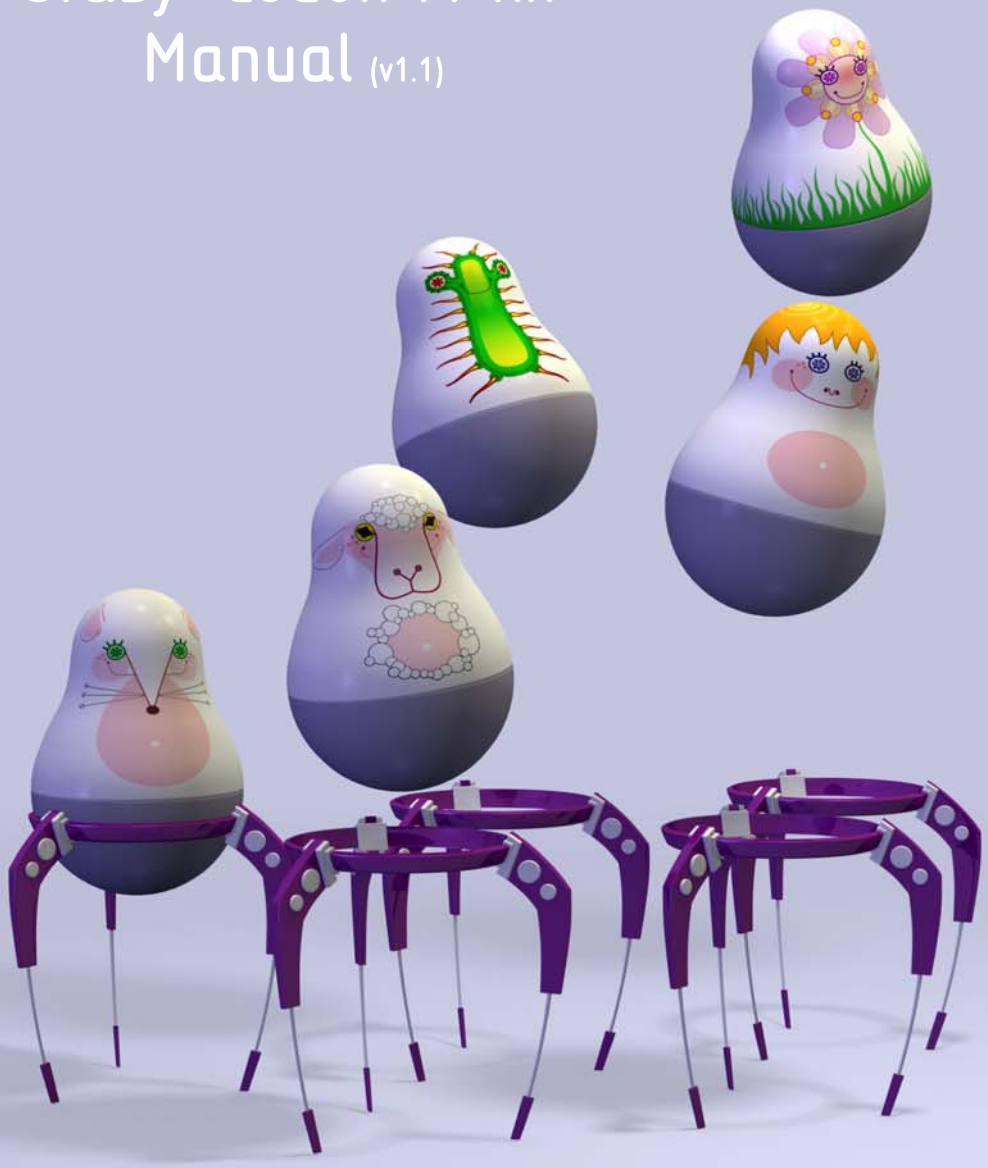
Staby™Codon T7 kit Manual (v1.1)



DELPHI genetics

EUROGENTEC

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Content and storage :

The Staby™ Codon T7 expression kit is shipped on **dry ice**.

Storage: -80°C

Two different types of Staby™ Codon T7 kits are available: one containing electrocompetent cells, and the other type containing chemically-competent cells. Electroporation is more efficient that chemical transformation.

