FAQ *StarGate®*

Frequently asked questions concerning *StarGate®*

(A) StarGate® technology

(1) What is StarGate®?

StarGate offers rapid and highly efficient subcloning of an arbitrary gene – initially cloned into a Donor Vector - to simultaneously combine it with many different genetic surroundings via transfer into Acceptor Vectors to generate Destination Vectors. The latter enable the efficient expression of your protein with various features (e.g. different tags and different promoters) in different hosts.

(2) For which hosts can the StarGate® system be used?

StarGate allows the expression of proteins in E. coli, mammalian, yeast and insect cells.

(3) Which genetic elements can be combined in the StarGate® system?

StarGate offers a multitude of genetic elements, including promoters for E.coli (T7 and Tet), yeast (CUP1), mammalia (CMV) and baculovirus (Polyhedrin) as well as N- and C-terminal Strep-, One-STrEP-, 6xHistidine-, GST- and FLAG-tag. For E. coli, mammalian and baculovirus vectors secretion signal sequences are also available.

(4) What is the genetic principle of combinatorial cloning using the StarGate® system?

The Donor Vector and Acceptor Vectors comprise combinatorial sites as well as corresponding recognition sites which enable the StarCombinaseTM to cut and relegate both vectors to create the desired Destination Vector.

(5) I have the combinatorial site sequence aatg several times in my gene of interest, can I use StarGate®?

Yes, you can use StarGate because the Donor Vector contributes the recognition sites and brings them into operative linkage with the combinatorial sites for the highly specific StarGate gene transfer process.

(6) What is the principle of the selection and transformation process of the Donor Vector and the Destination Vectors?

Transformation is performed using competent E. coli Top10 cells which are included in the Standard Entry Cloning Set. The reaction efficiency of the blue/white selection process is far above 99% due to a directed reaction during the ligation. For the Donor Vectors a kanamycin selection, for the Destination Vectors an ampicillin selection is applied.

(7) What do I need to start with StarGate®?

The StarGate Newcomer Set consists of the Standard Entry Cloning Set and the Transfer Reagent Set (each 20 rxns), which in combination with the appropriate Acceptor Vector(s) allow the optimal start with StarGate. The Entry Set generates up to 20 different Donor Vectors (each comprising one of your genes of interest) and with the Transfer Reagent Set in combination with the selected Acceptor Vector(s) one has the opportunity to transfer the genes in parallel in these Acceptor Vectors to obtain Destination Vectors for protein expression.

(8) Is it possible to adapt a commercial expression vector to the StarGate system ?

Yes, IBA offers a custom cloning, expression and purification service for StarGate. This covers e.g. simply the generation of a desired Donor Vector, the adaptation of a commercial vector to a StarGate Acceptor Vector or the covering of the entire process of protein production.

(9) Is there a site directed mutagenesis tool available for StarGate?

Yes, StarGate offers a Mutagenesis Kit allowing site directed mutation of your GOI.

(10) Is there a fusion cloning tool available for StarGate® to combine multiple genes from different Donor Vectors?

Yes, the StarGate Fusion Cloning system allows the fusion of two genes from two Donor Vectors by means of special Fusion Vectors as well as the bicistronic expression of genes by providing ribosomal binding sites (Shine-Dalgarno or IRES).

(11) How many genes can be combined with the StarGate® Fusion Cloning System?

There is no technical limitation known concerning the number of genes that can be combined. Up to 6.5 kb fragments have been transformed so far.

(12) How can I determine the amount of PCR product for the Donor Vector ligation?

The easiest way is to quantify the PCR product via gel electrophoresis through band intensity comparison with the DNA Ruler included in the set.

(13) How can I verify the sequence of the generated PCR product?

Sequence the inserted PCR product with the included forward/reverse sequencing primers for the Donor Vector.

(14) Is StarGate® compatible with the Gateway® system?

No, the StarGate and Gateway systems deal with different combinatorial sites based on completely different recombinatorial systems. Finally, the StarGate Destination Vector comprises 4 additional bases at the 5' and 3' end of your GOI (aatg /ggga), whereas Gateway comprises different additional bases.

(B) Gene expression in E. coli

(15) What is the strength of the tet promoter?

The strength of the tet promoter is comparable to the lacUV5 promoter.

(16) Do the pASG-IBA vectors confer resistance against tetracycline?

No. The pASG-IBA vectors contain the tet repressor and the tet promoter only (anhydrotetracycline inducible promoter), the tetracycline resistance gene is not encoded. Please note: If tetracycline resistant strains are used or vectors with a tetracycline resistance marker you will induce the protein expression by growing them under selective conditions in the presence of tetracycline.

(17) Which E. coli strain do you recommend?

The pASG-IBA vectors are compatible with virtually any E. coli hosts like XL1-Blue, BL21, DH5a, MG1655, W3110, JM83, WK6 and E. coli B. This is possible because the tet promoter is independent from the genetic background of E. coli.

