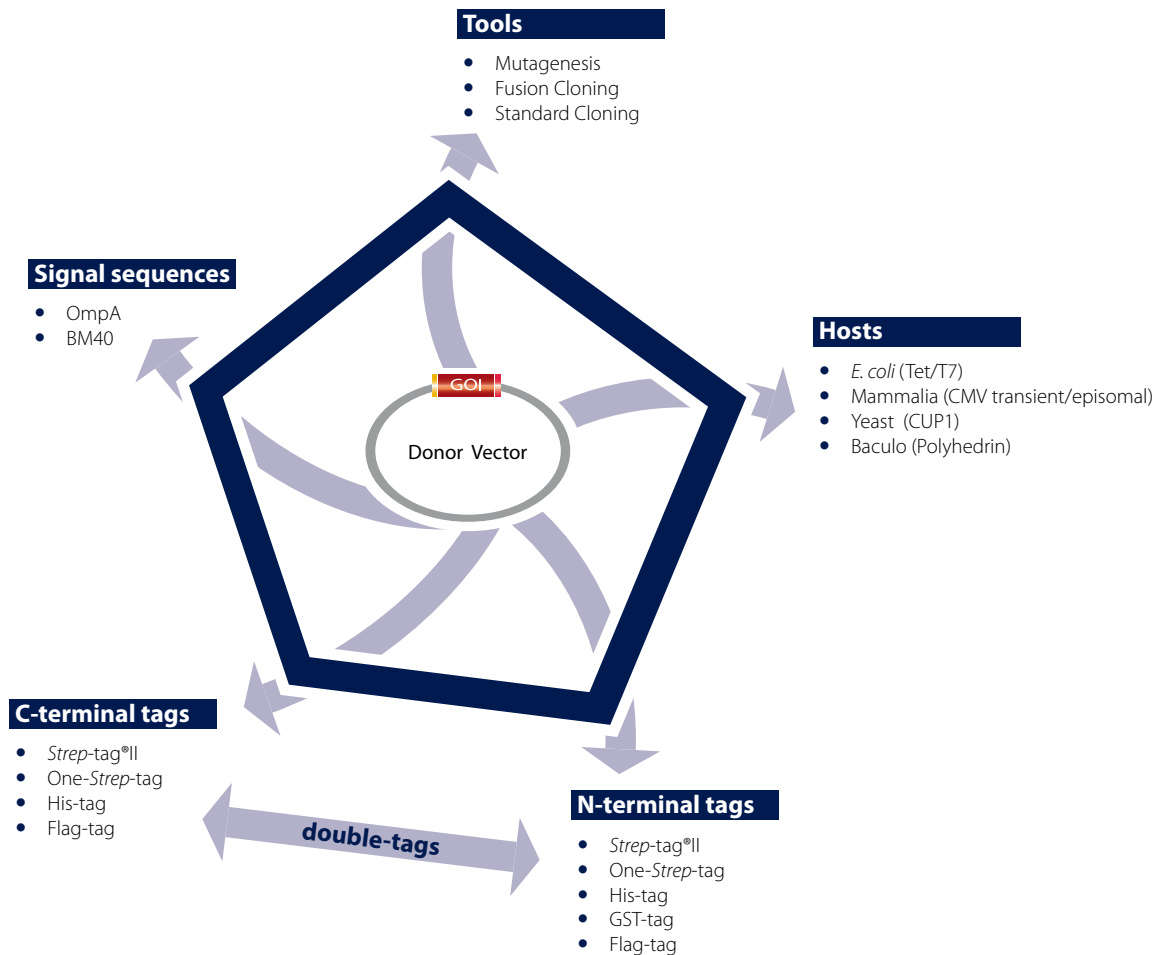


StarGate[®] for fast protein expression cloning

Combine optimal expression elements for best protein yields



StarGate provides a simple cloning procedure for a multitude of expression vectors

In this Issue :

