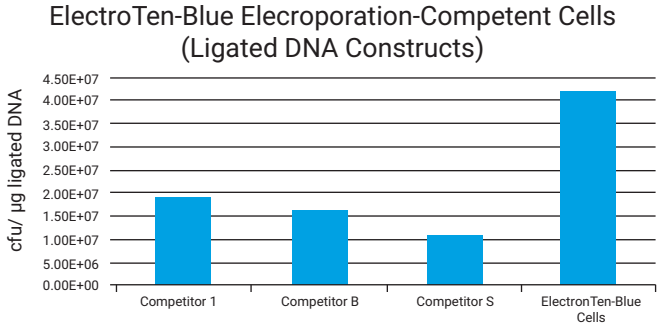


Figure 2. ElectroTen-Blue cells and ligated DNA. Agilent ElectroTen-Blue electroporation-competent cells consistently outperform "highest efficiency" electroporation-competent cells from other suppliers. The pBluescript cloning vector was ligated to a 0.8 kb orange fluorescent protein (OFP) fragment 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 bias against large inserts.

Large DNA

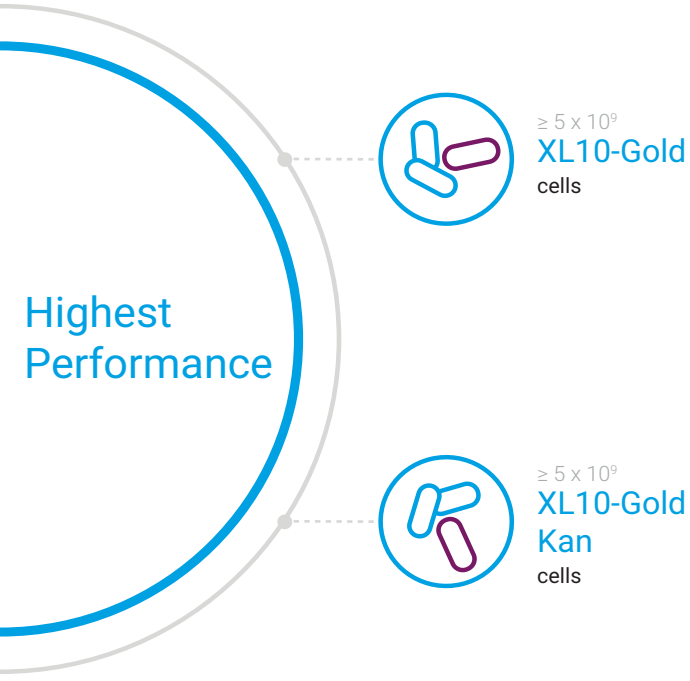
XL10-Gold cells are the only chemically competent cells that allow you to efficiently transform large DNA molecules, including expression vectors and genomic DNA. XL10-Gold ultracompetent cells are the host cells of choice when you need the highest transformation efficiencies for large constructs.

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.

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 libraires. 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).



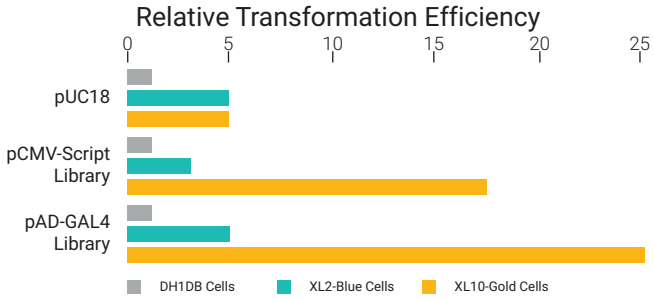


Figure 3. XL10-Gold cells demonstrate superior transformation of large DNA. Relative transformation efficiency comparison between Agilent XL10-Gold ultracompetent cells, Agilent XL2-Blue ultracompetent cells, and DH10B cells. XL10-Gold cells reduce bias against transformation of large DNA compared to alternative cells. 30 ng of the pCMV-Script vector (4.2 kb) or the pAD-GAL4 vector (7.2 kb) was ligated to 10 ng cDNA for construction of a plasmid library. Of the 15 µL ligation reactions, 1 µL aliquots were used to transform 100 µL of DH10B, XL2-Blue, or XL10-Gold competent cells. Supercoiled pUC18 plasmid was used as a transformation control.

Newly Improved ElectroTen-Blue Cells

Highest-efficiency electroporation-competent cells

Agilent ElectroTen-Blue electroporation-competent cells offer high transformation efficiencies of ≥ 3.0 x 10^{10} for 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.

