StabyCloning ™ kit Manual (v1.5)

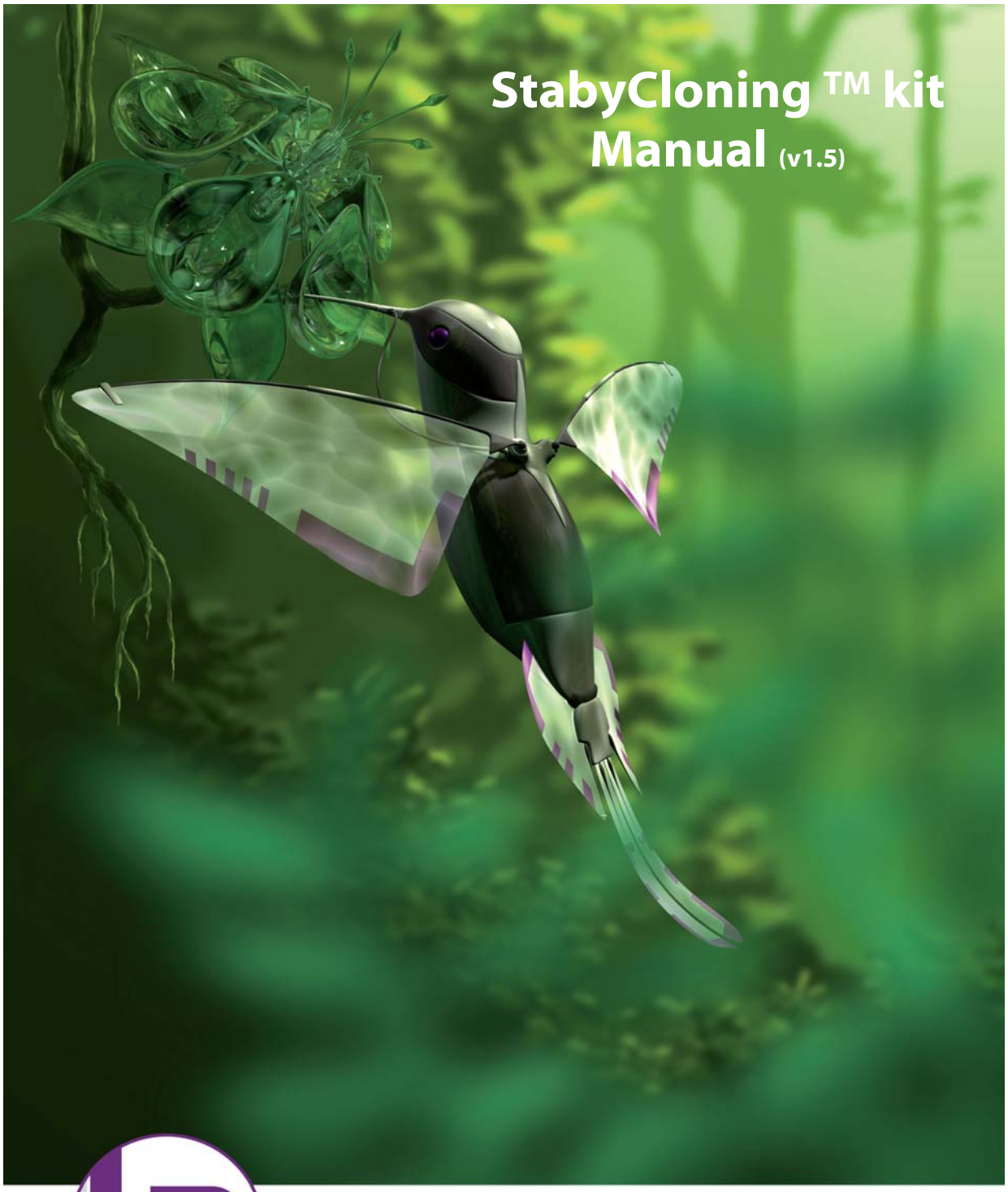

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Table of contents

- Content and storage 2
- Material Safety and Data Sheet 3
- Licenses 4
- User Guide 4
  - Overview of the Stabycloning™ system 4
  - Overview of the stabilization principle 5
  - Benefits of the StabyCloning™ system 6
  - The 3 step-cloning procedure 7
    - Step 1. PCR amplification and purification 7
    - Step 2. Ligation 10
    - Step 3. Transformation 12
  - Analysis of the clones 13
  - Troubleshooting 14
- References 15
- Related Staby™ products and services 16
- Staby™ products ordering information 17
- Worldwide ordering 18

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Content and storage :

The StabyCloning™ kit is shipped on **dry ice**.

Storage: -70 to -80°C. After the first use, we recommend to store the ligase and its buffer at -20°C.

Two different types of StabyCloning™ kits are available: one containing electrocompetent cells (in non self-standing tubes), and the other type containing chemically-competent cells (in self-standing tubes). Electroporation is more efficient that chemical transformation.