NEW Combi Entry Cloning

– improved high efficiency entry cloning step



StarGate® protein expression cloning system

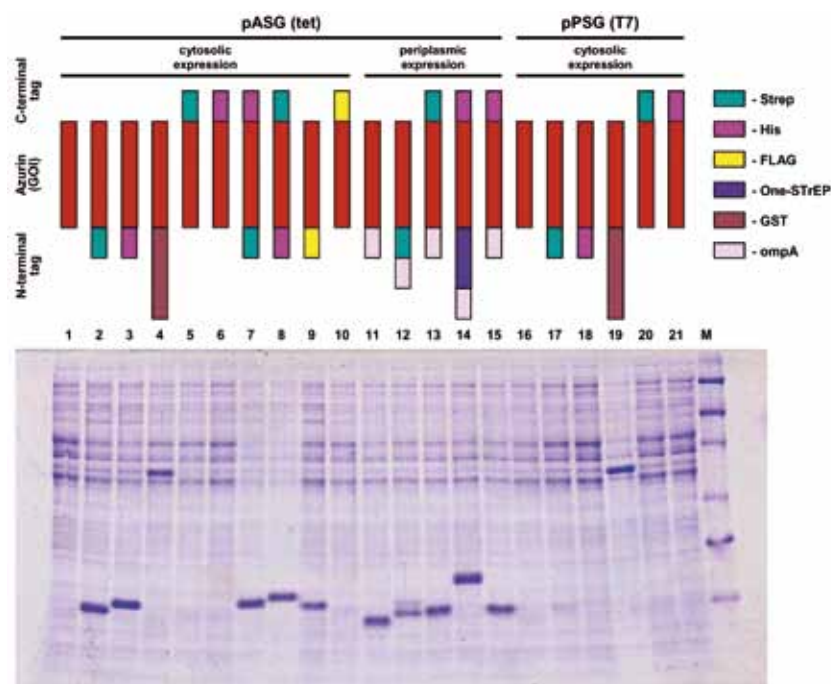
- time saving, easy to handle and efficient one-tube cloning

Due to the diverse nature of proteins the requirements for the optimal expression conditions have to be evaluated for each protein individually. The most important features in this process are the expression host, the promoter and the affinity tags.

The expression example (right) of the 14kDa protein (Azurin) illustrates the importance of finding the right expression features (nature of the tag its position and the promoter).

Don't waste your time with inappropriate expression features!

Screening for the best expression features in the beginning can save a lot of time for the complete protein expression project later on.



With Stargate we developed a cloning system which allows a fast and convenient screening for the optimal expression features. Rapid and standardized sub-cloning of an arbitrary gene into a wide collection of expression vectors (Acceptor Vectors) is enabled without time consuming planning.

StarGate: the cutting-edge cloning technology for protein expression

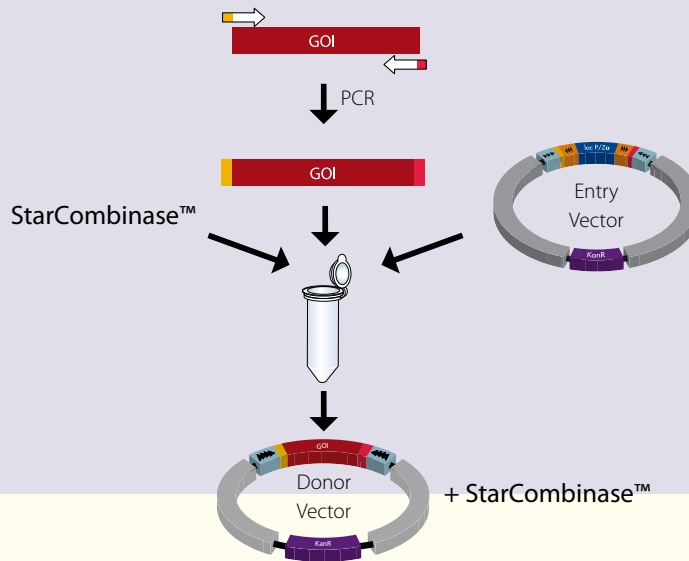
- Optimize the first step – **don't waste time later on with inappropriate features**
- Saves time – **no planning of cloning strategies or complicated primer design**
- Systematic portfolio of expression vectors – **allows direct comparison of different features**
- Flexible gene fusion tool – **combine your genes in any (variable) order**
- StarPrimer D'Signer software – **is doing the work for you**
- Site-directed mutation – **very easy with StarPrimer D'Signer software**