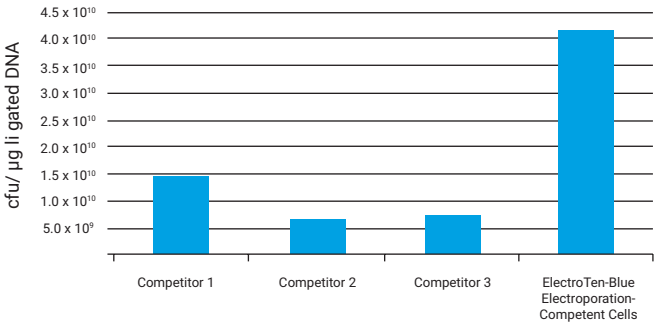


Figure 4. ElectroTen-Blue electroporation-competent cells versus the competition using supercoiled pUC DNA. Agilent ElectroTen-Blue electroporation-competent cells consistently outperform "highest efficiency" electroporation-competent cells from other suppliers. Supercoiled pUC was electroporated following manufacturer's instructions.

Easily transform large and ligated DNA

ElectroTen-Blue electroporation-competent cells exhibit the Hee phenotype. This phenotype improves the cell survival rate, increasing cloning efficiency of large plasmids and ligated DNA. Derived from XL1-Blue electroporation-competent 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 three-fold higher efficiency over our previous electroporation-competent cell line (Figure 5). These cells are perfect for 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.

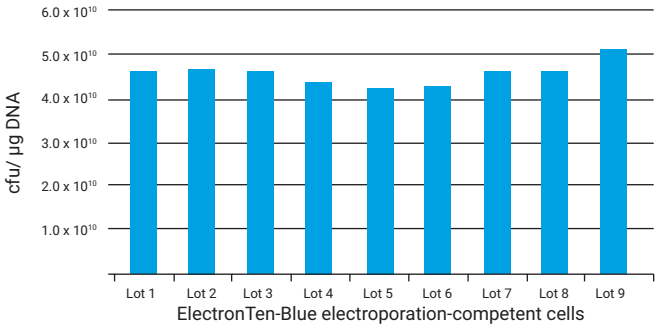


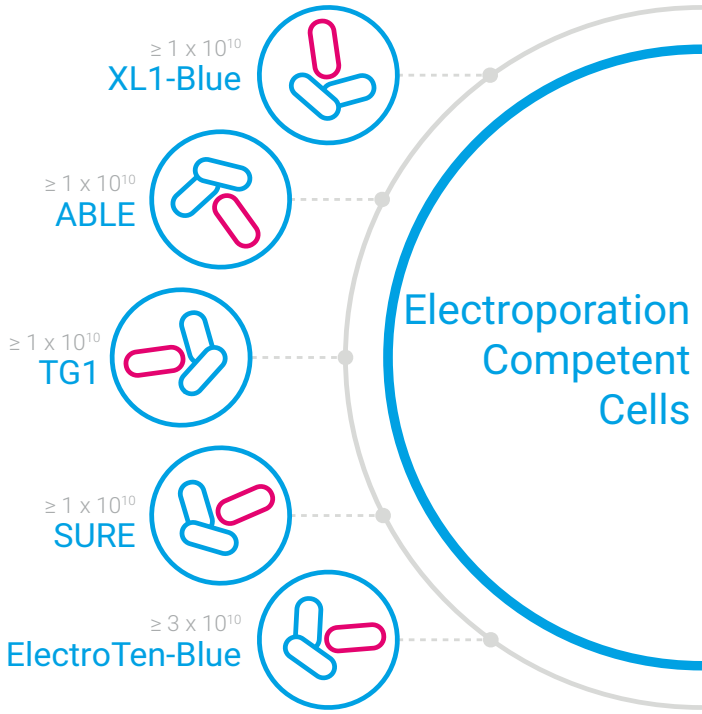
Figure 5. Lot-to-lot efficiency. We compared transformation efficiencies across several lots of Agilent ElectroTen-Blue electroporation-competent cells. Consistent lot-to-lot results ensure success in all of your cloning projects.

Spend less time preparing electroporation-ready DNA

Before electroporation, ligated DNA must be purified to remove DNA ligase, a potential inhibitor of electroporation. Agilent StrataClean resin dramatically simplifies this process. Because of its high affinity for proteins, StrataClean resin removes protein contamination with extraction complete in only five 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, SURE, ABLE, and TG1 cells.



Cloning Difficult DNA

Unstable DNA / toxic DNA / methylated DNA

We have created strains that solve some of the toughest cloning challenges. The Agilent SURE series is engineered to improve cloning of unstable DNA. The Agilent ABLE series offers a simplified approach for propagating toxic DNA. The Agilent 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. Some 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. Agilent SURE competent cells[13] 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, Δ(*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 (≥1 x 10^{10} transformants/µg DNA), as competent-grade (≥5 x 10^{8} transformants/µg DNA), and as a highly efficient derivative, Agilent SURE competent cells[14] (≥ 1 x 10^{9} transformants/µg DNA).