Each kit contains one box with the following items:

Name	**Concentration/remarks**	**Amount (STC1-10, STC1-12)**	**Amount (STC1-20, STC1-22)**	**Amount (STCB-20, STCB-22)**
pSTC1.3 DNA Yellow cap	0.1 µg/µl The vector is linearized (blunt ends)	1 tube of 25 µl	2 tubes of 25 µl	0
CYS21 bacteria* Pink cap	Competent cells	10 tubes	20 tubes	20 tubes
STC1 forward primer Red cap	0.1 µg/µl in water 5′ TGC-AGC-GCG-TTA-GAA-TAC 3′	1 tube of 20 µl	1 tube of 20 µl	0
STC1 reverse primer Red cap	0.1 µg/µl in water 5′ TGA-GGT-CGC-CCG-GTT-TAT-TG 3′	1 tube of 20 µl	1 tube of 20 µl	0
Ligation buffer Blue cap		1 tube of 150µl	1 tube of 150µl	0
T4 DNA ligase Gray cap		1 tube of 25µl	2 tubes of 25µl	0
Primer control mix Orange cap		1 tube of 40µl	1 tube of 40µl	0
Regeneration medium White cap	2% Tryptone, 0.5% Yeast extract, 0.05% NaCl, 2.5 mM KCl, 10mM $MgCl_2$	10 tubes of 1.5ml	20 tubes of 1.5ml	20 tubes of 1.5ml
Manual		1	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*lac*ZΔM15, Δ*lac*X74, *rec*A1, Δ(*ara*, *leu*)7697, *ara*D139, *galU*, *galK*, *nup*G, *rps*l, *ccdB*$^+$.

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Material Safety Data Sheet:

Product and company identification:

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B-6041 Charleroi, Belgium
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Fax: +32.71.37.60.57
e-mail: delphigenetics@delphigenetics.com

Hazards identification

There is no specific hazard concerning the products of the Stabycloning kit.

First aid measures

- Inhalation: If one of the products of the StabyCloning 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 StabyCloning 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 Stabycloning 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.

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Licenses

The StabyCloning™ cloning 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 SA at delphigenetics@delphigenetics.com).

User Guide

Overview of the StabyCloning™ system:

The *CYS21* bacterium, included in the StabyCloning kit, contains a natural bacterial poison gene (encoding the poison protein CcdB) into its chromosome. 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 antidote gene encoding an active protein (CcdA) able to counteract the action of the poison. The 14-bp sequence is incorporated to the DNA fragment using one modified PCR primer (the appropriate 14-base tail is added at the 5'-end of one of the two PCR primers). As illustrated in the figures 1 and 2, this process allows *(i)* the selection of recombinant plasmids that incorporate the fragment of interest (non-recombinant plasmids contain an inactive, truncated, CcdA* gene), and *(ii)* orientation of the fragment of interest (only one of the two possible orientations will restore an active, non-truncated, *ccd*A gene). Moreover, restoration of the *ccdA* gene also stabilizes the plasmid into the bacterial population without the need to use antibiotics (see below). Finally, the StabyCloning™ system eliminates the background due to the electroporation of the plasmid used as PCR template. Even after purification of the PCR product, traces of the plasmid used as PCR template are mixed with the PCR product and this plasmid is also electroporated into the bacteria. In conventional systems, when both the plasmid used as PCR template and the cloning vector carry the same antibiotic resistance gene, the bacteria containing the original plasmid without insert will survive and, hence, will increase the background of false positives. In the StabyCloning™ system, the selection is based not only on the antibiotic resistance but also on the resistance to the CcdB poison. Consequently, only bacteria containing the cloning vector with an insert in the correct orientation survive and the bacteria containing the original plasmid with the antibiotic-resistance gene (but without the antidote gene) are killed.