StarGate® overview

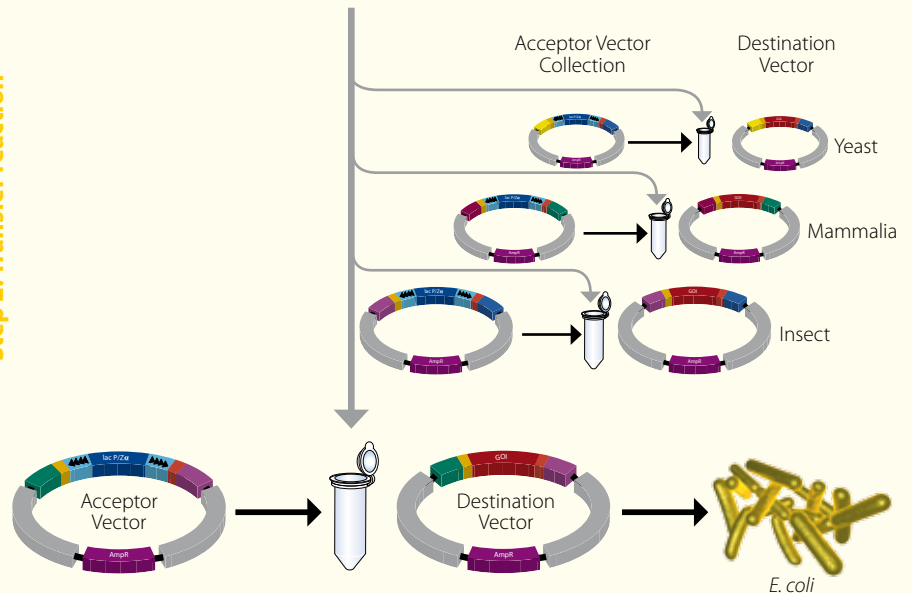
Easy cloning procedure:

- ▶ Design the primers with the free StarPrimerD'Signer software
- ▶ One-tube cloning of the PCR product to generate the Donor Vector

Step 1: Entry reaction



Step 2: Transfer reaction



- ▶ Generate the Destination Vector by mixing the Donor Vector with the selected Acceptor Vector(s) (overview page 6)

Key advantages

- Extremely **short combinatorial sites** have minimal effect on the gene of interest* (GOI)
- **High level cloning efficiency** due to a directed reaction (no equilibrium)
- Fast **one tube reaction**
- Multitude of expression vectors **for four different expression hosts** available

NEW StarGate Combi Entry Cloning

with improved high efficiency Entry Cloning Step

Provides the combination of Standard and Mutagenesis Entry Cloning in one Set

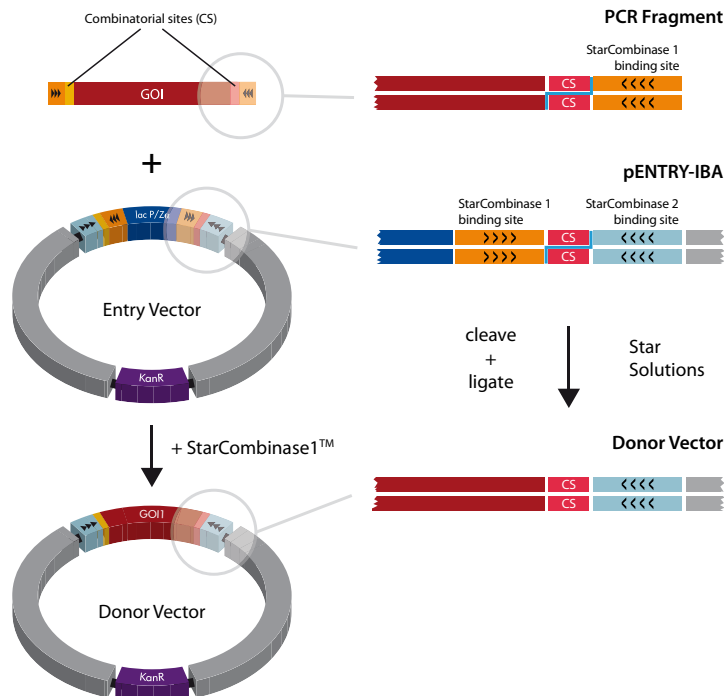
Step 1a: Donor Vector generation

The gene of interest (GOI) has to be equipped in a PCR-step at both termini with **combinatorial sites** (CS) and a recognition area for StarCombinase1™ which are important for oriented insertion of the PCR fragment into the Entry Vector.