Toxic DNA

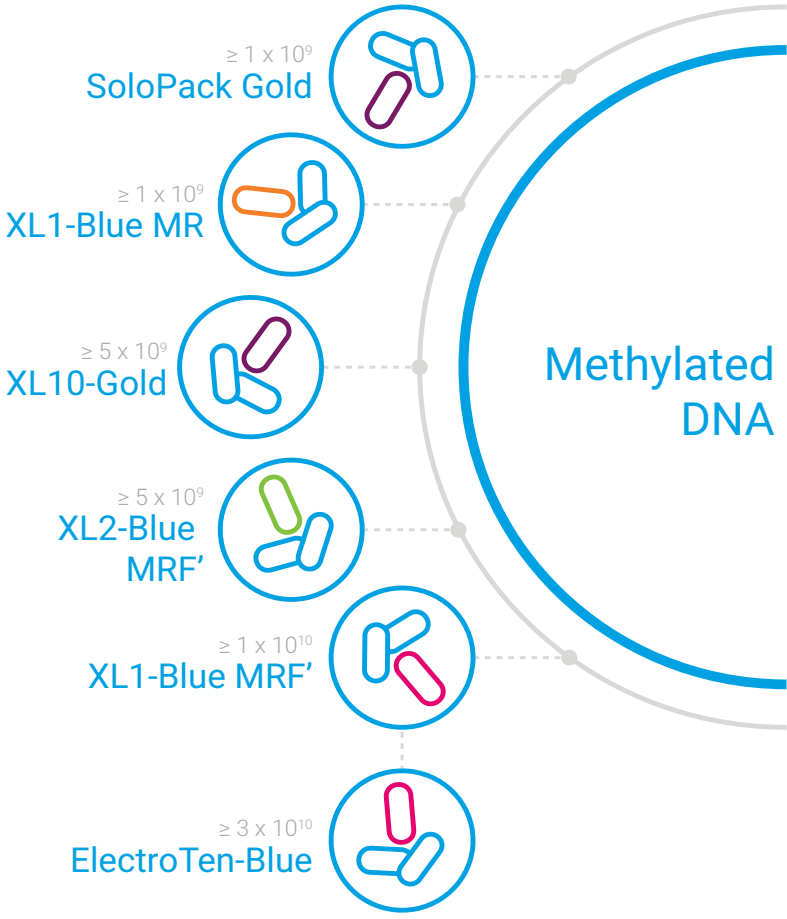
Many genes are difficult to clone in *E. coli*. Sometimes, the insert codes for a protein that is toxic or just difficult to clone. The high copy number of most commonly used cloning vectors amplifies this cloning problem.

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 K strain provides an easy alternative to recloning projects by reducing the copy number of plasmids by 10-fold. Reducing plasmid copy number can decrease the level of cloned protein product, resulting in increased cell viability while avoiding mutations to the gene of interest that arise from protein toxcity. Try this strain to obtain the highest copy number that still allows growth of your construct.

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. Cloning methylated DNA is more efficient when you use our restriction-minus competent cells. When DNA is methylated unlike bacterial host patterns, it is cleaved by *E. coli* host restriction enzymes. Cleavage of DNA before host replication creates libraries that lack complete representation. The bacterial strains in our MR 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 for the highest efficiency cloning of a variety of plasmids, XL1-Blue MRF' electroporation-competent cells for electroporation, XL1-Blue MR supercompetent cells 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 system 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 usage in the recombinant gene differs from that in the host cells. Forced high-level expression of a gene with codons 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). The Agilent 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

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.

The 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 strain has 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.

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

** 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/*

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*.

Save two days with BL21-Gold competent cells

Agilent BL21-Gold competent cells incorporate major improvements over the original BL21 series. BL21-Gold cells feature the Hte phenotype which contributes to a 100-fold increase in transformation efficiency for greater than $1x10^8$ 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 two 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