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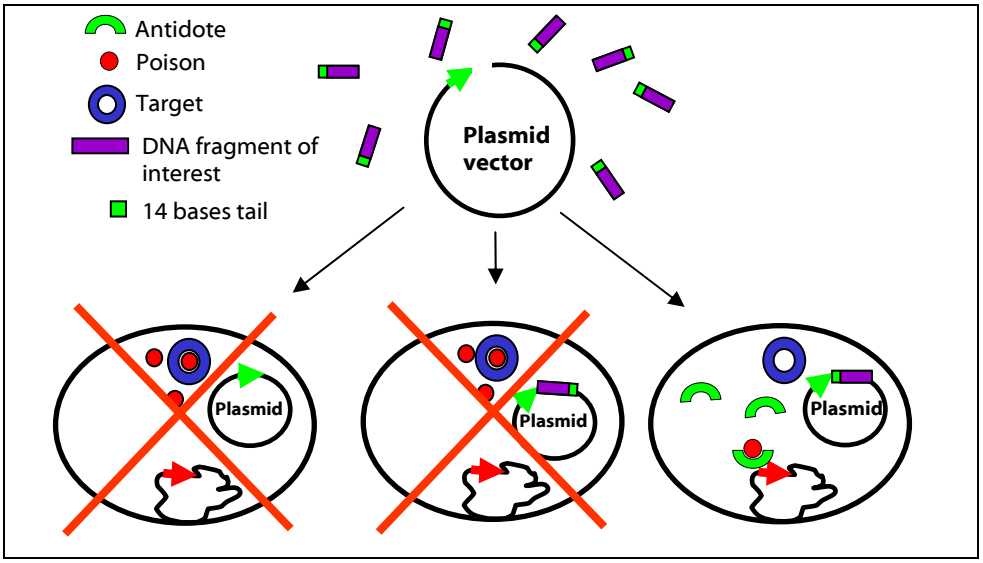
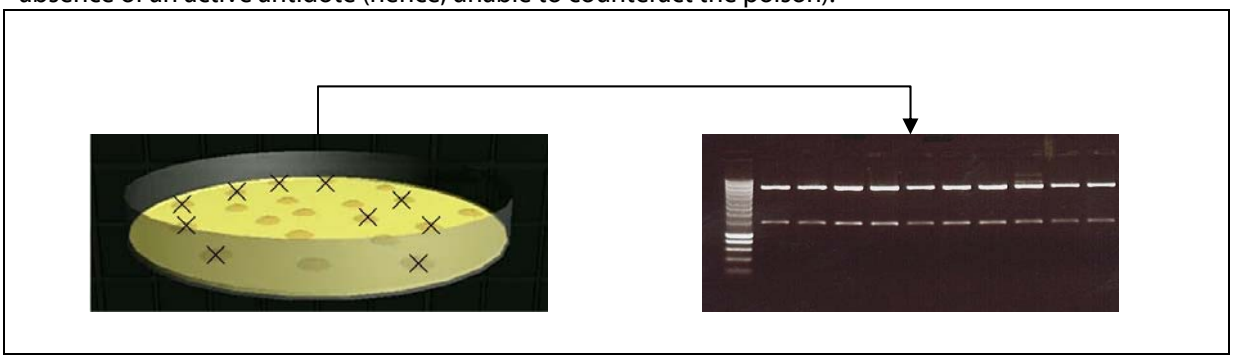
Figure 1: Principle of the StabyCloning™ system



Using the StabyCloning™ kit, the complete cloning procedure (including plating) is performed in 1 hour. All plated colonies are guaranteed as independent clones because no growth phase is required between transformation and plating.

Figure 2: All the colonies growing on the plates contain a vector with a correctly oriented insert. The bacteria without insert and the bacteria with the insert in the wrong orientation are killed due to the absence of an active antidote (hence, unable to counteract the poison).



Overview of the stabilization principle:

Higher plasmid stability = More DNA.

In the StabyCloning™ kit, the antidote gene (*ccdA*) is localized in the plasmid DNA under the control of a constitutive promoter. On the other hand, the toxic gene (*ccdB*) is localized in the chromosome of the bacteria (cf. figure 3) and its expression is under the control of a promoter strongly repressed in the presence of the plasmid. When the plasmid is lost, the antidote is degraded and the production of the toxin is induced, causing cell death.

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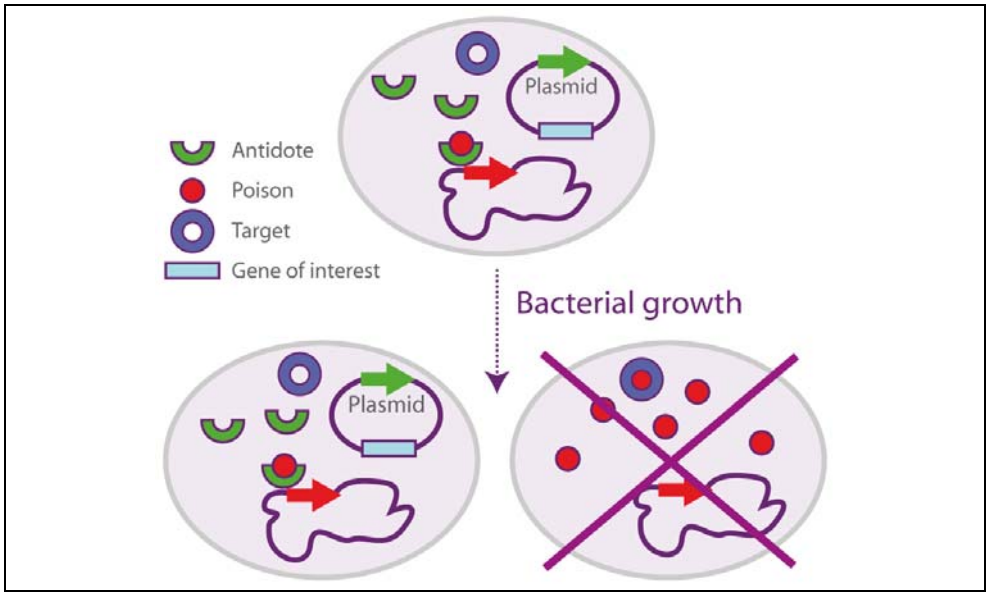
Figure 3: Principle of the stabilization system



If some bacteria lose the vector, they will not obtain a selective (growth speed) advantage, but will die. In practice, this means that during bacterial growth, 100% of the bacteria will carry the vector.
Some inserts, when cloned into a classical vector, may cause instability of this vector resulting in low yield of DNA extraction. The reasons of this instability are, for example, the toxicity of the insert and/or the secondary structure and/or the size of the insert. On the other hand, when using the unique selection technology of the StabyCloning™ system, all the recombinants will be stabilized after selection and will yield high amounts of DNA.

