3rd Edition

Capture the Gene

Competent Cell Guide

High Efficiency Cloning and Protein Expression

A Meridian Life Science® Company

Bioline Competent Cell Selection Table

Efficient DNA transformation of competent cells is essential for successful cloning and protein expression applications. Bioline offers a wide range of *E.coli* host strains to meet your requirements. We maintain rigorous quality control standards to ensure lot-to-lot consistency and the highest transformation efficiencies possible.

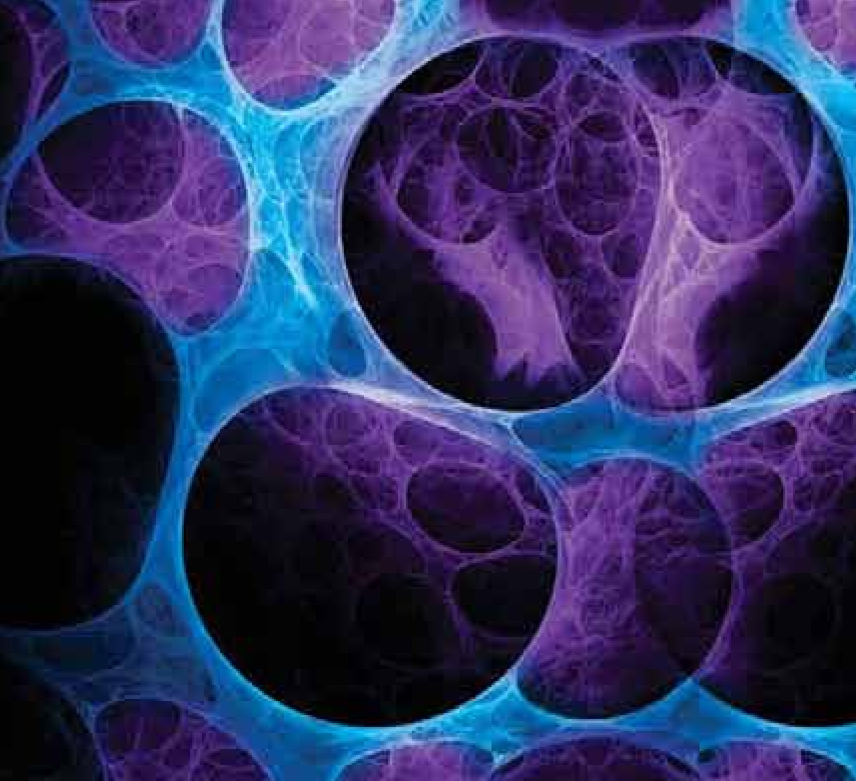
The Bioline Competent Cell Guide is designed to help you select the most appropriate competent cells for your cloning or expression application. Each *E.coli* host has different characteristics and for optimal results, it is important to use the strain that best suits your application. The Bioline Competent Cell Selection Table below provides a summary of the efficiencies, traits and ideal applications for each Bioline competent cell strain.

Cloning

Strain	Efficiency cfu/µg pUC19	Competency: Chemical C Electrocomp E	Strain background	Blue white screening (*lacZ*)	Recombination deficient (*rec*A)	Endonuclease deficient (*end*A)	Restriction deficient (*hsd*R_{K-})
α-Select Gold Efficiency	≥1 x 10^9	C	K12	✓	✓	✓	✓
α-Select Silver Efficiency	≥1 x 10^8	C	K12	✓	✓	✓	✓
α-Select Bronze Efficiency	≥1 x 10^7	C	K12	✓	✓	✓	✓
α-Select Electrocompetent	≥5 x 10^9	E	K12	✓	✓	✓	✓
α-Select Gold Efficiency T1-Res	≥1 x 10^9	C	K12	✓	✓	✓	✓
α-Select Silver Efficiency T1-Res	≥1 x 10^8	C	K12	✓	✓	✓	✓
CH3-Blue 10^8 Chemically competent cells	≥1 x 10^8	C	K12	✓	✓	✓	
CH3-Blue 10^9 Chemically competent cells	≥1 x 10^9	C	K12	✓	✓	✓	
ElectroSHOX Chemically competent cells	≥1 x 10^{10}	E	K12	✓	✓	✓	
BIO*Blue* 10^8 Chemically competent cells	≥1 x 10^8	C	K12	✓	✓	✓	✓
BIO*Blue* 10^9 Chemically competent cells	≥1 x 10^9	C	K12	✓	✓	✓	✓
dam-/dcm- Chemically competent cells	≥1 x 10^7	C	K12				✓

Protein Expression

Strain	Efficiency cfu/µg pUC19	Competency: Chemical C Electrocomp E	Strain background	Restriction deficient (*hsd*S_B)	Protease deficient (*omp*T)	T7 RNA polymerase	Deficient in cytosine metabolism (*dcm*)
BL21	≥1 x 10^7	C	B	✓	✓		✓
BL21 (DE3)	≥1 x 10^7	C	B	✓	✓	✓	✓
BL21 (DE3) PlysS	≥1 x 10^7	C	B	✓	✓	✓	✓
BL21 (DE3) PlysE	≥1 x 10^7	C	B	✓	✓	✓	✓



	Methyl restriction deficient (*mcr*A, *mcr*B, *mrr*)	Single-strand ability (F' episome)	Phage resistance (*fhu*A, *ton*A or T1R)	Unmethylated DNA (*dam dcm*)
			✓	
			✓	
	✓			
	✓			
	✓			
		✓		
		✓		
	✓		✓	✓

	Deficient in galactose metabolism (*gal*)	Ideal applications
	✓	Non T7 promoter expression
	✓	T7 promoter expression
	✓	Regulation of T7 promoter expression
	✓	Regulation of T7 promoter expression

Contents

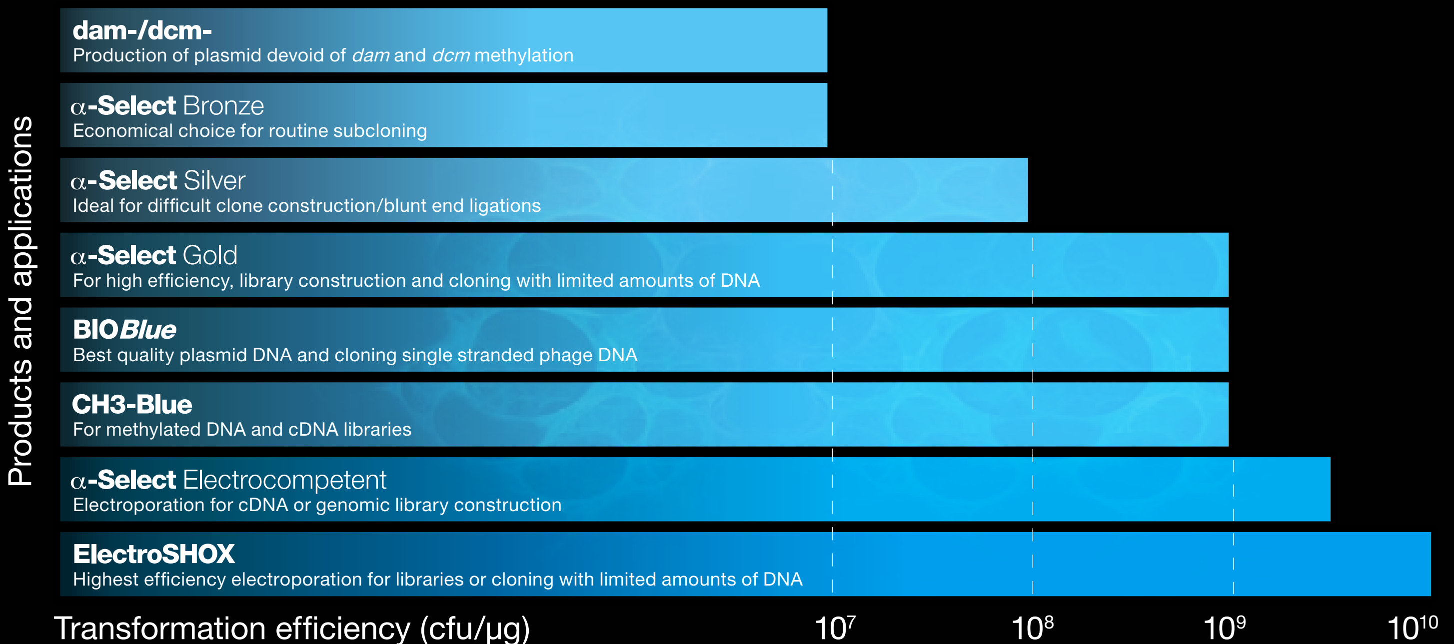
Cloning	04
Chemical Transformation	05
α-Select Competent Cells	06
α-Select Competent Cells (T1-Resistant)	07
dam-/dcm- Chemically Competent Cells (T1-Resistant)	07
CH3-Blue Chemically Competent Cells	08
BIO*Blue* Chemically Competent Cells	08
Electrotransformation	09
ElectroSHOX Competent Cells	09
α-Select Electrocompetent Cells	09
Cloning Reagents	10
Quick-Stick Ligase	10
VELOCITY PCR Kit	10
ISOLATE PCR & Gel Kit	11
SureClean/ SureClean Plus	11
Antibiotic Solutions	12
SOC Medium	12
Associated Cloning Reagents	12
Protein Expression in *E.coli*	14
BL21 Competent Cells	16
BL21 Competent Cells	16
BL21 (DE3) Competent Cells	16
BL21 (DE3) pLysE Competent Cells	17
BL21 (DE3) pLysS Competent Cells	17
BL21 Combo Pack	17
Associated Protein Expression Reagents	17
Technical Tips for Efficient Transformation	18
Chemical Transformation Protocol	19
Electrotransformation Protocol	19
Technical Tips for Successful Protein Expression	20
Genotype Table	21
Frequently Asked Questions	22
General Information	23
Ordering Information	23
Technical Support	23

Cloning

Bioline offers a wide range of cloning *E.coli* host strains for high-efficiency transformation, which are derivatives of a single strain known as K12. In 1922, K12 was first isolated and has undergone a series of selective mutations in order to produce competent cells with desirable genetic markers.

