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2.5 Gene Cloning & Expression in *E. coli*

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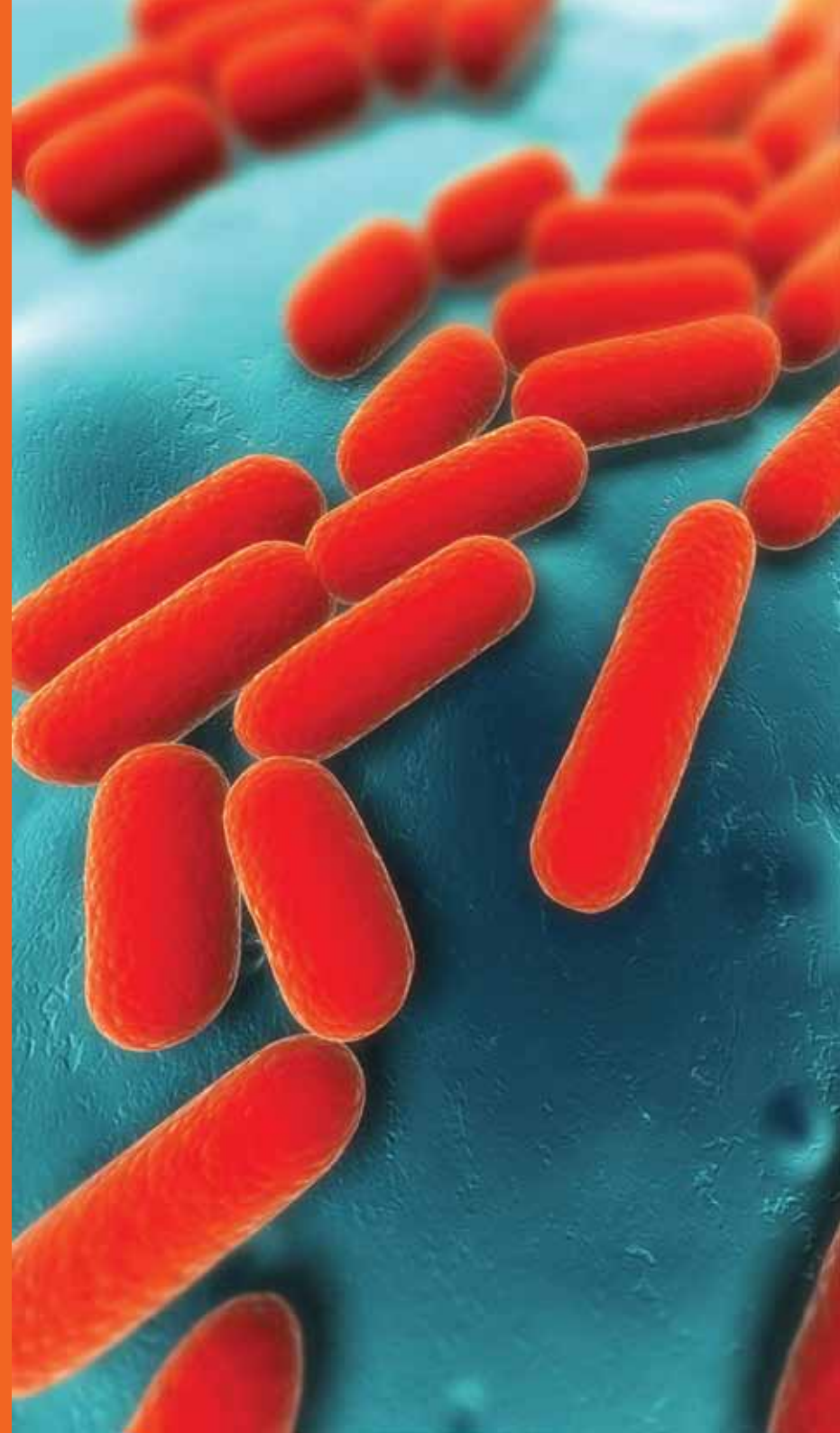
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General Principle of the Staby™ Operating System

Introduction

The Staby™ technology, relying on *ccdB/ccdA* - poison/antidote coding genes, allows you to get rid of the traditional antibiotic selection while cloning or expressing your gene of interest in *E. coli* (Figure 1).

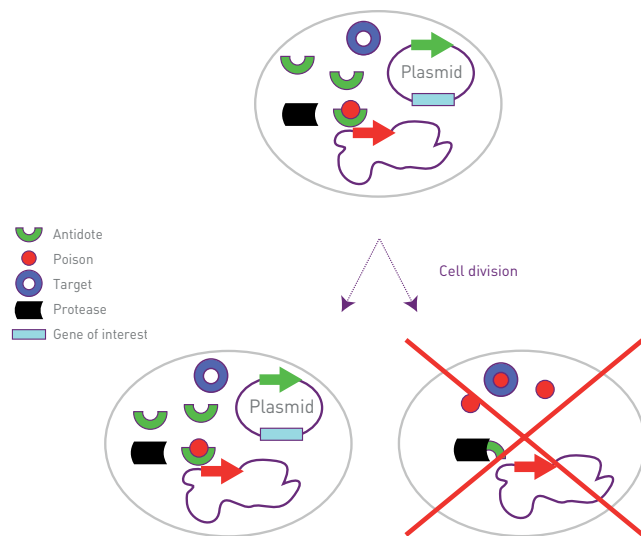


Figure 1: Expression of the poison gene located on the bacterial chromosome is under the control of a promoter, which is strongly repressed in the presence of the plasmid carrying the antidote gene. When the plasmid is lost, the antidote protein is degraded and the production of the poison is induced, causing cell death.

Staby™ Operating System Applications

Gene cloning

The insert may cause instability when cloned in classical vectors resulting in low yield of DNA or low protein production (for protein expression vectors). There are various reasons leading to vector instability, such as the toxicity of the insert, its secondary structure, the size of the vector... **Instability is fully controlled by the Staby™ technology.**

Indeed, by using the provided CYS21 *E. coli* strain containing in its chromosome the toxic gene (*ccdB*), you will strictly select and stabilize the cells containing the plasmid expressing the *ccdA* antidote gene. **The Staby™ cloning plasmids are designed to easily (sub-) clone your gene of interest in the right orientation as well as to export the antidote cassette in any other system.**

Protein expression

Plasmid instability is a significant concern in protein production in *E. coli*. Indeed, production processes require the use of bacterial plasmids as vectors carrying the gene to be overexpressed. Because the gene-of-interest overexpression represents a significant burden on metabolism and reduces the cellular growth rate, selectable markers are used to avoid plasmid-free cells to dominate the culture in fermentation, commonly antibiotic resistance genes. However, contamination of the product or biomass by antibiotics or use of genes encoding an antibiotic resistance is less and less tolerated (also mentioned in FDA guidelines).