Each kit contains one box with the following items:

Name	**Concentration/remarks**	**Amount** (SCT70505 and SCT70707)	**Amount** (SCT71010 and SCT71212)
pSCodon1 DNA	0.1µg/µl	1 tube of 100µl	1 tube of 100µl
CYS21 strain (for cloning) Pink cap	Competent cells	5 tubes	10 tubes
SE1 strain (for expression) Blue cap	Competent cells	5 tubes	10 tubes
Staby reverse primer Red cap	0.1µg/µl in water 5'-CCA ACT CAG CTT CCT TTC G-3'	1 tube of 20µl	1 tube of 20µl
Staby forward primer Red cap	0.1µg/µl in water 5'-GCG TCC GGC GTA GAG GAT C-3'	1 tube of 20µl	1 tube of 20µl
Expression control	SE1 bacteria carrying a plasmid with an insert encoding a His-tagged protein of 70kDa	1 tube: glycerol stock	1 tube: glycerol stock
Regeneration medium White cap	2% Tryptone 0.5% Yeast extract 0.05% NaCl 2.5mM KCl 10mM $MgCl_2$	5 tubes of 1.5 ml	9 tubes of 1.5 ml
Manual		1	1

The genotype of the CYS21 strain is: F^-, Cm^R, *mcr*A, *end*A1, Δ(*mrr-hsd*RMS-*mcr*BC) (restriction-, modification-), Φ80*lacZ*ΔM15, Δ*lacX*74, *rec*A1, Δ(*ara*, *leu*)7697, *ara*D139, *galU*, *galK*, *nup*G, *rps*L, *ccdB*^{r}.
The genotype of the SE1 strain is: derivates from *E. coli* B strain, F^-, Cm^R, *ompT*, *lon*, $hsdS_B$ (restriction$^-$, modification$^-$), *gal*, *dcm*, DE3 (*lacI*, T7polymerase under the control of the PlacUV5 promoter), *ccdB*^{r}.

Material Safety Data Sheet:

Product and company identification:

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Hazards identification

No specific hazard concerning the products of the StabyCodon T7 kit.

First aid measures

- Inhalation: If one of the products of the StabyCodon T7 kit is inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.
- Ingestion: Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. If large quantities of the products of the StabyCodon T7 kit are swallowed, call a physician immediately.
- Skin contact: In case of contact, immediately flush skin with plenty of water. Remove contaminated clothing and shoes. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention.
- Eye contact: In case of contact with one of the products of the StabyCodon T7 kit, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention.

Fire-fighting measures

Use foam or all purpose dry chemicals to extinguish. Fire fighters should wear positive self-contained breathing apparatus and full turnout gear.

Accidental release measures

Immediately contact emergency personnel. Use suitable protective equipment (see below exposure controls and personal protection). For small spills add absorbent, scoop up material and place in a sealed, liquid-proof container for disposal. For large spills dike spilled material or otherwise contain material to ensure runoff does not reach a waterway. Place spilled material in an appropriate container for disposal. Minimize contact of spilled material with soils to prevent runoff to surface waterways.

Handling and storing

Keep the container tightly closed, in a cool and well-ventilated area.

Personal protection

The occupational exposure limits were not determined. Protect your skin and body using uniform or laboratory coat, chemical resistant, impervious gloves. Use safety glasses, face shield or other full-face protection if potential exists for direct exposure to aerosols or splashes.

Disposal consideration

Waste must be disposed of in accordance with federal, state and local environmental control regulations.

N.B.: Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. To the best of our knowledge, the information contained herein is accurate. However, neither Delphi Genetics SA nor any of its subsidiaries assumes any liability whatsoever for the accuracy or completeness of the information contained herein.

Licenses

The Staby™Codon T7 expression kit is covered by worldwide patents. The kit is sold under a license from the Université Libre de Bruxelles (Belgium). **The kit is sold for research purpose only**. A license from Delphi Genetics SA is required for any commercial use.
(Please, contact Delphi Genetics at delphigenetics@delphigenetics.com)

T7 expression kit is based on technology developed at Brookhaven National Laboratory under contract with the U.S. Department of Energy and is the subject of patent applications assigned to Brookhaven Science Associate (BSA) in the United States of America.

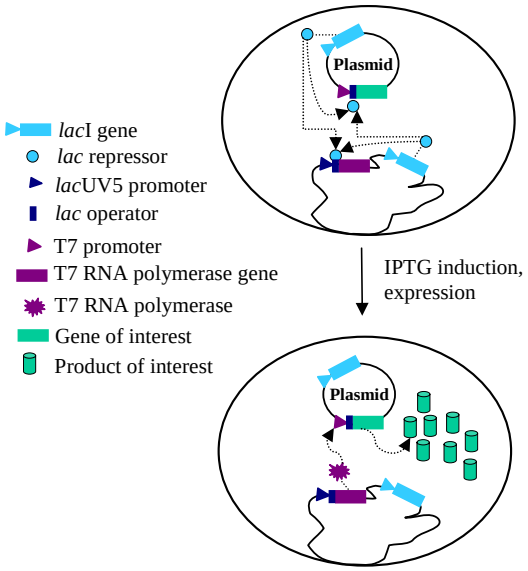
User Guide

The Staby™Codon T7 kit combines three technologies: T7 expression, plasmid stabilization and efficient supply of rare tRNAs to obtain a high yield of heterologous-protein expression even when the protein contains rare codons.

