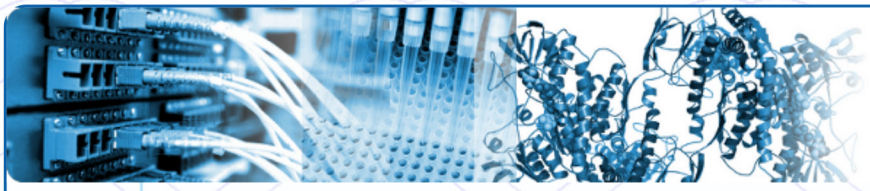


ATG:biosynthetics
Solutions
in biosynthetics

CODONEWS Issue45

14 December 2013

FlexTEC - pA0 - *basic vector*

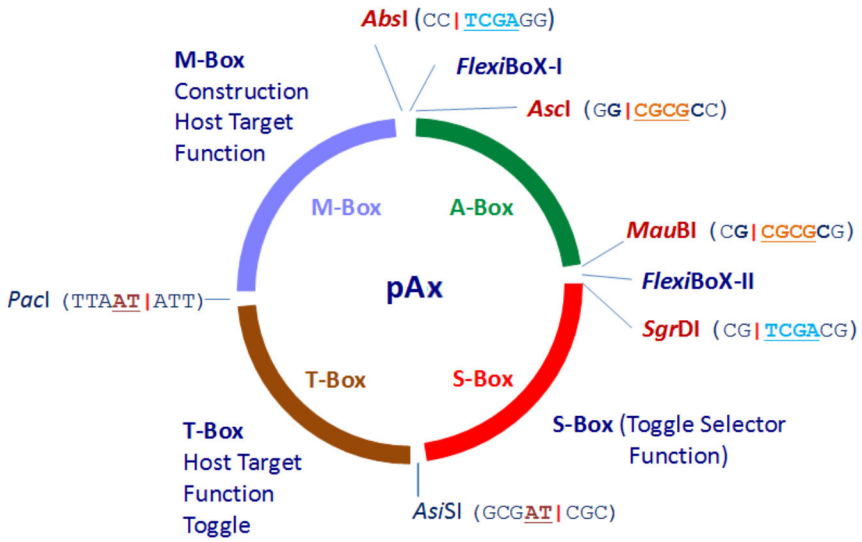
Introduction Offer: 50?* each**

INQUIRE/ORDER

* plus VAT and shipping cost. VAT only applies for German customers.

** **S-BoX**: available as Kanamycine, **KmR**-Chloramphenicol, **CmR** and Gentamicin, **GmR**

Synthetic genes and more ... regarding synthetic gene clusters today the most important question to be addressed is not the syntheses itself but the after synthesis handling. Therefore stringent formal and standardized frameworks for sequence handlings are required. For complex constructs the assembly strategy is still an issue. Addition and substitution of gene cluster functional sub-elements and the de-assembly of whole clusters for rearrangements are open questions to be addressed. **The pA0 - vector (no A-BoX-element) is the basis of the *Flex*TEC gene cluster - construction system.**



This vector is the most simple basis for all related vector constructions for the design of applied synthetic genetics. Is has only **S-BoX** and **M-BoX**-functions. In its basic accomplishment **S-BoX**:Kanamycin, Chloramphenicol and Gentamicin resistant marker genes are available. The **A-BoX** is reduced to the *Flex***BoX-I** and **-II** separated by the central ***BssH*II site**. Ligation of a **DONOR A-BoX** flanked by ***Asc*I** and ***MauB*I** sites into the **6**mer core ***BssH*I** sequence of **pA0** conserves these sites. For the addition of additional **A-BoX**-elements into the ***MauB*I** site of the **ACCEPTOR** the ligation fusion site leaves a ***BssH*II** site and the ***MauB*I** site is regenerated for further addition ligations. Any new insert **A-BoX**-element is needed to be designed for restoring the core-**A-BoX**-element of **pA0** with flanking *Asc*I and ***MauB*I**-sites if cloned into ***BssH*II**. Only if the systematic and logic of the *Flex***TEC** construction system is carefully maintained the advantageous sequence features can be exploited which allow the recovery of reproducible compatible sequence elements not only in the **pBoX**-vectors but in addition between individuals of a community working taking advantage of the system. An additional substitution design allows exchange of core CDS and inter-gene regulatory regions.