E.coli strains for cloning and transformation typically offer blue/white screening, are efficiently transformed and provide *rec*A1 and *end*A1 markers to minimize recombination and enhance the quality of plasmid DNA. These are the cells that are most commonly used in molecular biology experiments.

Bioline offers a wide range of chemically competent and electrocompetent cells for cloning which include Bronze Efficiency Competent Cells for subcloning, Gold Efficiency Competent Cells for library construction and competent cells for specialized applications. In general, lower efficiency competent cells are suitable for most purposes, such as transforming purified plasmid DNA, whereas high efficiency competent cells are suitable for more difficult cloning and library construction.



Chemical Transformation

Chemical transformation of competent cells is achieved by initially suspending the cells and the target DNA in a calcium chloride based ice-cold buffer. The transformation occurs if the cells are warmed briefly (heat-shock) at 42°C. Cells are then diluted in SOC media (Cat No. BIO-86033) and incubated for 1 hour. Following the incubation, cells are plated onto media that specifically selects for transformants. Transformation efficiencies typically vary from 10^7 to 10^9 transformants per µg of pUC19 DNA using Bioline chemically competent cells.

Chemical transformation is a cost-effective choice of transfection and involves a simple procedure that does not require any specialized equipment.

α-Select Chemically competent cells
α-Select Chemically competent cells (T1-Resistant)
dam-/dcm- Chemically competent cells (T1-Resistant)
BIO*Blue* Chemically competent cells
CH3-Blue Chemically competent cells

Electroporation

Electroporation of *E.coli* strains requires a very high cell density in addition to a non-ionic buffer. The frozen competent cells are thawed on ice, mixed with sample DNA and placed in an electroporation chamber. The Competent Cells and DNA are then subjected to a short and very intense electric field. The pulse of electricity disrupts the membranes of the cells allowing the uptake of plasmid DNA.

Using Bioline ElectroSHOX Competent Cells, transformation efficiencies of 10^{10} are expected. The most important factor to consider for successful electroporation is the conductivity of the sample. Conductivity should be as close to 0 as possible. For optimal results, the sample DNA should be in either sterile water or low ionic strength buffer such as TE.

Electrocompetent formats provide the highest transformation efficiencies, but require an electroporation device.

α-Select Electrocompetent
ElectroSHOX Chemically competent cells

α-Select Competent Cells

PRODUCT	PACK SIZE	EFFICIENCY	CAT NO.
α-Select Bronze Efficiency	2ml (10 x 200µl)	>1 x 10^7 cfu/µg pUC19	BIO-85025
α-Select Silver Efficiency	2ml (10 x 200µl)	>1 x 10^8 cfu/µg pUC19	BIO-85026
α-Select Gold Efficiency*	1ml (20 x 50µl)	>1 x 10^9 cfu/µg pUC19	BIO-85027

*Single use aliquots

FEATURES

- **Comparable to DH5α™**
- **≥10^7, ≥10^8, ≥10^9 transformation efficiencies available**
- **Reduced recombination of cloned DNA (*rec*A)**
- **endA1 mutation for improved plasmid quality**

APPLICATIONS

- **Transformation of cloned DNA into bacterial cells**
- **Blue/white color screening**

α-Select Chemically Competent Cells contain a *lac*Z marker that provides α-complementation of the ß-galactosidase gene for blue/white color screening. The cells are ideal for generating cDNA libraries and subcloning. pUC19 DNA is also provided as a positive control.

Genotype F^- *deo*R *end*A1 *rec*A1 *rel*A1 gyrA96 *hsdR*17($r_k^- m_k^+$) *sup*E44 *thi*-1 *pho*A Δ(*lacZ*YA-*argF*)U169 Φ80*lacZ*ΔM15 λ^-

Comparison of the transformation efficiency of α-Select Competent Cells with increasing size of DNA.

DNA	α-Select Silver Chemically Competent	α-Select Gold Chemically Competent	α-Select Electrocompetent
pUC19 - 2.7Kb	2.2 x 10^8	1.8 x 10^9	2.8 x 10^9
8.2Kb plasmid	3.6 x 10^7	3.8 x 10^8	6.2 x 10^8
13.3Kb plasmid	2.8 x 10^7	3.0 x 10^8	4.4 x 10^8
50Kb cosmid	3.6 x 10^5	3.8 x 10^7	5.6 x 10^7

Comparison of the transformation efficiency of α-Select Competent Cells with increasing size of DNA. Transformations were performed with supercoiled DNA of the indicated size using 50-100µl of α-Select Chemically Competent Cells (100µl for Silver and 50µl for Gold) and 40µl of α-Select Electrocompetent Cells. Results are in colony-forming units (cfu)/µg of DNA and represent the average of three or more tests.

Selection of pUC19 with inserts (white colonies) from those with no inserts (blue) using transformed α-Select competent cells grown in agar containing ampicillin, X-GAL and IPTG.

α-Select Competent Cells (T1-Resistant)

PRODUCT	PACK SIZE	EFFICIENCY	CAT NO.
α-Select Silver Efficiency T1-Resistant	2ml (10 x 200µl)	>1 x 10^8 cfu/µg pUC19	BIO-85029
α-Select Gold Efficiency T1-Resistant	1ml (20 x 50µl)	>1 x 10^9 cfu/µg pUC19	BIO-85030

FEATURES

- **Bacteriophage T1-Resistant**
- **≥10^8 and ≥10^9 transformation efficiencies available**
- **Reduced recombination of cloned DNA (*rec*A)**
- **endA1 mutation for improved plasmid quality**

APPLICATIONS

- **Construction of gene banks**
- **Generation of cDNA libraries using plasmid-derived from vectors**
- **Blue/white color screening**
- **High-quality plasmid preparation**
- **Hosting H13mp cloning vectors**

The Silver and Gold Efficiency Chemically Competent Cells are also available as bacteriophage T1-resistant strains. Many laboratories have experienced bacteriophage T1 outbreaks, as T1 attacks *E.coli* and spreads rapidly. α-Select T1-Resistant Cells protect samples from bacteriophage infection.

Genotype F^- *deo*R *end*A1 *rec*A1 *rel*A1 *gyr*A96 *hsd*R17(r_k^-,m_k^+) *sup*E44 *thi*-1 *pho*A Δ(*lacZ*YA-*arg*F) U169 Φ80*lacZ*ΔM15 λ^-

dam-/dcm-Chemically Competent Cells (T1-Resistant)

PRODUCT	PACK SIZE	EFFICIENCY	CAT NO.
dam-/dcm- T1-Resistant cells	1ml (10 x 100µl)	>10^7 cfu/µg	BIO-85044

FEATURES

- **Lacks *dam* and *dcm* methylases**
- **Bacteriophage T1-Resistant**
- **≥10^7 transformation efficiency**
- **Convenient 100µl aliquots**

APPLICATIONS

- **Generate plasmid DNA devoid of *dam* and *dcm* methylation**
- **Enable restriction digestion of plasmid DNA by methylation-sensitive endonucleases**
- **Stud methylation on expression or DNA repair**

dam-/dcm- Chemically Competent Cells are an ideal host to generate plasmid DNA lacking in *dam* and *dcm* methylation. The absence of Dam and Dcm methylases in this strain prevents methylation at GATC and CC(A/T)GG sites. Plasmid DNA propagated and purified from this host can be digested by the many restriction enzymes otherwise inhibited by *dam* or *dcm* methylation. pUC19 DNA is also provided as a positive control.