Overview of the T7 expression system

The T7 expression system is based on the use of the T7 bacteriophage promoter and RNA polymerase. The T7 RNA polymerase is useful for synthesizing large amounts of RNA selectively: the T7 RNA polymerase only recognizes the T7 promoter and not the *E. coli* promoters. Conversely, the *E. coli* RNA polymerase does not recognize the T7 promoter (see below). The T7 RNA polymerase is able to transcribe genes five times faster than the *E. coli* RNA polymerase (Chamberlin and Ring, 1973; Golomb and Chamberlin, 1974). The gene encoding the T7 RNA polymerase is inserted into the chromosome of the expression bacteria (SE1, figure 1). The expression of this gene is under the control of the *lacUV5* promoter and therefore is basically controlled by the same mechanisms as the *lac* operon. Thus, the expression of the T7 RNA polymerase is repressed by the binding of the *lac* repressor (encoded by the *lacI* gene) to the *lacO* operator sequence. The gene encoding the repressor is present in the bacterial chromosome and also in the pSCodon1 plasmid to ensure high amount of repressor molecules. Consequently, in normal conditions, the T7 RNA polymerase is not or very weakly expressed. An additional repression of the *lac* promoter can be obtained using medium containing glucose. The presence of glucose in the medium (especially in the stationary phase) induces the metabolic repression: the bacteria will first use glucose as a carbon source and will reduce the concentration of cyclic AMP, ensuring a better repression of the *lac* promoter (cyclic AMP stimulates the *lac* and *lacUV5* promoters). Moreover, Studier et al. (1990) have shown that a better regulation of the expression of the gene of interest is obtained by adding the *lacO* operator sequence between the T7 promoter and the beginning of the gene of interest. This sequence is present in the pSCodon1 vector. Consequently, the *lac* repressor will also repress the expression of the gene of interest.

Figure 1: The T7 expression system used in the SE1 strain



Adding isopropyl-β-D-thiogalactoside (IPTG) to the medium will induce the expression of (*i*) the T7 RNA polymerase and of (*ii*) the gene of interest by removing the *lac* repressor bound to the *lacO* sequence (figure 1).
A powerful feature of the T7 expression system is the ability to clone the gene of interest under conditions of extremely low or no transcriptional activity, that is, in the absence of the T7 RNA polymerase (as the CYS21 genetic background). The expression of the gene of interest is minimal in the absence of the T7 RNA polymerase because this gene is under the control of the T7 promoter which is only recognized by the T7 RNA polymerase and not by the *E. coli* RNA polymerase. If the target gene is cloned directly into the expression strain, even a low basal expression of the T7 RNA polymerase can interfere with growth and selection of the right construct. After the cloning step into a cloning strain lacking the T7 RNA polymerase (CYS21), the plasmid construct is transferred into the expression strain encoding the T7 RNA polymerase (SE1) to produce the protein of interest.
As the the Staby™Codon T7 system is based on commonly used T7 promoter, you can easily change between your existing expression system and Staby™Codon.

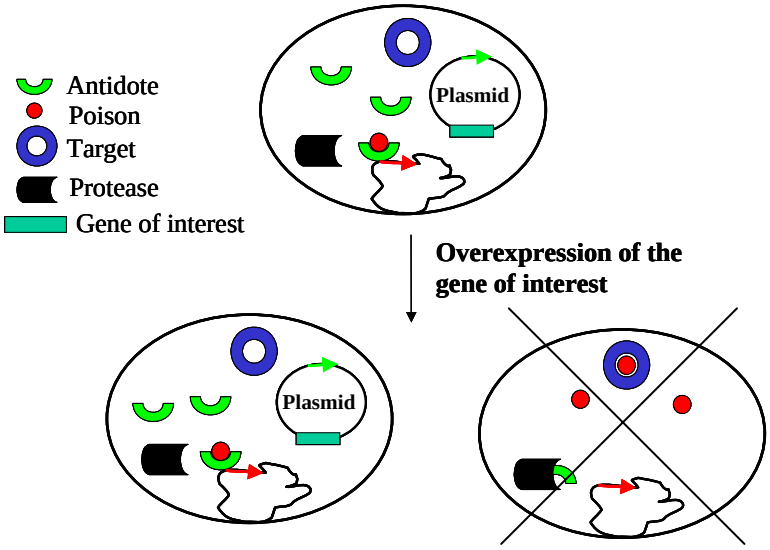
Overview of the stabilization system:

Higher plasmid stability= More proteins

Principle: The stabilization system is based on the use of bacterial antidote/poison *ccdA/ ccdB* genes naturally found in the F plasmid of *Escherichia coli* (for more information about this system, see Bernard *et al.*, 1992 and 1994 or Gabant *et al.* 1998 and 2002). In the Staby™Codon system, the antidote gene (*ccdA,* 218bp) is introduced in the plasmid DNA under the control of a constitutive promoter. On the other hand, the bacterial toxic gene (*ccdB*, 305bp) is introduced in the chromosome of the bacteria (cf. fig. below). Expression of the poison gene is under the control of a promoter strongly

repressed in the presence of the plasmid. When the plasmid is lost, the antidote protein is degraded and the production of the toxin is induced, causing cell death.