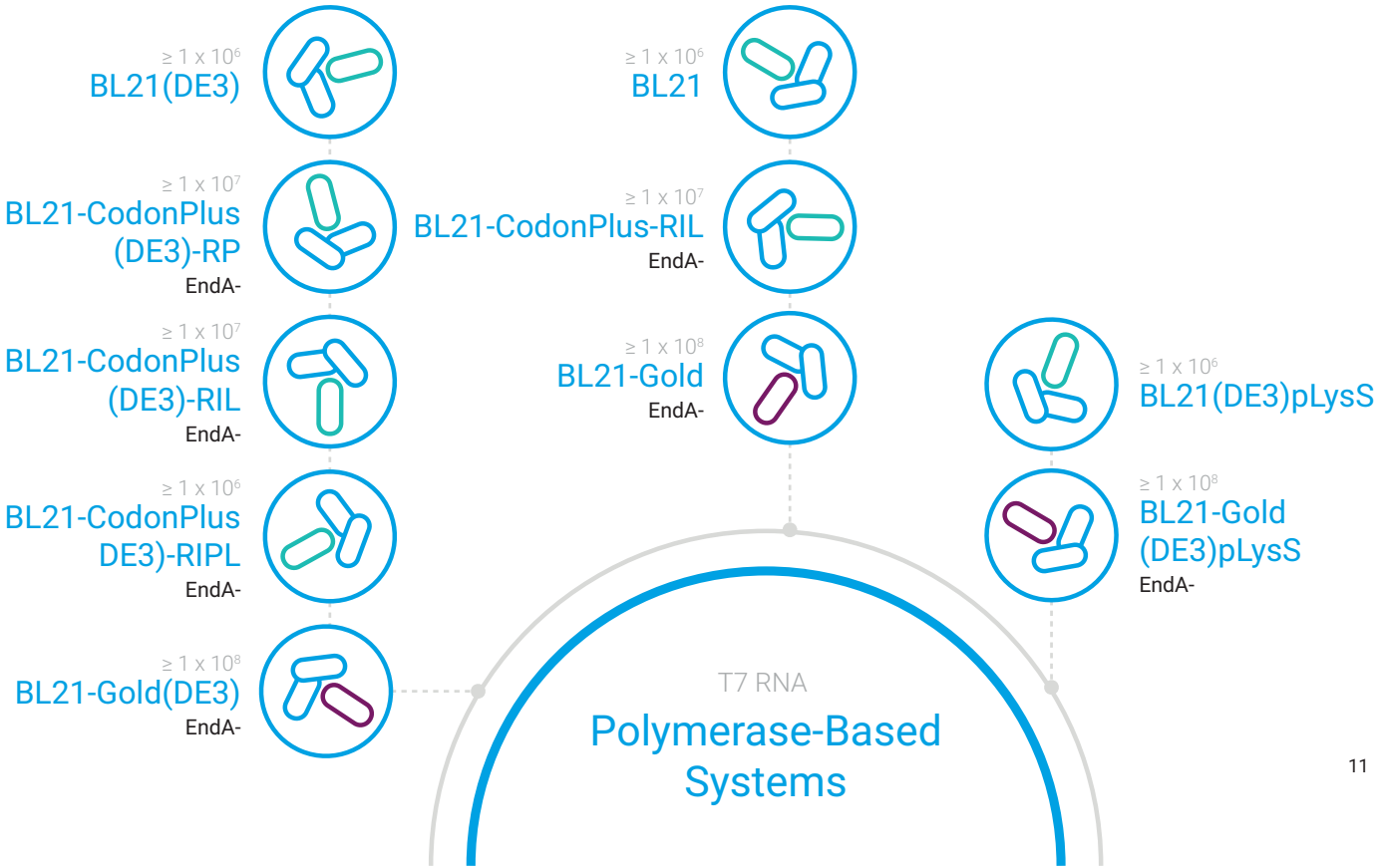
The basic BL21 strain does not contain the T7 RNA polymerase gene and can be used with non-T7 RNA polymerase protein expression systems. To induce protein expression from T7 promoter-driven vectors, the host is infected with lambda CE6 bacteriophage, which provides the T7 RNA polymerase. Since induction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

BL21(DE3), BL21-Gold(DE3), and BL210CodonPlus(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.

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 ≥ 1 x 10^{10} transformants/µg of DNA, and chemically competent XL2-Blue cells give you ≥ 5 x 10^{9} transformants/µg of DNA. When ultimate efficiency is not as critical, try the supercompetent-grade (≥ 1 x 10^{9} transformants/µg DNA) competent-grade ≥ 1 x 10^{8} transformants/µg DNA), or the subcloning-grade (≥ 1 x 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' (≥ 5 x 10^{9} transformants/µg DNA), or as supercompetent (≥ 1 x 10^{9} transformants/µg DNA) cells. When the F' episome and blue-white screening are unnecessary, use XL1-Blue MR supercompetent cells (≥ 1 x 10^{9} transformants/µg DNA).