Recombination of the PCR product and the Entry Vector at the combinatorial sites (red and orange) leads to generation of the Donor Vector under loss of all StarCombinase1™ binding areas (dark orange with arrowheads) making the recombination reaction unidirectional and thereby highly efficient.

In the resulting Donor Vector the same combinatorial sites are now under control of StarCombinase2™ (light blue) enabling the specific StarGate gene transfer into Acceptor Vectors in a similar manner.

Standard Cloning



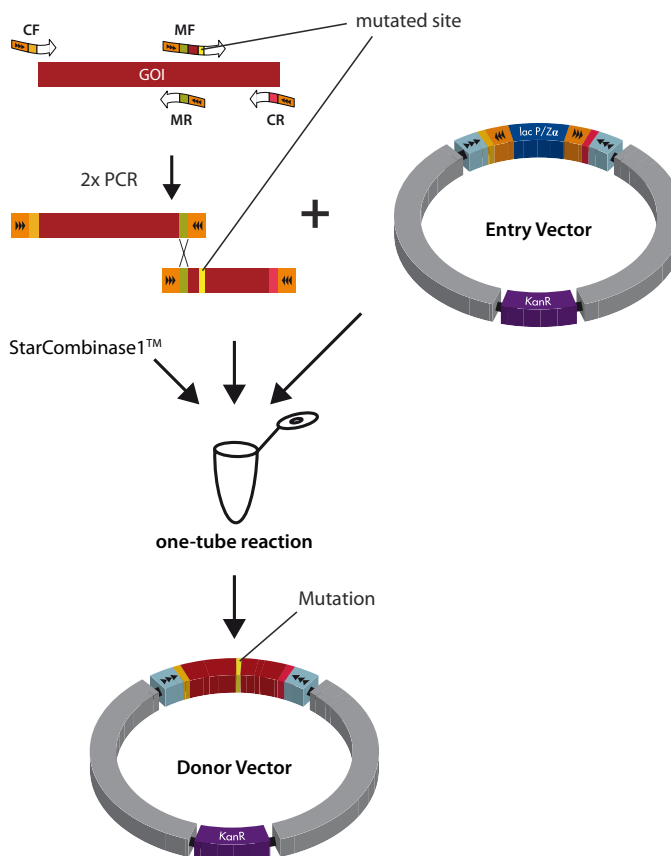
Step 1b (optional):

Donor Vector with mutated GOI

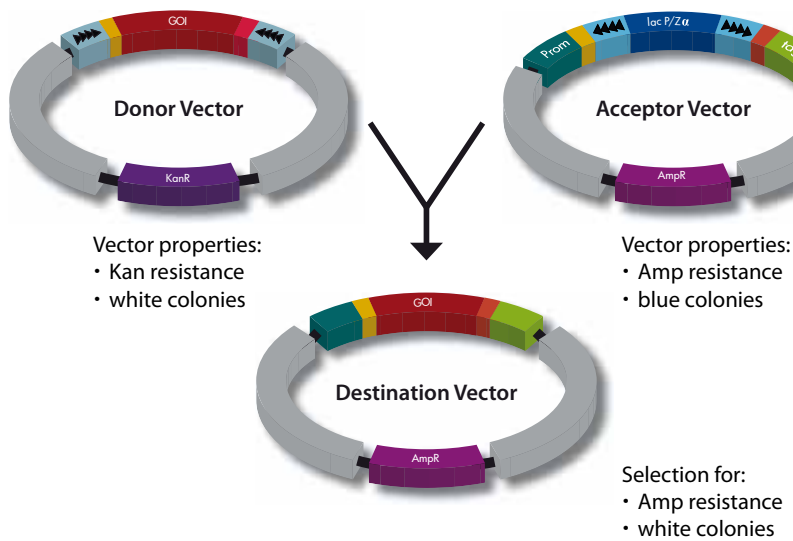
For site-directed mutagenesis of the gene of interest two additional internal primers are required (MF/MR) introducing the base changes (yellow).

Primer design is achieved by the free StarPrimer D`Signer software. Accordingly mutated gene fragments are generated by PCR and then assembled in a one-tube reaction in the Donor Vector.