The Staby™ technology allows you to stabilize your recombinant plasmids without the use of antibiotics and to produce higher yield of your protein of interest.



Benefits of the Staby™ Operating System

- High plasmid stability
- Higher expression yield (3-5 times)
- No antibiotic selection needed
- No more satellite colonies and false positive
- Faster cloning (1 hour)
- For any culture medium

Gene cloning

StabyCloning™ kit

The StabyCloning™ kit allows the selection of the recombinant plasmids that incorporate the fragment of interest, and the orientation of the fragment of interest as only one of the two possible orientations will restore an active, non-truncated, *ccdA* gene.

Principle

A truncated, inactive, antidote gene (*ccdA*) is present in the plasmid vector (pSTC1.3). This vector is linearized at the end of the truncated antidote gene. The ends of the vector are blunt. When a sequence of 14 base pairs (encoding the last 4 codons and the stop codon of the antidote gene) is added to the 5'-end of the DNA fragment to be cloned, the fusion of this sequence with the truncated gene restores an active *ccdA* gene encoding an active antidote protein. The 14-bp sequence is incorporated to the DNA fragment by PCR using one modified PCR primer.

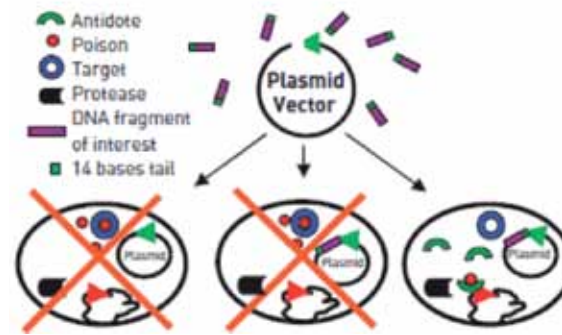


Figure 2: *ccdB* cloning strain bacteria

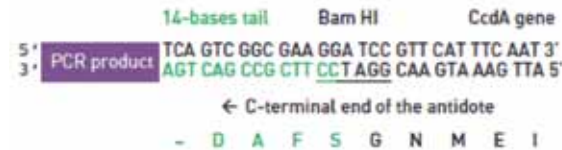


Figure 3: The introduction of the PCR fragment creates a *Bam*HI restriction site at the end of the insert.

Results

With the StabyCloning™ kit, the complete cloning procedure is performed in 1 hour including plating. All growing bacteria contain the cloning vector with an insert in the correct orientation. Restoration of an active *ccdA* gene allows the selection of the recombinants and also stabilizes the plasmid into the bacterial population: if some bacteria lose the vector, they will die due to the action of the bacterial poison.

StabyCloning™ vs competitors

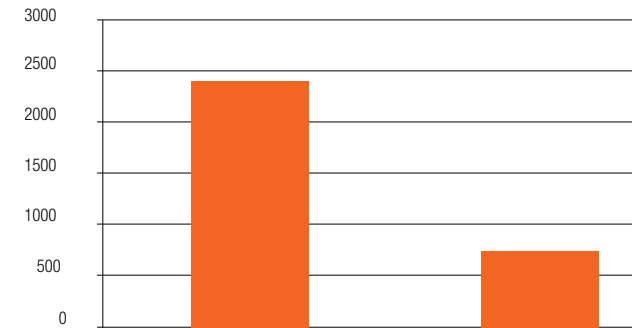
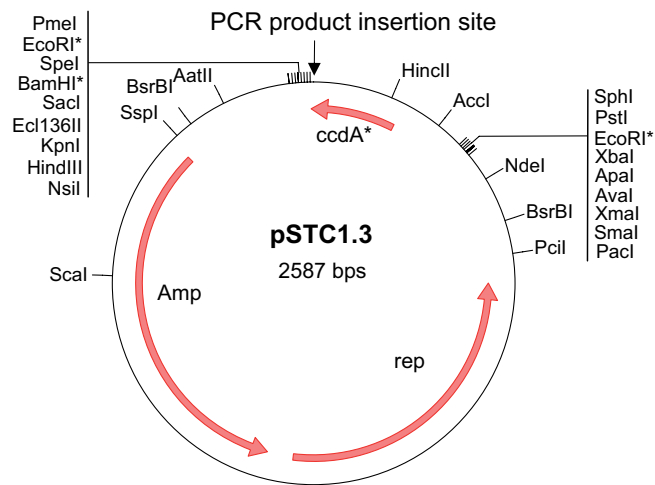


Figure 4: 100 µl transformed bacteria have been plated in each case. The insert size was 500 bp. Using StabyCloning™, 2400 independent clones are obtained, i.e., three times more than when using TOPO® cloning (Invitrogen).

(Tzika A. IBMM, Université libre de Bruxelles, Belgium)

Vector map



Kit content

- pSTC1.3 blunt ended vector
 - CYS21 competent bacteria (chemically or electro)
 - T4 DNA ligase and reaction buffer
 - Regeneration medium
 - Forward and reverse sequencing primers
 - Primer Control mix (positive control)
 - Instruction manual
- Additional competent bacteria can be purchased separately (see page 165)

Stabycloning™

Description	RXNs	References
Stabycloning™ kit with cloning bacteria CYS21, electro-competent cells	10	GE-STC1-10
Stabycloning™ kit with cloning bacteria CYS21, chemically-competent cells	10	GE-STC1-12
Stabycloning™ kit with cloning bacteria CYS21, electro-competent cells	2 x 10	GE-STC1-20-
Stabycloning™ kit with cloning bacteria CYS21, chemically-competent cells	2 x 10	GE-STC1-22-

Stabycloning™ Benefits

- **Speed:**
 - Ligation + transformation + plating in one hour
 - Recombinants are all independent clones (direct plating)
 - No topoisomerase needed
- **Precision:** Vectors without insert are eliminated (background < 1 %) and the DNA fragment is correctly oriented in the vector
- **Mobility:** Stabilized insert is easily exported into other vectors
- **Efficiency:** Efficient cloning even with small inserts

Customer publications

1. Tzika A. *et al.* (2009) *Conserv. Genet.* 10, 69-77
2. Tzika A. *et al.* (2008) *Molecular Ecology* 17, 533-544
3. Amstutz U. *et al.* (2008) *Journal of Experimental & Clinical Cancer Research* 27,54

Staby™ SubCloning kits

The Staby™ SubCloning kits allow you to transfer your gene of interest inserted in pSTC1.3 into expression vectors with new features: N-term-His tag, N-term-Cherry tag or rare codon tRNAs.

Principle

Using restriction sites from the pSTC1.3 vector, it is easy to transfer the gene of interest (linked to the *ccdA* antidote gene) into one subcloning expression vector. Since Staby™ selection is very efficient, it is not required to purify your insert linked to *ccdA* gene before subcloning. After subcloning, the vector is ready for protein expression in SE1 bacteria.

