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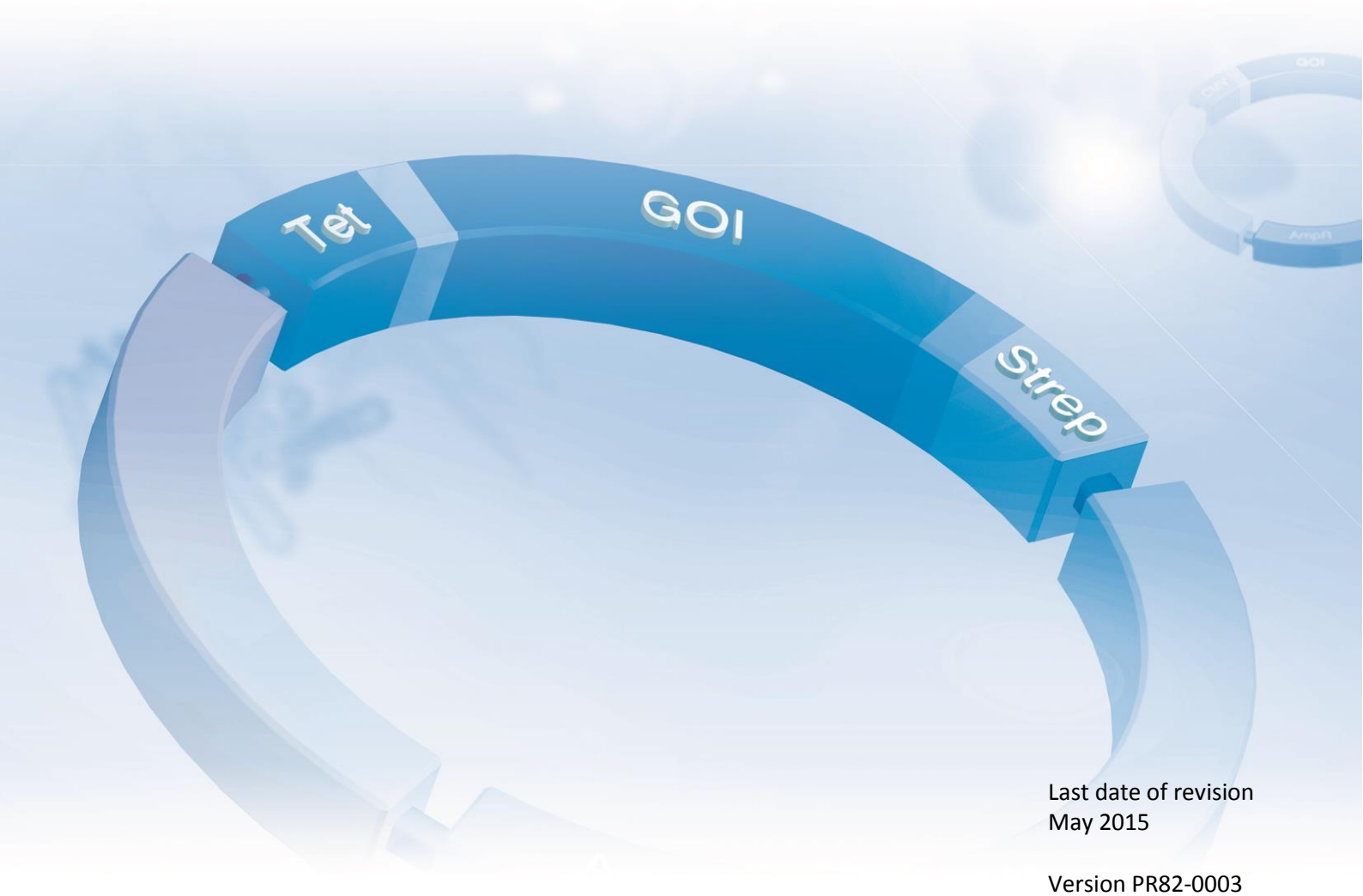
StarGate®
Direct transfer cloning

Instruction manual



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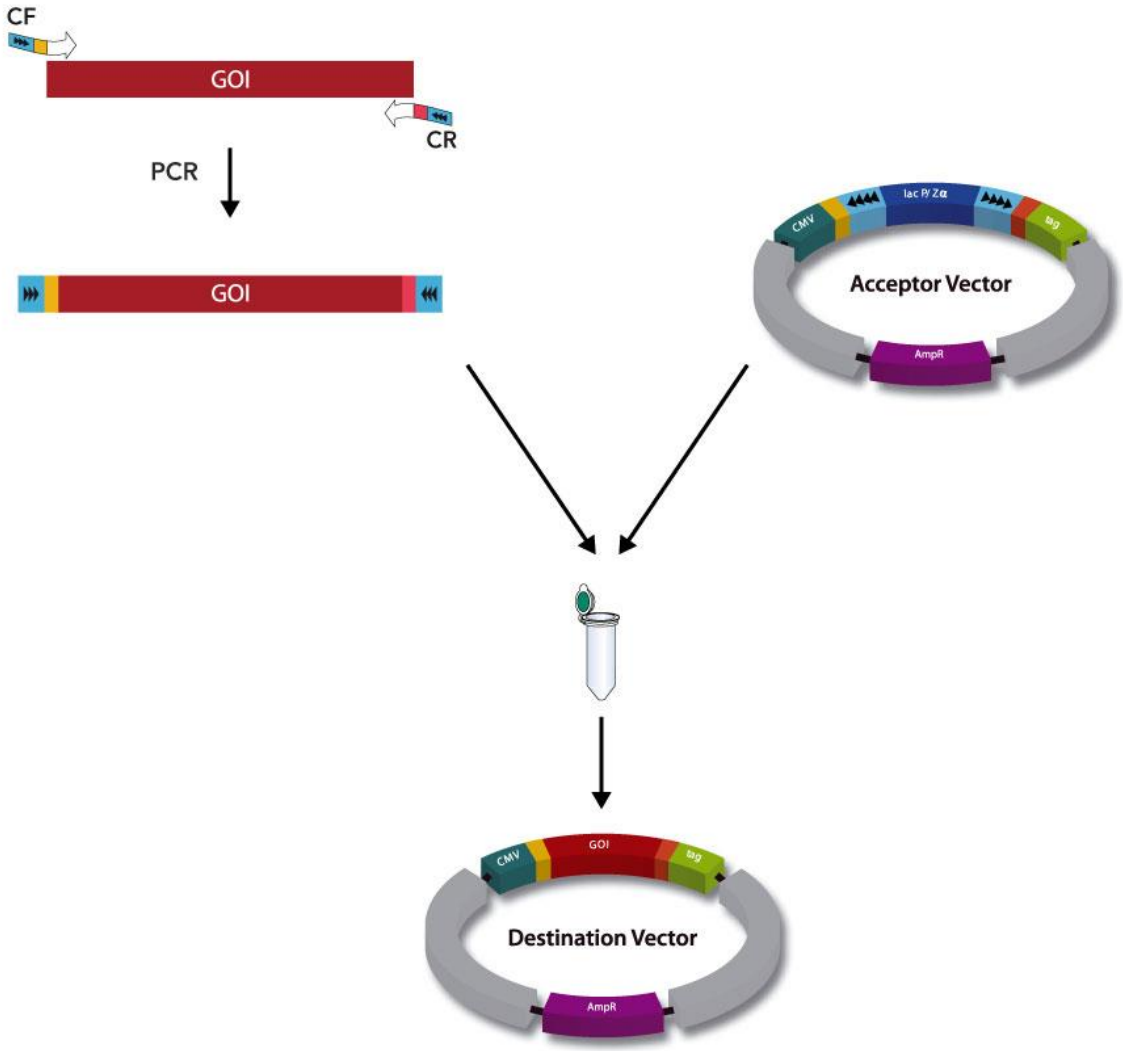
This manual can be downloaded under **www.iba-lifesciences.com/technical-support.html**.