Genotype F^- *dam*-13:*Tn*9(Cam^R) *dcm*-6 *ara*-14 *his*G4 *leu*B6 *thi*-1 *lac*Y1 *gal*K2 *gal*T22 *gln*V44 *hsd*R2 *xyl*A5 *mtl*-1 *rps*L 136(Str^R) *rtb*D1 *ton*A31 *tsx*78 *mcr*A *mcr*B1

CH3-Blue Chemically Competent Cells

PRODUCT	PACK SIZE	EFFICIENCY	CAT NO.
CH3-Blue	1ml (20 x 50µl)	>1 x 10^9 cfu/µg pUC19	BIO-85040

FEATURES

- **Lack *mcr*A, *mcr*BC, *mrr* and *hsd*RMS restriction systems**
- **≥10^9 transformation efficiencies available**
- **Convenient single use 50µl aliquots**
- **Blue/white color screening**

APPLICATIONS

- **Cloning of methylated DNA**
- **Ideal for subcloning and generating cDNA libraries**

CH3-Blue Chemically Competent Cells are ideal for the construction of cDNA libraries using plasmid-derived vectors. To facilitate the cloning of DNA that contains methylcytosine or 5-hydroxymethylcytosine, CH3-Blue lacks the *E.coli* restriction systems *mcr*A, *mcr*BC, *mrr* and *hsd*RMS. The *lac*Z mutation allows blue/white color screening and α-complementation of recombinants. pUC19 DNA is also provided as a positive control.

Genotype F^- Δ*mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lacZ*ΔM15 Δ*lac*X74 *rec*A1 *end*A1 *ara*Δ139 Δ(*ara*, *leu*)7697 *gal*U *gal*K *rps*L (Str^R) *nup*G λ^-

BIO*Blue* Chemically Competent Cells

PRODUCT	PACK SIZE	EFFICIENCY	CAT NO.
Bio*Blue*	1ml (20 x 50µl)	>1 x 10^9 cfu/µg pUC19	BIO-85037

FEATURES

- **No need to select on minimal media plates**
- **≥10^9 transformation efficiencies available**
- **Convenient single use 50µl aliquots**
- **Premium quality DNA**

APPLICATIONS

- **Blue/white color screening**
- **Single-stranded plasmid rescue**
- **Excellent host for M13 and related filamentous phage**
- **Ideal strain for preparation of high-quality plasmid DNA**
- **Routine cloning, using Lambda DNA or plasmid vectors**

BIO*Blue* Chemically Competent Cells provide an ideal host for optimal preparation of both high-quality plasmid and Lambda phage vectors. BIO*Blue* is also an excellent host for M13 and related filamentous phage. Single-stranded DNA can be produced from plasmids containing a phage f1 origin. Maintenance of the F' episome in this strain is facilitated via selection with tetracycline, unlike strains such as JM101 which require growth on minimal media.

The BIO*Blue* strain allows blue/white color screening through α-complementation of the ß-Galactosidase gene. pUC19 DNA is also provided as a positive control.

Genotype *rec*A*1* *end*A*1* *gyr*A96 *thi*-1 *hsd*R17($r_k^- m_k^+$) *sup*E44 *rel*A1 *lac* [F' *pro*AB *lac*I^qZΔM15 Tn*10*(Tet^r)]

ElectroSHOX Competent Cells

PRODUCT	PACK SIZE	EFFICIENCY	CAT NO.
ElectroSHOX	1ml (10 x 100µl)	>1 x 10^{10} cfu/µg pUC19	BIO-85038

FEATURES

- **Highest efficiency available: >10^{10} cfu/µg**
- ***rec*A1 and *end*A1 markers to minimize recombination events**
- **Lacks *E.coli* K restriction-modification system, to facilitate cloning of methylated genomic DNA**
- **Convenient aliquot size**

APPLICATIONS

- **Construction of cDNA and genomic DNA libraries**
- **Ideal for transformation of large plasmids (>30Kb)**
- **Blue/white color screening**
- **Construction of gene banks**
- **Efficient plasmid rescue of eukaryotic genomes**

ElectroSHOX Competent Cells are highly efficient *E.coli*, ideal for the construction of cDNA or genomic libraries using electroporation. The *lac*Z mutation allows blue/white color screening and α-complementation of recombinants. The *rec*A1 and *end*A1 markers minimize recombination events and improve the quality and yield of plasmid DNA. In order to facilitate cloning of methylated genomic DNA, ElectroSHOX lacks *E.coli* K restriction-modification systems, and is ideal for the transformation of large plasmids (>30Kb).

ElectroSHOX Competent Cells electroporated with plasmid and grown on MacConkey's agar.

Genotype F^- *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lacZ* M15 Δ*lac*X74 *rec*A1 *end*A1 *ara* Δ139 Δ(*ara*, *leu*)7697 *gal*U *gal*K *rps*L(Str^R) *nup*G λ^-

α-Select Electrocompetent Cells

PRODUCT	PACK SIZE	EFFICIENCY	CAT NO.
α-Select Electrocompetent Cells	1ml (10 x 100µl)	>5 x 10^9 cfu/µg pUC19	BIO-85028

FEATURES

- **Comparable to DH5α™**
- **>5 x 10^9, transformation efficiencies available**
- **Reduced recombination of cloned DNA (*rec*A)**
- **endA1 mutation for improved plasmid quality**

APPLICATIONS

- **Transformation of cloned DNA into bacterial cells**
- **Blue/white color screening**
- **Ideal for subcloning and generating cDNA libraries**

α-Select Electrocompetent Cells contain a *lacZ* marker that provides α-complementation of the ß-galactosidase gene for blue/white color screening. The cells are ideal for generating cDNA libraries and subcloning. pUC19 DNA is also provided as a positive control.

Genotype F^- *deo*R *end*A1 *rec*A1 *rel*A1 *gyr*A96 *hsd*R17($r_k^- m_k^+$) *sup*E44 *thi*-1 *pho*A Δ(*lacZ*YA-*arg*F)U169 Φ80*lacZ*ΔM15 λ^-

Quick-Stick Ligase

PRODUCT	PACK SIZE	CAT NO.
Quick-Stick Ligase	50 Reactions (10u/µl)	BIO-27027
Quick-Stick Ligase	100 Reactions (10u/µl)	BIO-27028
T4 DNA Ligase	500 Units (10u/µl)	BIO-27026

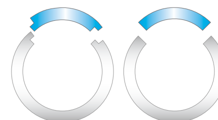
FEATURES

- **Dramatically decreases the time required for DNA cloning**
- **Rapid 5 to 15 minute protocol at room-temperature**
- **Efficient and reliable ligations of cohesive and blunt-ended DNA fragments**
- **No loss of transformation efficiency**

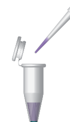
APPLICATIONS

- **Cloning of DNA from: PCR fragments, plasmids, cosmids, genomic, phage and viral DNA**
- **Linker ligation**
- **Re-ligation of linearized plasmids**
- **Ligation of double-stranded oligonucleotides into vectors (plasmid and phage)**

Quick-Stick Ligase is designed to carry out fast and efficient ligation of both cohesive and blunt-ended DNA at room temperature. Quick-Stick Ligase is a T4 DNA Ligase that has been mutated to improve enzyme activity, and contains a specially developed 4x Quick-Stick buffer. The enzyme catalyzes the joining of two strands of DNA between the 5'-phosphate and the 3'-hydroxyl groups of adjacent nucleotides in either a blunt-ended or cohesive-ended configuration.

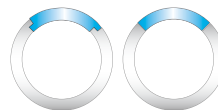


Perform vector and insert purification, mix in correct ratio in final volume of 14µl



Add 1µl Quick-Stick Ligase and 5µl 4x Quick-Stick Buffer, mix by pipetting

Incubate at room temp. for 5 mins (15 mins for blunt-end ligation)



The ligated DNA is now ready to be used in further downstream applications

e.g. add 2µl of ligation reaction to 50-100µl of competent cells for transformation

VELOCITY PCR Kit

PRODUCT	PACK SIZE	CAT NO.
VELOCITY PCR Kit	250 Units	BIO-21104

FEATURES

- **Vastly improves the efficiency of cloning**
- **Robust performance with problematic GC and AT rich targets**
- **Includes high-speed, high fidelity VELOCITY DNA polymerase**
- **Uses exonuclease inhibitors so no post PCR purification required**

APPLICATIONS

- **Cloning techniques where high fidelity is desirable**

The VELOCITY PCR Kit contains all necessary components for high-fidelity PCR and subsequent preparation of the PCR product for TA cloning. The unique properties of VELOCITY DNA Polymerase, combined with an optimized buffer system also allows superior results even with problematic templates such as high GC or AT content (Fig. 1).

VELOCITY PCR products are blunt ended due to the proofreading activity of the polymerase and since 3'-A overhangs are necessary for TA cloning, the kit contains Bioline's PCR Tailing Mix, which adds a single adenine base whilst simultaneously inhibiting the proofreading activity of VELOCITY. Consequently, no purification steps are required, allowing direct TA cloning of the DNA using the newly synthesized 3'-A overhang.

