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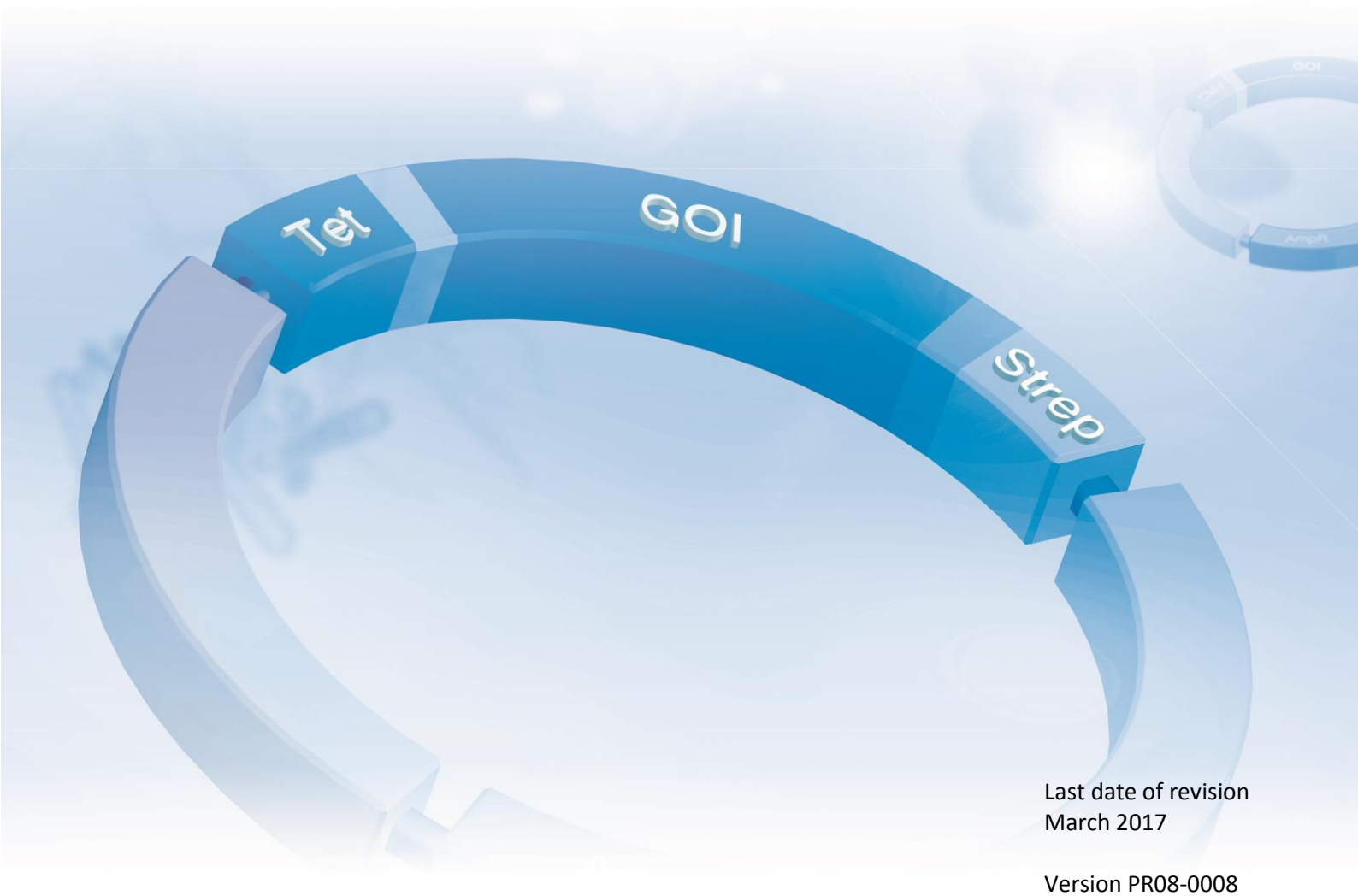
For Life Sciences

Classic cloning with pASK-IBA and pEXPR-IBA vectors

General protocol



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Content

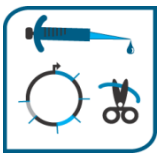
This manual can be downloaded under **www.iba-lifesciences.com/technical-support.html**.