Figure 2: Principle of the stabilization system



Practically this means that when during the pre-induction phase bacteria are grown, 100% of the bacteria will carry the vector. If they lose the vector, they will not obtain a growth advantage, but will die. **Upon induction 100% of the bacteria will start producing the recombinant protein leading to higher yields of the target protein and less background caused by unwanted proteins**.

For manufacturers of recombinant proteins this system offers a great benefit because it is an antibiotic free expression system. Therefore the manufactured protein will also be free of traces of antibiotics.

Expression of heterologous genes in *E. coli*:

In all organisms, most amino acids are encoded by more than one codon: 61 codons are available for 20 amino acids. But each organism is characterized by a specific "codon bias" (see table below), *i.e.* it preferentially uses some codons over others. 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*. Whereas, the presence of only a small number of rare codons might not severely depress target protein synthesis, the presence of clusters of and/or numerous rare codons generates a demand for one or more rare tRNAs. In turn, the rarity of some tRNAs leads to very low expression of the target protein due to premature translation termination, translation frameshifting, amino acid misincorporation, growth inhibition and plasmid instability. Six rare codons can cause problems in *E. coli* B (*e.g.;* BL21(DE3) or SE1): AGG and AGA (both encoding arginine using the *argU* tRNA), AUA (isoleucine, *ileX* tRNA), CUA (leucine, *leuW* tRNA), GGA (glycine, *glyT* tRNA), and CCC (proline, *proL* tRNA). An analysis of your gene-of-interest can be performed using **Staby™Soft**.

Amino acid	Codon	Frequency in E. coli B (SE1) (%)	Frequency in Homo sapiens (%)	Frequency in Arabidopsis thaliana (%)	Frequency in Saccharomyces cerevisiae (%)
Arginine	CGT	35	8	17	14
	CGC	40	19	7	6
	CGA*	5*	11	12	7
	CGG	11	20	9	4
	AGA	5	21	35	48
	AGG	4	21	20	21
Glycine	GGT	30	16	34	47
	GGC	41	34	14	19
	GGA	10	25	37	22
	GGG	18	25	15	12
Isoleucine	ATT	48	36	41	46
	ATC	44	47	35	27
	ATA	7	17	24	27
Leucine	TTA	14	8	13	28
	TTG	15	13	22	28
	CTT	11	13	26	13
	CTC	12	20	17	6
	CTA	3	7	11	14
	CTG	45	40	11	11
Proline	CCT	14	28	38	31
	CCC	6	33	11	16
	CCA	18	27	33	41
	CCG	61	11	18	12

*: CGA codon does not cause problem because large amounts of the corresponding tRNA are present

In the **Staby™Codon T7 kit**, we solve the problem by the use of the pSCodon1 expression plasmid encoding the tRNA genes of the six rare codons. Hence, this plasmid contains the T7 promoter for a strong expression, the *ccdA* gene for plasmid stabilization and supplies the rare tRNAs.

Benefits of the Staby™Codon system:

- High yield of heterologous-protein expression even when the protein contains rare codons;
- Not necessary to mutate each rare codon;
- Recombinant plasmid perfectly stabilized even without the use of antibiotics;
- Reduced background of "parasite proteins";
- No additional plasmid in bacteria;
- The promoters typically used in protein production remain available;
- System is usable in any culture medium.

Experimental outline: Easy 4 steps procedure.

1. Cloning of your gene of interest in the pSCodon1 vector

↓

2. Transformation into the CYS21 *E. coli* cells and selection of the desired construction

↓

3. Transformation of your plasmid DNA into the SE1 *E. coli* competent cells

↓

4. Expression of your gene of interest without antibiotic

Step 1: Cloning of your gene of interest in the pSCodon1 vector:

Many strategies can be used for cloning your gene of interest (goi) into the pSCodon1 vector. The most convenient strategy is to use restriction enzymes: the single-cutter enzymes from the multiple cloning sites are indicated on the map (see below: from BamHI to XhoI). Use the buffer and incubation conditions provided by the restriction enzyme manufacturer. After restriction, the DNA fragment encoding the gene of interest is inserted by ligation using compatible ends.