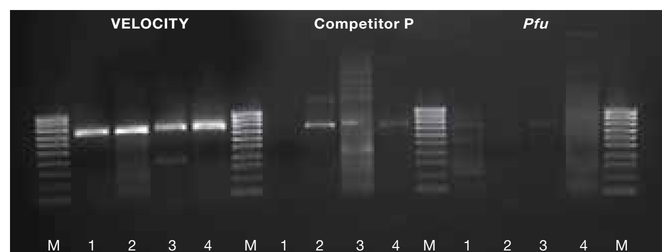
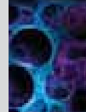


Fig. 1. Amplification of different types of GC-rich DNA fragments from human genomic DNA. VELOCITY, a competitor DNA polymerase (P) and wild type *Pfu* were compared. Lanes 1–4 are a 728bp fragment of the GP150 gene (76.9% GC), a 724bp fragment of the MRGRE gene (68% GC), a 723bp fragment of the NM_022372.3 gene (66.9% GC) and a 788bp fragment of the NM_033178.2 gene (70.9% GC) respectively. Reactions were set up in 50µl using 25ng human genomic DNA and 0.2µM of each primer, 0.5µl dNTPs, 5% DMSO and the recommended PCR buffer and incubated at 95°C for 5min, followed by 30 cycles at 95°C for 30s, 60°C for 30s, and 72°C for 40s. 5µl was then run on a 1.5% TAE agarose gel. HyperLadder™ IV (M) (Cat No. BIO-33029).



ISOLATE PCR & Gel Kit

PRODUCT	PACK SIZE	CAT NO.
ISOLATE PCR & Gel Kit	10 Preps	BIO-52028
ISOLATE PCR & Gel Kit	50 Preps	BIO-52029
ISOLATE PCR & Gel Kit	250 Preps	BIO-52030

FEATURES

- 3-minute protocol for purification of PCR products
- 15-minute protocol for DNA isolation from gels
- Excellent recovery rate
- Isolated DNA is ready for downstream applications

APPLICATIONS

- Purification of PCR products
- Isolation of DNA from TAE and TBE agarose gels
- Purification of DNA from contaminants (enzymes, dNTPs, etc.)

ISOLATE PCR & Gel Kit is designed for the purification of PCR products (Fig. 1) and for the isolation of DNA fragments from TAE and TBE agarose gel slices.

PCR products are purified in 3 minutes using simple binding and elution steps. Concentrated PCR products ranging between 60bp and 30Kb can be eluted in as little as 10µl buffer with a recovery rate of 75-95%. DNA fragments between 100bp and 30Kb can be extracted from agarose gel slices with an excellent recovery rate of 75-90%.

The isolated DNA is suitable for downstream applications such as transformation, cloning, sequencing, restriction analysis, etc.

Fig. 1 Purification of PCR products.
PCR was performed to amplify 500bp, 1.2Kb and 5Kb fragments of Lambda DNA. The products were purified with ISOLATE PCR and Gel Kit and run on a 1.5% TAE agarose gel. The gel shows complete cleanup of primer-dimers combined with a high percentage of recovery. Lanes: HyperLadder I (M), PCR products before cleanup (1, 3, 5), PCR products after cleanup using ISOLATE PCR and Gel Kit (2, 4, 6).

SureClean/SureClean Plus

PRODUCT	PACK SIZE	CAT NO.
SureClean	1 x 5ml	BIO-37042
SureClean	2 x 12.5ml	BIO-37046
SureClean Plus	1 x 5ml	BIO-37047
SureClean Plus	2 x 12.5ml	BIO-37048

FEATURES

- Column-free PCR clean-up
- Post-PCR recovery up to 98%
- Cost-effective and simple protocol
- Isolated products are suitable for downstream applications

APPLICATIONS

- Removes primers, non-specifics, dNTPs and enzymes
- DNA or dsRNA purification or concentration
- Buffer exchange

SureClean is a novel, inexpensive solution, which provides a column-free method for nucleic-acid purification. Using a simple and rapid procedure, SureClean can be used to purify or concentrate DNA or dsRNA from PCR reactions or any enzymatic digests.

SureClean Plus incorporates a pink co-precipitant that offers the distinct advantage of easy visualization of the purified pellet, since this acquires a pink color.

For full information about Biolines Nucleic Acid Isolation products please see www.bioline.com/isolate

Antibiotic Solutions

FEATURES

- Cost effective and time saving
- Ready-to-use solutions
- Avoids handling of toxic or harmful substances
- No sterile filtration required

APPLICATIONS

- Cell culture
- Plasmid selection
- Gene regulation

PRODUCT	PACK SIZE	EFFICIENCY	CAT NO.
Ampicillin	10ml	100mg/ml	BIO-87025
Carbenicillin	10ml	100mg/ml	BIO-87026
Chloramphenicol	10ml	50mg/ml	BIO-87027
Kanamycin	10ml	100mg/ml	BIO-87028
Neomycin	10ml	50mg/ml	BIO-87029
Tetracycline	10ml	12.5mg/ml	BIO-87030

SOC Medium

FEATURES

- Improved stability of cells
- Maximize transformation efficiency
- Sterile, ready-to-use solution
- Time saving and cost effective

APPLICATIONS

- For use in the recovery step of bacterial-cell transformation

SOC Medium is a rich medium used primarily to aid recovery of bacterial competent cells following transformation. Use of SOC medium improves the molecular uptake whilst stabilizing the cells rapidly and so maximizing the transformation efficiency.

PRODUCT	PACK SIZE	CAT NO.
SOC Medium	10 x 10ml	BIO-86033

Associated Cloning Reagents

Blue/white selection
Addition of a piece of foreign DNA into *lacZα* gene of a vector disrupting the production of functional β-galactosidase, this prevents the metabolism of X-gal in the presence of IPTG (a Lac operon inducer). Hydrolysis of X-gal by the β-galactosidase causes the characteristic blue color in the colonies, so white colonies indicate vectors carrying the inserted foreign DNA.

PRODUCT	PACK SIZE	CONCENTRATION	CAT NO.
Agarose	100g	-	BIO-41026
Agarose	500g	-	BIO-41025
Agarose Tablets	300 x 0.5g	-	BIO-41028
Agarose Tablets	600 x 0.5g	-	BIO-41027
Co-Precipitant, Pink	1.5ml	5mg/ml	BIO-37075
IPTG	5g	-	BIO-37036
IPTG Solution	10ml	1M	BIO-37082
IPTG Solution	5 x 10ml	1M	BIO-37083
Proteinase K	100mg	-	BIO-37037
Proteinase K	1000mg	-	BIO-37039
Proteinase K Solution	5ml	20mg/ml	BIO-37084
Proteinase K Solution	5 x 5ml	20mg/ml	BIO-37085
X-Gal	1g	-	BIO-37035