1 Introduction – Cloning of an arbitrary gene into pASK-IBA and pEXPR-IBA expression vectors

The multiple cloning sites of pASK-IBA and pEXPR-IBA vectors include many standard unique restriction sites like *Eco*RI or *Bam*HI for the introduction of foreign genes after PCR. However, the reading frame of the corresponding vector has to be considered if such restriction sites are used. In some vectors with N-terminal *Strep*-tag II, *Strep*-tag II is followed by the linker sequence 5'-GGCGCC. This sequence is recognized by three different restriction enzymes generating 5'-overhangs. Cleavage with the suitable enzyme and, if necessary, a subsequent filling reaction makes the production of blunt ends possible in all reading frames. Using standard restriction sites, additional polylinker derived amino acids are appended at the respective end of the recombinant protein. To avoid the fusion of such polylinker derived amino acids pASK-IBA and pEXPR-IBA vectors offer a general cloning strategy via Type IIS restriction enzymes, *Bsa*I or *Eco*31I (NEB, MBI Fermentas). They allow the precise fusion of the structural gene with the vector encoded functional elements (depending on the vector, *Strep*-tag II and/or 6xHis-tag, *Omp*A/BM40-signal sequence, protease cleavage site, start codon, or stop codon). To accomplish this it is necessary to adapt the structural gene at both ends of the coding region via PCR (see cloning scheme at www.iba-lifesciences.com). In order to avoid the incorporation of base substitutions, PCR should be performed with a proof reading DNA polymerase (e.g. *Pfu*, Stratagene) using phosphorothioate protected primers. The essential primer sequences to introduce the *Bsa*I restriction site into the PCR fragment for the cloning with a certain vector can be easily determined with our "Primer Design Software" which is free of charge and can be downloaded at IBA's web site.

Note: The general cloning strategy via Type IIS restriction enzymes is not applicable for vectors **pASK-IBA63a/b/c-plus and pASK-IBA65b-plus**. Please use the standard cloning procedures with restriction enzymes present in the multiple cloning site.

2 PCR with *Pfu* DNA polymerase



Important notes

- **Annealing and melting temperature of Primers:**
  Primers should have a theoretical melting temperature between 60 °C and 70 °C. This will be achieved automatically if the Primer D'Signer-Software is used. Otherwise 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). Additionally to the priming sequence a non-priming 5' extension for target vector specific cloning has to be appended to each primer as further specified in paragraph 4.1 for pASK-IBA3. This target vector correlated extension is respected when using Primer D'Signer.
  The annealing temperature should be chosen at least 5°C below the melting temperature of each primer. For primers designed by Primer D'Signer the recommended annealing temperature is 55 °C.

- **Cycles:**
  The number of cycles should be kept as low as possible in order to minimize base substitutions.

- **Polymerases:**
  - PCR instructions given in this protocol are recommended for the use of *Pfu* polymerase. When using another Polymerase than *Pfu*, please refer to the recommendations of the respective manufacturer.
  - hot-start: DNA polymerase is inactive until the initial denaturation step of PCR cycling. This reduces non-specific priming or the formation of primer dimers.
  - PTO protected primers: The use of 3' phosphorothioate (PTO) protected oligonucleotides is recommended to protect against the 3'-exonuclease activity of proof-reading polymerases.

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

Protocol

1. **Mix the following reagents in a 500 µl reaction tube and a total volume of 50µl** (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

2. **Use a heated lid when available. Alternatively, overlay the sample with 50 µl mineral oil.**

3. **For initial denaturation heat the sample at 94 °C for 3 min.**

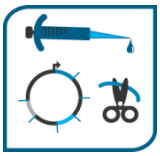
4. **Start temperature cycling:**

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

5. **Perform a final 60-72°C incubation step for 5 min in order to obtain full length products.**

6. **Store samples at 4°C until further analysis (e.g. agarose gel electrophoresis).**

3 Cloning of a PCR product via *Bsa*I or *Eco*31I (Type IIS restriction enzymes)



3.1 PCR product purification

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 clean up spin kit (according to the instructions of the manufacturer). If multiple bands are visible, it is recommended to isolate the PCR product by preparative agarose gel electrophoresis. **2. If a spin kit is used and the PCR fragment is eluted in H_2O, *Eco*31I (*Bsa*I) restriction can be performed immediately.** **1. Quantify PCR fragment by analytical agarose gel electrophoresis through band intensity comparison with a DNA standard.** Applying two different amounts of PCR product in separate lanes is recommended to find a band of equal intensity with a band of the DNA standard which has to be applied on the same gel for exact quantification.

3.2 Restriction digest of PCR fragment and Vector using *Bsa*I or *Eco*31I

Important notes	The vectors pASK-IBA and pEXPR-IBA can be digested with the isoschizomers *Bsa*I or *Eco*31I. Since both enzymes show different cutting efficiencies regarding the DNA source (vector DNA or PCR fragment) and incubation time, we determined the cloning efficiency for different digestion protocols. According to our results, we recommend using *Bsa*I for 1 hour or *Eco*31I for 16 hours for the cleavage of both the PCR fragment and the vector. **Note:** The general cloning strategy via Type IIS restriction enzymes is not applicable for vectors pASK-IBA63a/b/c-plus and pASK-IBA65b-plus. Please use standard cloning procedures with restriction enzymes present in the multiple cloning site. • For a detailed cloning scheme see Appendix 4.1.

