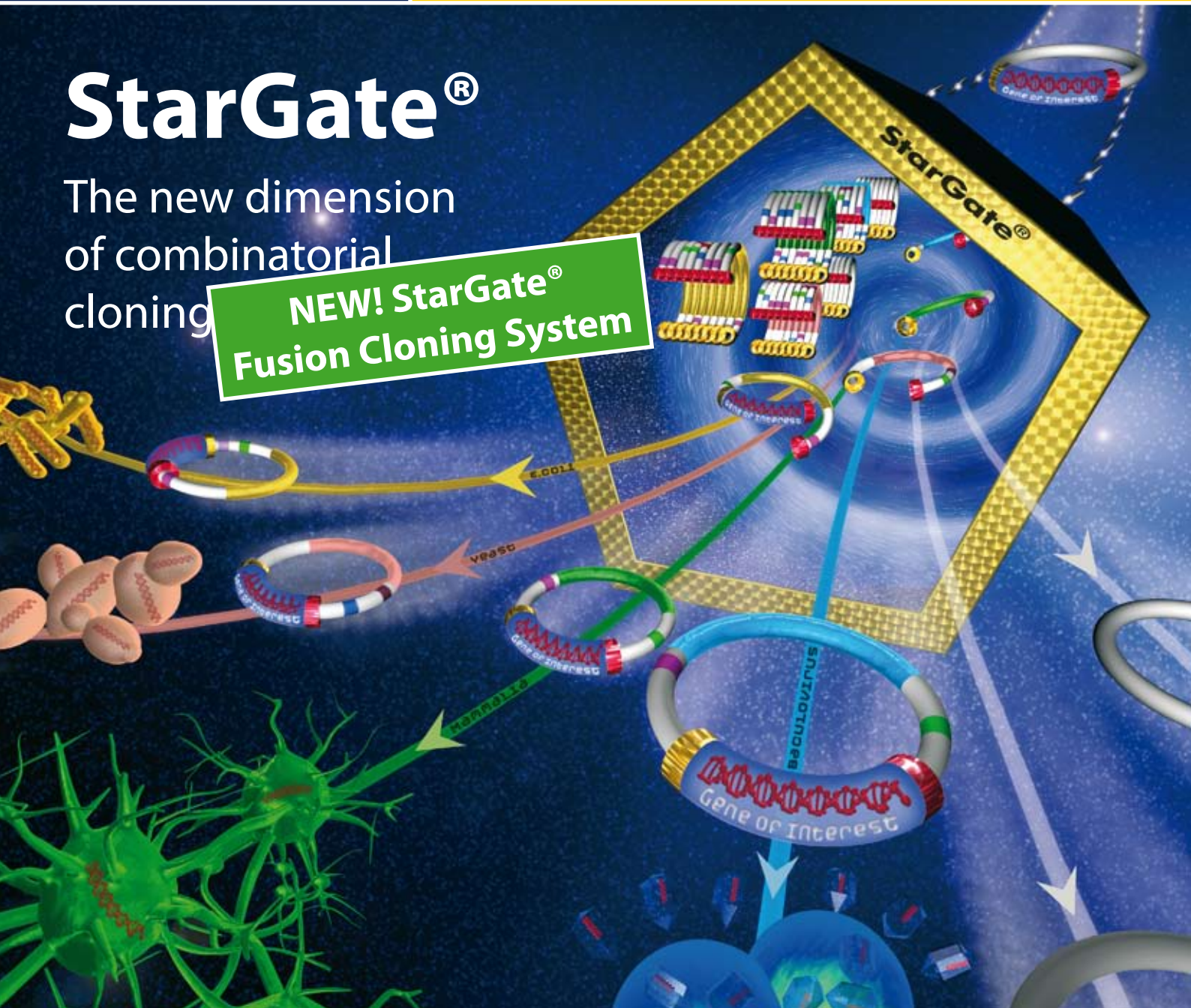


StarGate®

The new dimension
of combinatorial
cloning

**NEW! StarGate®
Fusion Cloning System**



In this issue

- New StarGate® Fusion Cloning Sets to construct fusion proteins or artificial operons
- Further StarGate® Acceptor Vectors
 - suitable for bacterial *in vitro* translation
 - with FLAG®-tag
 - with One-STREP/FLAG®-tag tandem technology for protein:protein interaction studies
 - for episomal mammalian expression with CMV promoter

StarGate® - now also available for Fusion Cloning

General StarGate® Technology

StarGate® is a technology that allows the systematic combination of promoters (i.e. hosts), purification tags or other genetic elements with any gene of interest (GOI) in a convenient cloning system.