1 Introduction

StarGate® has been developed for the rapid systematic screen of the optimal expression (regarding expression host and/or promoter) and purification (regarding the fusion tag) system for a given gene of interest (GOI). To this end, the GOI is cloned in a Donor Vector from which it can be subcloned by a standardized procedure into a variety of Acceptor Vectors providing the corresponding features without the need for sequencing each individual Acceptor Vector.

If, however, the optimal expression and purification system is already known a PCR product containing the gene of interest can be directly inserted into the appropriate Acceptor Vector without the need for prior generation of a Donor Vector. This “Direct Transfer Cloning” approach using appropriately designed PCR primers is described in this manual.

2 Direct Transfer Cloning Procedure



In a first step the PCR fragment is generated using the respective forward (CF) and reverse (CR) primer extending the gene of interest (GOI) with the corresponding integration sites. This PCR product is in a next step integrated into the appropriate Acceptor Vector (see page 14) resulting in the final expression construct, the so called Destination Vector. The formation of the correct Destination Vector is monitored via blue-white screening on LB-Agar plates containing X-Gal. The validated Destination Vector can be directly used for transformation or transfection of the expression host.

A list of available Acceptor Vectors is given on page 14 and on our homepage
http://www.iba-lifesciences.com/strep-tag-expression-vectors-technology.html

3 List of necessary components

Acceptor Vector (Expression vector)
Acceptor Vectors with respective Cat. No. are listed on page 14 *ff*. The vector is available as 20 µl aliquot [250 ng/µl].

Products from other suppliers
dNTP (containing dATP, dTTP, dGTP, dCTP)
Pfu DNA Polymerase and supplied buffer
T4 DNA Ligase [1 U/µl]
Esp3I [10 U/µl] restriction enzyme and supplied buffer (e.g. Thermo Scientific; The enzyme must be active at 37°C. Do not use the isoschizomer BsmBI.)

Complementary products	Cat.no
Competent cells *E. coli* TOP10 (20 rxns)	5-1600-020
Sequencing primers for *E. coli* vectors:	
Forward sequencing primer for pASG-IBA and pASK-IBA vectors (HPLC purified)	5-0000-101
Reverse sequencing primer for pASG-IBA and pASK-IBA vectors (HPLC purified)	5-0000-102
Forward and Reverse sequencing primers for pASG-IBA and pASK-IBA vectors (HPLC purified)	5-0000-104
Forward sequencing primer for pPSG-IBA and pPR-IBA vectors (HPLC purified)	5-0000-111
Reverse sequencing primer for pPSG-IBA and pPR-IBA vectors (HPLC purified)	5-0000-112
Forward and Reverse sequencing primers for pPSG-IBA and pPR-IBA vectors (HPLC purified)	5-0000-114
Sequencing primers for mammalian vectors	
Forward sequencing primer for pESG-IBA, pCSG-IBA, pDSG-IBA and pEXPR-IBA vectors (HPLC purified)	5-0000-121
Reverse sequencing primer for pESG-IBA, pCSG-IBA, pDSG-IBA and pEXPR-IBA vectors (HPLC purified)	5-0000-122
Forward and Reverse sequencing primers for pESG-IBA, pCSG-IBA, pDSG-IBA and pEXPR-IBA vectors (HPLC purified)	5-0000-124

Sequencing primers for yeast vectors	
Forward sequencing primer for pYSG-IBA vector (HPLC purified)	5-0000-141
Reverse sequencing primer for pYSG-IBA vector (HPLC purified)	5-0000-142
Forward and Reverse sequencing primers for pYSG-IBA vector (HPLC purified)	5-0000-144
Sequencing primers for insect cells	
Forward sequencing primer for pLSG-IBA vector (HPLC purified)	5-0000-161
Reverse sequencing primer for pLSG-IBA vector (HPLC purified)	5-0000-162
Forward and Reverse sequencing primers for pLSG-IBA vector (HPLC purified)	5-0000-164

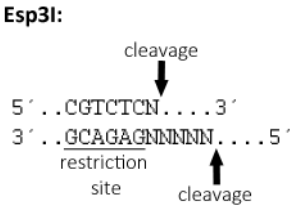
4 Direct Transfer Cloning Protocol and Recommendations

4.1 Amplification of GOI using PCR

4.1.1 Primer design

Important notes

- In case of using a proof reading polymerase, which is highly recommended (e.g., *Pfu)*, 3'-phosphorothioate protected primers should be used. Otherwise, proof reading activity may degrade the primers from the 3'-end during PCR thereby impairing annealing and consequently reduces the yield of PCR product.
- Initial hybridizing regions of primers (marked with | in the scheme below) should have a theoretical melting temperature between 60°C and 63°C. The primer melting temperatures can be derived by adding the single base melting temperatures of consecutive bases using 4°C for each GC pairing and 2°C for each AT pairing (and 1°C for each GT pairing).
- The integration site that needs to be attached by PCR to the 3'- and 5'-end of the GOI consists of an Esp3I recognition site. Esp3I is a type IIS restriction enzyme that cleaves the DNA in double strand outside the recognition site (4 bases, here NNNN (see scheme below)). Thereby the digestion with only one single enzyme can generate two different independent sticky ends with 4-base 5'-overhangs allowing directional cloning. In addition, after digestion reaction the recognition sequence is removed completely and therefore the encoded amino acid sequence is not affected by remaining restriction enzyme sites. Hence, even the expression of authentic proteins is possible.