Mutagenesis Cloning



StarGate® transfer reaction



Step2:

Gene transfer into Acceptor Vector

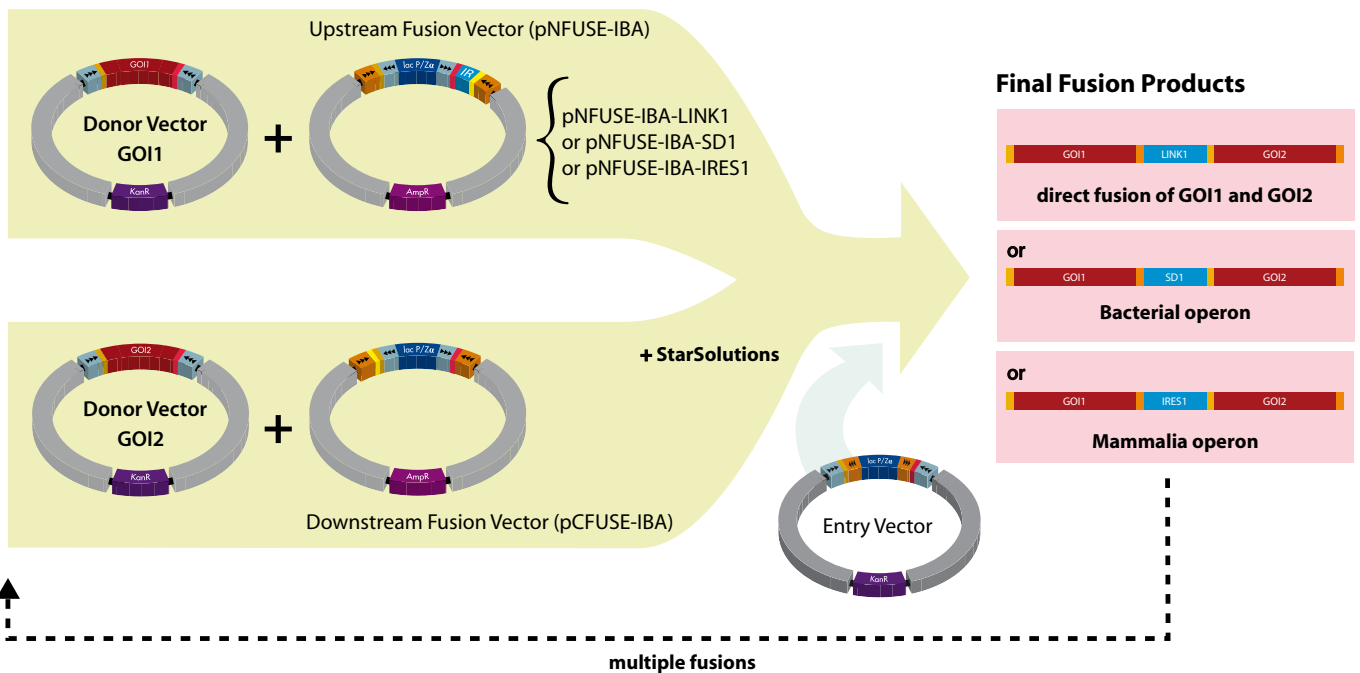
In the second step, the GOI may be transferred from the Donor Vector into a variety of Acceptor Vectors providing the desired genetic surroundings (i.e. tag, promoter (Prom), signal sequence etc.) by means of StarCombinase2.

The resulting Destination Vector of this example places the GOI under control of the CMV promoter allowing GOI expression in mammalian cells. In addition, a tag is fused to the C-terminal end of the GOI expression product.

StarGate® Fusion Cloning

Fuse genes from any Donor Vector in any combination to make single fusion proteins or even to construct artificial operons for multiple gene expression in bacterial or mammalian cells.

The StarGate Fusion Cloning system allows easy and fast fusion of two different genes of interest (GOI-1 and GOI-2) by an intergenic region (IR). Only **two sequential StarGate® subcloning reactions** with dedicated Fusion Vectors are required. The procedure can be repeated to fuse more than two GOI's.



StarPrimer D'Signer NEW Edition

- Let it work for you !



The FREE StarPrimer D'Signer software allows the convenient design of the appropriate primers for Standard as well as for Mutagenesis cloning by simply copying the gene sequence(s) in the corresponding windows. Additionally it guides you through the entire PCR procedure.

www.stargate-cloning.com

Acceptor Vector Collection

Acceptor Vectors for four different hosts with five different tags in various single-tag and double-tag versions are currently available.