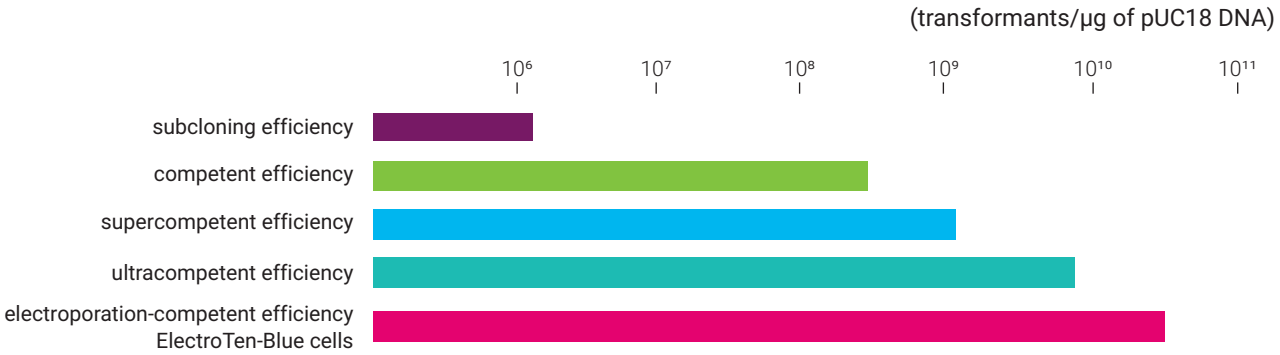
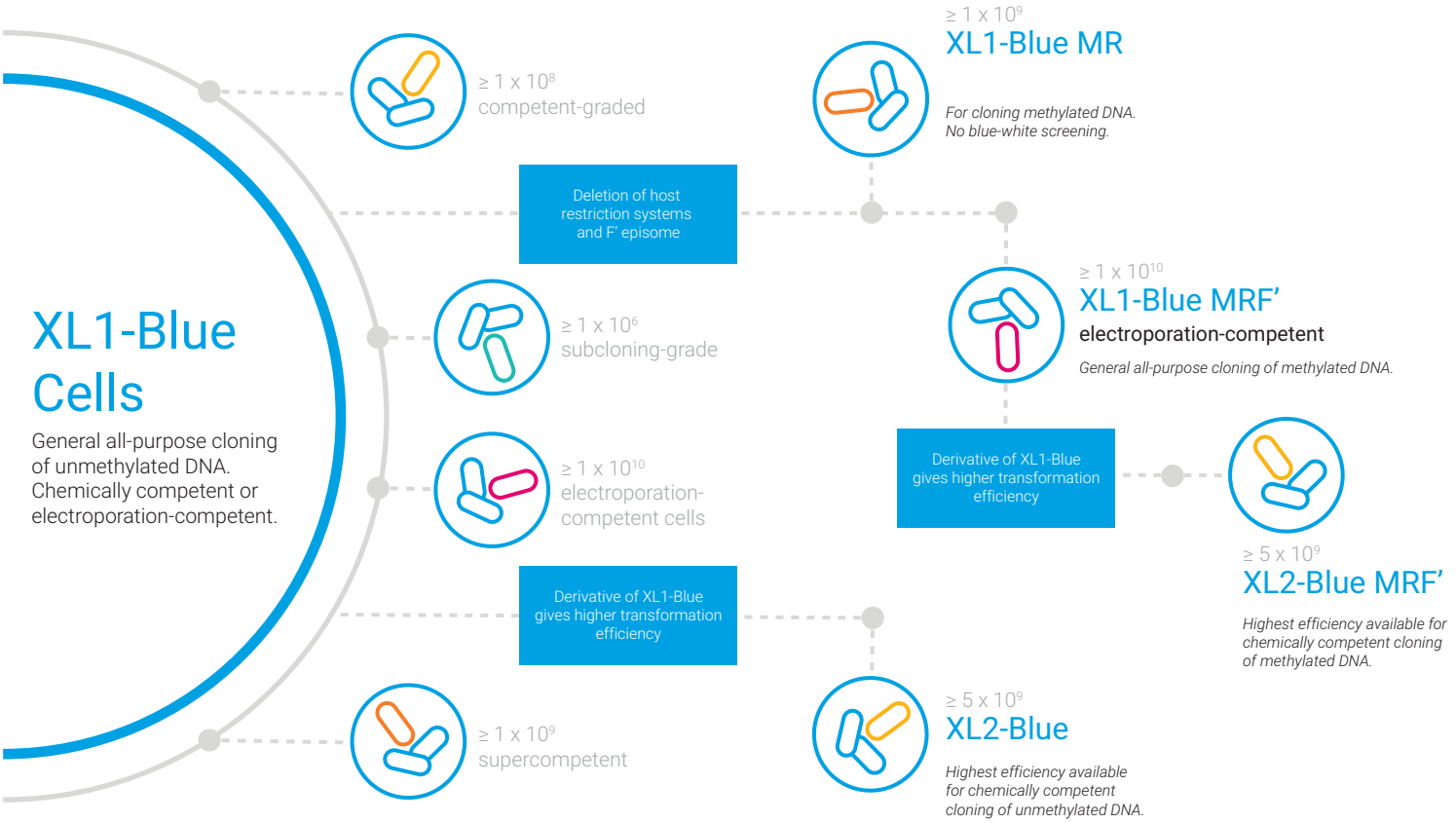


Figure 6. Competent cell efficiencies. The Agilent XL1-Blue series of competent cells is available in every efficiency so you can choose the derivative that matches the demands of your cloning experiment.