- Example:
  If the subsequent sequence would represent a GOI (start and stop codon are left out)

```
5'-TTGACCTGCAACAGCTGCATAGCC-3'
3'-AACTGGACGTTGTCGACGTATCGG-5'
   LeuThrCysAsnSerCysIleAla
```

Important notes, continued

then the following primers have to be designed for PCR to equip GOI with the needed sites:

```
        (CF: forward primer)
5'-AGCGCGTCTCCAATGTTGACCTGCAACAGCTGCAT-3'
                  ||||||||||||||||||||
               5'-TTGACCTGCAACAGCTGCATAGCC-3'
               3'-AACTGGACGTTGTCGACGTATCGG-5'
                       ||||||||||||||||||||
                    3'-GACGTTGTCGACGTATCGGCCCTCCTCTGCGCGA-5'
                                   (CR: reverse primer)
```

5' end of the CF primer (forward primer) is elongated by the AATG combinatorial site (italic and bold) and the Esp3I recognition site (underlined) and
5' end of the CR primer (reverse primer) is elongated by the reverse complement (CCCT) of the downstream combinatorial site GGGA and again with the Esp3I recognition site (underlined).

The resulting PCR product then has the following structure:

```
5'-AGCGCGTCTCCAATGTTGACCTGCAACAGCTGCATAGCCGGGAGGAGACGCGCT-3'
3'-TCGCGCAGAGGTTACAACTGGACGTTGTCGACGTATCGGCCCTCCTCTGCGCGA-5'
               MetLeuThrCysAsnSerCysIleProLeu
```

4.1.2 PCR amplification of the GOI

Important notes

Essential parameters for optimization are annealing temperature, duration of synthesis and template concentration.

Protocol

1. **PCR-Reaction:**

   Mix the following reagents in a 500 µl reaction tube and in a total volume of 50 µl (based on standard protocols for *Pfu* Polymerase PCR)**:**

200 µM	dNTP (each)
0.1-0.5 µM	forward primer
0.1-0.5 µM	reverse primer
5 µl	10x buffer (supplier)
20-200 pg/µl (plasmid DNA) **0,1-1 ng/µl** (cDNA library)	Template DNA
2.5 U	*Pfu* DNA polymerase (depending on the recommendations of the manufacturer. *Pfu* can also be added after the initial denaturation step)
ad 50 µl	distilled H_2O

Protocol, continued

2. PCR-Program:

- Use a heated lid when available. Alternatively, overlay the sample with 50 µl mineral oil.
- For initial denaturation heat the sample at 94 °C for 3 min.
- Start temperature cycling:

94 °C	30 s	Denaturation	Use 15 - 20 cycles for plasmid DNA and 30-40 cycles for cDNA library
55 - 65 °C	30 s - 1 min	Annealing	
72 °C	30 s - 4 min	DNA synthesis	

- Perform a final 60-72 °C incubation step for 5 min in order to obtain full length products.
- Store samples at 4 °C until further analysis (e.g., agarose gel electrophoresis)

4.1.3 Purification of PCR product

Protocol

1. Purify PCR product to remove *Pfu* polymerase and primers.

If PCR reaction produced a single product of the expected size the product can be purified using a PCR purification kit (according to the instructions of the manufacturer).

If multiple bands are visible, it is recommended to isolate the PCR product by preparative gel electrophoresis.

2. Quantify PCR fragment

- by analytical agarose gel electrophoresis through band intensity comparison with a DNA Ruler or
- by NanoDrop measurement

4.2 GOI Transfer Reaction into the Acceptor Vector

Protocol

1. **Prepare a DTT/ATP-Mix: 250 mM DTT, 12.5 mM ATP**
   (The mix can be stored in small aliquots at -20 °C.)

2. **Mix the reagents below for insertion of the GOI into the chosen Acceptor Vector:**

7.5 µl	Acceptor Vector (5 ng; dilute in distilled water)
2.5 µl	Buffer supplied with Esp3I
12.5 µl	PCR product solution [2 nM]; (from section 4.1.2)
1 µl	DTT/ATP-Mix
1 µl	T4 DNA-Ligase [1 U/µl]
0.5 µl	Esp3I [10 U/µl]

3. **Close the reaction vessel thoroughly, mix gently and incubate at 30 °C for 1 h.**

4. **Thaw a vial of competent *E. coli* cells on ice.**

5. **After incubation, pipet off an aliquot of 10 µl from the reaction mixture (25 µl) from step 2 and add it to the thawed competent *E. coli* cells.**
   Continue incubation of the residual reaction mixture (15 µl) in the refrigerator at 2-8 °C for backup purposes.

6. **Mix gently (do not vortex) and incubate for 30 min on ice.**

7. **Mix gently (do not vortex) and incubate for 5 min at 37 °C.**

8. **Mix gently (do not vortex) and incubate 2-5 min on ice.**

9. **Plate 10 µl (mixed with 90 µl LB medium) and 100 µl on LB agar containing 100 mg/l ampicillin and 50 mg/l X-gal.**

10. **Incubate plates over night at 37 °C (upside down).**

4.3 Destination Vector Identification

Protocol	1. Pick 3 white colonies and perform DNA mini preparation. 2. pASG-IBA, pPSG-IBA, pESG-IBA, pCSG-IBA, pDSG-IBA and pYSG-IBA have *Xba*I/*Hin*dIII restriction sites that flank the expression cassette and, therefore, may be used for confirmation of GOI integration. 3. Due to an additional *Hin*dIII site downstream to GOI, an additional fragment of 456 bp will be generated after *Xba*I/*Hin*dIII cleavage of pLSG-IBA vectors. 4. For exact calculation of expected restriction fragment length please refer to the appropriate Acceptor Vector data sheet. 5. Sequencing step for further validation In order to validate the correct sequence of your GOI you can use the sequencing primers specific for the Acceptor Vector listed on page 7/8. Now you are ready to transfect or to transform your host of choice with the respective Destination Vector.