Benefits of the StabyCloning™ system:

- Ligation + transformation + plating in one hour;
- Vectors without insert are eliminated (background <1%);
- The DNA fragment is correctly oriented in the vector;
- The recombinants are all independent clones (direct plating);
- The insert, or the [stabilization cassette + insert] are easily exportable to other vectors;
- No "satellite" colonies observed even after extended incubation;
- No risk of background when using Amp^R plasmids as PCR template;
- Recombinant plasmids are perfectly stabilized without the use of antibiotics;
- The system is usable in any culture medium.

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The 3 step-cloning procedure.

1) PCR amplification of your DNA fragment (addition of a 14-bases tail to one PCR primer) and purification (if necessary)

↓

2) Ligation of your (purified) PCR fragment into the *pSTC1.3* vector

↓

3) Transformation into the *CYS21 E. coli* cells and plating of the bacteria

Total cloning time (steps 2 + 3) = 1 hour

Step 1: PCR amplification of your DNA fragment and purification:

The StabyCloning™ kit is specifically designed to clone DNA fragments generated by PCR. We recommend using a proof-reading polymerase or a polymerase mix containing a proof-reading activity to avoid (*i*) the addition of A nucleotides at the ends of the PCR product and (*ii*) polymerization errors.
It is not necessary to add 5'-phosphates to your primers for PCR. However, the addition of 5'-phosphates does not cause any problem during the ligation step.
Please, follow the polymerase manufacturer's instructions and recommendations for producing PCR products. Optimize conditions to produce your target PCR product because small artifactual amplicons will be preferentially cloned (over long PCR products).
We recommend that you perform the control reaction (reagents provided) the first time you use the kit.

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Figure 4: Restriction map of the *pSTC1.3* vector. All restriction sites represented are unique in the vector excepted *Hin*dIII and *Eco*RI (note that the introduction of the PCR fragment will create a *Bam*HI restriction site, 5′ end of the insert). These sites are asterisked (*). Restriction map and sequence are available on line at www.delphigenetics.com

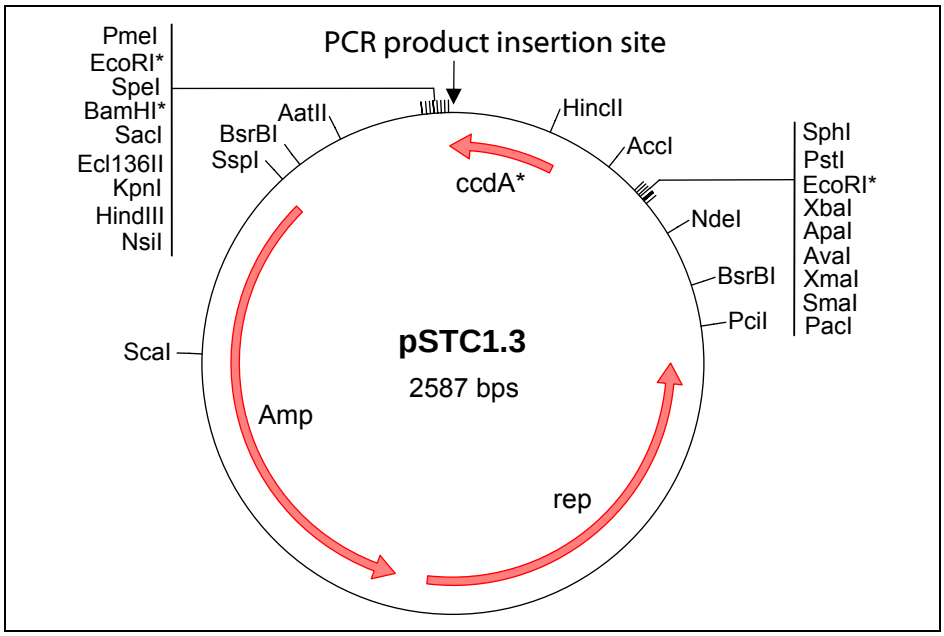

Figure 5: DNA sequence after the insertion of the PCR product.

```
                                                    Ecl136II
        STC1 forward primer          NsiI  HindIII KpnI SacI BamHI  SpeI EcoRI* PmeI
5'CATTAATGCAGCGCGTTAGAATACTCAAGCTATGCATCAAGCTTGGTACCGAGCTCGGATCCACTAGTGAATTCGTTTAA
3'GTAATTACGTCGCGCAATCTTATGAGTTCGATACGTAGTTCGAACCATGGCTCGAGCCTAGGTGATCACTTAAGCAAATT

                                    BamHI*
ACGAT   Insert   TCA GTC GGC GAA GGA TCC GTT CAT TTC AAT AAA CCG GGC GAC CTC AGC
TGCTA            AGT CAG CCG CTT CCT AGG CAA GTA AAG TTA TTT GGC CCG CTG GAG TCG
                                                        STC1 reverse primer
                 -   D   A   F   S   G   N   M   E   I   F   R   A   V   E   A
                                  ← C-terminal end of the CcdA antidote
```