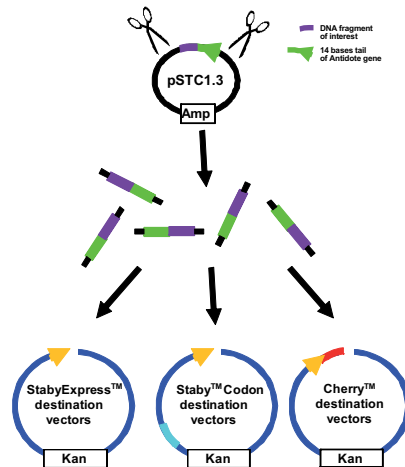


Figure 5: These vectors carry compatible restriction sites with those of pSTC1.3 plasmid and allow placing your gene in frame with the protein tags.

Kit content

- Staby™ SubCloning vectors
- CYS21 competent bacteria (for cloning)
- SE1 competent bacteria (for expression)
- Regeneration medium
- Forward and Reverse sequencing primers
- Expression control
- Instruction manual

Additional competent bacteria can be purchased separately (see page 165)

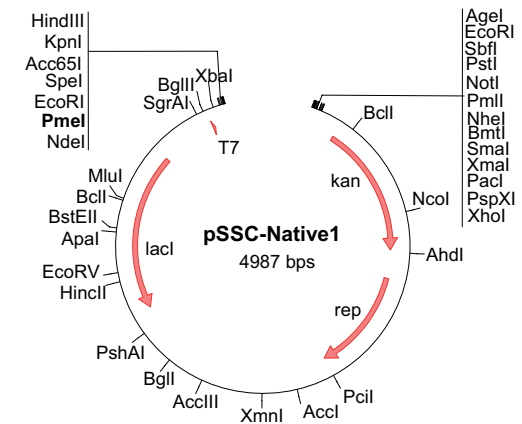
Staby™ subcloning

Description	RXNs	References
pSSC-Native1, pSSC-His1 and pSSC-Cherry1 vectors, electro-competent cells	10	GE-SSC1-10
pSSC-Native1, pSSC-His1 and pSSC-Cherry1 vectors, chemically-competent cells	10	GE-SSC1-12
pSSC-Native2, pSSC-His2 and pSSC-Cherry2 vectors, electro-competent cells	10	GE-SSC2-10
pSSC-Native2, pSSC-His2 and pSSC-Cherry2 vectors, chemically-competent cells	10	GE-SSC2-12

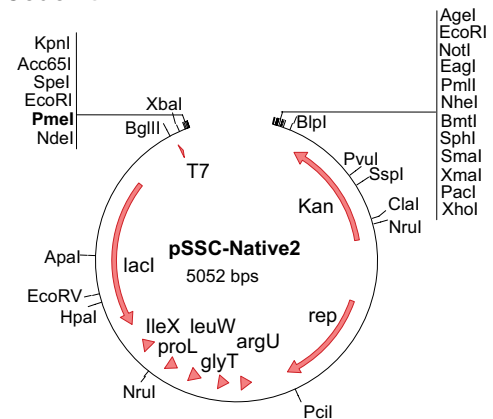
Vector maps

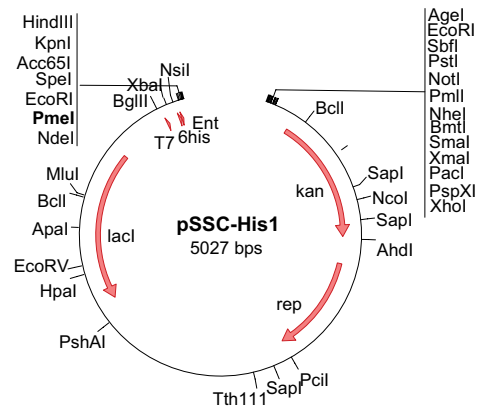
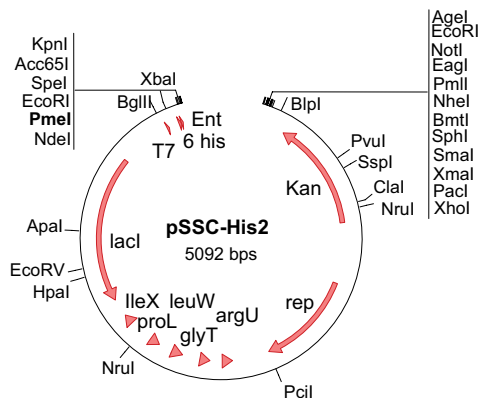
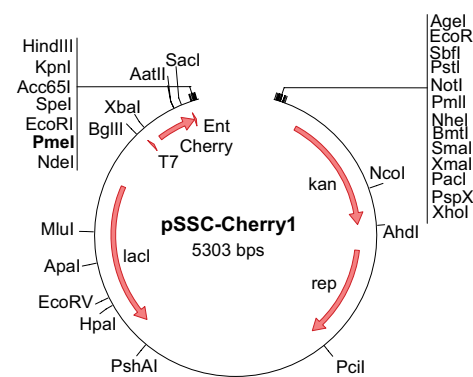
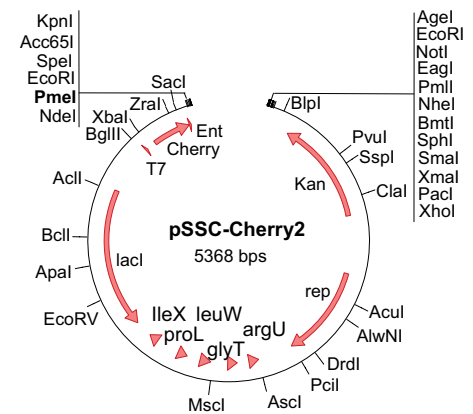
No tag

T7 promoter, Kan resistance, *lacI* repressor to reduce basal expression, plasmid stabilization in Cys21 and SE1 bacteria after subcloning



+ rare Codon tRNA



His tag**+ rare Codon tRNA****Cherry tag****+ rare Codon tRNA****Staby SubCloning™ Benefits**

- Add a tag with no unwanted sequences fused to your protein
- Efficient subcloning (background < 1 %)
- No need to purify the insert
- Subcloning in several compatible vectors in one time
- No deletion in the insert

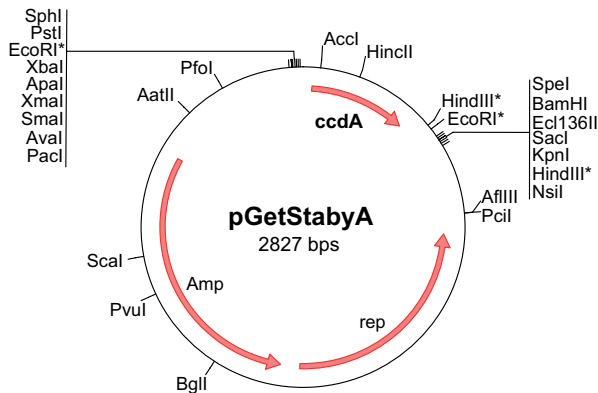
GetStaby™ kit

The GetStaby™ kit is made for integration of the DNA fragment encoding the CcdA antidote protein into your favourite vector. This results in the stabilization of the vector in bacteria expressing the CcdB poison protein.