5 Acceptor Vector Collection and Description

5.1 Overview

Expression Vector (Acceptor Vector)

Host	*E.coli*			Mammalia			Yeast	Baculo	Name	Secretion	N-term	GOI	C-term	Resistance
Promoter	tet	T7	CMV	CMV	CMV		CUP1	Polyhedrin						
Vector	pASG	pPSG	pDSG (MEXi)	pCSG	pESG		pYSG	pLSG						
Cat.no.	5-4000-001	5-4200-001	5-5200-001	5-5000-001	5-4400-001		5-4600-001	5-4800-001	IBAwt1	no				Amp
	5-4005-001	5-4205-001		5-5005-001	5-4405-001		5-4605-001	5-4805-001	IBA5	no				Amp
	5-4105-001	5-4305-001	5-5222-001	5-5105-001	5-4505-001		5-4705-001	5-4905-001	IBA105	no				Amp
	5-4035-001	5-4235-001		5-5035-001	5-4435-001		5-4635-001	5-4835-001	IBA35	no				Amp
	5-4025-001	5-4225-001		5-5025-001	5-4425-001		5-4625-001	5-4825-001	IBA25	no				Amp
	5-4003-001	5-4203-001		5-5003-001	5-4403-001		5-4603-001	5-4803-001	IBA3	no				Amp
	5-4103-001	5-4303-001	5-5220-001	5-5103-001	5-4503-001		5-4703-001	5-4903-001	IBA103	no				Amp
	5-4033-001	5-4233-001	5-5209-001	5-5033-001	5-4433-001		5-4633-001	5-4833-001	IBA33	no				Amp
	5-4045-001	5-4245-001	5-5214-001	5-5045-001	5-4445-001		5-4645-001	5-4845-001	IBA45	no				Amp
	5-4145-001	5-4345-001		5-5145-001	5-4545-001		5-4745-001	5-4945-001	IBA145	no				Amp
	5-4043-001	5-4243-001	5-5211-001	5-5043-001	5-4443-001		5-4643-001	5-4843-001	IBA43	no				Amp
	5-4143-001	5-4343-001		5-5143-001	5-4543-001		5-4743-001	5-4943-001	IBA143	no				Amp
	5-4023-001	5-4223-001		5-5023-001	5-4423-001		5-4623-001	5-4823-001	IBA23	no				Amp
	5-4123-001	5-4323-001		5-5123-001	5-4523-001		5-4723-001	5-4923-001	IBA123	no				Amp
	5-4065-001	5-4265-001		5-5065-001	5-4465-001		5-4665-001	5-4865-001	IBA65	no				Amp
	5-4063-001	5-4263-001		5-5063-001	5-4463-001		5-4663-001	5-4863-001	IBA63	no				Amp
	5-4062-001	5-4262-001	5-5220-001	5-5062-001	5-4462-001		5-4662-001	5-4862-001	IBA62	no				Amp
	5-4162-001	5-4362-001	5-5209-001	5-5162-001	5-4562-001		5-4762-001	5-4962-001	IBA162	no				Amp
	5-4064-001	5-4264-001	5-5214-001	5-5064-001	5-4464-001		5-4664-001	5-4864-001	IBA64	no				Amp
	5-4164-001	5-4364-001		5-5164-001	5-4564-001		5-4764-001	5-4964-001	IBA164	no				Amp
	5-4167-001	5-4367-001		5-5167-001	5-4567-001		5-4767-001	5-4967-001	IBA167	no				Amp
	5-4168-001	5-4368-001		5-5168-001	5-4568-001		5-4768-001	5-4968-001	IBA168	no				Amp
	5-4001-001		5-5201-001	5-5001-001	5-4401-001			5-4801-001	IBAwt2	yes				Amp
	5-4004-001								IBA4	yes				Amp
	5-4104-001		5-5221-001	5-5104-001	5-4504-001			5-4904-001	IBA104	yes				Amp
	5-4002-001								IBA2	yes				Amp
	5-4102-001		5-5219-001	5-5102-001	5-4502-001			5-4902-001	IBA102	yes				Amp
	5-4044-001								IBA44	yes				Amp
	5-4144-001			5-5144-001	5-4544-001			5-4944-001	IBA144	yes				Amp
				5-5142-001	5-4542-001			5-4942-001	IBA142	yes				Amp

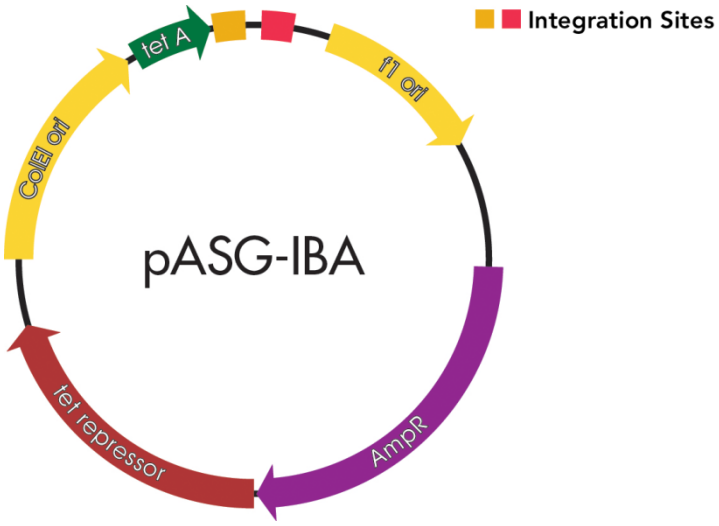
Secretion signal in *E. coli*: OmpA
Secretion signal in mammalia: BM40

Strep-tag®II
6xHis-tag

Twin-Strep-tag®
GST-tag
Flag-tag

Table 1: **IBA Expression Vector (Acceptor Vector) Overview.** A detailed overview of the current Acceptor Vectors is given here. The list provides information about the expression host, used promoter, available secretion signal and cloning site including N- or C-terminal tag as well as the cat.no of each expression vector. The vector name (e.g. pASG-IBA5) comprises the expression system (pASG = *E. coli*/tet) and the expression cassette (affinity-tag/position/secretion signal) IBA5 = *Strep*-tag®II/N-term/no secretion signal).

5.2 pASG-IBA



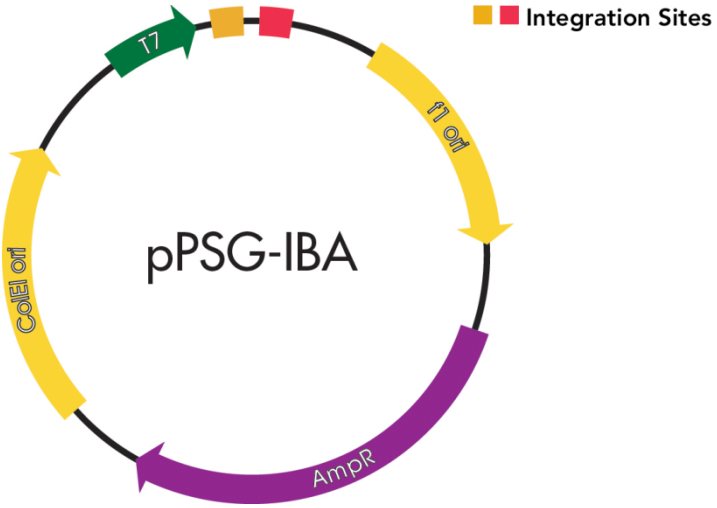
The production of a heterologous protein is often accompanied by an impaired growth of *E. coli* cells. Consequently, regulation of heterologous biosynthesis is generally recommended by the use of a promoter whose activity can be blocked by a repressor. If the foreign protein is cytotoxic, even the production of minute quantities can result in a dramatic selection against the *E. coli* cells which harbor the expression plasmid. In such cases, tight repression of the promoter is required. Synthesis of the gene product is then switched on in a controlled manner simply by adding a chemical inducer. pASG-IBA vectors which are similar to pASK-IBA vectors carry the promoter/operator region from the *tet*A resistance gene and are the optimal solution for such an inducible expression system (Skerra, 1994). The strength of the *tet*A promoter is comparable with that of the *lac*-UV5 promoter. Some vectors carry the ompA signal sequence for secretion of the recombinant protein into the periplasmic space which is crucial for functional expression of proteins with structural disulfide bonds.