Protocols

Cleavage of PCR-Fragment:

1. **Mix the following reagents in a 500 µl reaction tube and a total volume of 50µl:**

X µl	PCR fragment in H_2O (spin eluate)
5 µl	10x *Eco*31I (or *Bsa*I) restriction buffer
10-20 U/µg DNA	Restriction enzyme *(Eco*31I or *Bsa*I)
ad 50 µl	distilled H_2O

1. **Incubate with *Bsa*I at 50 °C for 1 hour (or *Eco*31I at 37 °C for 16 hours). Take measures to avoid evaporation.**

2. **Purify the desired fragment by using a spin column.**

3. **Quantify PCR fragment concentration to determine the appropriate vector:insert-ratio. Compare the band intensity of both the PCR fragment and the corresponding vector with a DNA standard on the same analytical agarose gel.**

Cleavage of Vector:

1. **Mix the following reagents in a 500 µl reaction tube and total volume of 50µl:**

2 µg	vector DNA
5 µl	10x *Eco*31I (or *Bsa*I) restriction buffer
10-20 U	Restriction enzyme *(Eco*31I or *Bsa*I)
ad 50 µl	distilled H_2O

2. **Incubate with *Bsa*I at 50 °C for 1 hour (or *Eco*31I at 37 °C for 16 hours). Take measures to avoid evaporation.**

3. **Incubate with *Pst*I for further 30 min at 37 °C or dephosphorylate linearized vector DNA with alkaline phosphatase according to the manufacturer's recommendations.**
   This step is to reduce background colonies which result from re-ligated vector.

4. **Purify vector fragment** using a preparative agarose gel with subsequent spin column purification

5. **Quantify vector fragment concentration to determine the appropriate vector:insert-ratio. Compare the band intensity of both the PCR fragment and the corresponding vector with a DNA standard on the same analytical agarose gel.**

3.3 Ligation reaction

Important notes

- To quantify background reactions we strongly recommend preparing a negative control without the addition of PCR fragment.
- For ligation use PCR fragment and vector in a molar ratio of 3:1

Protocol

1. **Mix the following reagents in a 500 µl reaction tube and total volume of 20µl:**

	Pos. control	Neg. control	
	100 ng	**100 ng**	Vector DNA, digested
	50 fmol	**-**	PCR fragment, digested
	2 µl	**2 µl**	Ligation buffer, 10x
	1 U	**1 U**	T4 DNA ligase
	ad 20 µl	**ad 20 µl**	distilled H_2O

2. **Incubate overnight at 16 °C.**
   Heat inactivation is not recommended and not necessary.

3. **Store the sample at 4 °C until transformation.**

4. **After initial clone selection (DNA mini preparation/restriction analysis), proceed to DNA sequencing.**

3.4 Sequencing

For validating correct vector insertion and sequence of the PCR fragment, the following sequencing primers can be used:

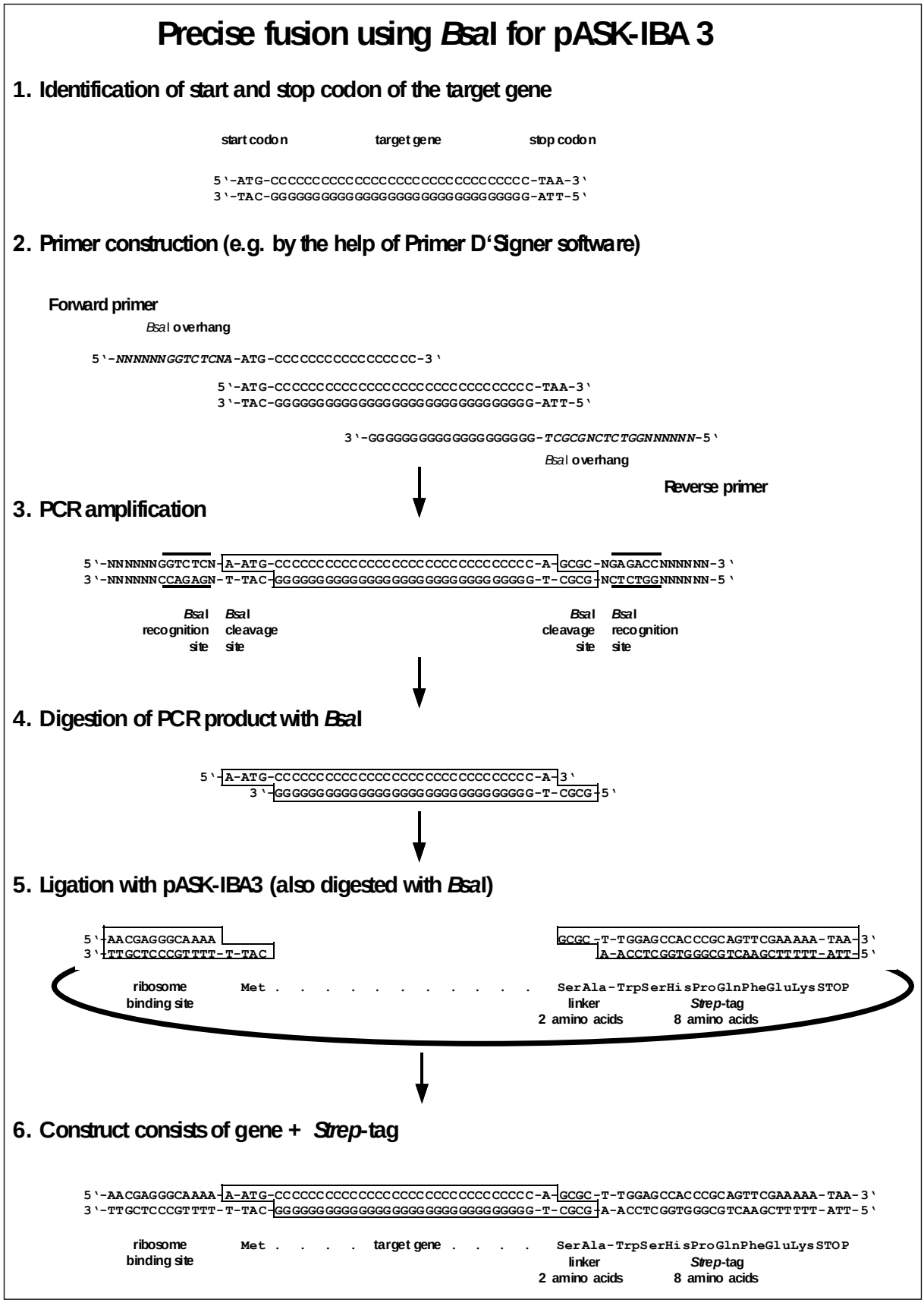
Sequencing primers for pASK-IBA vectors (cat. No. 5-0000-103):
Forward: 5’-GAGTTATTTTACCACTCCCT-3’
Reverse: 5’-CGCAGTAGCGGTAAACG-3’