Key Advantages of StarGate® are

- Easy-to-handle subcloning procedure
- Minimal modification of the gene of interest due to short combinatorial sites (≤ 2 aa)
- Inherent high level cloning efficiency due to a directed reaction (no equilibrium)
- High protein expression due to optimal host selection
- Highest protein purity due to optimal purification tag selection
- Versatile combination of GOIs using the new StarGate® Fusion Cloning technology

Standard StarGate® Procedure

Step 1: Entry reaction

In a first step, the gene of interest (GOI) is equipped at both ends with combinatorial sites (4 bases) by PCR and is inserted into an Entry Vector by a simple one-tube reaction (see figure on page 3). The opened Entry Vector contributes the recognition sites and provides an operative linkage with the combinatorial sites for the highly specific StarGate® gene transfer process (step 2).

Step 2: Transfer reaction

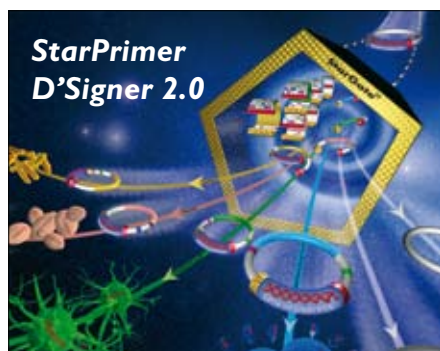
After sequence confirmation, the resulting Donor Vector is the source for the highly parallel subcloning of the GOI by a second simple one tube reaction into a multitude of Acceptor Vectors, each providing a different genetic surrounding like host specific promoters and different purification tags. The resulting Destination Vectors are then transformed into the corresponding host cells for further experiments.

Acceptor Vectors are listed in the Acceptor Vector Overview Table (see page 7). Please refer to www.stargate-cloning.com for the most up-to-date version.

Optional intermediate step: Fusion Cloning

Via an optional intermediate step between Entry and Transfer reaction (see page 3) you can generate fusion proteins or construct artificial operons for multiple gene expression in bacterial or mammalian cells.

Create primers for Entry reaction with StarPrimer D'Signer Software



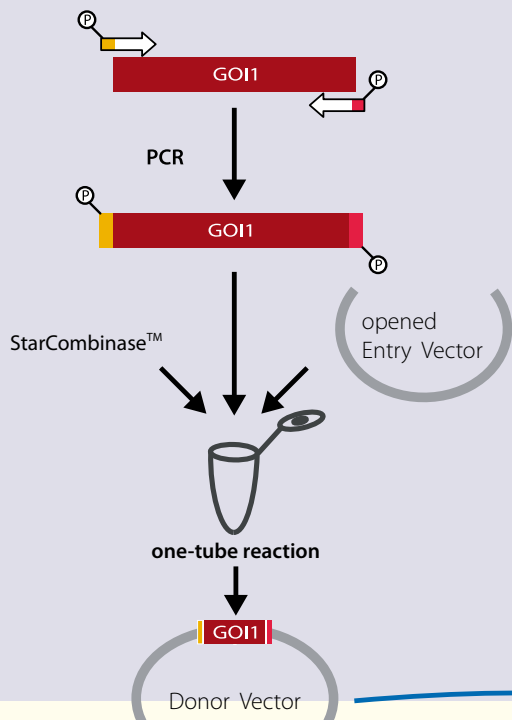
The StarPrimer D'Signer is an easy-to-use software facilitating the design of primers for standard StarGate® Entry Cloning as well as the introduction of mutations into a gene of interest (GOI) by StarGate® Mutagenesis Entry Cloning (details see www.stargate-cloning.com).