Antibiotic Properties

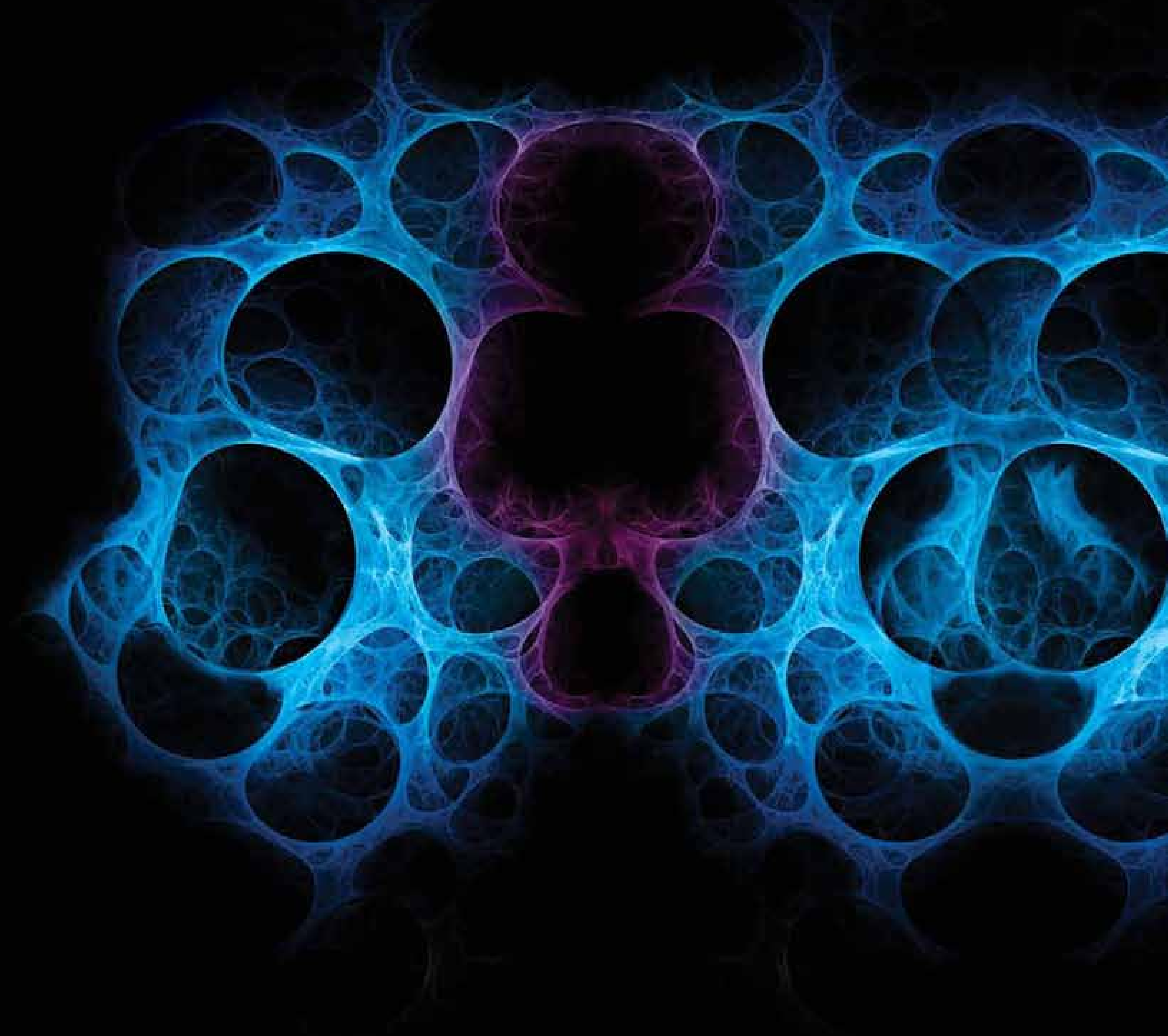
Antibiotics	Mode of Action	Mechanism of Resistance	Working Concentration	Stock Solution
Ampicillin	Ampicillin is a derivative of penicillin that causes cell death by interfering with bacterial cell wall synthesis.	Ampicillin resistance is mediated by cleavage of the ß-lactam ring by ß-lactamase (*bla* gene).	50-200µg/ml	100mg/ml in water
Carbenicillin	Carbenicillin is an ampicillin analogue that inhibits bacterial cell wall synthesis, and is commonly used in place of ampicillin to reduce the production of satellite colonies. Carbenicillin is more stable than ampicillin.	Carbenicillin resistance is mediated by cleavage of the ß-lactam ring by ß-lactamase (*bla* gene).	20-200µg/ml	100mg/ml in 50% ethanol
Chloramphenicol	Chloramphenicol is a bacteriostatic agent that inhibits translation on the 50S ribosomal subunit, preventing peptide bond formation.	Chloramphenicol resistance is mediated by acetyltransferase (*cat* gene), which inactivates chloramphenicol by acetylation.	25-170µg/ml	50mg/ml in 100% ethanol
Kanamycin	Kanamycin sulfate causes cell death by binding to 70S ribosomal subunits, which inhibits ribosomal translocation and causes miscoding.	Kanamycin resistance is mediated by aminoglycoside phosphotransferase (*kan* gene), which inactivates kanamycin by phosphorylation.	10-50µg/ml	100mg/ml in water
Neomycin	Neomycin causes cell death by blocking protein synthesis.	Neomycin resistance is mediated by aminoglycoside phosphotransferase (*nptII* gene), which inactivates neomycin by phosphorylation.	50µg/ml	50mg/ml in water
Tetracycline	Tetracycline inhibits protein synthesis by preventing binding of aminoacyl-tRNA to the 30S ribosomal subunit.	Tetracycline resistance is mediated by a protein (*tet* gene), which modifies the bacterial membrane and prevents transport of tetracycline into the cell.	12.5-50µg/ml	12.5mg/ml in 90% ethanol

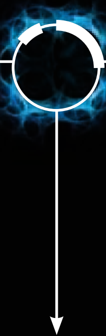
Antibiotics are supplied in convenient ready-to-use solutions

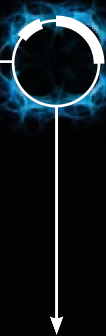
Protein Expression in *E.coli*

Especially engineered *E.coli* cells are often used to express a given construct and synthesize large amounts of the encoded protein. In such experiments, the main points to consider are, the type of promoter being used and the level of promoter control required.

BL21 is a hardy strain, which grows in minimal media and is deficient in key proteases. Bioline offers a series of BL21 Competent Cells for optimal protein expression and expression control from T7 and non-T7 *E.coli* promoters.







Non-T7 Promoter Expression

BL21 Competent Cells, derivatives of *E.coli* Strain B, are suitable for high-level expression of a variety of recombinant proteins and are ideal hosts for the expression of proteins from vectors utilizing *E.coli* promoters.

T7 Promoter Expression

BL21 (DE3) Competent Cells contain the T7 Polymerase gene and support the T7 promoter expression system, which is normally capable of producing more protein than any other bacterial expression system.

Regulation of T7 Promoter Expression

For the expression of proteins that may be toxic and therefore lethal to *E.coli*, the use of a system with tighter control of the T7 promoter is recommended, provided by the BL21 (DE3) pLysS, or BL21 (DE3) pLysE Competent Cells. Recombinant proteins that are non-toxic to *E.coli* are generally expressed at higher levels in BL21 (DE3) cells rather than in BL21 (DE3) pLysS or BL21 (DE3) pLysE.

The pLysE plasmid provides the highest level of repression of the T7 RNA Polymerase gene prior to induction.

Protein Expression

Strain	Efficiency cfu/µg pUC19	Ideal applications
BL21	≥1 x 10^7	Non T7 promoter expression
BL21 (DE3)	≥1 x 10^7	T7 promoter expression
BL21 (DE3) pLysS	≥1 x 10^7	Regulation of T7 promoter expression
BL21 (DE3) pLysE	≥1 x 10^7	Regulation of T7 promoter expression

BL21 Competent Cells

PRODUCT	PACK SIZE	EFFICIENCY	CAT NO.
BL21	1ml (10 x 100µl)	>1 x 10^7 cfu/µg pUC19	BIO-85031
BL21 (DE3)	1ml (10 x 100µl)	>1 x 10^7 cfu/µg pUC19	BIO-85032
BL21 (DE3) pLysS	1ml (10 x 100µl)	>1 x 10^7 cfu/µg pUC19	BIO-85033
BL21 (DE3) pLysE	1ml (10 x 100µl)	>1 x 10^7 cfu/µg pUC19	BIO-85034
BL21 Combo Pack	1.5ml (15 x 100µl)	>1 x 10^7 cfu/µg pUC19	BIO-85035

FEATURES

- High-level protein expression
- Protease deficient
- Transformation efficiency: ≥1 x 10^7 cfu/µg of pUC19
- IPTG inducibility help miminize toxic effects of some proteins

APPLICATIONS

- Non-T7 promotor protein expression: BL21
- T7 promotor expression: BL21 (DE3)
- Regulation of basal T7 promotor expression: BL21 (DE3) pLysS, BL21 (DE3) pLysE

REGULATION OF T7 PROMOTER EXPRESSION

T7 RNA Polymerase expression in BL21 (DE3) cells is repressed by lacI, however, a small amount of T7 RNA Polymerase is produced in the absence of IPTG induction. When expressing a protein that may be toxic to the cells it is essential to have tighter promoter control and the BL21 (DE3) pLysS and BL21 (DE3) pLysE strains are recommended for such applications.

BL21 Non-T7 promoter expression

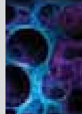
This is an all purpose strain used for protein expression from vectors containing *E.coli* promotors such as *trc*, *tac*, λPL and *ara*D. (This strain lacks a T7 polymerase gene and can be used for non-T7 RNA polymerase protein expression systems. For T7 promotor driven protein expression, this strain requires infection with Lambda CE6 bacteriophage, which provides the T7 RNA polymerase).

Genotype F- *omp*T *hsd*$S_B(r_B^- m_B^-)$ *gal dcm*

BL21 (DE3) T7 promoter expression

This is a general purpose host for T7 vector protein expression. This strain contains the T7 polymerase gene controlled by the *lac*UV5 promoter. T7 polymerase expression is induced by IPTG, which then targets the T7 promotor in the expression vector.

Genotype F- *omp*T *hsd*$S_B(r_B^- m_B^-)$ *gal dcm* (DE3)



BL21 (DE3) pLysE — Regulation of T7 promoter expression

This strain contains the T7 RNA polymerase gene, but also carries the plasmid pLysE that constitutively expresses T7 lysozyme, a natural inhibitor of T7 RNA Polymerase. This strain is used to minimize basal level expression of potentially toxic gene products before induction with IPTG. When induced with IPTG, the inhibition of T7 RNA polymerase expression by T7 lysozyme is overcome by the stronger T7 promoter.

The pLysE plasmid provides the highest level of repression of the T7 RNA Polymerase gene prior to induction, and confers a chloramphenicol resistance.