Sequencing primers for pEXPR-IBA vectors (cat. No. 5-0000-123):
Forward: 5’-GAGAACCCACTGCTTACTGGC-3’
Reverse: 5’-TAGAAGGCACAGTCGAGG-3’

The sequencing primers are also suitable for cycle sequencing.

4 APPENDIX

4.1 Cloning Scheme for the use of *Bsa*I or *Eco31*I



4.2 Multiple Cloning Sites of pEXPR-IBA vectors

pEXPR-IBA3

```
                  Eco31I PshAI                                                    PshAI Eco31I
XbaI              BsaI SacII     EcoRI       KpnI     BamHI XhoI                  BsaI  Eco47III                          HindIII
TCTAGACCCACaatgGGAGACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTCAgcgcTTGGAGCCACCCGCAGTTCGAAAAATAATAAGCTT
            MetGlyAspArgGlyProGluPheGluLeuGlyThrArgGlySerLeuGluValAspLeuGlnGlyAspHisGlyLeuSerAlaTrpSerHisProGlnPheGluLysEnd
                                                                                        Link       Strep-tag II
```

pEXPR-IBA5

```
                                          Eco31I PshAI                                                    PshAI Eco31I
XbaI           NheI                       BsaI SacII     EcoRI       KpnI     BamHI XhoI                  BsaI    EcoRV      HindIII
TCTAGACCCACCATGGCTAGCTGGAGCCACCCGCAGTTCGAAAAAGgcgccCGAGACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTCTgataTCTAACTAAGCTT
            MetAlaSerTrpSerHisProGlnPheGluLysGlyAlaGluThrAlaValProAsnSerSerSerValProGlyAspProSerArgSerThrCysArgGlyThrMetValSerAspIleEnd
                Link       Strep-tag II       Link  ArgProArgSerArgIleArgAlaArgTyrProGlyIleProArgGlyArgProAlaGlyGlyProTrpSerLeuIleSerAsnEnd
                                                   AspArgGlyProGluPheGluLeuGlyThrArgGlySerLeuGluValAspLeuGlnGlyAspHisGlyLeuEnd
```

pEXPR-IBA7

```
                                                Eco31I PshAI                                                    PshAI Eco31I
XbaI           NheI                             BsaI SacII     EcoRI       KpnI     BamHI XhoI                  BsaI    EcoRV      HindIII
TCTAGACCCACCATGGCTAGCTGGAGCCACCCGCAGTTCGAAAAAATCGAAGGgcgcCGAGACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTCTgataTCTAACTAAGCTT
            MetAlaSerTrpSerHisProGlnPheGluLysIleGluGlyArgArgAspArgGlyProGluPheGluLeuGlyThrArgGlySerLeuGluValAspLeuGlnGlyAspHisGlyLeuEnd
                Link       Strep-tag II       Factor Xa   GluThrAlaValProAsnSerSerSerValProGlyAspProSerArgSerThrCysArgGlyThrMetValSerAspIleEnd
                                                          ArgProArgSerArgIleArgAlaArgTyrProGlyIleProArgGlyArgProAlaGlyGlyProTrpSerLeuIleSerAsnEnd
```