To design the primers the sequence of the gene of interest (GOI) as present in the template and - in case of Mutagenesis Entry Cloning - the necessary mutated gene sequence simply have to be pasted into the respective StarPrimer D'Signer sequence windows. The software displays the sequence of the desired primers, which can then be ordered at the IBA Gene TAGnology division (see www.iba-bioTAGnology.com).

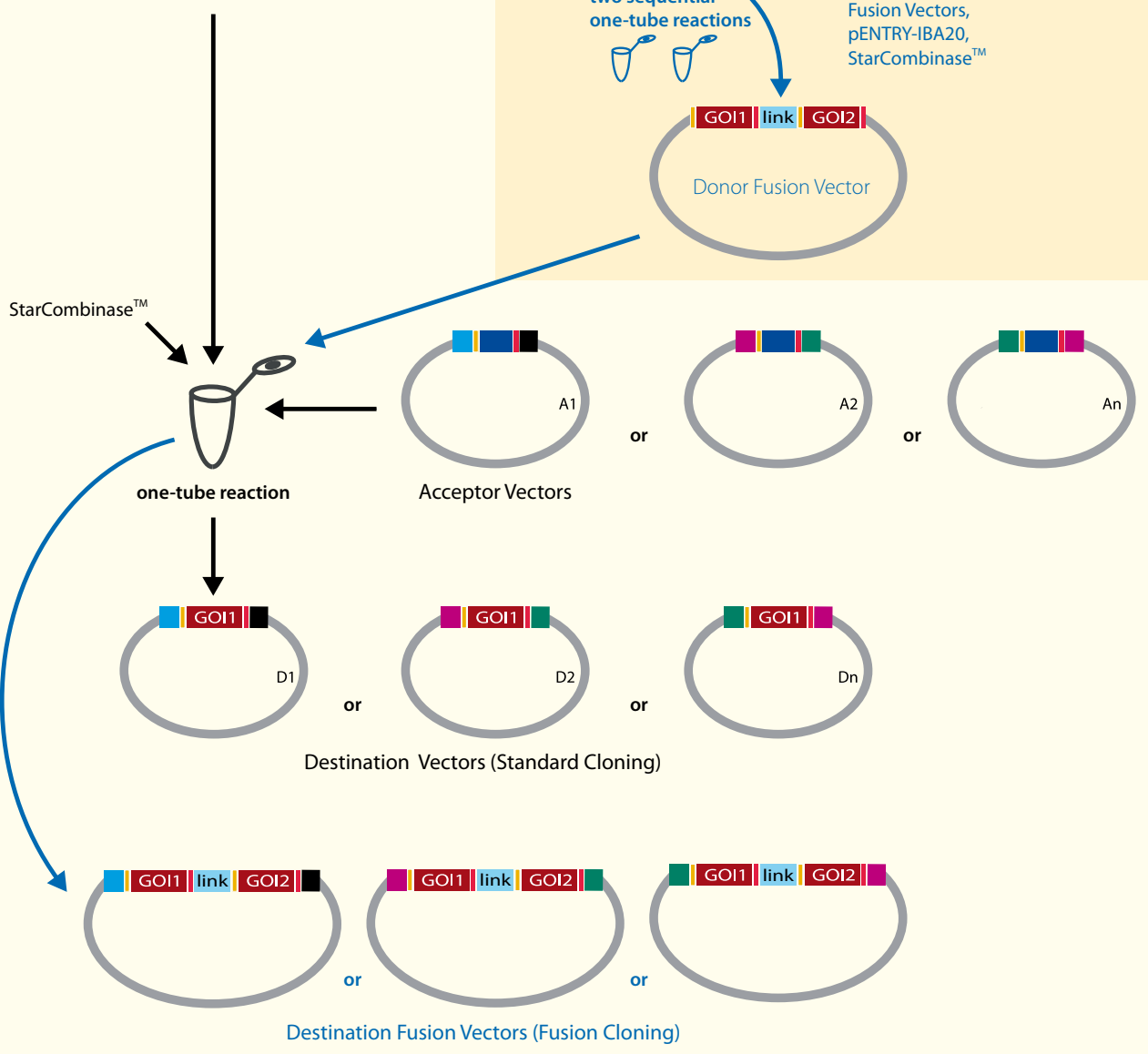
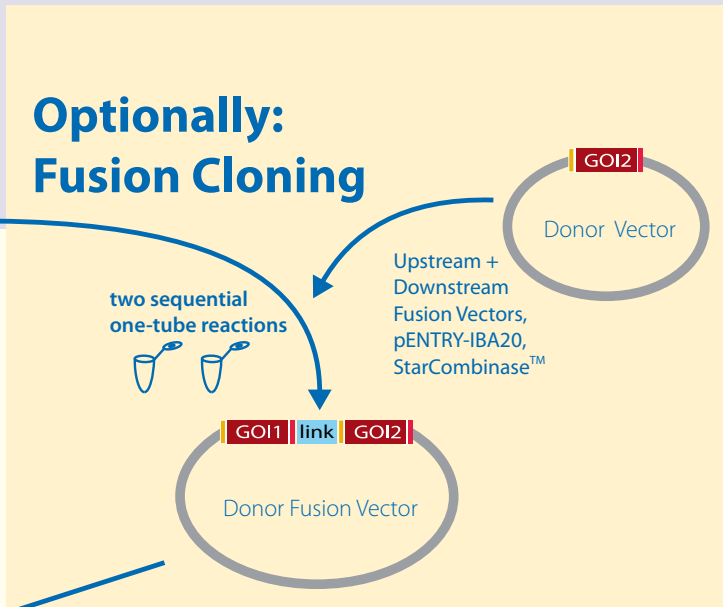
The Microsoft Windows software comes free of charge with the system. It is also available for download at <http://www.stargate-cloning.com/download.html> (<1 MB).