The *tet* promoter can be fully induced by adding anhydrotetracycline at a concentration that is not antibiotically effective (200 ng/ml). The constitutive expression of the *tet* repressor gene, which is also encoded on the expression plasmids, guarantees the repression of the promoter in the absence of the inducer. In a Western blot, no expression is detectable under these conditions (Skerra, 1994). In contrast to the *lac* promoter, which is susceptible to catabolite repression (cAMP-level, metabolic state) and chromosomally encoded repressor molecules, the *tet*A promoter/operator is not coupled to any cellular regulation mechanisms. Therefore, when using the *tet* system, there are basically no restrictions in the choice of culture medium or *E. coli* expression strain. For example, glucose minimal media and even the bacterial strain XL1-Blue, which carries an episomal copy of the tetracycline resistance gene, can be used for expression.

Plasmid propagation is supported by a ColEI ori (pUC) and the ampicillin resistance gene.

Note: Please refer to the corresponding data sheet for detailed information on a given vector.

5.3 pPSG-IBA



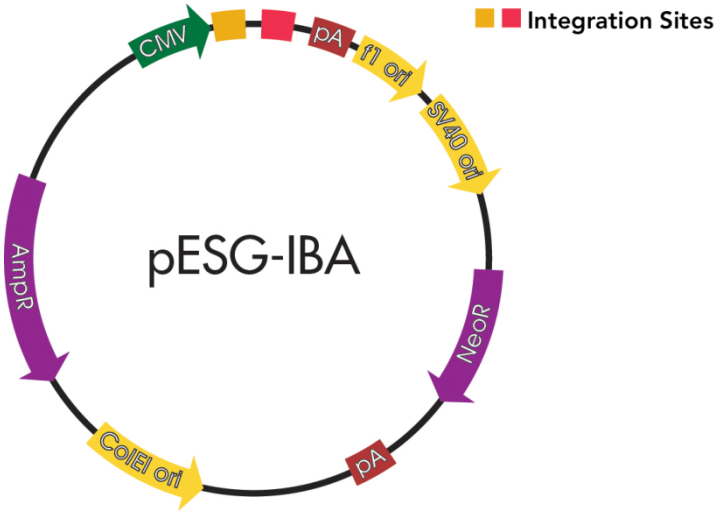
pPSG-IBA vectors which are similar to the former pPR-IBA vectors from IBA use the T7 promoter and T7 RNA polymerase for high-level transcription of the gene of interest (Studier *et al*., 1990). As the T7 promoter is stronger than the *tet* promoter, pPSG-IBA vectors are recommended in cases where expression with the *tet* promoter does not lead to significant yields of the recombinant protein. In other cases, strong T7 expression may cause insoluble inclusion bodies. In such cases the *tet* promoter might be a good alternative when expression of soluble protein is desired.

Expression of the target genes is induced by providing a source of T7 RNA polymerase in the *E. coli* host cell. This is accomplished by using, e.g., an *E. coli* host which contains a chromosomal copy of the T7 RNA polymerase gene (e.g., BL21 (DE3) which has the advantage to be deficient of *lon* and *ompT* proteases). The T7 RNA polymerase gene in BL21 (DE3) is under control of the lacUV5 promoter which can be induced by addition of IPTG.

Plasmid propagation is supported by a ColEI ori (pUC) and the ampicillin resistance gene.

Note: Please refer to the corresponding data sheet for detailed information on a given vector.

5.4 pESG-IBA

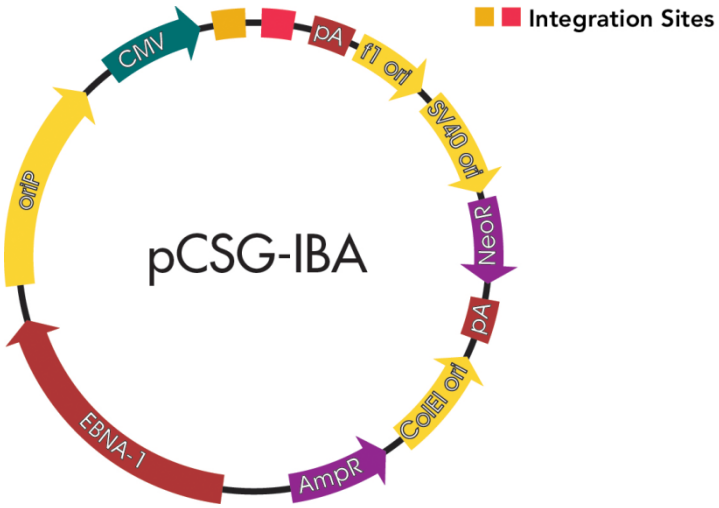


pESG-IBA vectors are designed for high-level constitutive expression of recombinant proteins in a wide range of mammalian host cells through the human cytomegalovirus immediate-early (CMV) promoter (Boshart *et al*., 1985; Nelson *et al*., 1987). To prolong expression in transfected cells, the vector will replicate in cell lines that are latently infected with SV40 large T antigen (e.g., COS1 or COS7). In addition, Neomycin resistance gene allows direct selection of stable cell lines.

Propagation in *E. coli* is supported by a ColEI ori and the ampicillin resistance gene. Some vectors carry the BM40 signal sequence for secretion of the recombinant protein into the medium.

Note: Please refer to the corresponding data sheet for detailed information on a given vector.