Description

Adding the DNA fragment encoding the CcdA antidote protein (Staby™ cassette) to any vector results in stabilization of this vector in CYS21 bacteria expressing the CcdB poison protein. The Staby™ cassette is isolated from the pGetStabyA vector using restriction sites or PCR amplification. The insertion of the Staby™ cassette into any vector is easily selected in bacteria encoding the CcdB poison protein.

Vector map



Kit content

- pGetStabyA vector
- CYS21 competent (chemically- or electro-) bacteria for cloning and selection
- Regeneration medium
- Forward and Reverse sequencing primers
- Instruction manual

Benefits

- **Compatible with any vector.** The stabilization cassette (linked to any part of the vector) is easily re-exported to other vectors
- **Usable in any culture medium** LB, SOC, SOB, YT or minimal media supplemented with a carbon source such as glucose or glycerol...
- **Increases 3 to 5 times the DNA or protein production**

Getstaby™

Description	RXNs	References
GetStaby™ kit with cloning bacteria CYS21, electro-competent cells	10	GE-GSA1-10
GetStaby™ kit with cloning bacteria CYS21, chemically-competent cells	10	GE-GSA1-12

Protein expression

Cherry™Express kit

The Cherry™Express kit easily allows expression and direct visualization of your protein of interest during the whole process of protein production and purification. A simple absorbance measurement at 413 nm is required for a specific and accurate calculation of the target protein concentration at each step.

Direct visualization of your protein of interest

When using the Cherry™Express vector, your gene of interest is fused to a small sequence encoding a red polypeptide (heme binding part of cytochrome, 11 kDa). This provides you with a visual aid for “live” estimation of the best conditions (temperature, time...) for protein expression.

Increase the solubility of your protein!



Figure 6: Cell pellet before protein expression and after 2h IPTG induction in *E. coli*.

Tube 1: $A_{430} = 0.33$ (uninduced)
 Tube 2: $A_{430} = 0.53$ (uninduced)
 Tube 3: $A_{430} = 0.88$ (uninduced)
 Tube 4: $A_{430} = 1.20$ (beginning of induction)
 Tube 5: $A_{430} = 2.30$ (end of induction)

It has been shown that the highly soluble Cherry™ tag can increase the solubility of target proteins, avoiding inclusion bodies and protein misfolding.

High yield of protein production using Staby™ technology

Using Cherry™Express the plasmid is perfectly stable before and after the induction period. Consequently, the production of the protein of interest is higher (3 to 5 times) with Cherry™Express than using a conventional expression system with antibiotics. Note that no over-production of the antidote is detectable.

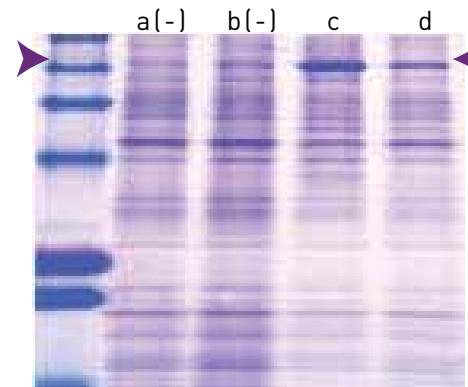


Figure 7: Expression protein level with Cherry™Express (lane c) or a conventional expression system (lane d). Lanes a and b are uninduced controls.

Cherry™Express Benefits

- ▣ Direct visualization of your protein of interest expression
- ▣ Increase the solubility of your protein!
- ▣ Easy-to-follow purification steps
- ▣ Reduced background of “non target proteins”

Customer publications

1. Gonzalez N. *et al.* (2007) *The Plant Journal* 51, 642-655
2. Taveneta A. *et al.* (2009) *PNAS* 106, 14265-14270

Easy-to-follow purification steps



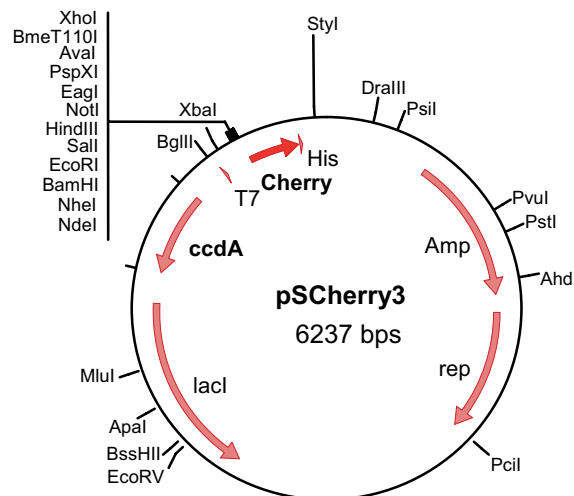
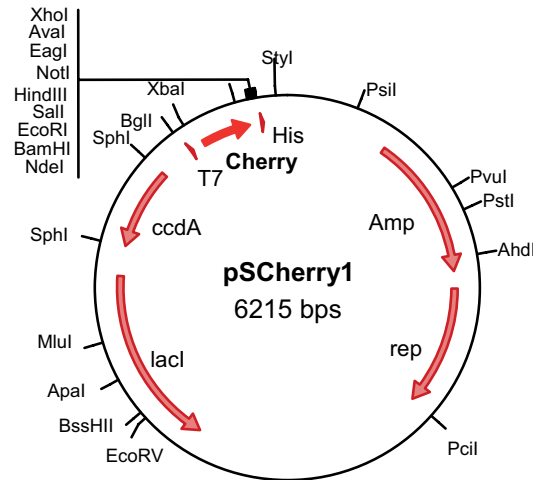
The red colour constitutes a visual marker throughout protein purification.

After purification, the tag can be cleaved using Enterokinase (a recognition site is inserted at the end of the tag sequence).

Figure 8: Protein visualization throughout protein purification.

Vector map

PSCherry1 and PSCherry3 vectors allow you to express your protein of interest with the Cherry™ tag at the N- and C-terminus, respectively. The Cherry™ tag was inserted in the StabyExpress™ T7 vector giving multiple advantages: T7 expression, plasmid stabilization without antibiotics and protein visualization.



Kit content

- ▣ pSCherry vector DNA
- ▣ CYS21 bacteria (for cloning)
- ▣ SE1 bacteria (for expression)
- ▣ Regeneration medium
- ▣ Fwd and Rev sequencing primers
- ▣ Expression control
- ▣ Instruction manual

Additional competent bacteria can be purchased separately (see page 165).