Entry reaction

Transfer reaction



Optionally:
Fusion Cloning



StarGate® Fusion Cloning example

Fig. 1 shows a bicistronic Destination Vector that consists of two genes, PR65 and GFP, cloned into 2 separate Donor Vectors that were fused by means of pNFUSE-IBA-IRES1. The insert of the resulting Fusion Donor Vector was transferred in a second step into pESG-IBAw1. Transcription of both genes in the bicistronic arrangement is controlled by the CMV promoter. While translation of upstream PR65 is cap-dependent, separate translation of downstream GFP is mediated by the intergenic IRES sequence. Since transfected cells are fluorescing green they can be detected easily and may be selected and enriched to optimize yields of the co-expressed PR65 target protein (Fig.2).

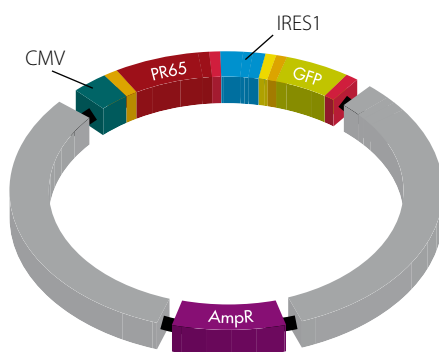


Fig. 1

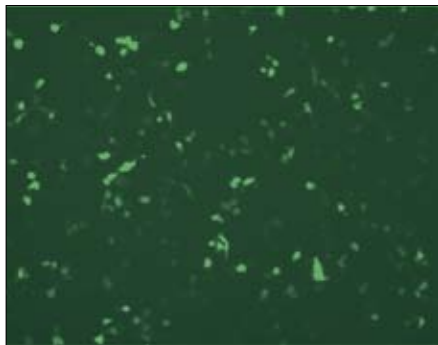


Fig. 2

StarGate® Fusion Cloning

For several applications, it is interesting to bring **two or more genes**, already cloned in separate Donor vectors, into **operative linkage** by an intergenic region. Such an **intergenic region** may e.g. code for an amino acid **linker sequence** directly connecting, after performing the fusion procedure, the gene product of a first GOI to the product of a second GOI. Alternatively, it may include a Shine Dalgarno or an IRES site for the construction of **synthetic operons** in bacterial or mammalian cells, respectively.