Versatile Cloning



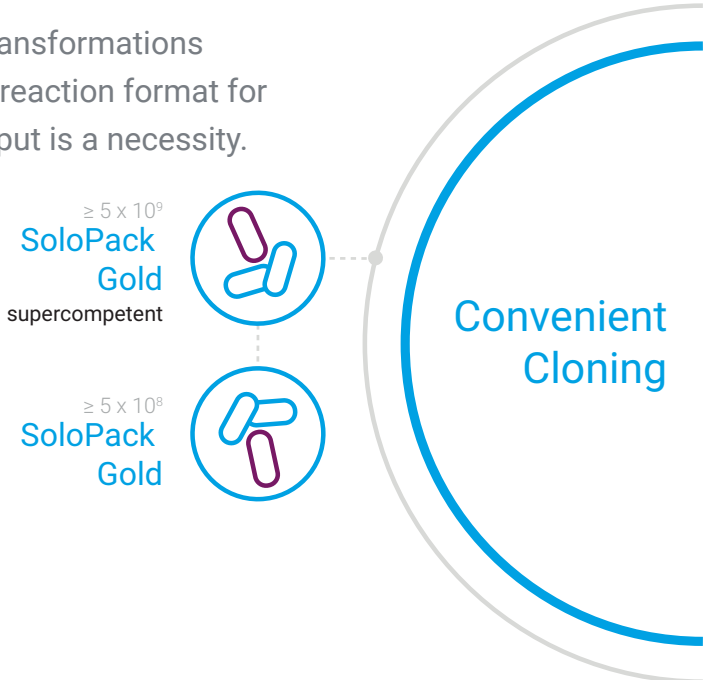
Convenient Cloning

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

Agilent 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, aliquoting, and refreezing. Thaw only the cells you need. There are fewer pipetting steps because the entire transformation reaction occurs in the tube supplied.



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 miniprep DNA.

SCS110
EndA-

JM110

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 Eco*R* II.

Mutagenesis

Fast, easy random mutagenesis

We created the Agilent 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.

XL1-Red

Wide 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.

High Efficiency

Our ultracompetent cells provide high transformation efficiencies and are your best insurance against troublesome cloning projects.

XL10-Gold ultracompetent cells provide more colonies than other commercially available competent cells. They're 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

- 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 x 10^8 transformants/µg.
- 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 Agilent 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.

Appendix Continued

- ***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 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 Agilent strains carry this mutation.
- ***hsdS*:** Specificity determinant for *hsdM* and *hsdR*. Mutation of this gene eliminates both HsdM and HsdR activity. Most Agilent 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 Agilent strains carry this mutation.
- ***mcrCB*:** *E. coli* restriction system that cleaves methylated DNA of sequence 5′ G^{5}*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, 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, 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 Agilent 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 Eco*R* 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.

- ***lac I*:** Repressor protein of lac operon. *lacIq* is a mutant of *lacI* that over-produces 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.
- ***lacZ*^{3}*M15*:** A specific N-terminal deletion which permits the α-complementation segment present on pUC-based plasmids, such as the pBluescript phagemid 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. ≥ 5 x 10^9 transformants/µg	Tetracycline resistant Chloramphenicol resistant*	#200314
	10 x 0.1 mL aliquots			#200315
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. ≥ 3 x 10^{10} transformants/µg	Tetracycline resistant Kanamycin resistant	#200159
Convenient Cloning				
SoloPack Gold Supercompetent Cells	15 single-tube transformations	For high-efficiency cloning. Convenient single-reaction format ≥ 1 x 10^9 transformants/µg	Tetracycline resistant Chloramphenicol resistant*	#230350
SoloPack Gold Competent Cells	15 single-tube transformations	For routine cloning. Convenient single-reaction format ≥ 1 x 10^8 transformants/µg	Tetracycline resistant Chloramphenicol resistant*	#230325
Routine Cloning				
XL1-Blue Electroporation-Competent Cells	5 x 0.1 mL aliquots	For electroporation. ≥ 1 x 10^{10} transformants/µg	Tetracycline resistant	#200228
XL1-Blue MRF' Electroporation-Competent Cells	5 x 0.1 mL aliquots	For electroporation. Restriction minus for cloning methylated DNA ≥ 1 x 10^{10} transformants/µg	Tetracycline resistant	#200158
XL2-Blue Ultracompetent Cells	10 x 0.1 mL aliquots	Highest cloning efficiency. ≥ 5 x 10^9 transformants/µg	Tetracycline resistant Chloramphenicol resistant*	#200150
XL2-Blue MRF' Ultracompetent Cells	10 x 0.1 mL aliquots	Restriction minus for cloning methylated DNA. Highest cloning efficiency. ≥ 5 x 10^9 transformants/µg	Tetracycline resistant Chloramphenicol resistant*	#200151
XL1-Blue Supercompetent Cells	5 x 0.2 mL aliquots	For high-efficincy cloning. ≥ 1 x 10^9 transformants/µg	Tetracycline resistant	#200236
XL1-Blue MR Supercompetent Cells	5 x 0.2 mL aliquots	Use for cloning without the F' episome. ≥ 1 x 10^8 transformants/µg		#200229
XL1-Blue Competent Cells	5 x 0.2 mL aliquots	For routine cloning. ≥ 1 x 10^8 transformants/µg	Tetracycline resistant	#200249
XL1-Blue Subcloning-Grade Competent Cells	8 x 0.5 mL aliquots	For cloning where DNA is not limited. ≥ 1 x 10^6 transformants/µg	Tetracycline resistant	#200130
Cloning Unstable DNA				
SURE 2 Supercompetent Cells	5 x 0.1 mL aliquots	High-efficiency derivative. ≥ 1 x 10^9 transformants/µg	Tetracycline resistant Chloramphenicol resistant* Kanamycin resistant	#200152
SURE Competent Cells	5 x 0.2 mL aliquots	≥ 5 x 10^8 transformants/µg	Tetracycline resistant Kanamycin resistant	#200238
SURE Electroporation-Competent Cells	5 x 0.1 mL aliquots	For electroporation. ≥ 1 x 10^9 transformants/µg	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 *lacUV5* promoter for easy induction of protein expression. ≥ 1 x 10^6 transformants/µg	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 λCE6 for extremely tight control of expression. ≥ 1 x 10^7 transformants/µg	Tetracycline resistant Chloramphenicol resistant*	#230240
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 *lacUV5* promoter for easy induction of protein expression. ≥ 1 x 10^7 transformants/µg	Tetracycline resistant Chloramphenicol resistant*	#230245