pEXPR-IBA13

```
                                                                        Eco31I PshAI                                                    PshAI Eco31I
XbaI           NheI                                                     BsaI SacII     EcoRI       KpnI     BamHI XhoI                  BsaI    EcoRV      HindIII
TCTAGACCCACCATGGCTAGCTGGAGCCACCCGCAGTTCGAAAAATCTGGTGGTGGTGGTGGTCTGGTTCCGCGTGGctccCGAGACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTCTgataTCTAACTAAGCTT
            MetAlaSerTrpSerHisProGlnPheGluLysSerGlyGlyGlyGlyGlyLeuValProArgGlySerArgAspArgGlyProGluPheGluLeuGlyThrArgGlySerLeuGluValAspLeuGlnGlyAspHisGlyLeuEnd
                Link       Strep-tag II           Spacer           Thrombin       GluThrAlaValProAsnSerSerSerValProGlyAspProSerArgSerThrCysArgGlyThrMetValSerAspIleEnd
                                                                                   ArgProArgSerArgIleArgAlaArgTyrProGlyIleProArgGlyArgProAlaGlyGlyProTrpSerLeuIleSerAsnEnd
```

pEXPR-IBA15

```
                                                                  Eco31I PshAI                                                    PshAI Eco31I
XbaI           NheI                                               BsaI SacII     EcoRI       KpnI     BamHI XhoI                  BsaI    EcoRV      HindIII
TCTAGACCCACCATGGCTAGCTGGAGCCACCCGCAGTTCGAAAAAGGCGCCGACGACGACGACAAGGGctccCGAGACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTCTgataTCTAACTAAGCTT
            MetAlaSerTrpSerHisProGlnPheGluLysGlyAlaAspAspAspAspLysSerArgAspArgGlyProGluPheGluLeuGlyThrArgGlySerLeuGluValAspLeuGlnGlyAspHisGlyLeuEnd
                Link       Strep-tag II           Enterokinase     Link  GluThrAlaValProAsnSerSerSerValProGlyAspProSerArgSerThrCysArgGlyThrMetValSerAspIleEnd
                                                                          ArgProArgSerArgIleArgAlaArgTyrProGlyIleProArgGlyArgProAlaGlyGlyProTrpSerLeuIleSerAsnEnd
```

pEXPR-IBA42

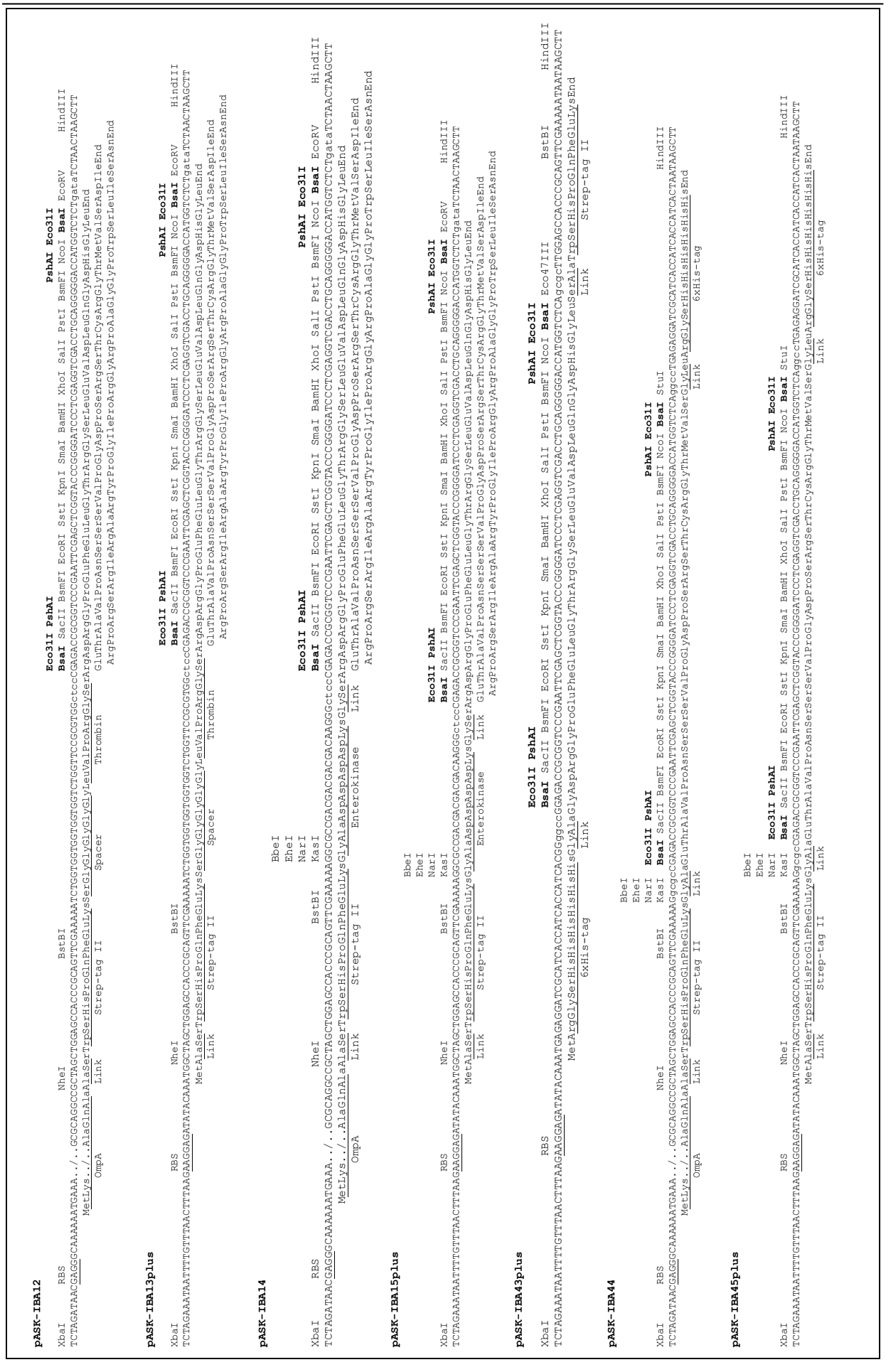
```
                                                Eco31I PshAI                                                    PshAI Eco31I
XbaI                     NheI                   BsaI SacII     EcoRI       KpnI     BamHI XhoI                  BsaI  Eco47III                          HindIII
TCTAGACCCACCATGAGG./.GCTCTGGCAGCTAGCAGAGGATCGCATCACCATCACCATCACGgcgcCGAGACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTCAgcgcTTGGAGCCACCCGCAGTTCGAAAAATAATAAGCTT
          MetArg./.AlaLeuAlaAlaSerArgGlySerHisHisHisHisHisGlyAlaGluThrAlaValProAsnSerSerSerValProGlyAspPrLeuGluValAspLeuGlnGlyAspHisGlyLeuSerAlaTrpSerHisProGlnPheGluLysEnd
                 BM40        Link          6xHis-tag          Link                                                                        Link       Strep-tag II
```