The 14-bp sequence added for activation of the antidote is shown in green. All restriction sites represented are unique in the vector except *Eco*RI and *Bam*HI. These sites are asterisked (*). Note that one *Bam*HI site is created by the introduction of the PCR fragment and another one is present in the vector backbone. Consequently, you can easily remove the cloned fragment from the vector by a single *Bam*HI restriction (please, first check for the absence of *Bam*HI restriction sites in your insert). It is also easy to export the stabilization cassette (antidote gene) together with your insert using one of the restriction sites in the 5′ end of the antidote gene (*Sph*I to *Pac*I, see figure 4 above) and one of the restriction sites in the 3′ end of your insert (*Nsi*I to *Pme*I). For example, an *Eco*RI site is present on both sides of the insert. It is then possible to select the entry of this excised fragment (stabilization cassette+insert) into another vector using the CYS21 strain and the antidote properties.

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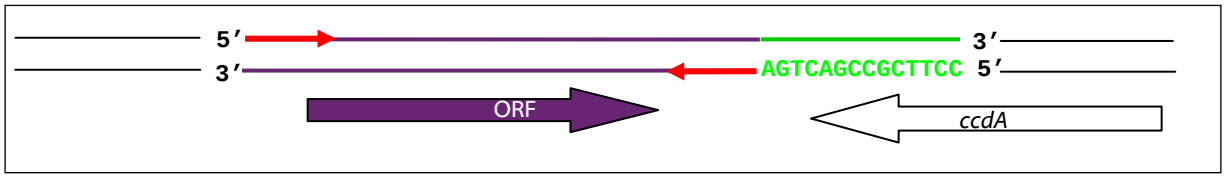
Protocol:

1. Design your specific PCR primers (oligonucleotides): 20 bases are often necessary to have sufficient specificity.

   Add the following 14-base-tail sequence to the 5'-end of one of the two PCR primers:
   5' CCT-TCG-CCG-ACT-GA 3'

   If the orientation is not important, you can add the 14-base tail to both primers. In that case, the orientation will not be selected.
   If the target DNA fragment encodes an ORF (open reading frame), it is preferable to add the 14-base tail to the primer positioned at the 3'-end of the ORF (see figure 6) to avoid undesirable expression of this gene from the *ccdA* promoter.

   Figure 6: Recommended primer positions for PCR amplification and cloning of an ORF



2. Order or synthesize your primers. Use preferentially purified primers (reverse-phase or PAGE purification and/or Mass-spectrometry analysis). Please note that the Staby™-Tag corresponding to the 14 bases-tail sequence is available as a 5' modification on some oligo web ordering interfaces.

3. Set up a 50 µl or 100 µl PCR reaction:
   - √ according to the manufacturer's recommendations;
   - √ using cycling parameters adapted to your specific primers and DNA template;
   - √ using (preferably) a proof-reading polymerase or a polymerase mix having a proof-reading activity.

   At the end of the PCR amplification, place the tube on ice.

4. Check 5 to 10 µl of the PCR product using agarose gel electrophoresis and ethidium bromide or another staining solution. The rest of the sample can be stored at -20°C for several days.

5. Purification of your DNA fragment.
   In most of the cases, purification of the DNA fragment on an agarose gel gives better results than dialysis or simple removal of PCR primers. However, the purification grade of your target DNA fragment depends on the quality of the PCR amplification, and on the DNA fragment size. Indeed, with a specific PCR amplification (high amount of your target DNA fragment and no other band visible on the agarose gel) and a fragment size between 300 and 1000 bp, you can readily proceed to ligation after a simple purification of your PCR product by dialysis (see point 6a. below) or by using a column for the removal of left-over primers (see point 6b below). On the other hand, a gel purification (see point 6c below) is highly recommended for DNA fragments >1000 bp or after a PCR amplification that produced non-specific bands in addition to the target amplicon.

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a) *Dialysis*. Dialyse at least 20 µl of your PCR product against sterile water using a filter with a 0.025µm pore size: add sterile water in a Petri dish and carefully place the filter on the water surface; delicately put the PCR product on the filter; wait 10 min; pipet back the PCR product and use it for ligation.

b) *Removal of the PCR primers.* Several kits are available to remove left-over primers from PCR products. Perform this purification according to the manufacturer's recommendations. Elute your DNA using a small volume (30-50 µl) of a solution **without** EDTA (because EDTA inhibits the ligase).

c) *Gel purification*. Several kits are available to purify DNA fragments after agarose gel electrophoresis (generally, the band corresponding to the target PCR product is cut out of an agarose gel and the DNA is eluted from the excised small block of agarose). Perform this purification according to the manufacturer's recommendations. Use 20 to 40 µl of the PCR product to load the gel. Excise the target band from the agarose gel with a clean, sharp scalpel. Minimize the size of the gel slice containing your DNA fragment and transfer it to a sterile tube. Perform the DNA visualization on the gel and the excision steps quickly to avoid degradation of your DNA fragment by UV light. Use sterile plastic ware and gloves to minimize the presence of nucleases.