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

Host	<i>E. coli</i>	<i>E. coli</i>	Mammalia	Mammalia	Insect cells	Yeast		Signal sequence	N-terminal tag	CS*	Gene of Interest	CS*	C-terminal tag
Promoter	Tet	T7	CMV	CMV	Polyhedrin	CUP1					GOI		
	pASG-	pPSG-	pESG-	pCSG-	pLSG-	pYSG-							
5-4000-005	5-4200-005	5-4400-005	5-5000-005	5-4800-005	5-4600-005	IBAwt1							
5-4005-005	5-4205-005	5-4405-005	5-5005-005	5-4805-005	5-4605-005	IBA5							
5-4105-005	5-4305-005	5-4505-005	5-5105-005	5-4905-005	5-4705-005	IBA105							
5-4035-005	5-4235-005	5-4435-005	5-5035-005	5-4835-005	5-4635-005	IBA35							
5-4025-005	5-4225-005	5-4425-005	5-5025-005	5-4825-005	5-4625-005	IBA25							
5-4003-005	5-4203-005	5-4403-005	5-5003-005	5-4803-005	5-4603-005	IBA3							
5-4103-005	5-4303-005	5-4503-005	5-5103-005	5-4903-005	5-4703-005	IBA103							
5-4033-005	5-4233-005	5-4433-005	5-5033-005	5-4833-005	5-4633-005	IBA33							
5-4045-005	5-4245-005	5-4445-005	5-5045-005	5-4845-005	5-4645-005	IBA45							
5-4145-005	5-4345-005	5-4545-005	5-5145-005	5-4945-005	5-4745-005	IBA145							
5-4043-005	5-4243-005	5-4443-005	5-5043-005	5-4843-005	5-4643-005	IBA43							
5-4143-005	5-4343-005	5-4543-005	5-5143-005	5-4943-005	5-4743-005	IBA143							
5-4023-005	5-4223-005	5-4423-005	5-5023-005	5-4823-005	5-4623-005	IBA23							
5-4123-005	5-4323-005	5-4523-005	5-5123-005	5-4923-005	5-4723-005	IBA123							
5-4065-005	5-4265-005	5-4465-005	5-5065-005	5-4865-005	5-4665-005	IBA65							
5-4063-005	5-4263-005	5-4463-005	5-5063-005	5-4863-005	5-4663-005	IBA63							
5-4062-005	5-4262-005	5-4462-005	5-5062-005	5-4862-005	5-4662-005	IBA62							
5-4162-005	5-4362-005	5-4562-005	5-5162-005	5-4962-005	5-4762-005	IBA162							
5-4064-005	5-4264-005	5-4464-005	5-5064-005	5-4864-005	5-4664-005	IBA64							
5-4164-005	5-4364-005	5-4564-005	5-5164-005	5-4964-005	5-4764-005	IBA164							
5-4167-005	5-4367-005	5-4567-005	5-5167-005	5-4967-005	5-4767-005	IBA167							
5-4168-005	5-4368-005	5-4568-005	5-5168-005	5-4968-005	5-4768-005	IBA168							
5-4001-005		5-4401-005	5-5001-005	5-4801-005		IBAwt2							
5-4004-005						IBA4							
5-4104-005			5-4504-005	5-5104-005	5-4904-005	IBA104							
5-4002-005						IBA2							
5-4102-005			5-4502-005	5-5102-005	5-4902-005	IBA102							
5-4044-005						IBA44							
5-4144-005			5-4544-005	5-5144-005	5-4944-005	IBA144							
			5-4542-005	5-5142-005	5-4942-005	IBA142							

signal sequence: OmpA

BM40 BM40 BM40

- FLAG-tag
- Strep-tag II
- One-STREP-tag
- 6xHistidine-tag
- GST-tag with PreScission™ (PSC) site

*CS signifies "combinatorial site"

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

Trademark information

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StarGate® Products

To perform the complete StarGate Cloning procedure the new Combi Entry Cloning Set and the products listed under Transfer reaction are required.

For the optional intermediate Fusion Cloning step one of the Fusion Cloning Sets is required.