Fusion Cloning Procedure

StarGate® Fusion Cloning is an **optional intermediate step** between the Entry reaction and the Transfer reaction described on page 2. It allows easy and fast fusion of two genes of interest (GOI-1 and GOI-2) present in separate Donor Vectors by performing **two sequential StarGate® subcloning reactions** (see page 5).

The **first subcloning reaction** is placing each GOI into a special Fusion Vector. One Fusion Vector (pNFUSE-IBA-derivative specifying the intergenic region) is designed for **upstream**, while the other (pCFUSE-IBA) is for **downstream** positioning of the respectively inserted GOI. The Downstream Fusion Vector is identical in each reaction. Upstream and Downstream Fusion Vectors with inserted GOI are then assembled in a directed manner within pENTRY-IBA20 thereby providing a new **Donor Vector** consisting of GOI1 fused to GOI2 by the intergenic region of the given pNFUSE-IBA derivative.

The fused GOIs are now ready to be subcloned into any of the StarGate® Acceptor Vectors available for expression ("**Transfer reaction**"). Upstream Fusion Vectors with different intergenic regions are included as part of our new Fusion Cloning Sets as described below. Acceptor Vectors are listed in the "**Acceptor Vector Overview Table**" (see page 7) or at www.stargate-cloning.com for the most up-to-date version.

StarGate® Fusion Cloning Sets

NEW!

Three different versions of StarGate® Fusion Cloning Sets are available to date. Please note, that also a combination of these three versions is possible allowing the construction of a large variety of genetic fusions. Your gene of interest could e.g. be fused with GFP using "LINK1" followed by "SD1" to construct an artificial operon of your GFP fusion reporter protein with further genes. For this purpose, the Upstream Fusion Vectors are also available separately on request.

StarGate® Fusion Cloning Set "IRES1"

Internal ribosomal entry site (IRES) for polycistronic gene expression in mammalian cells from one expression vector

StarGate® Fusion Cloning Set "SD1"