Cherry™ Express

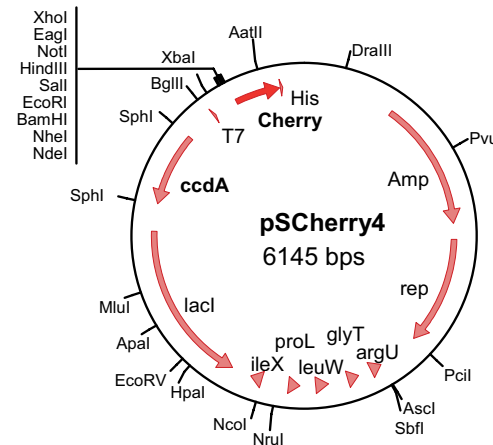
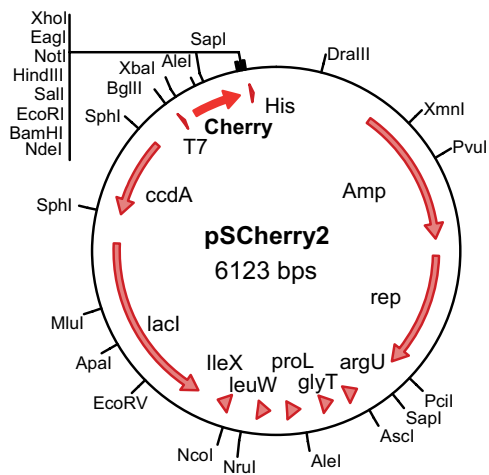
Description	RXNs	References
Cherry™ Express T7 expression kit, N-terminal tag, electro-competent cells	5	GE-CET7-05
Cherry™ Express T7 expression kit, C-terminal tag, electro-competent cells	5	GE-CET7-06
Cherry™ Express T7 expression kit, N-terminal tag, chemically-competent cells	5	GE-CET7-07
Cherry™ Express T7 expression kit, C-terminal tag, chemically-competent cells	5	GE-CET7-08
Cherry™ Express T7 expression kit, N-terminal tag, electro-competent cells	10	GE-CET7-10-
Cherry™ Express T7 expression kit, C-terminal tag, electro-competent cells	10	GE-CET7-11
Cherry™ Express T7 expression kit, N-terminal tag, chemically-competent cells	10	GE-CET7-12-
Cherry™ Express T7 expression kit, C-terminal tag, chemically-competent cells	10	GE-CET7-13

Cherry™Codon kit (Cherry™Express kit + rare tRNA)

The Cherry™Codon kit solves low recombinant protein expression problem by the use of the pSCherry2 or pSCherry4 expression plasmids encoding the tRNA of the six rare codons in *E. coli*.

Vector map

PSCherry2 and PSCherry4 vectors allow you to efficiently express your protein of interest with the Cherry™ tag at the N- and C-terminus, respectively providing *E. coli* with rare tRNAs. The Cherry™ tag was inserted in the Staby™Codon T7 vector giving multiple advantages: T7 expression, plasmid stabilization without antibiotics, efficient supply of rare tRNAs and protein visualization.



Cherry™Codon

Description	RXNs	References
Cherry™Codon T7 expression kit, N-terminal tag, electro-competent cells	5	GE-CCT7-05
Cherry™Codon T7 expression kit, C-terminal tag, electro-competent cells	5	GE-CCT7-06
Cherry™Codon T7 expression kit, N-terminal tag, chemically-competent cells	5	GE-CCT7-07
Cherry™Codon T7 expression kit, C-terminal tag, chemically-competent cells	5	GE-CCT7-08
Cherry™Codon T7 expression kit, N-terminal tag, electro-competent cells	10	GE-CCT7-10-
Cherry™Codon T7 expression kit, C-terminal tag, electro-competent cells	10	GE-CCT7-11
Cherry™Codon T7 expression kit, N-terminal tag, chemically-competent cells	10	GE-CCT7-12-
Cherry™Codon T7 expression kit, C-terminal tag, chemically-competent cells	10	GE-CCT7-13

Cherry™Codon Benefits

- High yield of heterologous-protein expression even when the gene contains rare codons
- Increase the solubility of your protein!
- Easy-to-follow purification steps
- Reduced background of “non target proteins”

Why providing rare codon tRNAs?

In practice, when a heterologous gene is expressed in *E. coli*, this gene might exhibit some codons that are common in the original host but are rarely used in *E. coli*. The presence of numerous rare codons (or in clusters) leads to very low expression of the target protein due to premature translation termination, translation frameshifting, amino acid misincorporation, growth inhibition and plasmid instability.

StabyExpress™ T7 kit

The StabyExpress™ T7 expression kit allows the cloning of your gene of interest into a stabilized plasmid and expression of your protein of interest.

Results

Several unstable vectors were stabilized in *E. coli* with our technology (Figure 9). In the StabyExpress™ T7 kit, the Staby™ technology is applied to protein production using the T7 promoter.

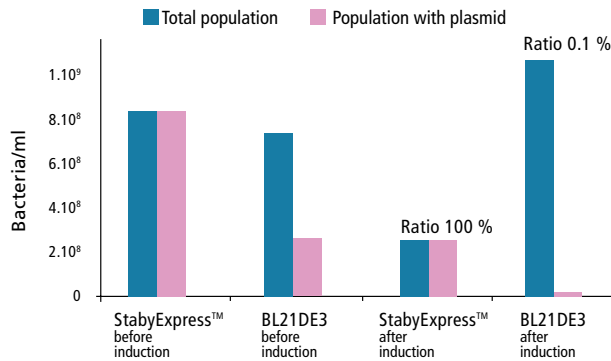


Figure 9: Comparison of plasmid stability with and without the use of StabyExpress™ technology. Without StabyExpress™ technology, only 1/1000 cell still possesses the plasmid after induction.

StabyExpress™ is better in producing two forms of HIV-RT proteins than competition

In order to assess new inhibitors of HIV-1 Reverse Transcriptase, the laboratories of Dr. A. Jacquet and Prof. C. Van Lint (ULB) had to produce two different forms of the recombinant enzyme (P66: complete form and P51: truncated version).

A visible band is obtained when using the StabyExpress™ whereas no expression is detected using competitors systems. The identity of the overexpressed proteins was confirmed by Western-blot (Figure 10).