Genotype F- *ompT hsdS*$_B$($r_B^- m_B^-$) *gal dcm* (DE3) pLysE (Cam^R)

BL21 (DE3) pLysS — Regulation of T7 promoter expression

This strain contains the T7 RNA polymerase gene but also carries the plasmid pLysS that constitutively expresses T7 lysozyme, a natural inhibitor of T7 RNA Polymerase. This strain is used to minimize basal level expression of potentially toxic gene products before induction with IPTG. When induced with IPTG, the T7 RNA polymerase is produced in excess, overcoming the inhibition of T7 RNA polymerase by T7 lysozyme.

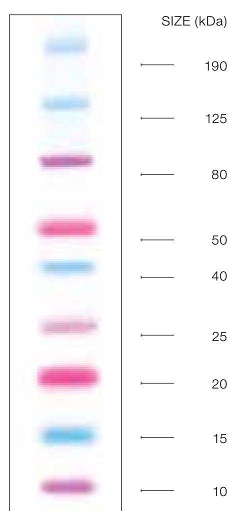
Genotype F- *ompT hsdS*$_B$($r_B^- m_B^-$) *gal dcm* (DE3) pLysS (Cam^R)

BL21 Combo Pack — Choice of expression

The BL21 Competent Cell Combo Pack provides everything you need for T7 promoter-driven protein expression, whether you are setting up new protein expression experiments or need to express a set of proteins with different properties.

The BL21 Competent Cell Combo Pack contains five aliquots of each of the following:
BL21 (DE3) Competent Cells
BL21 (DE3) pLysS
BL21 (DE3) pLysE

Associated Protein Expression Reagents



HyperPAGE Prestained Protein Marker
Band sizes are approximate

PRODUCT	PACK SIZE	CAT NO.
Protein Purification		
His-Catch Metal Chelating Cellulose	5ml	BIO-75036
His-Catch Metal Chelating Cellulose	25ml	BIO-75031
His-Catch Metal Chelating Cellulose	50ml	BIO-75032
Glutathione Cellulose	5ml	BIO-75034
Glutathione Cellulose	25ml	BIO-75027
Glutathione Cellulose	50ml	BIO-75028
Heparin Cellulose	5ml	BIO-75033
Heparin Cellulose	25ml	BIO-75025
Heparin Cellulose	50ml	BIO-75026
Protein Electrophoresis		
HyperPAGE	10 Lanes	BIO-33065
HyperPAGE	50 Lanes	BIO-33066
Crystal 1x TG Buffer	10 Pouches	BIO-37106
Crystal SDS Reagent	50 Tablets	BIO-37109

IMPORTANT FACTORS TO CONSIDER

AMOUNT OF DNA

Typically, one would expect that if more DNA were added to a transformation reaction, the number of transformants would increase. However, one must consider the point of diminishing returns in both chemical transformation and electroporation. The use of more than 10ng of pUC19 DNA does not result in significantly more transformants in chemical transformation. Nor does the use of more than 100ng pUC19 result in more transformants with electrocompetent cells.

PURITY OF DNA

For the most efficient transformation possible, sample DNA should not contain detergent, alcohol, PEG, phenol, or DNA-binding protein. Unfortunately, some of these components are commonly used in ligation reactions. We recommend the use of SureClean (Cat. No. BIO-37042) to purify the sample DNA following a ligation reaction. Alternatively, the ligation mixture can be diluted 2-3 fold using TE buffer and 1µl can then be used in the transformation.

SOURCE OF DNA

DNA from eukaryotic cells is heavily methylated and *E.coli* have intrinsic restriction systems which can degrade this DNA. When cloning genomic DNA, it is important to use a *mcr* mutant such as Bioline ElectroSHOX (Cat No. BIO-85038) and CH3-Blue Chemically Competent Cells (Cat No. BIO-85039, BIO-85040). DNA generated by PCR is unmethylated, therefore cloning a PCR fragment from genomic DNA does not require a *mcr* mutant.

STORAGE AND HANDLING

Competent cells should be stored at -80°C. Thaw competent cells on ice prior to transformation. Never thaw the cells under running water or in a water bath. If is critical to speed up the thawing procedure, rub the tube containing the competent cells between your fingers. If the cells are left on ice for as long as 1 hour prior to transformation, the transformation efficiency will decrease 2 fold. Competent cells should never be vortexed.

TRANSFORMATION EFFICIENCY CALCULATION FOR CONTROL DNA

Transformation efficiency indicates the capability of the competent cells to incorporate and duplicate the DNA of interest. Transformation efficiency is measured as colony-forming units (cfu's) per input DNA, and the unofficial standard is cfu/µg pUC19 DNA.

Transformation Efficiency can be calculated as follows:

$$\text{(cfu/µg pUC19 DNA)} = \frac{\text{Number of colonies (colony-forming units)}}{\text{pg pUC19 transformed}} \times \frac{10^6\ \text{pg}}{\text{µg}} \times \frac{\text{Final volume (µl) of transformation mix}}{\text{Volume plated (µl)}}$$

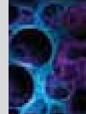
For example, if 40 colonies were obtained after transforming 20pg of pUC19 and plating 5µl of the final 1ml transformation mixture, the calculated transformation efficiency would be:

$$\frac{\text{40cfu}}{\text{20pg pUC19}} \times \frac{10^6\ \text{pg}}{\text{µg}} \times \frac{\text{1000µl}}{\text{5µl}} = 4 \times 10^8\ \text{cfu/µg pUC19}$$

BLUE/WHITE SCREENING

Blue/White screening can be used with various vectors in conjunction with all Bioline Cloning Competent cells. To screen for transformants, plate the transformed cells on LB plates containing 0.08mM IPTG and 60ng/ml X-Gal, as well as the appropriate antibiotic(s). Incubate the plates O/N at 37°C.

- Colonies that carry the wild type plasmid contain active ß-galactosidase. These colonies are pale blue in the center and dense blue at their periphery.
- Colonies that carry recombinant plasmids do not contain active ß-galactosidase. These colonies are creamy-white or eggshell blue, sometimes with a faint blue spot in the center.
- Blue/white color screening can be performed without IPTG (X-Gal only) when using α-select, CH3-Blue and ElectroSHOX competent cells. However, the absence of IPTG may require slightly longer incubation times for comparable color development.



CHEMICAL TRANSFORMATION PROTOCOL

Suggested Transformation Procedure for Optimal Results

1. Remove cells from -80°C and thaw on wet ice (5-10 minutes).

2. Mix cells gently by lightly flicking tube. Aliquot 50-100µl of cells into chilled microcentrifuge tube(s).

3. Unused cells may be refrozen, but a small drop in efficiency may occur. For optimal recovery, refreeze cells in a dry ice/ ethanol bath prior to -80°C storage.

   Add DNA solution (1-5µl per 50-100µl cells) to cell suspension and swirl tube(s) gently for a few seconds to mix. If a control is desired, repeat this step with 2µl of the provided pUC19 control DNA in a separate tube.

4. Incubate on ice for 30 minutes (shorter incubation times can be applied, but will result in a decrease in efficiency).

5. Place tube(s) in water bath at 42°C for 30 to 45 seconds without shaking. For 50µl aliquots in 15ml Falcon 2059 tubes, 30 seconds is recommended for maximum efficiency.

6. Replace tube(s) on ice for 2 minutes.

7. Dilute transformation reaction(s) to 1ml by addition of SOC medium (Cat. No. BIO-86033). Using SOC medium, as opposed to LB medium, can result in a 2-fold higher transformation efficiency.

8. Shake tube(s) at approximately 200rpm for 60 minutes at 37°C.

9. Plate by spreading 5-200µl of the cell transformation mixture on LB agar plates containing appropriate antibiotic and incubate overnight at 37°C.

When performing the pUC19 control transformation, plate 5µl of the transformation mixture on an LB agar plate containing 100µg/ml ampicillin. To facilitate cell spreading, place a pool of SOC (100µl) onto the surface of the plate prior to addition of transformation mixture.

ELECTROTRANSFORMATION PROTOCOL

Suggested Transformation Procedure for Optimal Results

1. Pre-chill electroporation cuvettes, electroporation chamber (if applicable), and microcentrifuge tubes on ice.

2. Remove cells from -80°C and thaw on wet ice (5-10 minutes).

3. Mix cells gently by lightly flicking tube. Aliquot 40-50µl of cells into chilled microcentrifuge tube(s). Unused cells may be refrozen, but a small drop in efficiency may occur. For optimal recovery, refreeze cells in a dry ice/ ethanol bath prior to -80°C storage.

4. Add DNA solution (1-5µl per 40-50µl cells) to cell suspension and swirl tube(s) gently for a few seconds to mix. For optimal results, sample DNA should be in sterile H_2O or a low ionic strength buffer such as TE. If a control is desired, repeat this step with 2µl of the provided pUC19 control DNA in a separate tube.

5. Transfer the cell mixture into a pre-chilled cuvette and pulse using settings recommended by manufacturer of electroporator. As a general guideline, maximum transformation efficiency is normally attained using cuvettes with a 0.1cm gap with an applied voltage of around 1800V (field strength of around 18kV/cm).