5.5 pCSG-IBA

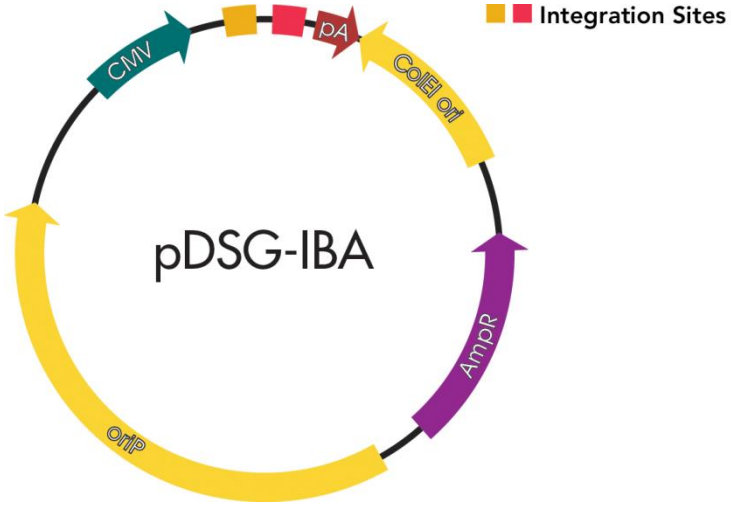


pCSG-IBA vectors are designed for high-level constitutive expression of recombinant proteins in a wide range of mammalian host cells through the human cytomegalovirus immediate-early (CMV) promoter (Boshart *et al*., 1985; Nelson *et al*., 1987). The Epstein Barr Virus replication origin (*oriP*) and nuclear antigen encoded by EBNA-1 provide extrachromosomal replication in human, primate and canine cells and the SV40 replication origin provides extrachromosomal replication in cell lines that express SV40 large T antigen (e.g., COS1 or COS7). Thus, by means of the NeoR marker, prolonged expression of the inserted GOI occurs in such cell lines under G418 selection without the need for making stable cell lines.

Propagation in *E. coli* is supported by a ColEI ori (pUC) and the ampicillin resistance gene. Finally, some vectors carry the BM40 signal sequence for secretion of the recombinant protein into the medium.

Note: Please refer to the corresponding data sheet for detailed information on a given vector.

5.6 pDSG-IBA



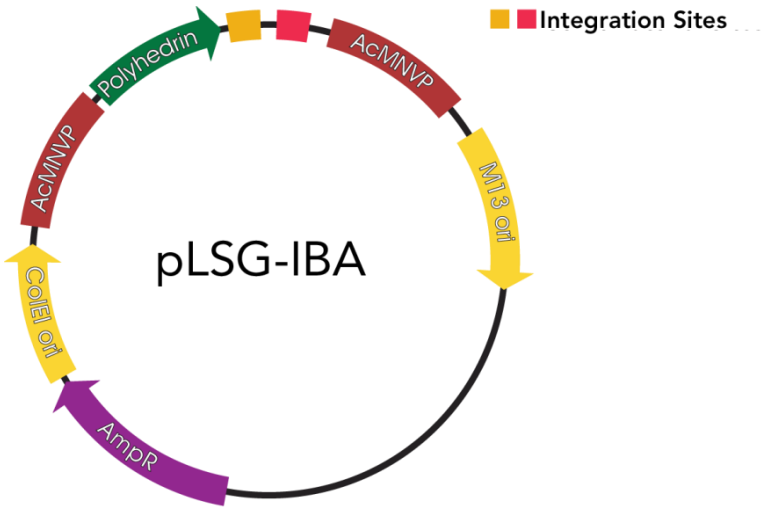
pDSG-IBA vectors are designed for high-level constitutive expression of recombinant proteins in a wide range of mammalian host cells through the human cytomegalovirus immediate-early (CMV) promoter (Boshart *et al*., 1985; Nelson *et al*., 1987). The Epstein Barr Virus replication origin (*oriP*) provides extrachromosomal replication in human, primate and canine cells which have the nuclear antigen encoded by EBNA-1 chromosomally expressed.

Propagation in *E. coli* is supported by a ColEI ori and the ampicillin resistance gene. Some vectors carry the BM40 signal sequence for secretion of the recombinant protein into the medium.

An advantage compared to pCSG is the small size of the vector.

Note: Please refer to the corresponding data sheet for detailed information on a given vector.

5.7 pLSG-IBA



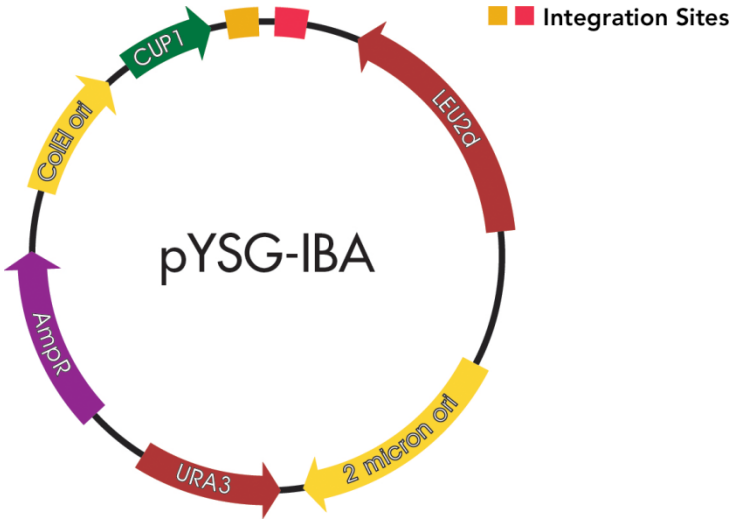
pLSG-IBA vectors are transfer vectors to introduce the GOI into the polyhedrin gene locus of AcMNPV DNA by homologous recombination. Co-transfection with BacPAK6 linearized AcMNPV DNA (Clontech) or circular *flash*BAC modified AcMNPV DNA (Oxford Expression Technologies) allows the generation of recombinant baculovirus at very high efficiency through reconstitution of an essential gene (ORF 1629) and elimination of wild type virus to great extent.

pLSG-IBA vectors provide the strong polyhedrin promoter for high level expression of an inserted GOI in insect cells.

Propagation in *E. coli* is supported by a ColEI ori (pUC) and the ampicillin resistance gene.

Note: Please refer to the corresponding data sheet for detailed information on a given vector.

5.8 pYSG-IBA



pYSG-IBA expression vectors are designed for high-level expression of recombinant proteins in yeast. Cloned genes are under the control of the Cu^{++}-inducible CUP1 promoter which means that expression is induced upon addition of copper sulfate. pYSG-IBA vectors favour correct protein folding and the production of soluble proteins — inclusion bodies rarely form.

In addition, all vectors include the yeast selectable markers leu2-d (a LEU2 gene with a truncated, but functional promoter) and URA3. Vectors including the leu2-d marker are maintained at high copy number to provide enough gene products from the inefficient promoter for cell survival during growth selection in minimal medium lacking leucine (Macreadie *et al*., 1991; Gietz & Sugino, 1989). For selection after transformation, the URA3 marker should be used instead of leu2-d to enable growth of transformants.

Optimal repression under non-inducing conditions is obtained with yeast strains carrying multiple $CUP1^r$ loci, while partially constitutive expression in strains lacking the $CUP1^r$ locus (ΔCUP1) is still enhanced upon the addition of copper through a trans-acting factor (Butt & Ecker, 1987).

Propagation in *E. coli* is supported by a ColEI ori (pUC) and the ampicillin resistance gene.