pEXPR-IBA44

```
                                                Eco31I PshAI                                                    PshAI Eco31I
XbaI                     NheI                   BsaI SacII     EcoRI       KpnI     BamHI XhoI                  BsaI                                                HindIII
TCTAGACCCACCATGAGG./.GCTCTGGCAGCTAGCTGGAGCCACCCGCAGTTCGAAAAAGgcgcCGAGACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTCAggccTGAGAGGATCGCATCACCATCACCATCACTAATAAGCTT
          MetArg./.AlaLeuAlaAlaSerTrpSerHisProGlnPheGluLysGlyAlaGluThrAlaValProAsnSerSerSerValProGlyAspProSerArgSerThrCysArgGlyThrMetValSerArgGlySerHisHisHisHisHisHisEnd
                 BM40        Link       Strep-tag II       Link                                                                        Link          6xHis-tag
```

Please note: Restriction sites in bold cut twice and are useful for precise and oriented insertion of the recombinant gene by one cleavage reaction only. All the regions denoted with “link” carry a restriction site which may be useful for cutting out the recombinant gene for e.g. subcloning into pASK-IBA vectors for bacterial expression. The restriction enzyme cleavage site is not mentioned at the “link” region when such enzyme has at least one further site in the vector.

4.3 Multiple Cloning Sites of pASK-IBA vectors


pASK-IBA12
Eco31I PshAI
PshAI Eco31I
BsaI SacII BsmFI EcoRI SstI KpnI SmaI BamHI XhoI SalI PstI BsmFI NcoI BsaI EcoRV
XbaI RBS NheI BstBI HindIII
TCTAGATAACGAGGGCAAAAAATGAAA../..GCGCAGGCCGCTAGCTGGAGCCACCCGCAGTTCGAAAAATCTGGTGGTGGTGGTTCCGCGTGGCtcccCGAGACCGGGTCCCGAATTCGAGCTCGACCTCGCAGGGGGACCATGGTCTCTgataTCTAACTAAGCTT
MetLys../..AlaGlnAlaAlaSerTrpSerHisProGlnPheGluLysSerGlyGlyGlyGlyLeuValProArgGlySerArgAspArgGlyProGluPheGluLeuGlyThrArgGlySerLeuGluValAspLeuGlnGlyAspHisGlyLeuEnd
OmpA Link Strep-tag II Spacer Thrombin ArgProArgSerArgIleArgAlaAlaArgTyrProGlyIleProArgGlyArgProAlaGlyGlyProTrpSerLeuIleSerAsnEnd
pASK-IBA13plus
Eco31I PshAI
PshAI Eco31I
BsaI SacII BsmFI EcoRI SstI KpnI SmaI BamHI XhoI SalI PstI BsmFI NcoI BsaI EcoRV HindIII
XbaI RBS NheI BstBI
TCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACAAATGGCTAGCTGGAGCCACCCGCAGTTCGAAAAATCTGGTGGTGGTGGTTCCGCGTGGCtccGAGACCGGGTCCCGAATTCGAGCTCGACCTCGCAGGGGGACCATGGTCTCTgataTCTAACTAAGCTT
MetAlaSerTrpSerHisProGlnPheGluLysSerGlyGlyGlyGlyLeuValProArgGlySerArgAspArgGlyProGluPheGluLeuGlyThrArgGlySerLeuGluValAspLeuGlnGlyAspHisGlyLeuEnd
Link Strep-tag II Spacer Thrombin GluThrAlaValProAsnSerSerSerValProGlyAspProSerArgSerThrCysArgGlyThrMetValSerAspIleEnd
ArgProArgSerArgIleArgAlaAlaArgTyrProGlyIlePro
pASK-IBA14
BbeI
EheI
NarI
Eco31I PshAI
PshAI Eco31I
XbaI RBS NheI BstBI KasI
BsaI SacII BsmFI EcoRI SstI KpnI SmaI BamHI XhoI SalI PstI BsmFI NcoI BsaI EcoRV HindIII