6. Dilute pulsed cells immediately to 1ml with SOC medium (Cat. No. BIO-86033) and transfer to a sterile culture tube. Using SOC medium, as opposed to LB medium, can result in a 2-fold higher transformation efficiency.

7. Shake tube(s) at approximately 200rpm for 60 minutes at 37°C.

8. Plate by spreading 5-200µl of cell transformation mixture on LB agar plates containing appropriate antibiotic and incubate overnight at 37°C.

When performing the pUC19 control transformation, plate 5µl of the transformation mixture on an LB agar plate containing 100µg/ml ampicillin. To facilitate cell spreading, place a pool of SOC (100µl) onto the surface of the plate prior to addition of transformation mixture.

When expressing a foreign protein in E.coli cells, a number of factors can affect the efficiency of protein expression. These include the stability of the construct, the solubility of the protein expressed and the effect the protein may have on the cells expressing it.

Following chemical transformation of the BL21 competent cells with the plasmid of choice, we suggest the following procedures for optimizing and expressing the desired protein.

PREPARING STOCK CULTURES (GLYCEROL STOCKS)

In order to obtain optimal expression from a single clone, we recommend initially making a stock culture of the BL21 cells transformed with the plasmid of choice.

1. Transform the BL21 strain of choice with your plasmid.

2. Pick a single transformant colony from a fresh plate and place in 30ml LB containing the appropriate antibiotic(s).

3. Incubate the cells overnight at room temperature. If a shaker is not available at room temperature and only a 37°C shaker is available we suggest you dilute your culture 100 times. For accuracy make two extra cultures at a 10-fold dilution each and use the weakest strength culture to produce your stocks. have intrinsic restriction systems which can degrade this DNA. When cloning genomic DNA, it is important to use a *mcr* mutant such as Bioline ElectroSHOX (Cat No. BIO-85038) and CH3-Blue Chemically Competent Cells (Cat No. BIO-85039, BIO-85040). DNA generated by PCR is unmethylated, therefore cloning a PCR fragment from genomic DNA does not require a *mcr* mutant.

   Note: At room temperature the cells may not reach saturation during an overnight incubation. However, the slower rate of bacterial metabolism at a lower temperature may prevent the formation of inclusion bodies.

4. Dilute 10ml of the overnight culture in 10ml LB-20% glycerol.

5. Distribute the culture into 1ml aliquots and store at -80°C.

6. Each time an expression is performed, thaw out a glycerol stock on ice and use that to start the culture.

   Note: It is important to note that cultures must not be stored as stabs on plates or in a tube in the refrigerator as most of the cells will die. Stocks should be made from cells in the log phase and never from induced cells. At log phase the T7 system is repressed and the cells are more or less competitive, whereas at stationary phase the cells are stressed and many of them will die.

OPTIMIZATION OF PROTEIN EXPRESSION

When expressing a protein in *E.coli* for the first time, the following must be determined:

- Is the desired protein expressed from your clone?
- How much protein is made?
- Is the protein soluble or insoluble?

When expressing a foreign protein in *E.coli* the rate of cell growth can have a great effect on the yield of expressed protein. It is therefore important to monitor the number of bacteria inoculated into the growth medium, the length of time the cells are grown before induction and the density to which cells are grown after induction. We recommend carrying out the following test expression prior to scaling up:

1. Dilute 1ml of stock culture in 100ml LB media containing the appropriate antibiotic(s).

2. Grow cells to 0.5 OD_{600} at 37°C (approx. 2-3 hours).

   Note: It is important to establish the optimum temperature for expression of a foreign protein. This temperature can very between 15-42°C, and the optimum temperature range for successful expression can be quite narrow, spanning 2-4°C.

3. For your induced control, harvest 10ml of the cells (prior to induction) in a 15ml conical centrifuge tube. Spin the cells for 20 minutes at 4,000rpm and store pellet at -20°C.

4. Add 0.9 ml of 100mM IPTG to the remaining cells (final conc. of 1mM). The concentration of IPTG can dramatically influence expression.

   Note: The suggested concentration of 1mM is a starting point and at the higher range of the scale. To establish the optimum concentration, vary the amount of IPTG concentration between 0.01mM and 5.0mM.

5. At various time points during the induction period (e.g. 1,2,4 and 6 hours), harvest a 10ml sample in a 15ml conical centrifuge tube. Spin the cells for 20 minutes at 4,000 rpm, and store pellet at -20°C.

6. Continue to induce overnight and harvest the cells in the morning, noting the time. Store pellet at -20°C.

ANALYSIS OF PROTEIN EXPRESSION

Analyze by SDS-PAGE. Compare the samples from each time-point after induction, including the non-induced control. Also run the soluble and total protein fractions.

- Cells are not inhibited by induction and produce a lot of soluble protein. Scaling up is recommended.
- Cells are not inhibited by induction and do not produce a lot of soluble protein. You can consider producing an amino-terminal protein fusion with a protein that *E.coli* expresses well, such as thioredoxin.
- Cells are inhibited by induction and do not make a lot of soluble protein. This indicates that the protein being produced is killing off the cells. Tighter regulation of the T7 promoter system can perhaps reduce the toxic effects of the protein on the cells. We recommend using the BL21 (DE3) pLysS or BL21 (DE3) pLysE strains.
- Cells are inhibited by induction and make a lot of soluble protein. This is a good result. We recommend inducing at the highest OD_{600} possible, which will depend on the media and aeration. Induce at 1/3 of the final OD_{600} obtained when not inducing.

SEPARATION OF SOLUBLE AND INSOLUBLE PROTEINS

1. Dilute samples to 2mg/ml protein.*
2. Sonicate samples to disrupt cells.
3. Remove 10ml for analysis by SDS-PAGE.
4. Spin the remaining cells for 5 minutes at 13,000rpm.
5. Remove 10ml of the supernatant for analysis by SDS-PAGE. This is the soluble fraction. The insoluble fraction remains with the pelleted cell debris.

*The protein content can be roughly estimated by the OD_{600}, (whereby total protein concentration. = $OD_{600}/2$). For a more accurate protein determination resuspend the frozen pellets in 1ml TE and measure the total protein of each sample using Bradford reagent or an equivalent with a Standard Curve. This is an important step as gel analysis will be easier if the same amount of protein is used.

SCALING UP YOUR EXPRESSION FOLLOWING OPTIMIZATION

1. Dilute 1ml of stock culture in 50ml LB media containing the appropriate antibiotic(s), and incubate overnight with shaking at 20-37°C.
2. The next day, inoculate 400-500ml of LB in a 2L flask with 5-50ml of the overnight culture. Incubate with shaking at 20-37°C until the culture has reached the mid-log phase of growth (OD_{600}= 0.5-1.0).
3. Induce expression of the target protein based on the optimal IPTG concentration, incubation time and temperature determined in the test expression.
4. Harvest the cells by centrifugation at 5,500 rpm for 15 minutes at 4°C and proceed with the purification protocol of choice.

The protocol presented is generalized and will vary depending on the bacterial strain, recombinant protein and parent plasmid.

Genotype Table

Genotype	Description
Δ(*lacZ*YA-*arg*F)U169 Φ80dlacΔ(*lacZ*)M15	Blue/White Screening. Selection of positive transformants through α-complementation of the beta-galactosidase gene
*rec*A	Recombination Deficient. Reduces the rearrangement of plasmids and the deletion of insertions which are normally caused from the homologous recombination of propagated DNA
*end*A	Endonuclease Deficient. A mutation in the DNA specific endonuclease I increases the yield and quality of plasmid DNA
*hsd*R	Restriction Deficient. Absence of this activity permits the introduction of DNA propagated from non *E.coli* sources
*mcr*A, *mcr*B, *mrr*	Methyl Restriction Deficient. Mutation in all three methyl restriction systems is essential for cloning DNA from eukaryotic cells
F' episome	Single-Strand Ability. Excellent host for M13 and related filamentous phage
*fhu*A, *ton*A or T1R	Phage Resistant. Provide resistance to T1 bacteriophage
dam, *dcm*	Methylation deficient. Mutation in these DNA methylases blocks methylation of adenine and cytosine residues and allows for methylation-sensitive restriction digest
*omp*T	Protease deficient. Reduces degradation of some heterologous proteins
DE(3)	T7 promoter based expression. Contains an inducible T7 RNA Polymerase under the control of the *lac*UV5 promoter
gal	Deficient in galactose metabolism. Prevents BL21 from using galactose as a carbon source
argA	Inhibition of N-Acetylglutamate synthase in the presence of arginine. Arginine required from growth in minimal media
deoR	Allows constitutive expression of genes for deoxyribose synthesis. Efficient propagation of large plasmids
*hsd*R (r_K-,m_K+)	Host DNA restriction and methylation system mutation. Allows cloning without cleavage of transformed DNA by endogenous restriction endonucleases.
ompT	Mutation of protease VII, an outer membrane protein. Reduces proteolysis of expressed proteins

WHY DID I NOT GET ANY TRANSFORMANTS?