Cat. no.	Name
Combi Entry Cloning	
5-1608-000 NEW!	StarGate® Combi Entry Cloning Set - StarGate® Combi Entry Reagent Set (Entry Vector pENTRY-IBA51 (20 rxns); StarSolution M1 - M3 (20 rxns); For/Rev Sequencing Primer; DNA Ruler) - Competent <i>E. coli</i> TOP10 cells (20 rxns)
Fusion Cloning	
5-1607-001	StarGate® Fusion Cloning Set "IRES1" (Internal ribosomal entry site (IRES) for polycistronic gene expression) - StarGate® Fusion Reagent Set (pENTRY-IBA51 (5 rxns), Downstream Fusion Vector pCFUSE-IBA11 (5 rxns), StarSolutions F1 to F6 (for 5 fusions) - Upstream Fusion Vector pNFUSE-IBA-IRES11 (5 rxns) - Competent <i>E. coli</i> TOP10 cells (15 rxns)
5-1607-002	StarGate® Fusion Cloning Set "SD1" (Shine-Dalgarno (SD) sequence for artificial operons) - StarGate® Fusion Reagent Set (pENTRY-IBA51 (5 rxns), Downstream Fusion Vector pCFUSE-IBA11 (5 rxns), StarSolutions F1 to F6 (for 5 fusions) - Upstream Fusion Vector pNFUSE-IBA-SD11 (5 rxns) - Competent <i>E. coli</i> TOP10 cells (15 rxns)
5-1607-003	StarGate® Fusion Cloning Set "LINK1" (GlySerGlyGlySerGlyGlySer) - StarGate® Fusion Reagent Set (pENTRY-IBA51 (5 rxns), Downstream Fusion Vector pCFUSE-IBA11 (5 rxns), StarSolutions F1 to F6 (for 5 fusions) - Upstream Fusion Vector pNFUSE-IBA-LINK11 (5 rxns) - Competent <i>E. coli</i> TOP10 cells (15 rxns)
5-1607-004 NEW!	StarGate® Fusion Cloning Set "LINK2" (GlySerGlyGlySerGlyGlySerGlyGlySer) - StarGate® Fusion Reagent Set (pENTRY-IBA51 (5 rxns), Downstream Fusion Vector pCFUSE-IBA11 (5 rxns), StarSolutions F1 to F6 (for 5 fusions) - Upstream Fusion Vector pNFUSE-IBA-LINK12 (5 rxns) - Competent <i>E. coli</i> TOP10 cells (15 rxns)
Transfer Cloning	
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 6	Acceptor Vectors (5rxns each) The more vectors you select, the higher the discount

StarGate Newcomer Set

For StarGate Newcomers! Save 45% on the individual set prices with the StarGate Combi Newcomer Set providing all products to perform the entire StarGate procedure.

Cat. no.	Newcomer Set	Discount
5-1600-996 NEW!	StarGate Combi Newcomer Set consisting of: - StarGate Combi Entry Reagent Set - StarGate Transfer Reagent Set - Competent <i>E. coli</i> Top10 cells - Control Reactions (PCR-fragment and Donor Vector)	45% compared to individual set prices
Choose catalog numbers from the "Acceptor Vector Overview Table" on page 6	Acceptor Vectors The more Vectors select the higher the discount (5rxns each)	up to 50%

StarGate® Components

Components supplied by IBA:

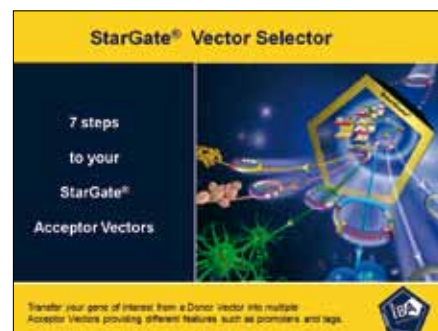
- Entry Vector pENTRY-IBA
- Forward and reverse sequencing primers
- Competent *E. coli* Top10 cells
- Individual custom primer synthesis for amplification of your GOI
- Acceptor Vectors:
pASG (*E. coli*, Tet promoter),
pPSG (*E. coli*, T7 promoter),
pESG / pCSG (Mammalia, CMV promoter),
pYSG (Yeast, 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 Amp agar plates and LB Kan agar plates with X-Gal