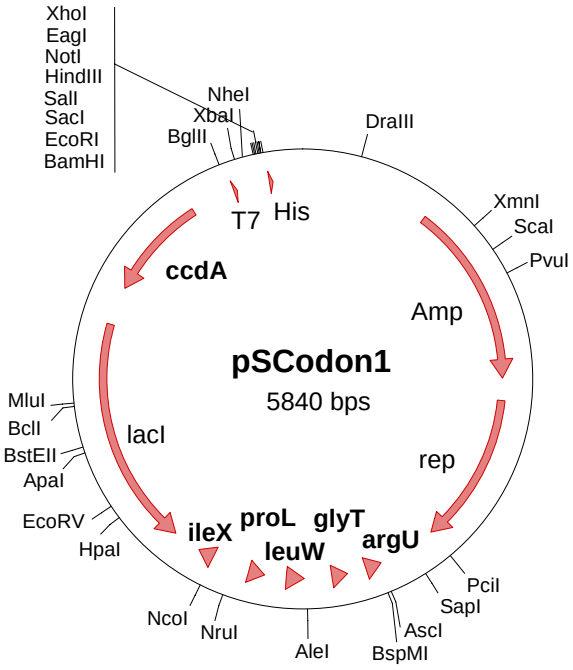


Figure 3: Restriction map of the pSCodon1 vector

Remarks:

If you want to use the ribosome binding site (RBS) from the vector to express your gene, you must take into account the reading frame and the position of the start codon (cf. figure 4 below).

If you need to avoid any fusion of amino acid at the N-terminal end of your protein, amplify your gene of interest (goi) by PCR and add the RBS region and an appropriate restriction site to the 5' end primer (see figure 5).

The pSCodon1 vector allows the **fusion** of 6 histidine residues at the C-terminal end of the protein. This tag facilitates the detection and the purification of the target protein. If needed, the C-terminal fusion can be skipped by including a stop codon at the end of the gene of interest. Please, note that the 6 histidine residues are not in the same frame than the start codon to avoid any fortuitous fusion (the 6 histidine codons are indicated in blue).

Fig. 4. Sequence of the cloning region: (unique restriction sites are indicated in bold or italized)

```
      .T7.promoter            lac operator            XbaI
gaaattaatacgactcactataggggaattgtgagcggataacaattcccctctagaaataattttgttt
cttaattatgctgagtgatatccccttaacactcgcctattgttaagggagatcttattaaaacaaa

         RBS          Start NheI
aactttaagaaggagatatacatatg gct agc atg act ggt gga cag caa atg ggt cgc
ttgaaattcttcctctatatgtatac cga tcg tac tga cca cct gtc gtt tac cca gcg

                Ecl136II                      EagI
 BamHI  EcoRI   SacI      SalI   HindIII    NotI      XhoI
gga tcc gaa ttc gag ctc cgt cga caa gct tgc ggc cgc act cga g cac cac
cct agg ctt aag ctc gag gca gct gtt cga acg ccg gcg tga gct c gtg gtg

 6 his          Stop
cac cac cac cac tgagatccggctgctaa
gtg gtg gtg gtg actctaggccgacgatt
```

Fig. 5. Structure of the 5'end primer in order to avoid any N-terminal fusion:

```
5'-restriction sitetttaagaaggagatatacatatggoi without the start codon-3'
```

Important:

Delphi Genetics can help you with a software-based optimization of the nucleotide sequence of your gene-of-interest for a best protein production (please contact us at delphigenetics@delphigenetics.com).

Step 2. Transformation into the CYS21 strain and selection of the desired construction:

Selection of the desired construction is performed in CYS21 *E. coli* cells lacking the T7 RNA polymerase gene (*). These cells contain the *ccdB* gene in their chromosome.

This enables:

(i) High efficiency of transformation (the transformation efficiency of SE1 - derivative of BL21 is lower than that of CYS21),
(ii) Stabilization of plasmids for high DNA production,
(iii) Selection of the desired construction without expression of the gene of interest (goi).

(*)Remark:
It is not recommended to clone directly the goi into the expression host containing the T7 RNA polymerase gene: the T7 gene basal expression, and the resulting goi basal expression, would reduce the efficiency of recovery of the desired construction.

Protocol:
Two different types of Staby™Codon T7 kits are available: one containing electrocompetent cells, and the other type containing chemically-competent cells. Electroporation is more efficient that chemical transformation.

a) Transformation by electroporation:

1) Prepare LB plates containing 100µg/ml Ampicillin. Let the plates dry and then warm them at 37°C.
2) Set up your electroporator for bacterial transformation. Use the manufacturer's instructions.
3) For each cloning reaction, place one vial of the CYS21 electrocompetent cells (pink cap) and one electroporation cuvette on ice. Allow the cells to thaw on ice for 5-10 minutes.
4) Add 1 or 2 µl of the ligation to the vial of the CYS21 electrocompetent cells (pink cap). Stir gently to mix. Do not mix by pipetting up and down.