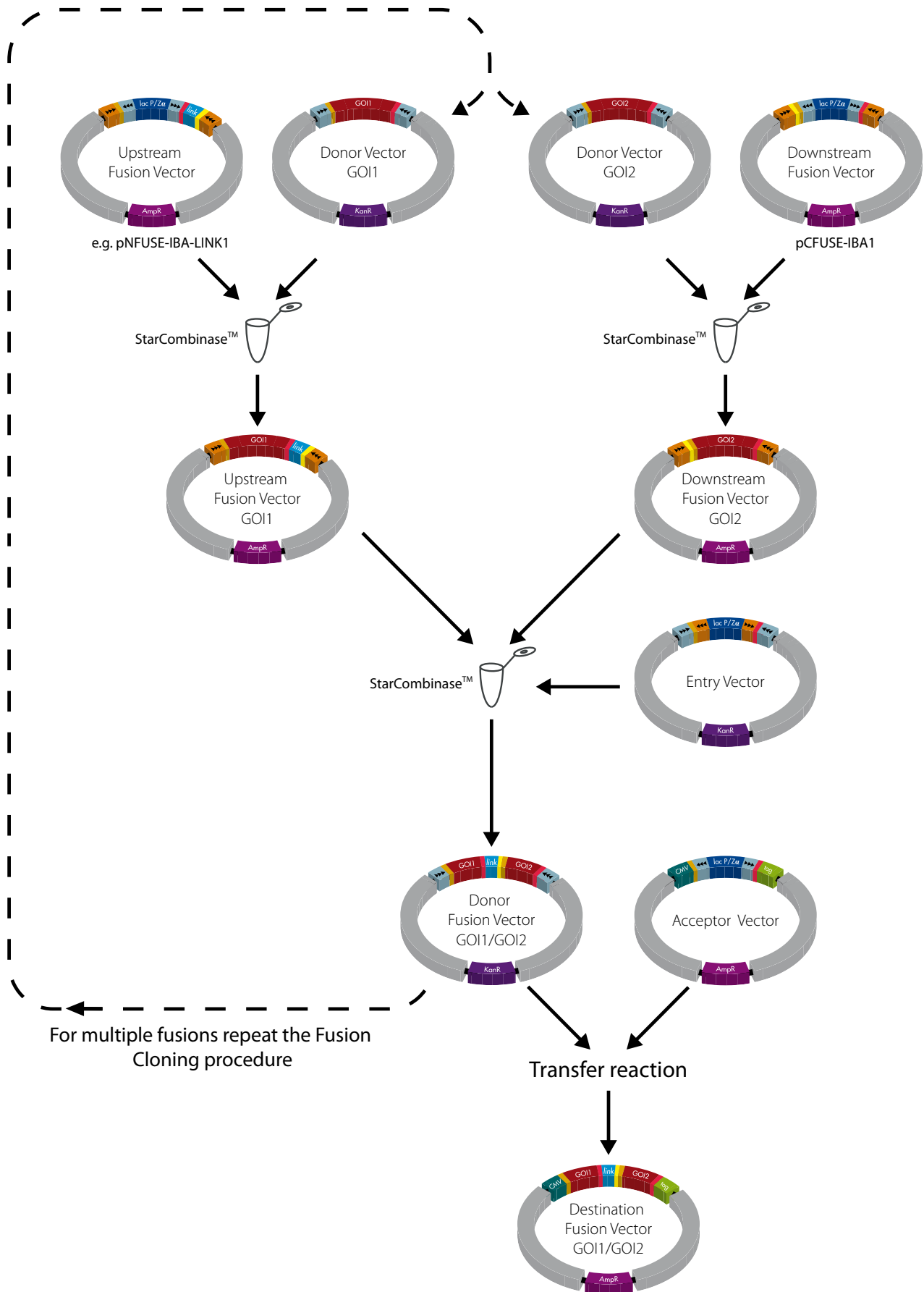
Shine-Dalgarno (SD) sequences for the construction of artificial operons in *E. coli*

StarGate® Fusion Cloning Set "LINK1"

Amino acid linker (GSGGGSGGGS) for the generation of fusion proteins

Upstream Fusion Vector	Sequence of intergenic region
pNFUSE-IBA-IRES1	AATG-GOI1-GGGAGCTAAGGG//IRES//ATGATAAAATG-GOI2-GGGA M -POI1-G S * M -POI2-G
pNFUSE-IBA-SD1	AATG-GOI1-GGGAGCTAACGAGGGCAAAAATG-GOI2-GGGA M -POI1-G S * RBS M -POI2-G
pNFUSE-IBA-LINK1	AATG-GOI1-GGGAGCGGCGGTGGCTCTGGTGGCGGTTCAATG-GOI2-GGGA M -POI1-G S G G G S G G G S M -POI2-G

The StarGate® Fusion Cloning Principle



StarGate® Components

Components supplied by IBA:

- Entry Vector pENTRY-IBA
- Forward and reverse sequencing primers
- Competent *E. coli* Top10 cells
- Individual custom primer synthesis for amplification of your GOI
- Acceptor Vectors:
pASG (*E. coli*, Tet promoter),
pPSG (*E. coli*, T7 promoter),
pESG / pCSG (Mammalia, CMV promoter),
pYSG (Yeast, CUP1 promoter),
pLSG (Insect cells, Polyhedrin promoter)
- StarSolutions
- StarPrimer D' Signer Software
- StarGate® Vector Selector Software
- DNA Ruler

Additionally required:

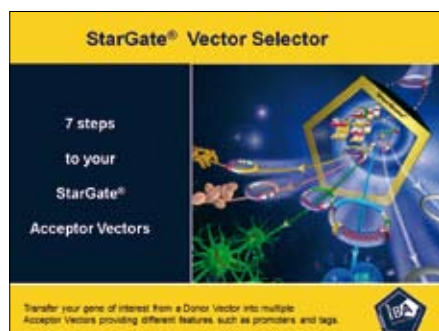
- Pfu DNA polymerase and reagents
- LB agar plates with Kanamycin (Ampicillin) and X-Gal

High quality, HPLC purified and PTO protected

custom cloning primers for StarGate®

can be obtained at the IBA Gene TAGnologies department; refer to www.iba-bioTAGnology.com.