Related Products available at ATG:biosynthetics:

*flex***Coli** ^{turbo}**-Kit** = basic highly flexible system for protein expression in ***E. coli* ... more**

*flex***BAC** ^{turbo}**-Kit** = **Baculovirus** based multi-gene expression system for **insect cells ... more**

*multi***Label-Kit** = multi-**gene expression** e.g. membrane proteins **in mammalian cells ... more**

FlexTEC for Constructive & Synthetic Biology

Assembly, **D**e-assembly, Substitution

The unique features of *flex***TEC** are based on the **pBoX-vector** system. This design fixes molecularfunctional positions to four main vector segments which are in a logical constructive order. The constructional backbone system is designed for the use of synthetic and mostly artificial sequence arrangements adaptable for any organism by use of specified molecular function. But moreover it is intended for collecting molecular building blocks which are of proved reproducible and robust functionality and in addition it allows standardization on the basis of a minimum of constructive elements.

The pA0-Vector

The **pA0T0** (**no A-BoX** and **no T-BoX- functional genetic elements**)**-vector is the core vector design for a highly flexible vector backbone**. This can be adapted easily for all purposes by use of the exchangeability of synthetic genes but also for complete de-assembly and recovery of sequence identical building blocks. The **A-BoX, S-BoX, T-BoX** and **M-BoXes** are the basis for highly flexible restriction ligation assembly system (see the **ATG:toggle assembly brochure**). **In addition the system can easily be adapted to recombination assembly and exo-nuclease assembly strategies**. It is designed for integrating specific functional genetic elements in each of the boxes. The order of genetic function is predetermined. After assembly the **A-BoX** contains gene clusters of genes with desired concerted function. The **S-BoX** is the selector box, the **T-BoX** can be equipped with multiple target host functions, like shuttle oris or recombination sites and the **M-BoX** is for DNA maintenance or expression in ***E. coli***.

The A-Box- Module for Gene Assembly and *multi*-Gene Expression

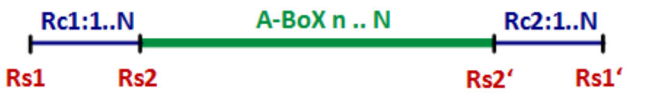


Figure-2-1. The *flex*BoXes R_{c1} and R_{c2} are *Abs*I(XhoI) - *Asc*I(*BssH*II) and *Mau*BI(*BssH*II) - *Sgr*DI(*Sal*I) freely designable intermediate sequences called ***flex*BoXes**. These are part of the **A-BoXes**. In these sequence elements construction relevant sequence motives can be placed like specific site directed, general recombination site sequences or homing nuclease sites for the transfer of whole clusters. In addition to simple cloning these can individually be adapted for recombination and exonuclease cloning or assembly strategies like toggle assembly. Sites can be restored by cloning ***Bss*HII**-fragments into **pA0**-vectors.

The **A-BoX** consists of three distinct regions marked by unique restriction sites with unique features:

1. *Flex***BoX-I**: definded by ***Abs*I**(XhoI)-***Asc*I**(*BssH*II)
2. *core* **A-BoX**: ***Asc*I**(*BssH*II)-***Mau*BI**(*BssH*II) also called **toggle-BoX**
3. *Flex***BoX-II**: definded by ***Mau*BI**(*BssH*II)-***Sgr*DI**(*Sal*I)

The **S-BoX** is physically delineated by the unique restriction sites ***Sgr*DI**(*Sal*I) and **AsiSI**(PvuI):

The **T-BoX** is separeated by **AsiSI**(PvuI) and **PacI**(-) and closing the vector cycle

The **M-BoX** is definded by **PacI**(-) and ***Abs*I**(XhoI)

Add1: The *Flex***BoX-I** and **-II** flanking the core **A-BoX** are designed for providing accessory molecular functionality for the **A-BoX** or **T-BoX** functions in terms of **CIS** and **TRANS** regulating, constructive elements or signals. In the formal example (see below) the *Flex***BoX-I** elements are a constructive homing nuclease site and the 5'-inverted repeat of **TN5**-transposase-integration site for recombination action. The *Flex***BoX-II** can be equipped for example with TRANS-regulator gene(s) or a reporter gene.
The **A-***core***-BoX** is designed for the integration of expression units of the desired application. The ligation of DNA with ***Asc*I/*Mau*BI** termini into **A-BoX**-box ***Mau*BI**-sites results in the fusions of ***Asc*I/*Mau*BI**-sites leaving just the **6**meric restriction core - function ***Bss*HII** to constructs of higher order. This integration can be designed for working by restriction-ligation strategies like Toggleassembly (see the ATG-toggle brochure) but in addition by using the flanking *Flex***BoX**es **-I** and **-II**. **In addition** the assembly system can be extended to **general recombination** and **exo-nuclease** by design. For this purpose the corresponding recombination or exo-nuclease target sequences of interest can be inserted into the *Flex***BoX**es.