   If you wish to use more than 2µl of the ligation mix, it is recommended to dialyze it against sterile water using a 0.025µm filter. Add the sterile water in a Petri dish and carefully place the filter on the water surface. Delicately, put the ligation mix on the filter. Wait 10min, pipet back the ligation mix and add the dialyzed solution to the electrocompetent cells.
5) Transfer all the content of the tube (cells+DNA) to the pre-chilled electroporation cuvette.
6) Electroporate the cells according to the manufacturer's instructions.
7) Quickly add 500µl of the regeneration medium (white cap) at room temperature and mix well.

8) Spread immediately 20 to 150µl on the pre-warmed plates containing ampicillin.
9) Incubate the plates overnight at 37°C.
10) Pick about 10 colonies and culture them overnight in 10ml of LB medium with or without ampicillin (100µg/ml).

    Note: The stabilization is now effective; the ccdB gene is activated. Consequently, the plasmid is stabilized in the CYS21 strain and no antibiotic is needed to select bacteria containing the plasmid. However, the ampicilline resistance is still available. The stabilization system will insure high yield of plasmid DNA.

11) Extract plasmid DNA and analyze the constructions using your method of choice (restriction, sequencing, ...). Sequencing primers (Staby reverse and Staby forward primers) are included in the kit (0.1 µg/µl). The complete sequence of the pSCodon1 vector is available on our website: http://www.delphigenetics.com or http://www.eurogentec.com
12) Choose one of the clones containing the desired construct. Mix well 800µl of the culture with 800µl of sterile glycerol and transfer to a cryo-vial. Store at -80°C.

b) Transformation using chemically competent cells:

1. Prepare LB plates containing 100 µg/ml Ampicillin. Let the plates dry and then warm them up at 37°C.
2. Set a water bath or a heating-bloc to 42°C
3. Thaw (bring to room temperature) one vial of regeneration medium (white cap) per cloning reaction.
4. For each cloning reaction, place one vial of the CYS21 chemically-competent cells (self-standing tube with pink cap) on ice. Allow the cells to thaw on ice for 5-10 minutes.
5. Add 5 µl of the ligation product to one vial of the CYS21 chemically competent cells (self-standing tube with pink cap). Stir gently to mix. Do not mix by pipetting up and down.
6. Incubate on ice for 30 minutes.
7. Heat-shock the bacteria by placing the vial at 42°C for 30 seconds without shaking.
8. Immediately transfer the tubes to ice.
9. Add 250µl of room-temperature regeneration medium (white cap) and mix well.
10. Spread immediately 10, 20 and 100µl of the product (from step 9) on different pre-warmed plates.

    If you wish to have more clones, incubate the product (from step 9) at 37°C for one hour for regeneration of the bacteria before spreading of 10, 20 and 100µl on different pre-warmed plates.

11. Incubate the plates overnight at 37°C.
12. Pick about 10 colonies and culture them overnight in 10ml of LB medium with or without ampicillin (100µg/ml).

Note: The stabilization is now effective; the ccdB gene is activated. Consequently, the plasmid is stabilized in the CYS21 strain and no antibiotic is needed to select bacteria containing the plasmid. However, the ampicilline resistance is still available. The stabilization system will insure high yield of plasmid DNA.

13. Extract plasmid DNA and analyze the constructions using your method of choice (restriction, sequencing, ...). Sequencing primers (Staby reverse and Staby forward primers) are included in the kit (0.1 µg/µl). The complete sequence of the pSCodon1 vector is available on our website: http://www.eurogentec.com or http://www.delphigenetics.com
14. Choose one of the clones containing the desired construct. Mix well 800µl of the culture with 800µl of sterile glycerol and transfer to a cryo-vial. Store at -80°C.

Step 3. Transformation in the expression host:

a) Transformation by electroporation:

1) Prepare LB plates containing 100µg/ml Ampicillin. Let the plates dry and then warm them at 37°C.

   Note: Addition of 1% glucose (from a sterile filtered 20% stock solution) in the plates can be useful to better repress the promoter and to avoid basal expression.