- Perhaps the ligation was completely inefficient. Try to evaluate your ligation mixture on an agarose gel prior to transformation.
- Try the pUC19 control DNA supplied with Bioline competent cells to ensure that your cells are competent.

THE TRANSFORMATION EFFICIENCY IS MUCH LOWER THAN EXPECTED. WHY?

- The transformation efficiency can be affected by the purity of the DNA. Try Bioline SureClean (Cat. No. BIO-37042) to purify DNA prior to transformation.
- If you are using electroporation the presence of PEG and salts, in the ligation mixture, are a major problem and we strongly suggest purification and suspension in TE. After this has been performed add 1µl of the resuspended ligation mixture per 50µl of ElectroSHOX or α-Select Electrocompetent Cells.

MY FREEZER TEMPERATURE DROPPED TO -40°C BEFORE I WAS ABLE TO TRANSFER THE COMPETENT CELLS. ARE THE CELLS STILL VIABLE?

- The cells can be used but you should expect a 2-5 fold loss in transformation efficiency.
- Competent cells are very sensitive to even small variations in temperature and must be stored at the bottom of a -80°C freezer. Transferring tubes from one freezer to another may result in a loss of efficiency.

CAN I FREEZE-THAW CELLS FREQUENTLY?

- Avoid more than two freeze-thaw cycles. We recommend aliquoting cells into smaller volumes and freeze cells quickly in a dry ice/ethanol bath.

FOLLOWING TRANSFORMATION AND PLATING, THERE ARE MANY SMALLER COLONIES SURROUNDING THE TYPICAL LARGER COLONIES. WHAT ARE THESE SMALL COLONIES?

- These are not transformants; they are satellite colonies. In order to eliminate satellite colonies, we suggest the use of more antibiotic or new plates.

WHICH BIOLINE COMPETENT CELL STRAINS ALLOW BLUE/WHITE SCREENING?

- Blue/white screening is a convenient tool for easy identification of recombinants and all of Bioline cloning strains carry the *lac*ZM15 marker necessary for blue/white screening (with the exception of dam-/dcm-Chemically Competent Cells (T1-Resistant)).

HOW CAN PROTEIN SOLUBILITY BE IMPROVED?

- Lowering the induction temperature to as low as 15°C can increase the solubility and decrease the formation of inclusion bodies.
- The solubility of the protein may also be increased by using a low-copy number plasmid, or a less rich medium than LB.

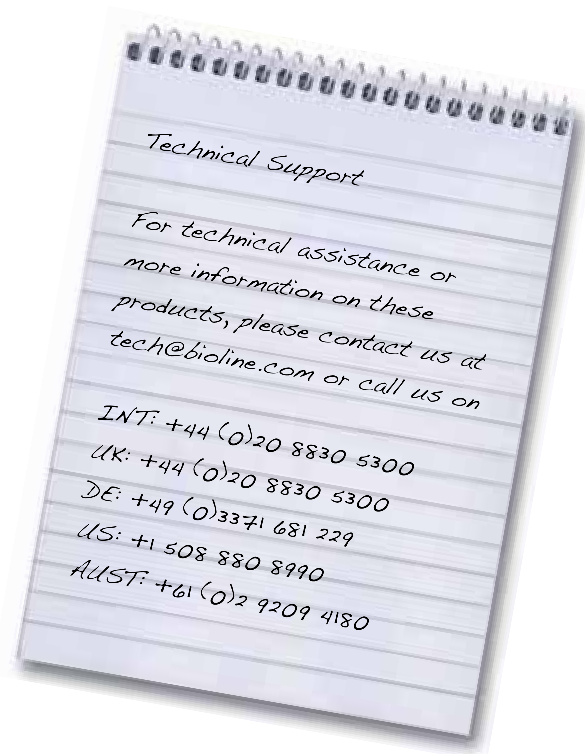
HOW CAN RECOMBINANT PROTEIN YIELD BE IMPROVED?

- When expressing a protein it is important that the culture is set up from a stock culture or a fresh bacterial plate.
- Including protease inhibitors in the purification buffers can increase the yield of the recombinant protein.
- The codon usage in the recombinant protein can also be considered, since replacing the rare codons with more commonly used codons can significantly increase the yield of the expressed protein.

ORDERING INFORMATION

PRODUCT	PACK SIZE	CAT NO.
Competent Cells		
α-Select Bronze Efficiency	2ml (10 x 200µl)	BIO-85025
α-Select Silver Efficiency	2ml (10 x 200µl)	BIO-85026
α-Select Gold Efficiency*	1ml (20 x 50µl)	BIO-85027
α-Select Silver Efficiency T1-Resistant	2ml (10 x 200µl)	BIO-85029
α-Select Gold Efficiency T1-Resistant	1ml (20 x 50µl)	BIO-85030
dam-/*dcm-* T1-Resistant cells	1ml (10 x 100µl)	BIO-85044
CH3-Blue	1ml (20 x 50µl)	BIO-85040
Bio*Blue*	1ml (20 x 50µl)	BIO-85037
ElectroSHOX	1ml (10 x 100µl)	BIO-85038
α-Select Electrocompetent Cells	1ml (10 x 100µl)	BIO-85028
BL21	1ml (10 x 100µl)	BIO-85031
BL21 (DE3)	1ml (10 x 100µl)	BIO-85032
BL21 (DE3) pLysS	1ml (10 x 100µl)	BIO-85033
BL21 (DE3) pLysE	1ml (10 x 100µl)	BIO-85034
BL21 Combo Pack	1.5ml (15 x 100µl)	BIO-85035

*Single use aliquots



ORDERING INFORMATION

PRODUCT	PACK SIZE	CAT NO.
Associated Products		
Agarose	100g	BIO-41026
Agarose	500g	BIO-41025
Agarose Tablets	300 x 0.5g	BIO-41028
Agarose Tablets	600 x 0.5g	BIO-41027
Ampicillin	10ml (100mg/ml)	BIO-87025
Carbenicillin	10ml (100mg/ml)	BIO-87026
Chloramphenicol	10ml (50mg/ml)	BIO-87027
Co-Precipitant, Pink	1.5ml	BIO-37075
Crystal 1x TG Buffer	10 Pouches	BIO-37106
Crystal SDS Reagent	50 Tablets	BIO-37109
Glutathione Cellulose	25ml	BIO-75027
Glutathione Cellulose	50ml	BIO-75028
Glutathione Cellulose	5ml	BIO-75034
Glycogen	1.0ml	BIO-37077
Heparin Cellulose	25ml	BIO-75025
Heparin Cellulose	50ml	BIO-75026
Heparin Cellulose	5ml	BIO-75033
His-Catch Metal Chelating Cellulose	25ml	BIO-75031
His-Catch Metal Chelating Cellulose	50ml	BIO-75032
His-Catch Metal Chelating Cellulose	5ml	BIO-75036
HyperPAGE	10 Lanes	BIO-33065
HyperPAGE	50 Lanes	BIO-33066
IPTG	5g	BIO-37036
IPTG Solution	10ml	BIO-37082
IPTG Solution	5 x 10ml	BIO-37083
ISOLATE PCR & Gel Kit	10 Preps	BIO-52028
ISOLATE PCR & Gel Kit	250 Preps	BIO-52030
ISOLATE PCR & Gel Kit	50 Preps	BIO-52029
Kanamycin	10ml (100mg/ml)	BIO-87028
Neomycin	10ml (50mg/ml)	BIO-87029
Proteinase K	1000mg	BIO-37039
Proteinase K	100mg	BIO-37037
Proteinase K Solution	5 x 5ml	BIO-37085
Proteinase K Solution	5ml	BIO-37084
Quick-Stick Ligase	100 Reactions (10u/µl)	BIO-27028
Quick-Stick Ligase	50 Reactions (10u/µl)	BIO-27027
SOC Medium	10 x 10ml	BIO-86033
SureClean	1 x 5ml	BIO-37042
SureClean	2 x 12.5ml	BIO-37046
SureClean Plus	1 x 5ml	BIO-37047
SureClean Plus	2 x 12.5ml	BIO-37048
T4 DNA Ligase	500 Units (10u/µl)	BIO-27026
Tetracycline	10ml (12.5mg/ml)	BIO-87030
VELOCITY PCR Kit	250 Units	BIO-21104
X-Gal	1g	BIO-37035

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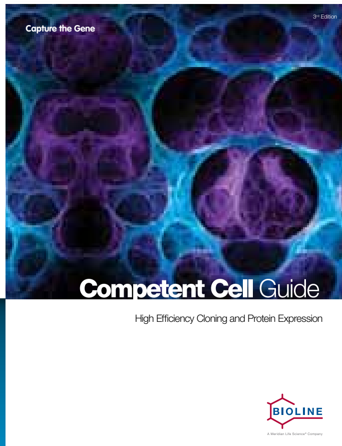

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