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OmpA Link Strep-tag II Enterokinase Link GluThrAlaValProAsnSerSerSerValProGlyAspProSerArgSerThrCysArgGlyThrMetValSerAspIleEnd
ArgProArgSerArgIleArgAlaAlaArgTyrProGlyIlePro ArgProArgGlyArgProAlaGlyGlyProTrpSerLeuIleSerAsnEnd
pASK-IBA15plus
BbeI
EheI
NarI
Eco31I PshAI
PshAI Eco31I
XbaI RBS NheI BstBI KasI
BsaI SacII BsmFI EcoRI SstI KpnI SmaI BamHI XhoI SalI PstI BsmFI NcoI BsaI EcoRV HindIII
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MetAlaSerTrpSerHisProGlnPheGluLysSerGlyAlaAspAspAspAspLysGlySerArgAspArgGlyProGluPheGluLeuGlyThrArgGlySerLeuGluValAspLeuGlnGlyAspHisGlyLeuEnd
Link Strep-tag II Enterokinase Link GluThrAlaValProAsnSerSerSerValProGlyAspProSerArgSerThrCysArgGlyThrMetValSerAspIleEnd
ArgProArgSerArgIleArgAlaAlaArgTyrProGlyIlePro ArgProArgGlyArgProAlaGlyGlyProTrpSerLeuIleSerAsnEnd
pASK-IBA43plus
Eco31I PshAI
PshAI Eco31I
XbaI RBS
BsaI SacII BsmFI EcoRI SstI KpnI SmaI BamHI XhoI SalI PstI BsmFI NcoI BsaI Eco47III BstBI HindIII
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6xHis-tag Link Link Strep-tag II
pASK-IBA44
BbeI
EheI
NarI
Eco31I PshAI
PshAI Eco31I
XbaI RBS NheI BstBI KasI
BsaI SacII BsmFI EcoRI SstI KpnI SmaI BamHI XhoI SalI PstI BsmFI NcoI BsaI StuI HindIII
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MetLys../..AlaGlnAlaAlaSerTrpSerHisProGlnPheGluLysGlyAlaGluThrAlaValProAsnSerSerSerValProGlyAspProSerArgSerThrCysArgGlyThrMetValSerArgGlySerHisHisHisHisHisEnd
OmpA Link Strep-tag II Link Link 6xHis-tag
pASK-IBA45plus
BbeI
EheI
NarI
Eco31I PshAI
PshAI Eco31I
XbaI RBS NheI BstBI KasI
BsaI SacII BsmFI EcoRI SstI KpnI SmaI BamHI XhoI SalI PstI BsmFI NcoI BsaI StuI HindIII
TCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACAAATGGCTAGCTGGAGCCACCCGCAGTTCGAAAAAGgcgcGAGACCGGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTCAggccTGAGAGGATCGCATCACCATCACTAATAAGCTT
MetAlaSerTrpSerHisProGlnPheGluLysGlyAlaGluThrAlaValProAsnSerSerSerValProGlyAspProSerArgSerThrCysArgGlyThrMetValSerArgGlySerHisHisHisHisHisEnd
Link Strep-tag II Link Link 6xHis-tag

pASK-IBA32

```
                                    Eco31I PshAI                                                                  PshAI Eco31I
XbaI      RBS                       BsaI NcoI BsmFI EcoRI SstI KpnI SmaI BamHI XhoI SalI PstI BsmFI NcoI BsaI Eco47III                                HindIII
TCTAGATAACGAGGGCAAAAAATGAAA../..GCGCAggccGGAGACCATGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTCAgcgcTAGAGGATCGCATCACCATCACCATCACTAATAAGCTT
                  MetLys../..AlaGlnAlaGlyAspHisGlyProGluPheGluLeuGlyThrArgGlySerLeuGluValAspLeuGlnGlyAspHisGlyLeuSerAlaArgGlySerHisHisHisHisHisHisEnd
            OmpA                                                                                                Link          6xHis-tag
```