2) Set up your electroporator for bacterial transformation. Use the manufacturer's instructions.
3) For each transformation, place one vial of the SE1 electrocompetent cells (blue cap) and one electroporation cuvette on ice. Allow the cells to thaw on ice for 5-10 minutes.
4) Add 1µl of the selected plasmid DNA (steps 11 and 12 above) to the SE1 cells and mix gently.
5) Transfer all the content of the tube (cells+DNA) to the pre-chilled electroporation cuvette.
6) Electroporate the cells according to the manufacturer's instructions.
7) Quickly add 500µl of the regeneration medium (white cap) at room temperature and mix well.
8) Spread immediately 20 to 150µl on the pre-warmed LB plates.
9) Incubate the plates overnight at 37°C.
10) Optional: Pick about 5 colonies and culture them overnight in 10ml of LB medium.

    Note: The plasmid is now stabilized in the SE1 strain using the Staby™ Codon system, no antibiotic is needed to select bacteria containing the plasmid. However, the ampicilline resistance gene is still available. ***Addition of 1% glucose (from a sterile filtered 20% stock solution) in the medium can be useful to better repress the promoter and to avoid basal expression.***

11) Optional: Extract plasmid DNA and analyze the constructions using your method of choice (restriction, sequencing, ...). Sequencing primers are included in the kit (0.1

µg/µl). The complete sequence of the pSCodon1 vector is available on our website: http://www.delphigenetics.com or http://www.eurogentec.com

12) Optional: Select one of the clones containing the desired construction. Mix well 800µl of the culture with 800µl of sterile glycerol and transfer to a cryovial. Store at -80°C.

b) Transformation using chemically competent cells:

1. Prepare LB plates containing 100 µg/ml Ampicillin. Let the plates dry and then warm them up at 37°C.

   Note: Addition of 1% glucose (from a sterile filtered 20% stock solution) in the plates can be useful to better repress the promoter and to avoid undesirable expression.

2. Set a water bath or a heating-bloc to 42°C
3. Thaw (bring to room temperature) one vial of regeneration medium (white cap) per cloning reaction.
4. For each transformation, place one vial of the SE1 chemically-competent cells (self-standing tube with blue cap) on ice. Allow the cells to thaw on ice for 5-10 minutes.
5. Add 1µl or 2µl of the selected plasmid DNA (steps 11 and 12 above) to one vial of the SE1 chemically competent cells (self-standing tube with blue cap). Stir gently to mix. Do not mix by pipetting up and down.
6. Incubate on ice for 30 minutes.
7. Heat-shock the bacteria by placing the vial at 42°C for 30 seconds without shaking.
8. Immediately transfer the tubes to ice.
9. Add 250µl of room-temperature regeneration medium (white cap) and mix well.
10. Spread immediately 10, 20 and 100µl of the product (from step 9) on different pre-warmed plates.
11. Incubate the plates overnight at 37°C.
12. Optional: Pick about 5 colonies and culture them overnight in 10ml of LB medium.

    Note: The plasmid is now stabilized in the SE1 strain using the Staby™ Codon system, no antibiotic is needed to select bacteria containing the plasmid. However, the ampicilline resistance gene is still available. ***Addition of 1% glucose (from a sterile filtered 20% stock solution) in the medium can be useful to better repress the promoter and to avoid basal expression.***

13. Optional: Extract plasmid DNA and analyze the constructions using your method of choice (restriction, sequencing, ...). Sequencing primers are included in the kit (0.1 µg/µl). The complete sequence of the pSCodon1 vector is available on our website: http://www.eurogentec.com or http://www.delphigenetics.com
14. Optional: Select one of the clones containing the desired construction. Mix well 800µl of the culture with 800µl of sterile glycerol and transfer to a cryovial. Store at -80°C.

Step 4. Expression of your gene of interest:

The T7 RNA polymerase is under the control of the PlacUV5 promoter (Studier and Moffat, 1986; Studier *et al.*, 1990). Both the SE1 strain and the pSCodon1 vector carry the *lacI* gene. LacI represses both the expression of the T7 RNA polymerase and the transcription of the gene of interest. Consequently, the expression of the T7 RNA polymerase is inducible by isopropyl-β-D-thiogalactoside (IPTG): addition of IPTG to the culture of the SE1 strain containing the pSCodon1 plasmid will induce the expression of the T7 RNA polymerase which, in turn, will transcribe the gene of interest.

Remark: Expression protocols already established using other T7 expression systems (with or without antibiotics) can be readily used with the Staby™Codon T7 system. Indeed, using Staby™Codon T7 does not modify expression and/or action of the T7 RNA polymerase. However, the Staby™Codon system will insure greater plasmid stability and will reduce the background of "parasite proteins", especially if your gene of interest codes for a toxic protein.

Protocol for a small-scale expression and analysis:

The small-scale protocol below will allow you to verify that the target protein is produced upon induction and to verify for the presence of detection tags in the target protein. The glycerol stock of the expression positive control (provided in the kit) will allow you to produce his-tagged test protein of 69kDa.