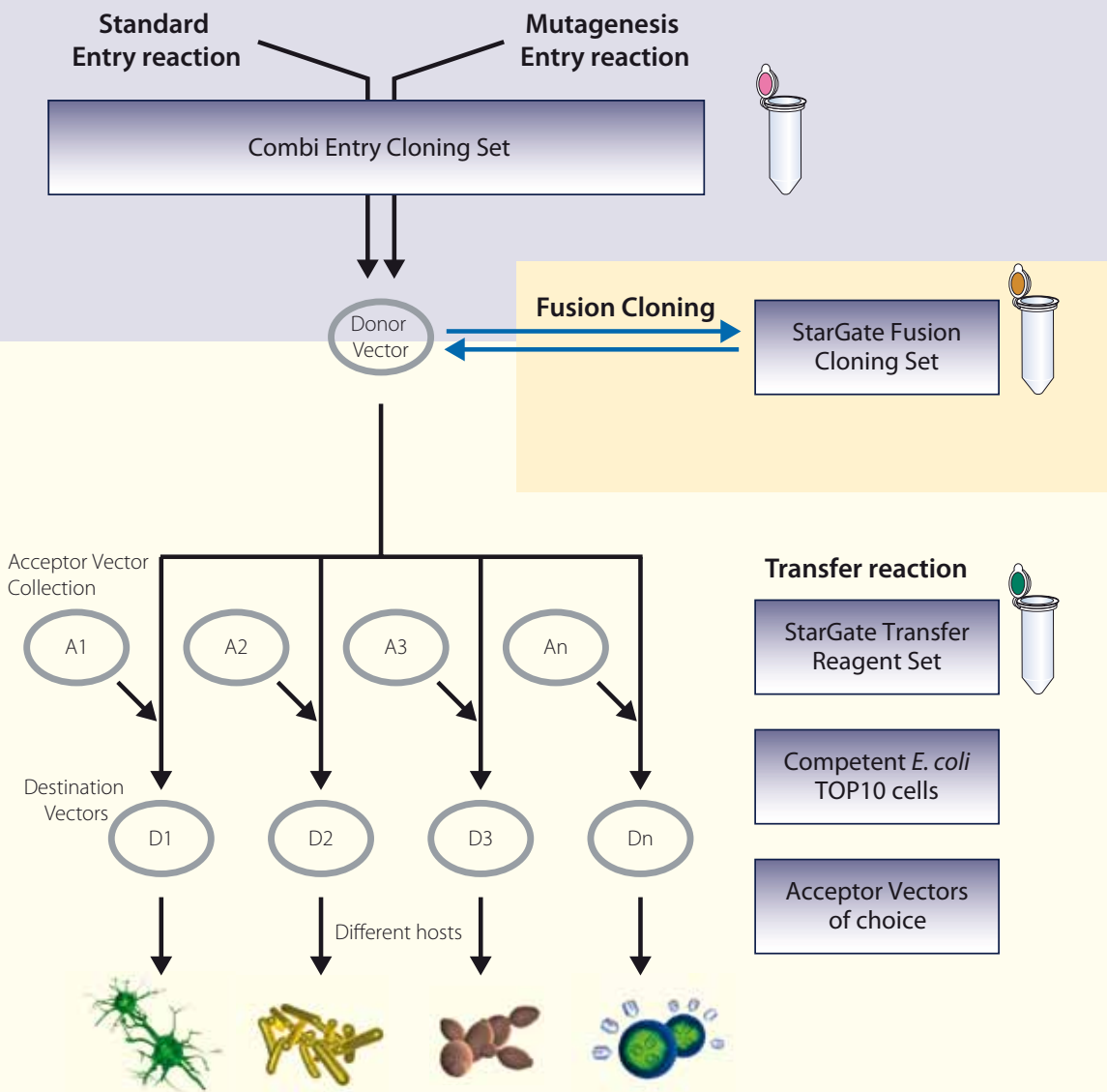
High quality, HPLC purified and PTO protected **custom cloning primers for StarGate®** can be obtained at the IBA Gene TAGnologies department; refer to www.iba-bioTAGnology.com.

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



The StarGate® Vector Selector allows the fast search of Acceptor Vectors based on the required features. See www.stargate-cloning.com.

StarGate® - a Fast and Flexible Expression Cloning System



IBA web shop for convenient ordering!



www.iba-shop.com

IBA is represented by a worldwide network of distributors

Please select your local distributor from our website:
www.iba-bioTAGnology.com/distributors.html



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 You can reach us from 8 am - 10 pm (CET)

Request further information as well as the complete IBA Portfolio Folder at www.stargate-cloning.com and subscribe to our e-newsletter