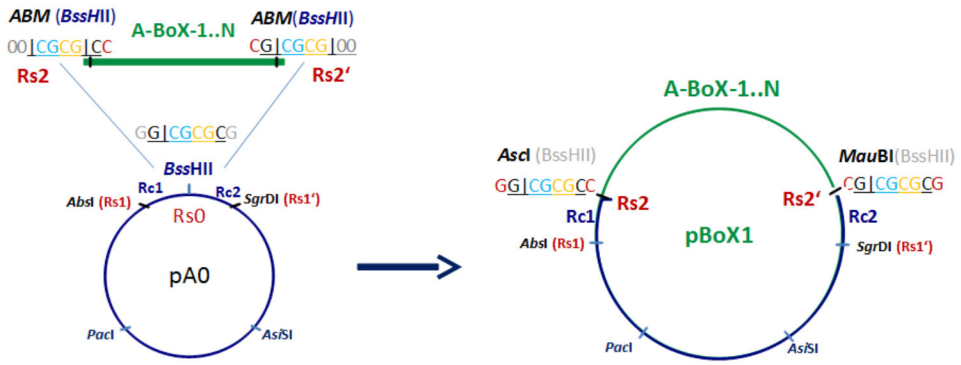


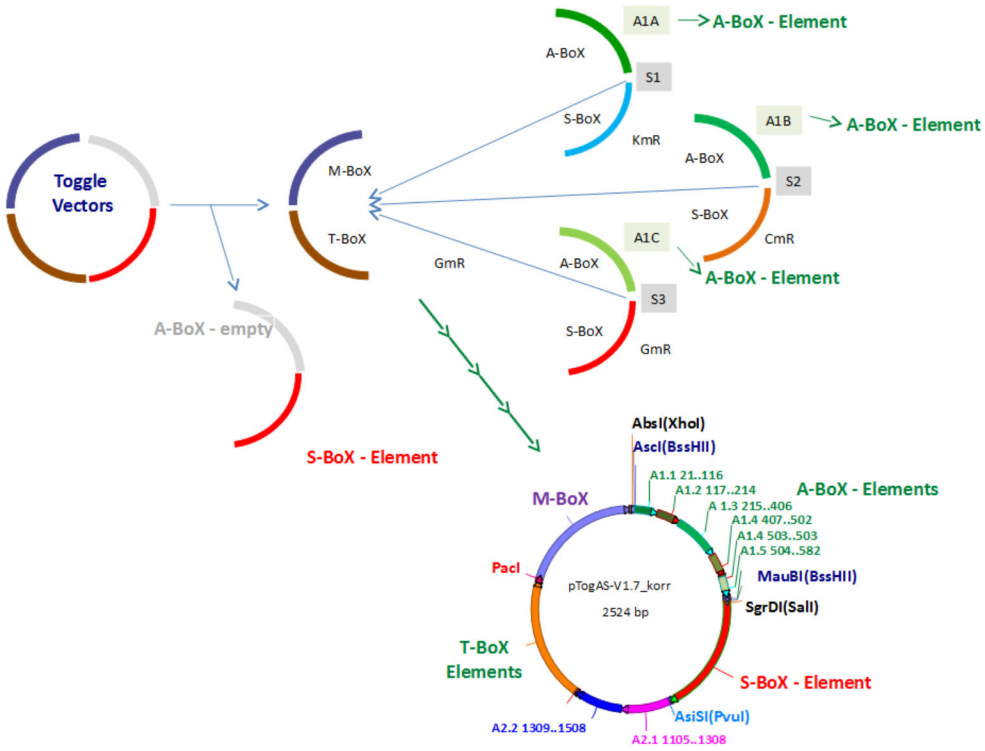
Figure-2-2. **The *flex*BoXes R^{c1} and R^{c2} are located between *Abs*I(XhoI) - *Asc*I(*Bss*HII)** and ***Mau*BI(*Bss*HII) - *Sgr*DI(*Sal*I)** respectively and are freely designable intermediate sequences. These are part of the **A-BoXes**. In these sequence elements construction relevant sequence motives can be placed like specific site directed, general recombination site sequences or homing nuclease sites for the transfer of whole clusters. In addition to simple cloning these can individually be adapted for **recombination** and **exonuclease cloning** or **assembly** strategies like toggle assembly. After recovery cleavage from multi-gene constructs with ***Bss*HII**-sites DNA-fragments can be sequence identically restored by cloning it into **pA0**-vectors.

Add2: The **S-BoX** selection box can be equipped with negative (antibiotic resistance) and positive selector genes (e.g. sacB) and extended towards multiple selector genes. This can be achieved by use of the compatible **AsiSI**- and **PacI-**sites using heterologous **5'-** and **3'**-termini of the insert fragments. This should result in the deletion of the **5'-** or **3'-** insertion **R**-site depending if the left or right orientation of the additional selector unit is determined for insertion.