Select your Acceptor Vectors conveniently with the new StarGate® Vector Selector Software



To select the vectors suited best for your application use our new online tool "StarGate® Vector Selector" helping you to choose your required vectors easily. See www.stargate-cloning.com.

Order Information Fusion Cloning

To perform the complete StarGate® Cloning procedure, you require one of the **Entry Cloning Sets** (Standard or Mutagenesis) and the products listed under "Transfer reaction".

For **Fusion Cloning**, you additionally require one of the Fusion Cloning Sets.

Cat. no.	StarGate® products
Entry reaction	
5-1601-000	StarGate® Standard Entry Cloning Set consisting of - StarGate® Entry Reagent Set (Entry Vector pENTRY-IBA10 (20 rxns), StarSolution E (20 rxns), For/Rev Sequencing Primer, DNA Ruler) - Competent <i>E. coli</i> Top10 cells (20 rxns)
5-1602-000	StarGate® Mutagenesis Entry Cloning Set consisting of - StarGate® Mutagenesis Reagent Set (Entry Vector pENTRY-IBA20 (5 rxns), StarSolution M1 (5 rxns), M2 (5 rxns), M3 (5 rxns), For/Rev Sequencing Primer, DNA Ruler) - Competent <i>E. coli</i> Top10 cells (5 rxns)
Fusion Cloning reaction	
5-1607-001	StarGate® Fusion Cloning Set "IRES1" consisting of - StarGate® Fusion Reagent Set (pENTRY-IBA20 (5 rxns), Downstream Fusion Vector pCFUSE-IBA1 (5 rxns), StarSolutions F1 to F6 (for 5 fusions)) - Upstream Fusion Vector pNFUSE-IBA-IRES1 (5 rxns) - Competent <i>E. coli</i> TOP10 cells (20 rxns)
5-1607-002	StarGate® Fusion Cloning Set "SD1" consisting of - StarGate® Fusion Reagent Set (pENTRY-IBA20 (5 rxns), Downstream Fusion Vector pCFUSE-IBA1 (5 rxns), StarSolutions F1 to F6 (for 5 fusions)) - Upstream Fusion Vector pNFUSE-IBA-SD1 (5 rxns) - Competent <i>E. coli</i> TOP10 cells (20 rxns)
5-1607-003	StarGate® Fusion Cloning Set "LINK1" consisting of - StarGate® Fusion Reagent Set (pENTRY-IBA20 (5 rxns), Downstream Fusion Vector pCFUSE-IBA1 (5 rxns), StarSolutions F1 to F6 (for 5 fusions)) - Upstream Fusion Vector pNFUSE-IBA-LINK1 (5 rxns) - Competent <i>E. coli</i> TOP10 cells (20 rxns)
Transfer reaction	
5-1603-001	StarGate® Transfer Reagent Set consisting of StarSolutions A1, A2, A3 (20 rxns)
5-1600-020	Competent <i>E. coli</i> TOP10 cells (20 rxns)
Choose catalog number from "Acceptor Vector Overview Table" on page 7	Acceptor Vectors The more vectors you select, the higher the discount (5 rxns each).

Note: Upstream Fusion Cloning Vectors are also available separately on request. See price list for Acceptor Vector sequencing primers.

StarGate® Newcomer Set: 50% Discount!

For StarGate® newcomers! Please enjoy a 50% discount for the StarGate® Newcomer Set which provides all products required to perform the entire StarGate® procedure. Please choose number and type of Acceptor Vectors according to your needs and order them separately.