Ordering Information Continued

BL2-CodonPlus (DE3)-RP Competent Cells	10 x 0.1 mL aliquots	Use to eliminate bias. Use with pET or pCAL vectors. Encodes T7 RNA polymerase under the control of the *lacUV5* promoter for easy induction of protein expression. ≥ 1 x 10^7 transformants/µg	Tetracycline resistant Chloramphenicol resistant*	#230255
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. ≥ 1 x 10^8 transformants/µg	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. ≥ 1 x 10^8 transformants/µg	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. ≥ 1 x 10^8 transformants/µg	Tetracycline resistant Chloramphenicol resistant*	#230134
BL21 Cells	5 x 0.2 mL aliquots	Use with non-T7 polymerase-based systems or with λCE6 for extremely toxic proteins. ≥ 1 x 10^6 transformants/µg		#200133
BL21(DE3) Cells	5 x 0.2 mL aliquots	Use with nontoxic proteins. ≥ 1 x 10^6 transformants/µg		#200131
BL21(DE3)pLysS Cells	5 x 0.2 mL aliquots	Use with both toxic and nontoxic proteins. ≥ 1 x 10^6 transformants/µg	Chloramphenicol resistant*	#200132
Cloning Sequences that Encode Toxic Proteins				
ABLE K Cells	5 x 0.2 mL aliquot ABLE K	≥ 5 x 10^6 transformants/µg	Tetracycline resistant Kanamycin resistant	#200172
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. ≥ 5 x 10^6 transformants/µg	Streptomycin resistant	#200247
JM110 Cells	5 x 0.2 mL aliquots	≥ 5 x 10^6 transformants/µg	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 ≥ 3 x 10^{10} transformants/µg	Tetracycline resistant Kanamycin resistant	#200159
XL1-Blue Electroporation-Competent Cells	5 x -0.1 mL aliquots	For all-purpose cloning ≥ 1 x 10^{10} transformants/µg	Tetracycline resistant	#200228
XL1-Blue MRF' Electroporation-Competent Cells	5 x 0.1 mL aliquots	Restriction minus for cloning methylated DNA. ≥ 1 x 10^{10} transformants/µg	Tetracycline resistant	#200158
SURE Electroporation-Competent Cells	5 x 0.1 mL aliquots	For cloning unstable DNA ≥ 1 x 10^{10} transformants/µg	Tetracycline resistant Kanamycin resistant	#200227
TG1 Electroporation-Competent Cells	5 x 0.1 mL aliquots	For phage display. ≥ 1 x 10^{10} transformants/µg		#200123
Classic Cells				
SCS1 Supercompetent Cells	5 x 0.1 mL aliquots	≥ 1 x 10^9 transformants/µg		#200231
JM101 Competent Cells	5 x 0.2 mL aliquots	≥ 1 x 10^8 transformants/µg		#200234
JM109 Competent Cells	5 x 0.2 mL aliquots	≥ 1 x 10^8 transformants/µg		#200235
Competent Cell Reagents				
AMP Tabs	200 x 25 mg tablets	Ampicillin in premeasured tablets.		#300021
IPTG	1 gram			#300127