Protocol for performing the control PCR:

1. Set up the following PCR reaction mix to amplify a 537 bps fragment of the ampicillin-resistance gene:

Control primer mix (orange cap)	4µl
DNA template: 0.1 µg of DNA of any vector resistant to ampicillin or 0.1 µg of DNA of the *pSTC1.3* vector provided with the kit (yellow cap).	1µl
Polymerase buffer (according to the manufacturer's instructions)	
Polymerase (see manufacturer's instructions)	
dNTPs (100 mM)	1µl
Sterile water to reach a total volume of 50µl	
TOTAL	50µl

   Follow the manufacturer's instructions and recommendations for amplification of a 537 bps fragment. Set up the annealing temperature to 55°C.

2. Check the result of the PCR amplification by agarose gel electrophoresis.

3. Purify the control PCR product (537bp) with the same method you use for the target (non-control) DNA fragment.

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Step 2. Ligation of your DNA insert in the *pSTC1.3* vector:

At this point, you should have your purified PCR product (and the control PCR product) ready for cloning into the vector.

Protocol:

1. Set a water bath or a heating-bloc to 65°C.

2. Estimate the DNA concentration of the PCR product on an agarose gel or, preferably, determine DNA concentration using a spectrophotometer.

3. Set up the following reaction in a sterile microcentrifuge tube. Remember to also set up a reaction for the control PCR product. We recommend to fully thaw (and mix) the ligase buffer. After the first use, it is preferable to store the ligase and its buffer at -20°C. During the manipulations, we recommend to keep the ligase at -20°C using a cold block.

pSTC1.3 vector supplied with the kit (200ng, ~0.11pmole, yellow cap)	2 µl
Quantity of PCR product to use (in ng) = size of the product in bp x 0.23 (this will correspond to ~0.33 pmole of PCR product)	X µl
Ligase buffer (blue cap)	2 µl
T4 DNA ligase (gray cap)	2 µl
H_20 to reach a total volume of 20µl	Y µl
TOTAl	20 µl

4. Mix gently and incubate for 50 minutes at room temperature.
   Incubations of several hours (or overnight) do not decrease efficiency.

5. Inactivate the T4 DNA ligase by incubating the tube at 65°C for 8 minutes.
   After inactivation of the ligase, the cloning reaction may be stored on ice or frozen at -20°C for several days. After storage at -20°C, you might observe a decrease in the total number of clones but the cloning efficiency should remain high.

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Step 3. Transformation into the CYS21 *E. coli* cells and immediate plating of the bacteria:

Selection of the recombinants is performed in the *CYS21 E. coli* strain. This strain contains the *ccdB* gene in its chromosome. Two different types of StabyCloning™ kits are available: one containing electrocompetent cells, and the other type containing chemically-competent cells. Electroporation is more efficient that chemical transformation.

Protocol:

a) Transformation by electroporation:

1. Prepare LB plates containing 100 µg/ml Ampicillin. Let the plates dry and then warm them up at 37°C.
2. Set up your electroporator for bacterial transformation. Use the manufacturer's instructions. Classically, electroporation conditions are: 2,5 kV, 25 µF, and 200 Ohms.
3. Thaw (bring to room temperature) one vial of regeneration medium (white cap) per reaction.
4. 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.
5. Add 1 or 2 µl of the ligation product to one 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 filter with a 0.025µm pore size. 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.
6. Transfer all the content of the tube (cells+DNA) to the pre-chilled electroporation cuvette.
7. Electroporate the cells according to the manufacturer's instructions.
   If you experience electric arcing during electroporation, try again with a dialyzed ligation or reduce the voltage (10 to 20% less, 2kV instead of 2.5kV for example).
8. Quickly add 1ml of the regeneration medium (white cap) at room temperature and mix well.
9. Spread immediately 10, 20 and 100 µl of the product of step 8 on the pre-warmed plates.
10. Incubate the plates overnight at 37°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.

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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.

Analysis of the clones:

Protocol

1. Pick about 10 colonies and culture them overnight in 10 ml of LB medium with or without ampicillin (100 µg/ml).

Note: The stabilization is now effective; the ccdA gene is activated. Consequently, the plasmid is stabilized in the CYS21 strain and no antibiotic is required to select bacteria containing the plasmid. The stabilization system will insure high yield of plasmid DNA. However, the ampicillin-resistance is still available if you want to use it.

2. Extract plasmid DNA and analyze the constructions using your method of choice (restriction, sequencing, …). Consult the literature to find the best adapted method (see Ausubel et al., 1995 or Sambrook et al., 1989). Sequencing primers (*StabyCloning forward* and *StabyCloning reverse* primers) are included in the kit (0.1 µg/µl). The complete sequence of the *pSTC1* vector is available on our website: http://www.delphigenetics.com.