Cat. no.	StarGate® product required to get started	Discount
5-1600-998	StarGate® Newcomer Set consisting of - StarGate® Entry Reagent Set - StarGate® Transfer Reagent Set - Competent <i>E. coli</i> Top10 cells (20 rxns) - Control for Entry and Transfer reaction	50% compared to individual set prices
Choose catalog number from "Acceptor Vector Overview Table" on page 7	Acceptor Vectors The more vectors you select, the higher the discount (5 rxns each).	up to 50%

StarGate® Acceptor Vector Overview Table

IBA extended its selection of StarGate® Acceptor Vectors with several new single and double-tag vector versions for *E. coli*, yeast, mammalia and insect cells.

FLAG®-tag **NEW!**

FLAG®-tag is now available individually or in combination with other tags. The FLAG®/One-STREP-tag tandem versions with two tags at one terminus are especially suited for protein:protein interaction studies.

Vectors suitable for *in vitro* Translation

For bacterial *in vitro* translation, the vectors pPSG-IBA are available with several tag combinations.

Episomal Mammalian Expression **NEW!**

The new pCSG-IBA vectors are suited for episomal plasmid propagation and stable expression of the recombinant protein under geneticin (G418) selection.

Name assembly: e.g. **pASG- IBAwt1**
(host/promoter) (expression cassette)

Host Promoter	<i>E. coli</i> Tet	<i>E. coli</i> T7	Mammalia CMV	Mammalia CMV	Insect cells Polyhedrin	Yeast CUP1		Signal sequence	N-terminal tag	CS*	Gene of Interest	CS*	C-terminal tag
	pASG-	pPSG-	pESG-	pCSG-	pLSG-	pYSG-					GOI		
5-4000-005							IBAwt1						
5-4005-005							IBA5						
5-4105-005							IBA105						
5-4035-005							IBA35						
5-4025-005							IBA25						
5-4003-005							IBA3						
5-4103-005							IBA103						
5-4033-005							IBA33						
5-4045-005							IBA45						
5-4145-005							IBA145						
5-4043-005							IBA43						
5-4143-005							IBA143						
5-4023-005							IBA23						
5-4123-005							IBA123						
5-4065-005							IBA65						
5-4063-005							IBA63						
5-4062-005							IBA62						
5-4162-005							IBA162						
5-4064-005							IBA64						
5-4164-005							IBA164						
5-4167-005							IBA167						
5-4168-005							IBA168						
5-4001-005							IBAwt2						
5-4004-005							IBA4						
5-4104-005							IBA104						
5-4002-005							IBA2						
5-4102-005							IBA102						
5-4044-005							IBA44						
5-4144-005							IBA144						
							IBA142						

signal sequence:	OmpA	BM40	BM40	BM40
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- FLAG-tag
- Strep-tag II
- One-STREP-tag
- 6xHistidine-tag
- GST-tag with PreScission™ (PSC) site

The list of vectors is constantly expanded. See www.stargate-cloning.com for the most up-to-date version.

IBA, IBA BioTAGnology, Strep-tag, StarGate, StarCombinase and TAGnologies are registered trademarks of IBA GmbH. FLAG is a registered trademark of Sigma-Aldrich Co.

Intellectual property rights for the GST-tag and for PreScission protease are owned by GE Healthcare. Refer to GE Healthcare AB, Sweden (www.ge.com), for purchasing reagents for further use of GST.

Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

Products are for research use only.

*CS signifies "combinatorial site"

StarGate® - a Combinatorial Cloning System

Standard Entry reaction

StarGate Standard Entry Cloning Set

see pages 2 and 6



Mutagenesis Entry reaction

StarGate Mutagenesis Entry Cloning Set

see page 6 and www.stargate-cloning.com



Donor Vector

Fusion Cloning

StarGate Fusion Cloning Set

see pages 4 and 6



Acceptor Vectors

A1

A2

A3

An

Destination Vectors

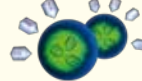
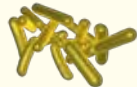
D1

D2

D3

Dn

Different hosts



Transfer reaction

StarGate Transfer Reagent Set

Competent *E. coli* TOP10 cells

Acceptor Vectors of choice

see pages 2, 6 and 7