Note: Please refer to the corresponding data sheet for detailed information on a given vector.

6 Acceptor Vector Expression Cassettes

```
IBAwt1: AATG-GOI-GGGAGCTAA
         M  -POI-G  S  *

IBAwt2:  ATGAAAAAGACA-OMPA-GCGCAGGCCGCAATG-GOI-GGGAGCTAA
         M  K  K  T  -OMPA-A  Q  A  A  M  -POI-G  S  *
                                  ↑
IBA2:    ATGAAAAAGACA-OMPA-GCGCAGGCCGCAATG-GOI-GGGAGCGCTTGGAGCCACCCGCAGTTCGAAAAATAA
         M  K  K  T  -OMPA-A  Q  A  A  M  -POI-G  S  A  W  S  H  P  Q  F  E  K  *
                                  ↑
IBA3:   AATG-GOI-GGGAGCGCTTGGAGCCACCCGCAGTTCGAAAAATAA
         M  -POI-G  S  A  W  S  H  P  Q  F  E  K  *

IBA4:    ATGAAAAAGACA-OMPA-GCGCAGGCCGCAATGGCTAGCGCATGGAGTCATCCTCAATTCGAAAAATCCGGAATG-GOI-GGGAGCTAA
         M  K  K  T  -OMPA-A  Q  A  A  M  A  S  A  W  S  H  P  Q  F  E  K  S  G  M  -POI-G  S  *
                                  ↑
IBA5:    ATGGCTAGCGCATGGAGTCATCCTCAATTCGAAAAATCCGGAATG-GOI-GGGAGCTAA
         M  A  S  A  W  S  H  P  Q  F  E  K  S  G  M  -POI-G  S  *

IBA23:   ATGTCCCCTATA-GST-CCTCCAAAAATGTCCGGAGGTGGCGGTGGGAGCCTGGAAGTTCTGTTCCAGGGGCCAATG-GOI-GGGAGCGCTTGGAGCCACCCG
         M  S  P  I  -GST-P  P  K  M  S  G  G  G  G  G  S  L  E  V  L  F  Q  G  P  M  -POI-G  S  A  W  S  H  P
                                                                                   ↑
         CAGTTCGAAAAATAA
         Q  F  E  K  *

IBA25:   ATGTCCCCTATA-GST-CCTCCAAAAATGTCCGGAGGTGGCGGTGGGAGCCTGGAAGTTCTGTTCCAGGGGCCAATG-GOI-GGGAGCTAA
         M  S  P  I  -GST-P  P  K  M  S  G  G  G  G  G  S  L  E  V  L  F  Q  G  P  M  -POI-G  S  *
                                                                                   ↑
IBA33:  AATG-GOI-GGGAGCGCTCACCATCACCATCACCATTAA
         M  -POI-G  S  A  H  H  H  H  H  H  *

IBA35:   ATGGCTAGCCATCACCATCACCATCACTCCGGAATG-GOI-GGGAGCTAA
         M  A  S  H  H  H  H  H  H  S  G  M  -POI-G  S  *

IBA43:   ATGGCTAGCCATCACCATCACCATCACTCCGGAATG-GOI-GGGAGCGCTTGGAGCCACCCGCAGTTCGAAAAATAA
         M  A  S  H  H  H  H  H  H  S  G  M  -POI-G  S  A  W  S  H  P  Q  F  E  K  *
```

```
IBA44:   ATGAAAAAGACA-OMPA-GCGCAGGCCGCAATGGCTAGCGCATGGAGTCATCCTCAATTCGAAAAATCCGGAATG-GOI-GGGAGCGCTCACCATCACCAT
         M  K  K  T  -OMPA-A  Q  A  A  M  A  S  A  W  S  H  P  Q  F  E  K  S  G  M  -POI-G  S  A  H  H  H  H
                                   ↑
         CACCATTAA
         H  H  *

IBA45:   ATGGCTAGCGCATGGAGTCATCCTCAATTCGAAAAATCCGGAATG-GOI-GGGAGCGCTCACCATCACCATCACCATTAA
         M  A  S  A  W  S  H  P  Q  F  E  K  S  G  M  -POI-G  S  A  H  H  H  H  H  H  *

IBA62:   ATGGCTAGCGATTACAAGGATGACGACGATAAGTCCGGAATG-GOI-GGGAGCGCTTGGAGCCACCCGCAGTTCGAAAAATAA
         M..A  S  D  Y  K  D  D  D  D  K  S  G  M  -POI-G  S  A  W  S  H  P  Q  F  E  K  *

IBA63:  AATG-GOI-GGGAGCGCTGATTACAAGGATGACGACGATAAGTAA
         M  -POI-G  S  A  D  Y  K  D  D  D  D  K  *

IBA64:   ATGGCTAGCGCATGGAGTCATCCTCAATTCGAAAAATCCGGAATG-GOI-GGGAGCGCTGATTACAAGGATGACGACGATAAGTAA
         M  A  S  A  W  S  H  P  Q  F  E  K  S  G  M  -POI-G  S  A  D  Y  K  D  D  D  D  K  *

IBA65:   ATGGCTAGCGATTACAAGGATGACGACGATAAGTCCGGAATG-GOI-GGGAGCTAA
         M  A  S  D  Y  K  D  D  D  D  K  S  G  M  -POI-G  S  *

IBA102:  ATGAAAAAGACA-OMPA-GCGCAGGCCGCAATG-GOI-GGGAGCGCTTGGAGCCACCCGCAGTTCGAAAAAGGTGGAGGTTCTGGCGGTGGATCGGGAGGT
         M  K  K  T  -OMPA-A  Q  A  A  M  -POI-G  S  A  W  S  H  P  Q  F  E  K  G  G  G  S  G  G  G  S  G  G
                                   ↑
         TCAGCGTGGAGCCACCCGCAGTTCGAGAAATAA
         S  A  W  S  H  P  Q  F  E  K  *

IBA103: AATG-GOI-GGGAGCGCTTGGAGCCACCCGCAGTTCGAAAAAGGTGGAGGTTCTGGCGGTGGATCGGGAGGTTCAGCGTGGAGCCACCCGCAGTTCGAGAAATAA
         M  -POI-G  S  A  W  S  H  P  Q  F  E  K  G  G  G  S  G  G  G  S  G  G  S  A  W  S  H  P  Q  F  E  K  *

IBA104:  ATGAAAAAGACA-OMPA-GCGCAGGCCGCAATGGCTAGCGCATGGAGTCATCCTCAATTCGAGAAAGGTGGAGGTTCTGGCGGTGGATCGGGAGGTTCAGCG
         M  K  K  T  -OMPA-A  Q  A  A  M  A  S  A  W  S  H  P  Q  F  E  K  G  G  G  S  G  G  G  S  G  G  S  A