pASK-IBA33plus

```
                                    Eco31I PshAI                                                                  PshAI Eco31I
XbaI                      RBS       BsaI SacII BsmFI EcoRI SstI KpnI SmaI BamHI XhoI SalI PstI BsmFI NcoI BsaI Eco47III                                HindIII
TCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACAaatgGGAGACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTCAgcgcTAGAGGATCGCATCACCATCACCATCACTAATAAGCTT
                                    MetGlyAspArgGlyProGluPheGluLeuGlyThrArgGlySerLeuGluValAspLeuGlnGlyAspHisGlyLeuSerAlaArgGlySerHisHisHisHisHisHisEnd
                                                                                                                Link          6xHis-tag
```

pASK-IBA35plus

```
                                                                                BbeI
                                                                                EheI
                                                                                NarI  Eco31I PshAI                                                  PshAI Eco31I
XbaI                      RBS             NheI                                  KasI  BsaI SacII BsmFI EcoRI SstI KpnI SmaI BamHI XhoI SalI PstI BsmFI NcoI BsaI EcoRV      HindIII
TCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACAAATGGCTAGCAGAGGATCGCATCACCATCACCATCACCATCACGgcgcCGAGACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTCTgataTCTAACTAAGCTT
                                    MetAlaSerArgGlySerHisHisHisHisHisHisGlyAlaGluThrAlaValProAsnSerSerSerValProGlyAspProSerArgSerThrCysArgGlyThrMetValSerAspIleEnd
                                        Link          6xHis-tag          Link  ArgProArgSerArgIleArgAlaArgTyrProGlyIleProArgGlyArgProAlaGlyGlyProTrpSerLeuIleSerAsnEnd
                                                                                  AspArgGlyProGluPheGluLeuGlyThrArgGlySerLeuGluValAspLeuGlnGlyAspHisGlyLeuEnd
```

pASK-IBA37plus

```
                                                                                  BbeI
                                                                                  EheI
                                                                                  NarI  Eco31I PshAI                                                  PshAI Eco31I
XbaI                      RBS             NheI                                    KasI  BsaI SacII BsmFI EcoRI SstI KpnI SmaI BamHI XhoI SalI PstI BsmFI NcoI BsaI EcoRV      HindIII
TCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACAAATGGCTAGCAGAGGATCGCATCACCATCACCATCACATCGAAGGgcgcCGAGACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTCTgataTCTAACTAAGCTT
                                    MetAlaSerArgGlySerHisHisHisHisHisHisIleGluGlyArgArgAspArgGlyProGluPheGluLeuGlyThrArgGlySerLeuGluValAspLeuGlnGlyAspHisGlyLeuEnd
                                        Link          6xHis-tag          Factor Xa   GluThrAlaValProAsnSerSerSerValProGlyAspProSerArgSerThrCysArgGlyThrMetValSerAspIleEnd
                                                                                     ArgProArgSerArgIleArgAlaArgTyrProGlyIleProArgGlyArgProAlaGlyGlyProTrpSerLeuIleSerAsnEnd
```

pPR-IBA1

```
                                    Eco31I PshAI                                                                  PshAI Eco31I
XbaI                        RBS     BsaI SacII BsmFI EcoRI SstI KpnI SmaI BamHI XhoI SalI PstI BsmFI NcoI BsaI Eco47III           BstBI        HindIII
TCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACAaatgGGAGACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTCAgcgcTTGGAGCCACCCGCAGTTCGAAAAATAATAAGCTT
                                    MetGlyAspArgGlyProGluPheGluLeuGlyThrArgGlySerLeuGluValAspLeuGlnGlyAspHisGlyLeuSerAlaTrpSerHisProGlnPheGluLysEnd
                                                                                                                Link       Strep-tag II
```

pPR-IBA2

```
                                                                                BbeI
                                                                                EheI
                                                                                NarI  Eco31I PshAI                                                  PshAI Eco31I
XbaI                        RBS             NheI                  BstBI       KasI  BsaI SacII BsmFI EcoRI SstI KpnI SmaI BamHI XhoI SalI PstI BsmFI NcoI BsaI EcoRV      HindIII
TCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGGCTAGCTGGAGCCACCCGCAGTTCGAAAAAGgcgcCGAGACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGACCTGCAGGGGGACCATGGTCTCTgataTCTAACTAAGCTT
                                    MetAlaSerTrpSerHisProGlnPheGluLysGlyAlaGluThrAlaValProAsnSerSerSerValProGlyAspProSerArgSerThrCysArgGlyThrMetValSerAspIleEnd
                                        Link       Strep-tag II       Link  ArgProArgSerArgIleArgAlaArgTyrProGlyIleProArgGlyArgProAlaGlyGlyProTrpSerLeuIleSerAsnEnd
                                                                              AspArgGlyProGluPheGluLeuGlyThrArgGlySerLeuGluValAspLeuGlnGlyAspHisGlyLeuEnd
```

5 References

For help with DNA ligation, *E. coli* transformation, restriction enzyme analysis, purification of DNA, DNA sequencing, and DNA biochemistry, please refer to “Molecular Cloning: A Laboratory Manual” (Sambrook et al., 1989) or “Current Protocols in Molecular Biology” (Ausubel et al., 1994).

Please refer to **www.iba-lifesciences.com/technical-support.html** for downloading this manual.

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