(18) Are there special culture media to be used for expression?

No. No catabolite repression exists for the tet promoter which makes even the use of glucose containing culture media possible.

(19) What is the function of the ompA signal peptide?

The ompA signal peptide directs the fusion protein to the periplasm of E. coli where it is cleaved off.

(20) Is it possible to express recombinant proteins with disulfide bonds in E. coli?

Yes. The oxidizing conditions in the periplasmic space lead to disulfide bond formation of proteins containing cysteins. Therefore, some of our vectors provide a N-terminal fusion of the ompA signal peptide, which mediates the secretion of recombinant proteins to the periplasm.

(21) How is expression induced in pASG-IBA vectors?

Standard induction is performed by the addition of 200 µg anhydrotetracycline per liter E.coli culture to ensure full induction. This concentration is not antibiotically effective.

(22) What expression level can be expected in pASG-IBA containing E. coli strains?

Up to 100 mg per litre E. coli culture. Please note, though, that the expression level strongly depends on the properties (e.g. folding kinetics; thermodynamic stability) of the recombinant protein itself.

(C) Gene expression in Mammalia

(23) What is the difference between pESG-IBA and pCSG-IBA vectors?

Whereas pESG-IBA vectors resist transiently in the mammalian cells after tranfection, pCSG-IBA vectors comprise an origin of replication for mammalian cells. Thus, due to episomal vector replication pCSG-IBA vectors remain even in proliferating cells allowing longer cultivation times and consequently higher protein yields.

(24) Which mammalian cell line do you recommend?

The pESG-IBA vectors are compatible with the following cell lines: Human: HeLa, Bowes Melanoma, HEK-293, IVEC, HBEC-90, CHU-2; Monkey: Cos-7; Chick: CEF; Rat: REMC, C6, Mouse: NIH-3T3, CV1, TR2, L929, PU5-1.8, LTK, N2A, 33.1.1, K46, J558L, IC11. In addition, many others should also be possible. The pCSG vectors are compatible with human, primate and canine cells. 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).

(25) How is expression induced in pESG-IBA and pCSG-IBA vectors?

The CMV promoter needs no induction since CMV is a strong promoter for high-level, constitutive expression.

(26) Is it possible to secrete recombinant proteins into the culture medium?

Yes. Some of our vectors provide a N-terminal fusion of the BM40 signal peptide, which mediates the secretion of recombinant proteins to the culture medium.

(27) What expression level can be expected in pESG-IBA and pCSG-IBA containing

mammalian cell lines? pESG-IBA and pCSG-IBA vectors allow high-level expression of heterologous proteins in many mammalian cell lines. Their expression level, though, strongly depends on the properties (e.g. folding kinetics; thermodynamic stability) of the recombinant protein itself.

(D) Gene expression in Yeast

(28) Which yeast strain do you recommend?

The pYSG-IBA vectors are compatible with the following strains: DY150, DBY745, DBY747, K2346, while other ura, leu deficient strains should also be possible. Please note, that ura selection should be performed for transformation, while leu selection should be performed for expression.

(29) How is expression induced in pYSG-IBA vectors?

Standard induction for the CUP1 promoter is performed using a final concentration of 0.5 mM copper sulphate.

(30) Is it possible to secrete recombinant proteins into the culture medium?

No. All of our yeast vectors are designed for cytosolic protein production, only. Please note, though, that the StarGate system include vectors for the secretion of recombinant protein in E. coli, mammalia cells and baculovirus.

(31) What expression level can be expected in pYSG-IBA containing yeast strains?

pYSG-IBA vectors allow high-level expression of heterologous proteins in yeast. Their expression level, though, strongly depends on the properties (e.g. folding kinetics; thermodynamic stability) of the recombinant protein itself.

(E) Gene expression in Baculovirus

(32) Which insect cell line do you recommend?

The pLSG-IBA vectors are compatible with all baculovirus expressing insect cell lines, e.g. Sf21, Sf9, Hi5.

(33) How is expression induced in pLSG-IBA vectors?

The polyhedrin promoter needs no induction since polyhedrin is a strong promoter for high-level, constitutive expression.

(34) Is it possible to secrete recombinant proteins into the culture medium?

Yes. Some of our vectors provide a N-terminal fusion of the BM40 signal peptide, which mediates the secretion of recombinant proteins to the culture medium. Please note: Insect cell media contains Biotin very often. To avoid the unspecific binding of the Biotin to Strep-Tactin you have to add certain amounts of Avidin (Table available).

(35) What expression level can be expected in pLSG-IBA containing insect cell lines?

Up to 500 mg per litre of culture. Please note, though, that the expression level strongly depends on the properties (e.g. folding kinetics; thermodynamic stability) of the recombinant protein itself.