Comparison of the production of a 69 kDa protein with and without the StabyExpress™ technology

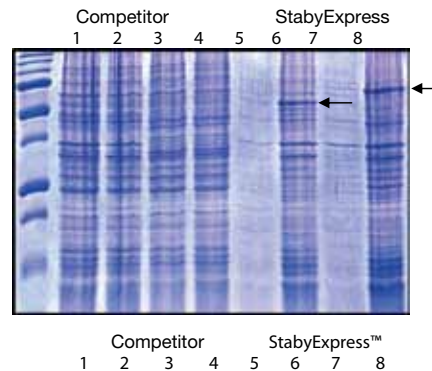
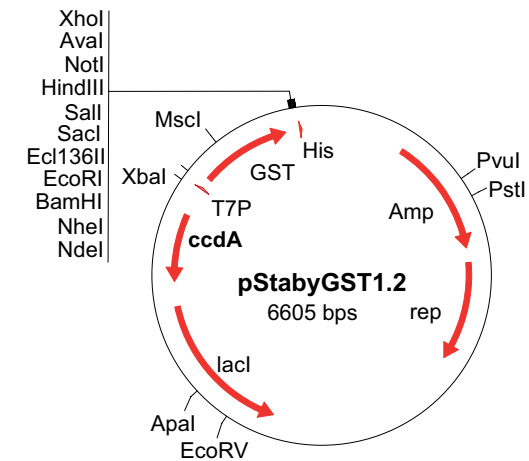
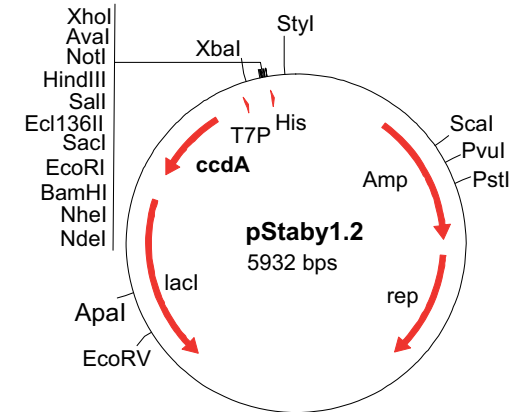


Figure 10: Lane 1 to 4, P66 & P51 expressed in competitor systems. Lane 5: P51, StabyExpress™ Supernatant, Lane 6: P51 StabyExpress™ Cellular Extract, Lane 7: P66 StabyExpress™ Supernatant, Lane 8: P66 StabyExpress™ Cellular extract.

Vector map



Subcloning with Staby™

If your gene was previously cloned with StabyCloning™, we can provide you with a StabyExpress™ kit specifically designed for subcloning. For more information, see the subcloning section of this catalogue.

StabyExpress™		
Description	RXNs	References
Without GST		
pStaby1.2 kit, electro-competent cells	5	GE-SET7-0505
pStaby1.2 kit, chemically-competent cells	5	GE-SET7-0707
pStaby1.2 kit, electro-competent cells	10	GE-SET7-1010-
pStaby1.2 kit, chemically-competent cells	10	GE-SET7-1212-
With GST		
pStabyGST1.2 kit, electro-competent cells	5	GE-SEGST-0505
pStabyGST1.2 kit, chemically-competent cells	5	GE-SEGST-0707
pStabyGST1.2 kit, electro-competent cells	10	GE-SEGST-1010
pStabyGST1.2 kit, chemically-competent cells	10	GE-SEGST-1212

Kit content

- ▣ pStaby™ vector DNA
- ▣ CYS21 bacteria (for cloning)
- ▣ SE1 bacteria (for expression)
- ▣ Regeneration medium
- ▣ Fwd and Rev sequencing primers
- ▣ Expression control
- ▣ Instruction manual

Additional competent bacteria can be purchased separately (see page 165).

Benefits of StabyExpress™

- ▣ High yields of expressed proteins
- ▣ Reduced background of non-target proteins
- ▣ Standardization of the protein-production protocol

Customer publications

1. Stevens R. *et al.* (2008) *PNAS* 105, 12780-12784
2. Walgraffe D. *et al.* (2009) *Journal of Allergy and Clinical Immunology* 123, 1150-1156

Staby™Codon T7 kit

The Staby™Codon T7 kit solves low recombinant protein expression problem by the use of the pSCodon expression plasmids encoding the tRNA of the six rare codons in E.coli. The plasmid also contains the T7 promoter for a strong expression and *lacI* repressor to reduce basal expression.

Results

To evaluate the efficiency of the Staby™Codon T7 kit, a DNA fragment encoding a human protein containing 24 rare codons (10 prolines, 7 arginines, 3 isoleucines, 3 leucines and 1 glycine) was cloned into pStaby1 and pSCodon1. These plasmids were transformed in SE1 bacterial strain. As illustrated (Figure 11), the protein is not or very poorly produced using pStaby1 but clearly visible using pSCodon1. The protein was purified and its integrity was confirmed by Mass Spectrometry.

Subcloning with Staby™

If your gene was previously cloned with StabyCloning™, we can provide you with a StabyExpress™ kit specifically designed for subcloning. For more information, see the subcloning section of this catalogue.

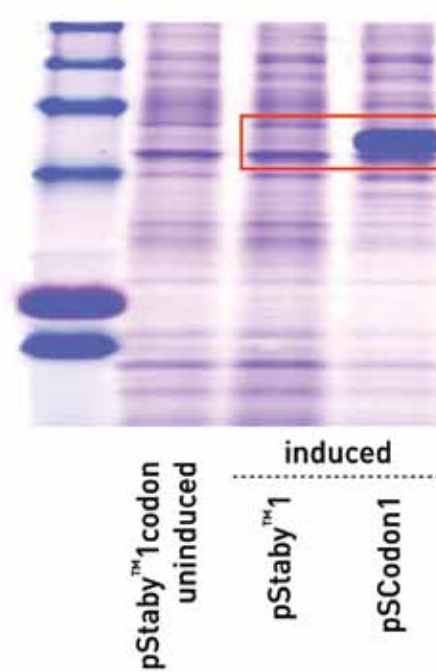
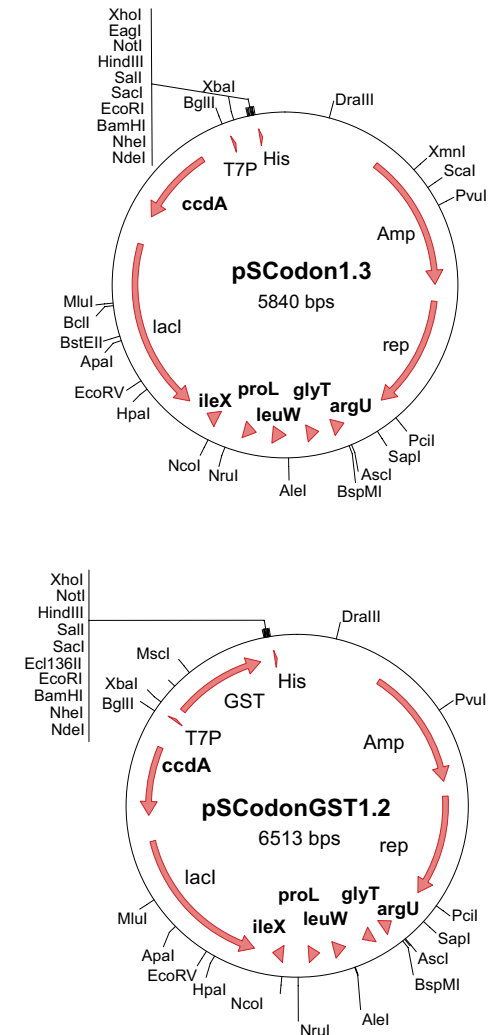