Add3: The **T-BoX** transfer box is reserved for all functions which are for the mobilization, the transfer or the maintenance or all establishing heterologous gene constructs into the desired host system.

Add4: The **M-BoX** for maintenance of defined construction-DNA in the constructional host-mostly ***E. coli*** or yeast.

A



B

Asc*I**----A0----Mau*BI** Cycle 0 (cleavage Cycle0 with ***Mau*BI** for A1)

Asc*I** ----A0----ABM----A1----Mau*BI** Cycle 1 (cleavage Cycle1 with ***Mau*BI** for A2)

Asc*I** ----A0----ABM----A1----ABM----A2----Mau*BI** Cycle 2 (cleavage Cycle2 with ***Mau*BI** for A3)

Asc*I** ----A0----ABM----A1----ABM----A2----ABM----A3----Mau*BI** Cycle 3 dito

*Asc*I ----A0----ABM----A1----ABM----A2----ABM----A3----ABM----A4----***Mau*BI** Cycle 4 dito

|

etc

|

CycleN

.

Figure-3. **Two types of addition assembly of A-BoX-Elements with A-BoX constructional elements and alternative selection pattern.** The cyclic sequence addition

reaction can follow the scheme in **Figure-3-B** or include the **T-BoX**-Selection marker **(A)** is based on the Toggle assembly technology for adding and exchanging new building blocks and as well as recover all building blocks (see Fig.2). Donor ***Asc*I-AsiSI** fragments are cloned into ***Mau*BI-AsiSI** Acceptor vectors and selected for the selector of the donor fragment. Please order the brochure for toggle assembly. Individual cycles of the sequence addition processes can be performed in parallel.

pBoX-vectors are designed for the easy exchange and assembly of molecular function of expression units, transfer functions, selector genes and even oris of replication. Especially **A-** and **T-BoX**es are extendable by the addition application relevant genes and transfer functions like **TRANS**-regulators or **CIS**-elements towards higher integrated function.

The assembly process can be performed in parallel for gene sub-clusters.

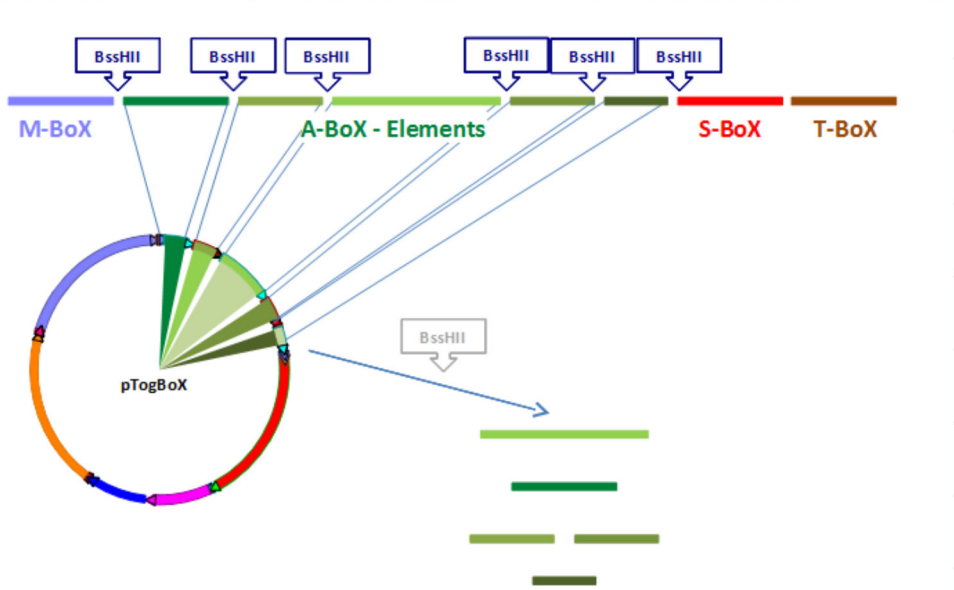


Figure-4. Toggle assembly schemes are generating *BssH*II sites by destruction of an 8mer specificity. *BssH*II sites are intermediate located between molecular modules like A-BoX-Elements. Gene clusters can be sequence identically de-assembled from existing constructs and recycled in other projects because the modules are separated systematically by **A-BoX** constructional ***BssH*II 6mer**-core sites for recovery of e.g. **A-BoX**-Elements. For identical module recovery these can be cloned into variant **pA0**-vectors into ***Asc*I** and ***Mau*BI** A0-sites.

For most efficient set up of multi-gene constructs it is most feasible to synthesize the construct according to the intrinsic logic of *flex***TEC** in order to maintain the design which warrantees the intended flexbility in the option of recovery of complete expression cassettes and building blocks. The final

---