   Notes:

   √ *Ligation of the PCR fragment will create a BamHI restriction site at the linearization site (5'-end of the insert) by the introduction of the 14 bp (see figure 5 above). Another BamHI is available in the vector backbone at the opposite end of the insert. Consequently, you can easily remove from the vector, or check the presence of, the cloned fragment by a single BamHI restriction (please, first check for the absence of BamHI restriction sites in your insert).*

   √ *It is also easy to export the stabilization cassette (antidote gene) together with your insert using one of the restriction sites in 5' of the antidote gene (SphI to PacI, see figure 4 above) and one of the restriction sites in 3' of your insert (HindIII to NsiI, see figure 4 above). For example, an EcoRI site is present on both sides of the insert. It is then possible to select the entry of this excised fragment (stabilization cassette+insert) into another vector using the CYS21 strain and the antidote properties.*

3. *Optional:* For long-term storage of a recombinant clone, choose the culture of one clone containing the desired construct. Mix well 800 µl of the liquid culture with 800µl of sterile glycerol and transfer to a cryo-vial. Store at -70°C.

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Troubleshooting:

Please note that problems with cloning efficiency can result from the following parameters. Most of these problems can be fixed as explained in the table below. However, due to intrinsic and specific properties of your DNA fragment, the number of recovered recombinants may vary.

Problem	Solution
No or Low amount of PCR product before cloning.	Check the sequence of your primers and the PCR conditions
Multiple amplification bands observed in the PCR product	√ Either change the PCR conditions (annealing temperature, salt concentration) for increasing specificity or gel-purify (step 1,point 6c) the amplified DNA of the expected size before cloning into the vector. √ Use purified primers. *Remark: cloning of non-purified PCR product will result of colonies containing non expected recombinants. The cloning efficiency is always much higher for small fragments. Consequently, if you have several amplification bands in the PCR product, the smaller ones will be preferentially cloned.*
Arcing during the electroporation step	Check your electroporation conditions. Classical conditions for bacterial electroporation are: 25µF, 2.5kV, 200 Ohms. Electroporate only 1 or 2 µl of your ligation mix. If you want to use more DNA or if you still experience arcing, dialyze your DNA sample using a 0.025µm filter and sterile water (cf. step 3, point 5).
Only a few or no colonies are observed after transformation of the ligation mix into the *CYS21* strain.	√ Check the sequence of your PCR primers; be sure that the 14-bp tail is included at the 5'-end of one of your two primers. Preferentially use purified primers. √ Check the DNA concentration of your insert. Be sure to use the adequate DNA quantity mentioned in the manual. √ Check the quality of your insert (one single band must be visible after agarose gel electrophoresis of the purified DNA fragment). √ During the DNA purification step, perform the visualization of the gel and the excision steps quickly to avoid degradation of your DNA fragment by UV light. √ Be sure that the DNA transformation was optimal. When using electrocompetent bacteria, check the electroporation conditions (see above). When using chemically competent bacteria: check the temperature of the water bath, incubate the transformation product (from step 9) during one hour at 37°C to allow regeneration of the bacteria before spreading. √ Check your plates with another strain which is resistant to the ampicillin antibiotic. If no growth is observed, check your antibiotic solution. √ Your cloned fragment could be toxic for the bacteria. Check the literature. Try to clone the insert in the opposite orientation (with the opposite primer containing the 14-bp tail).

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	√ Use a proof-reading polymerase to avoid addition of A nucleotides to the 3' ends of the PCR product. *Remark: You can check the cloning and electroporation efficiencies by using the positive control (PCR primers for amplification of the control DNA fragment are included in the kit).*
The colonies contain insert but not the expected fragment	√ Check the quality of your insert (visualize by agarose gel electrophoresis the size and quantity of the purified DNA fragment). √ Purify your PCR product on gel to remove small non-specific amplifications. √ Your cloned fragment could be toxic for the bacteria. Check the literature. Try to clone the insert in the opposite orientation (with the opposite primer containing the 14 bp tail). *Remark: cloning of a non-purified or not completely purified PCR product can yield colonies containing non expected recombinants. Cloning efficiency is always much higher for small fragments. Consequently, the smaller (unspecific) PCR fragments will be preferentially cloned (even if they are not visible on agarose gel). Higher cloning efficiency is reached using target PCR products purified on gel.*
No restriction of the vector after cloning	Check the expiration date of the enzymes used. If you are using two different enzymes, check the buffer compatibility with these enzymes. Test this compatibility by restriction in the same conditions but with the separate enzymes.

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.
- 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.

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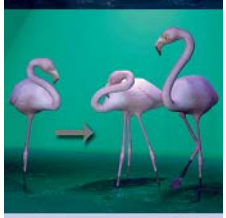
Related Staby™ products and services:

The **StabyCloning™ kit** is designed for the rapid, precise and efficient DNA cloning of PCR products. The complete cloning procedure is performed in one hour (including plating), the background is basically nil (the bacteria containing vectors without insert are killed), the PCR product is oriented, the plasmid is stabilized, and the export of the insert to another vector is easily selected.