Figure 11: Protein expression comparison between strain carrying pSCodon and pStaby plasmids

Vector map



Staby™ Codon T7		
Description	RXNs	References
Without GST		
pSCodon1.3 kit, electro-competent cells	5	GE-SCT7-0505
pSCodon1.3 kit, chemically-competent cells	10	GE-SCT7-0707
pSCodon1.3 kit, electro-competent cells	5	GE-SCT7-1010-
pSCodon1.3 kit, chemically-competent cells	10	GE-SCT7-1212-
With GST		
pSCodonGST1.2 kit, electro-competent cells	5	GE-SCGST-0505
pSCodonGST1.2 kit, chemically-competent cells	10	GE-SCGST-0707
pSCodonGST1.2 kit, electro-competent cells	5	GE-SCGST-1010
pSCodonGST1.2 kit, chemically-competent cells	10	GE-SCGST-1212

Kit content

The Staby™Codon T7 kit combines three technologies: T7 expression, plasmid stabilization and efficient supply of rare tRNAs.

- pSCodon vector DNA,
- CYS21 bacteria (for cloning)
- SE1 (for expression),
- Regeneration medium,
- Fwd and Rev sequencing primers,
- Expression control.

Additional competent bacteria can be purchased separately (see page 165).

Benefits of Staby™ Codon

- High yield of heterologous-protein expression even when the gene contains rare codons
- Not necessary to optimize your gene
- Reduced background of “non target proteins”

Customer publications

1. Gonzalez N. *et al.* (2007) *The Plant Journal* 51, 642-655
2. Taveneta A. *et al.* (2009) *PNAS* 106, 14265-14270

Auto-inducible medium

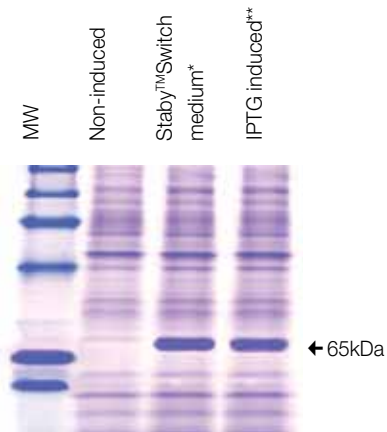
Staby™Switch

The Staby™Switch medium is designed for high-level protein expression using Staby™ products or any IPTG-inducible bacterial expression system (lac or tac promoters, T7 expression systems based on the use of SE1 or BL21 (DE3) bacteria).

Principle

Staby™Switch is an auto-inducible medium: metabolization of medium components during bacterial growth will automatically induce protein expression at high cell density.

Results



Take it easy with your protein expression and get better results

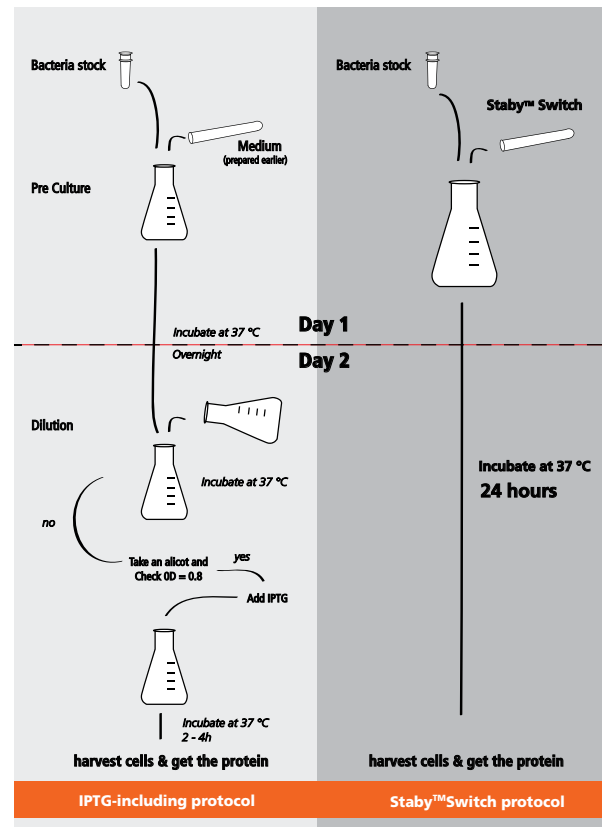


Figure 12: Comparison of protein production using classical IPTG induction or Staby™Switch auto-inducible media.

* After 20h at 37 °C.

** After 20h + 2h of IPTG induction at 37 °C.

Benefits

- High protein expression levels
- No need to monitor cell growth and add IPTG
- Compatible with all IPTG-inducible *E. coli* expression systems
- Compatible with cultures grown in:
 - ❖ Flasks
 - ❖ Tubes
 - ❖ Deep-well plates (ideal for the screening of clones)
 - ❖ Fermentors
- Ideal for Staby™ and Cherry™ systems (no more antibiotics selection)
- Convenient 2 x 1 l format, ready to use

Staby™Switch

Description	Quantity	Reference
Staby™Switch auto-inducible Medium	2 x 1 l final	GE-AIME-04

Competent bacteria

For Staby™ technology

CYS21 strain

Competent bacteria used with the Staby™ technology for cloning, plasmid preparation and library construction

Description

CYS21 strain

CYS21 *E. coli* strain contains the selection gene (*ccdB*) in its chromosome. This strain is recommended for cloning applications when using the Staby™ system. In CYS21 bacteria, plasmids encoding a *ccdA* gene are selected and perfectly stabilized even without antibiotics.

CYS21 genotype

F⁻, Cm^R, *endA1*, Δ (*mrr-hsdRMS-mcrBC*) (restriction- modification-), Φ 80*lacZ* Δ M15, Δ *lacX74*, *recA1*, Δ (*ara, leu*)7697, *araD139*, *galU*; *galk*, *nupG*, *rpsI*, *ccdB*⁻

CYS21C

Products	RXNs	References
Set of cloning bacteria CYS21, electro-competent cells	20 x 50 μ l	GE-STCB-20
Set of cloning bacteria CYS21, chemically-competent cells	20 x 100 μ l	GE-STCB-22

Kit content

- The competent bacteria are shipped with SOC regeneration medium.