Genotypes

Host Strain	Genotype
ABLE K Strain	*E. coli* C lac(LacZω–) [Kan^r McrA– McrCB– McrF– Mrr– HsdR (r_K – m_K –)] [F´ *proAB lacI^{q}ZΔM15 Tn10* (Tet^r)]
BL21-Gold Starin	*E. coli* B F– *dcm*+ Hte *ompT hsdS*(r_B – m_B –) *gal endA* Tet^r [a]
BL21-Gold(DE3) Strain	*E. coli* B F– *dcm*+ Hte *ompT hsdS*(r_B – m_B –) *gal* λ (DE3) *endA* Tet^r [a]
BL21-Gold(DE3)pLysS Strain	*E. coli* B F– *dcm*+ Hte *ompT hsdS*(r_B – m_B –) *gal* λ(DE3) [pLysS Cam^r]* *endA* Tet^r [a]
BL21 Strain	*E. coli* B F– *dcm ompT hsdS*(r_B – m_B –) *gal*
BL21(DE3) Strain	*E. coli* B F– *dcm ompT hsdS*(r_B – m_B –) *gal* λ(DE3)
BL21(DE3)pLysS Strain	*E. coli* B F– *dcm ompT hsdS*(r_B – m_B –) *gal* λ(DE3) [pLysS Cam^r]*
BL21-CodonPlus (DE3)-RIPL Strain	*E. coli* B F– *ompT hsdS*(r_B – m_B –) *dcm*+ Tet^r *gal* λ(DE3) *endA* Hte [*argU proL* Cam^r] [*argU ileY leuW* Strep/Spec^r]
BL21-CodonPlus RIL Strain	*E. coli* B F– *ompT hsdS*(r_B – m_B –) *dcm*+ Tet^r *gal endA* Hte [*argU ileY leuW* Cam^r]*[a]
BL21-CodonPlus(DE3)-RIL Strain	*E. coli* B F– *ompT hsdS*(r_B – m_B –) *dcm*+ Tet^r *gal* λ(DE3) *endA* Hte [*argU ileY leuW* Cam^r]*[a]
BL21-CodonPlus (DE3)-RP Strain	*E. coli* B F– *ompT hsdS*(r_B – m_B –) *dcm*+ Tet^r *gal* λ(DE3) *endA* Hte [*argU proL* Cam^r]*[a]
ElectroTen-Blue Strain	Δ(*mcrA*)*183* (*mcrB-hsdSMR-mrr*)*173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac* Kan^r Hee [F´*proAB lacI^{q}ZΔM15Tn10*(Tet^r)]**
JM101 Strain	*supE thi*-1 *Δ(lac-proAB) [*F´ *traD36 proAB lacI^{q}ZΔM15]*
JM109 Strain	*e14–*(McrA–*) recA1 endA1 gyrA96 thi-1 hsdR17 (r_K – m_K+) supE44 relA1 Δ(lac-proAB) [*F´ *traD36 proAB lacI^{q}ZΔM15]*
JM110 Strain	*rpsL* (Str^r) *thr leu thi-1 lacY galK galT ara tonA tsx dam dcm supE44 Δ(lac-proAB) [*F´ *traD36 proAB lacI^{q}ZΔM15]*
SCS1 Strain	*recA1 endA1 gyrA96 thi-1 hsdR17* (r_K – m_K +) *supE44 relA1*
SCS110 Strain	*rpsL (Strr) thr leu endA thi-1 lacY galK galT ara tonA tsx dam dcm supE44 Δ(lac-proAB) [*F´ *traD36 proAB lacI^{q}ZΔM15]*
SURE Strain	*e14–*(McrA–) Δ*(mcrCB-hsdSMR-mrr)171 endA1 supE44 thi-1 gyrA96 relA1 lac recB recJ sbcC umuC::*Tn*5* (Kan^r) *uvrC* [F´ *proAB lacI^{q}ZΔM15 Tn10* (Tet^r)*]*
SURE 2 Strain	*e14–*(McrA–) Δ*(mcrCB-hsdSMR-mrr)171 endA1 supE44 thi-1 gyrA96 relA1 lac recB recJ sbcC umuC::*Tn*5* (Kan^r) *uvrC* [F´ *proAB lacI^{q}ZΔM15 Tn10* (Tet^r) Amy Camr]*
TG1 Strain	*supE thi-1* Δ*(lac-proAB)* Δ*(mcrB-hsdSM)5*(r_K – m_K –) [F´ *traD36 proAB lacI^{q}ZΔM15*]
XL1-Blue Strain	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F´ *proAB lacI^{q}ZΔM15* Tn*10* (Tet^r)]
XL1-Blue MR Strain	Δ*(mcrA)183* Δ*(mcrCB-hsdSMR-mrr)173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac*
XL1-Blue MRF' Strain	Δ*(mcrA)183* Δ*(mcrCB-hsdSMR-mrr)173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac* [F´ *proAB lacI^{q}ZΔM15 Tn10* (Tet^r)]
XL2-Blue Strain	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F´ *proAB lacI^{q}ZΔM15 Tn10* (Tet^r) Amy Cam^r]*
XL2-Blue MRF' Strain	Δ*(mcrA)183* Δ*(mcrCB-hsdSMR-mrr)173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac* [F´ *proAB lacI^{q}ZΔM15 Tn10* (Tet^r) Amy Cam^r]*
XL10-Gold Strain	Tet^r Δ*(mcrA)183* Δ*(mcrCB-hsdSMR-mrr)173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac Hte* [F´ *proAB lacI^{q}ZΔM15 Tn10* (Tet^r) Amy Cam^r]*
XL1-Red Strain	*endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac mutD5 mutS mutT Tn10* (Tet^r)

* *Chloramphenicol resistant at concentrations of < 40 µg/mL, but sensitive at concentrations of 100 µg/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.*

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