1) Inoculate two Erlenmeyer flasks containing 10ml of LB medium with a few microliters from a glycerol stock. Alternatively, pick two single colonies from a plate streaked with the SE1 bacteria containing your construction; inoculate two flasks containing 10ml of LB medium.
2) Incubate with shaking at 37°C until OD_{600} reaches 0.4-1 (the best range is between 0.6 and 0.8).
3) In one of the two flasks, add IPTG (100µl of a fresh 100mM stock solution) to reach a final concentration of 1mM. The other flask is used as a non-induced control. Continue incubation of both flasks for 2-3 hours.
4) Measure the Optical Density at 600nm for each culture. Transfer 1ml sample of each flask in a microcentrifuge tube. Add 50µl of cold 100% Trichloroacetic acid (TCA) (w/v) to each sample and vortex for a few seconds.

   Note: The TCA precipitation allows the analysis of the total protein content of the cells. Other methods can be used to specifically analyze different fractions (soluble, insoluble, periplasm, ...) in order to identify the cellular localization of the target protein. For more information, please, check specialized literature or protocols (e.g., Sambrook et al., Ausubel et al.).
5) Place on ice for 10 min.

6) Centrifuge at maximum speed (13000 g) for 10 min (if possible at 4°C).
7) Remove carefully and discard the supernatant.
8) Wash the pellet with cold acetone (+4°C): add 500µl of acetone, vortex, and centrifuge for 5 min at maximum speed (if possible at 4°C).
9) Repeat steps 7 and 8
10) Remove carefully the supernatant. Air dry the final pellet: leave the tube opened on the bench or use vacuum drying.
11) Add (OD_{600} x 200)µl of 1X sample buffer (2X sample buffer= 100mM DTT, 2% SDS, 80mM Tris-HCl, pH 6.8, 0.006% bromophenol blue, 15% glycerol). Vortex vigorously to resuspend the pellet.

    Note: Taking into account the OD_{600} allows comparison of Coomassie-stained band intensities between samples.

12) Heat the samples at 70°C-100°C (10min.) to resuspend and denature the proteins. The samples can be used directly for SDS-PAGE analysis or stored at -20°C.
13) Load 4 to 10 µl of each sample in a SDS-PAGE gel containing the appropriate concentration of polyacrylamide (according to the size of the overproduced protein). Add a molecular size marker.

    Note: The sample volume that needs to be loaded will depend on the gel size, the expression level, and the extraction efficiency.

14) After migration, visualize the proteins with Coomassie-blue staining or continue the analysis with western blot.

    Note: Western blot analysis is a more specific and sensitive method but needs protein-specific antibodies or fusion tag-specific antibodies. For more information, please, check specialized literature or protocols (e.g., Sambrook et al, Ausubel et al.).

References:

- Ausubel F., Brent R., Kingston R., Moore D., Seidman J.G., Smith J.A., Struhl K. 1995. Current Protocols in Molecular Biology. John Wiley and Sons edition. USA.
- Bernard P. and Couturier M. 1992. Cell killing by the F plasmid CcdB protein involves poisoning of DNA-topoisomerase II complexes. J. Mol. Biol. 226: 735-745.
- Bernard P., Gabant P., Bahassi El M., Couturier M. 1994. Positive-selection vectors using the F plasmid *ccdB* killer gene. Gene 148: 71-74.
- Chamberlin M., and Ring J. 1973. Characterization of T7-specific ribonucleic acid polymerase. 1. General properties of the enzymatic reaction and the template specificity of the enzyme. J. Biol. Chem. 248:2235-2244.
- Gabant P., Szpirer C.Y., Couturier M., Faelen M. 1998. Direct selection cloning vectors adapted to the genetic analysis of Gram-negative bacteria and their plasmids. Gene 207: 87-92.
- Gabant P., C.Y. Szpirer, Van Melderen L. 2002. Plasmid poison-antidote systems: functions and technological applications. In "Recent advances in plasmid science" ed.: S.G. Pandalai (Transworld research network edition). Vol1, pg: 15-28.
- Golomb M. and Chamberlin M. 1974. Characterization of T7-specific ribonucleic acid polymerase. IV. Resolution of the major in vitro transcripts by gel electrophoresis. J. Biol. Chem. 249:2858-2863
- Sambrook J., Fritsch E., Maniatis T. 1989. in Molecular Cloning: a laboratory manual, Second edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Studier W. and Moffat B.A. 1986. Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. J. Mol. Biol. 189:113-130.
- Studier W., Rosenberg A., Dunn J., Dubendorff J. 1990. Use of T7 RNA polymerase to direct expression of cloned genes. Methods in Enzymology vol. 185: 60-89.

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