SE1 strain

Competent bacteria used with the Staby™ technology for protein expression under T7 promoter

Description

SE1 strain

The SE1 *E. coli* strain contains the T7 ARN polymerase for expression and the *ccdB* gene for higher yields and efficient plasmid stabilization even without antibiotics. This strain is recommended for protein expression when using the Staby™ technology.

SE1 genotype

Derivates from *E. coli* B strain, F⁻, Cm^R, *ompT*, *lon*, *hsdB* (restriction-modification-), *gal*, *dcm*, DE13 (*lacI*, T7 polymerase under the control of the *PlacUV5* promoter), *ccdB*⁺.

SE1

Products	RXNs	References
Set of StabyExpress™ T7 expression bacteria SE1, electro-competent cells	20 x 50 μ l	GE-SET7-0020
Set of StabyExpress™ T7 expression bacteria SE1, chemically-competent cells	20 x 100 μ l	GE-SET7-0022

Kit content

- The competent bacteria are shipped with SOC regeneration medium.

Benefits

Transformation efficiency

- CYS21 >1.10⁹ cfu/ug DNA
- SE1 >1.10⁹ cfu/ug DNA

Custom gene synthesis service



- Any Gene in any vector
- Up to 50 000 bp
- Gene Optimization
- Synthesis of Complex Genes
- 100 % Guaranteed Sequence

See page 112

For general cloning and expression

DG1 Strain

DG1 competent bacteria is used for cloning, plasmid preparation and library construction

Product Description

DG1 chemically competent *E. coli* are offered either in the 24, 48 or 96 individual tubes packed in a tray. Each 1.2 ml tube contains 50 µl of competent bacteria, which are sufficient for one transformation.

This high-efficiency DG1 competent *E. coli* strain is convenient for use in cloning, plasmid preparation and library construction. It is endonuclease and recombination minus. It allows *lacZ*alpha complementation for blue / white screening.

The DG1 competent bacteria transformation efficiency is higher than 10⁹ cfu/µg of DNA

DG1 genotype: *mcrA* Δ(*mrr-hsdRMS-mcrBC*, modification-, restriction-) φ80*lacZ*DM15 Δ*lacX74 recA1 araD139* Δ(*ara-leu*)7697 *galU galK rpsL endA1 nupG*

DG1 Chemically-competent bacteria

Quantity	References
24 x 50 µl	GE-DG1C-24
48 x 50 µl	GE-DG1C-48
96 x 50 µl	GE-DG1C-96

BL21(DE3) Strain

Competent bacteria used for protein expression under T7 promoter

Product Description

BL21(DE3) chemically competent *E. coli* are offered either in the 24, 48 or 96 individual tubes packed in a tray. Each 1.2 ml tube contains 100 µl of competent bacteria, which are sufficient for one transformation. BL21(DE3) bacteria are convenient for protein expression using the T7 promoter. The (DE3) designation indicates that the host is a lysogen of λ prophage (DE3), and therefore carries a chromosomal copy of the T7 RNA polymerase gene under control of the *lacUV5* promoter (inducible by addition of IPTG). BL21(DE3) is the most widely used host for protein expression because it is deficient in the Lon and OmpT proteases.

The BL21(DE3) competent bacteria transformation efficiency is 8.10⁷ cfu/µg DNA (at least 4 times higher than competent bacteria from competitors).

BL21 (DE3) genotype:

F-ompT lon hsdSB (rB·mB) *gal dcm* (DE3)

BL21(DE3) Chemically- competent bacteria

Quantity	References
24 x 100 µl	GE-BL21C-24
48 x 100 µl	GE-BL21C-48
96 x 100 µl	GE-BL21C-96

Specifications

Affordable chemically-competent bacteria in a convenient format

- ▣ Freedom to use anywhere from 1 to 96 reactions
- ▣ No wasted reactions
- ▣ Convenient for high-throughput use
- ▣ High efficiency
 - ↳ 10⁹ cfu/µg of DNA (DG1)
 - ↳ 8.10⁷ cfu/µg of DNA (BL21)
- ▣ High protein expression level (BL21)

Quality control

Each production batch is tested regarding the transformation efficiency. We chose our test conditions to be on the safe side when the bacteria are used to transform a ligation. Usually, after a ligation, 10 to 100 ng of DNA are transformed using a high or medium copy number plasmid. Consequently, all the tests are performed with 10 ng DNA of a 6 kb plasmid (medium copy number).

Manipulation tips

The box that holds the 96 transformation tubes can be used as a rack during the transformation procedure. Remove the bottom panel of the box to thaw the cells on ice or to immerse the tubes in a water bath (during heat shock).

Shipping and storage conditions

Competent cells are supplied on dry ice.

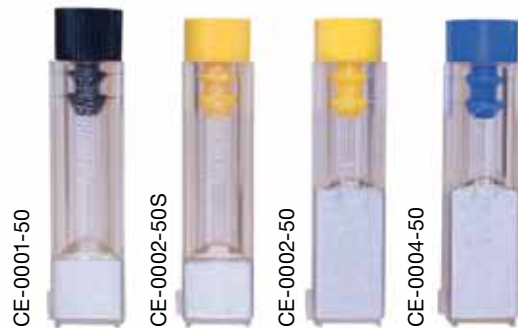
To maintain high competency, the bacteria must be stored at between -70 °C and -80 °C.

Electroporation Cuvettes

Features and benefits

Compatible with all common electroporation devices (*i.e.* Bio-Rad, BTX, Eppendorf).

- ▣ Extremely precise electrode gaps to avoid electric arcs
- ▣ Available in 3 sizes: 1 mm (black cap), 2 mm (yellow caps) and 4 mm (blue cap)
- ▣ Conical bottoms compatible with the use of very low electrocompetent cell volume (*i.e.* 10 μ l) for 1 & 2 mm cuvettes
- ▣ Caps with a double internal seal, ensuring perfect sterility and absence of aerosols
- ▣ Individually packaged and sterilized by gamma irradiation
- ▣ Electrically and biologically controlled



Electroporation cuvettes

Description	Quantity	References
1 mm black cap	50 pcs	CE-0001-50
2 mm yellow cap with big electrode (Universal cuvettes)	50 pcs	CE-0002-50
2 mm yellow cap with small electrode (for older Bio-Rad electroporator versions)	50 pcs	CE-0002-50S
4 mm blue cap	50 pcs	CE-0004-50

Typical transformation parameters

For *E. coli* and other bacteria transformation

- ▣ Field strength up to 25 kV/cm
- ▣ Use 1 mm cuvette

For Yeast transformation

- ▣ Field strength up to 12.5 kV/cm
- ▣ Use 2 mm cuvette

For Mammalian cells

- ▣ Field strength up to 6.25 kV/cm
- ▣ Use 4 mm cuvette

Try it ! ask for a free sample