                                   ↑
         TGGAGCCACCCGCAGTTCGAAAAATCCGGAATG-GOI-GGGAGCTAA
         W  S  H  P  Q  F  E  K  S  G  M  -POI-G  S  *

IBA105:  ATGGCTAGCGCATGGAGTCATCCTCAATTCGAGAAAGGTGGAGGTTCTGGCGGTGGATCGGGAGGTTCAGCGTGGAGCCACCCGCAGTTCGAA
         M  A  S  A  W  S  H  P  Q  F  E  K  G  G  G  S  G  G  G  S  G  G  S  A  W  S  H  P  Q  F  E

         AAATCCGGAATG-GOI-GGGAGCTAA
         K  S  G  M  -POI-G  S  *
```

```
IBA123:  ATGTCCCCTATA-GST-CCTCCAAAAATGTCCGGAGGTGGCGGTGGGAGCCTGGAAGTTCTGTTCCAGGGGCCAATG-GOI-GGGAGCGCTTGGAGCCACCCGCAG
         M  S  P  I  -GST-P  P  K  M  S  G  G  G  G  G  S  L  E  V  L  F  Q  G  P  M  -POI-G  S  A  W  S  H  P  Q
                                                                                       ↑
         TTCGAAAAAGGTGGAGGTTCTGGCGGTGGATCGGGAGGTTCAGCGTGGAGCCACCCGCAGTTCGAGAAATAA
         F  E  K  G  G  G  S  G  G  G  S  G  G  S  A  W  S  H  P  Q  F  E  K  *

IBA142:  ATGAGGGCCTGG-BM40-GCTCTGGCAGCAATGGCTAGCCATCACCATCACCATCACTCCGGAATG-GOI-GGGAGCGCTTGGAGCCACCCGCAGTTCGAAAAA
         M  R  A  W  -BM40-A  L  A  A  M  A  S  H  H  H  H  H  H  S  G  M  -POI-G  S  A  W  S  H  P  Q  F  E  K
                                    ↑
         GGTGGAGGTTCTGGCGGTGGATCGGGAGGTTCAGCGTGGAGCCACCCGCAGTTCGAGAAATAA
         G  G  G  S  G  G  G  S  G  G  S  A  W  S  H  P  Q  F  E  K  *

IBA143:  ATGGCTAGCCATCACCATCACCATCACTCCGGAATG-GOI-GGGAGCGCTTGGAGCCACCCGCAGTTCGAAAAAGGTGGAGGTTCTGGCGGTGGATCGGGAGGT
         M  A  S  H  H  H  H  H  H  S  G  M  -POI-G  S  A  W  S  H  P  Q  F  E  K  G  G  G  S  G  G  G  S  G  G
         TCAGCGTGGAGCCACCCGCAGTTCGAGAAATAA
         S  A  W  S  H  P  Q  F  E  K  *

IBA144:  ATGAAAAAGACA-OMPA-GCGCAGGCCGCAATGGCTAGCGCATGGAGTCATCCTCAATTCGAGAAAGGTGGAGGTTCTGGCGGTGGATCGGGAGGTTCAGCG
         M  K  K  T  -OMPA-A  Q  A  A  M  A  S  A  W  S  H  P  Q  F  E  K  G  G  G  S  G  G  G  S  G  G  S  A
                                    ↑
         TGGAGCCACCCGCAGTTCGAAAAATCCGGAATG-GOI-GGGAGCGCTCACCATCACCATCACCATTAA
         W  S  H  P  Q  F  E  K  S  G  M  -POI-G  S  A  H  H  H  H  H  H  *

IBA145:  ATGGCTAGCGCATGGAGTCATCCTCAATTCGAGAAAGGTGGAGGTTCTGGCGGTGGATCGGGAGGTTCAGCGTGGAGCCACCCGCAGTTCGAA
         M  A  S  A  W  S  H  P  Q  F  E  K  G  G  G  S  G  G  G  S  G  G  S  A  W  S  H  P  Q  F  E
         AAATCCGGAATG-GOI-GGGAGCGCTCACCATCACCATCACCATTAA
         K  S  G  M  -POI-G  S  A  H  H  H  H  H  H  *

IBA162:  ATGGCTAGCGATTACAAGGATGACGACGATAAGTCCGGAATG-GOI-GGGAGCGCTTGGAGCCACCCGCAGTTCGAAAAAGGTGGAGGTTCTGGCGGTGGATCG
         M  A  S  D  Y  K  D  D  D  D  K  S  G  M  -POI-G  S  A  W  S  H  P  Q  F  E  K  G  G  G  S  G  G  G  S
         GGAGGTTCAGCGTGGAGCCACCCGCAGTTCGAGAAATAA
         G  G  S  A  W  S  H  P  Q  F  E  K  *

IBA164:  ATGGCTAGCGCATGGAGTCATCCTCAATTCGAGAAAGGTGGAGGTTCTGGCGGTGGATCGGGAGGTTCAGCGTGGAGCCACCCGCAGTTCGAAAAATCCGGAATG-GOI-
         M  A  S  A  W  S  H  P  Q  F  E  K  G  G  G  S  G  G  G  S  G  G  S  A  W  S  H  P  Q  F  E  K  S  G  M  -POI-
         GGGAGCGCTGATTACAAGGATGACGACGATAAGTAA
         G  S  A  D  Y  K  D  D  D  D  K  *
```

```
IBA167:  ATGGCTAGCGATTACAAGGATGACGACGATAAGGGTGCATCTAGCGCATGGAGTCATCCTCAATTCGAGAAAGGTGGAGGTTCTGGCGGTGGATCGGGAGGTTCAGCG
         M  A  S  D  Y  K  D  D  D  D  K  G  A  S  S  A  W  S  H  P  Q  F  E  K  G  G  G  S  G  G  G  S  G  G  S  A

         TGGAGCCACCCGCAGTTCGAAAAATCCGGAATG-GOI-GGGAGCTAA
         W  S  H  P  Q  f  E  K  S  G  M  -POI-G  S  *

IBA168: AATG-GOI-GGGAGCGCTTGGAGCCACCCGCAGTTCGAAAAAGGTGGAGGTTCTGGCGGTGGATCGGGAGGTTCAGCGTGGAGCCACCCGCAGTTCGAGAAAGGT
         M  -POI-G  S  A  W  S  H  P  Q  F  E  K  G  G  G  S  G  G  G  S  G  G  S  A  W  S  H  P  Q  F  E  K  G

         GCTTCTGGTGAAGATTACAAGGATGACGACGATAAGTAA
         A  S  G  E  D  Y  K  D  D  D  D  K  *
```

7 References

For up-to-date references see www.iba-lifesciences.com/StarGate_Cloning.html

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