The **StabyExpress™ T7 kit** contains all the key elements for cloning of a gene-of-interest and its expression in *Escherichia coli*. The kit combines two technologies (T7 expression and plasmid stabilization) that allow high-yield protein expression and standardization of the production-protocol.



The **GetStaby™ kit** allows easy addition of Delphi-Genetics' stabilization technology into your favourite vector. The technology is compatible with any expression system. Using this technology, your vectors are perfectly stabilized even without antibiotics.

The **Staby™Codon T7 kit** combines three technologies to ensure high-yield and standardized expression of eukaryote proteins in *Escherichia coli*. These technologies are *(i)* T7-controled expression, *(ii)* plasmid stabilization, and *(iii)* codon-usage adaptation of *E. coli* for the efficient expression of proteins that contain rare codons.

The **Cherry™Express kit** allows direct visualization (by eye!) of your protein of interest during protein production in *E. coli* and protein purification. Special requirements or reagents are not needed. It is also possible to quantify the protein concentration at any step by spectral measurement. The Cherry™Express kit combines multiple advantages: protein visualization, T7 expression, plasmid stabilization and codon-usage adaptation.

The **Staby™Switch** medium is an auto-inducible medium (ready-to-use) designed for high-level protein expression using Staby™ products or any other IPTG-inducible bacterial expression system. Using Staby™Switch medium, protein expression is automatically induced when high cell density is reached. Thus, it is neither necessary to add IPTG nor to monitor optical density during bacterial growth.

Staby™ Soft was specifically designed by Delphi Genetics to support the users of the Staby™ Operating System. This software package can perform customized gene-of-interest analysis to choose the most adapted kit and to optimize protein production.

For more information, please, consult www.delphigenetics.com

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Staby™ products ordering information:

StabyExpress™		
GE-SET7-0505	StabyExpress T7 expression kit, electro-competent cells	5 reactions
GE-SET7-0707	StabyExpress T7 expression kit, chemically-competent cells cells	5 reactions
GE-SET7-1010	StabyExpress T7 expression kit, electro-competent cells	10 reactions
GE-SET7-1212	StabyExpress T7 expression kit, chemically-competent cells	10 reactions
GE-SET7-1111	Set of 10 cloning bacteria (CYS21) and 10 expression bacteria (SE1), electro-competent cells	10 reactions
GE-SET7-1313	Set of 10 cloning bacteria (CYS21) and 10 expression bacteria (SE1), chemically-competent cells	10 reactions
GE-SET7-2020	StabyExpress T7 expression kit, electro-competent cells	20 reactions
GE-SET7-2222	StabyExpress T7 expression kit, chemically-competent cells	20 reactions
GE-SET7-0020	Set of 20 expression bacteria (SE1), electro-competent cells, 50µl/tube	20 reactions
GE-SET7-0022	Set of 20 expression bacteria (SE1), chemically-competent cells, 100µl/tube	20 reactions
GetStaby™		
GE-GSA1-10	GetStaby kit, electro-competent cells	10 reactions
GE-GSA1-12	GetStaby kit, chemically-competent cells	10 reactions
StabyCloning™		
GE-STC1-10	StabyCloning kit, electro-competent cells	10 reactions
GE-STC1-12	StabyCloning kit, chemically-competent cells	10 reactions
GE-STC1-20	StabyCloning kit, electro-competent cells	20 reactions
GE-STC1-22	StabyCloning kit, chemically-competent cells	20 reactions
GE-STCB-20	Set of 20 cloning bacteria (CYS21) electro-competent cells (50µl/tube)	20 reactions
GE-STCB-22	Set of 20 cloning bacteria (CYS21) chemically-competent cells (100µl/tube)	20 reactions
Staby™Codon		
GE-SCT7-0505	StabyCodon T7 expression kit, electro-competent cells	5 reactions
GE-SCT7-0707	StabyCodon T7 expression kit, chimio-competent cells	5 reactions
GE-SCT7-1010	StabyCodon T7 expression kit, electro-competent cells	10 reactions
GE-SCT7-1212	StabyCodon T7 expression kit, chimio-competent cells	10 reactions
Staby™Switch		
GE-AIME-04	Auto-induction medium	2L
Cherry™Express		
GE-CET7-05	CherryExpress T7 expression kit, electrocompetent	5 reactions
GE-CET7-07	CherryExpress T7 expression kit, chimio-competent cells	5 reactions
GE-CET7-10	CherryExpress T7 expression kit, electrocompetent	10 reactions
GE-CET7-12	CherryExpress T7 expression kit, chimio-competent cells	10 reactions
Cherry™Codon		
GE-CCT7-05	CherryCodon T7 expression kit, electrocompetent	5 reactions
GE-CCT7-07	CherryCodon T7 expression kit, chimio-competent	5 reactions
GE-CCT7-10	CherryCodon T7 expression kit, electrocompetent	10 reactions
GE-CCT7-12	CherryCodon T7 expression kit, chimio-competent	10 reactions

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