Introduction Offer: 50?* each**

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

** S-Box: available as Kanamycin, 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 synthesizes 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 FlexTEC 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 FlexiBox-I and -II separated by the central BssHII site. Ligation of a DONOR A-Box flanked by Ascl and MauBI sites into the 6mer core BssHII sequence of pA0 conserves these sites. For the addition of additional A-Box elements into the MauBI site of the ACCEPTOR the ligation fusion site leaves a BssHII site and the MauBI 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 Ascl and MauBI sites if cloned into BssHII. Only if the systematic and logic of the FlexTEC 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.

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**FlexTEC for Constructive & Synthetic Biology**

**Assembly, De-assembly, Substitution**

The unique features of flexTEC are based on the **pBoX-vector** system. This design fixes molecular functional 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 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](image)

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 exo-nuclease cloning or assembly strategies like toggle assembly. Sites can be restored by cloning *BssHII* fragments into pA0-veectors.

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

1. **FlexBoX-I**: defined by *Absi(XhoI)-Asci(BssHII)*
2. **core A-Box**: *Asci(BssHII)-MauBl(BssHII)* also called toggle-Box
3. **FlexBoX-II**: defined by *MauBl(BssHII)-SgrDI(SalI)*

The S-Box is physically delineated by the unique restriction sites SgrDI(SalI) and AsISi(PvuII).

The T-Box is separated by AsISi(PvuII) and PacI(-) and closing the vector cycle.

The M-Box is defined by PacI(-) and Absi(XhoI).

**Add1**: The **FlexBoX-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 FlexBoX-I elements are a constructive homing nuclease site and the 5' inverted repeat of TNS transposase-integration site for recombination action. The FlexBoX-II can be equipped for example with Trans-regulator genes 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 Asci/MauBl termini into A-Box box MauBl-sites results in the fusions of Asci/MauBl-sites leaving just the 6meric restriction core - function *BssHII* to construct 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 FlexBoXes -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 FlexBoXes.
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.

Figure 2-2. The flexiBoxes f[3] and f[4] are located between Absl(Xhol) - Ascl(BssHII) and MauBI(BssHII) - SgrDI(SalI) 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 hoisting 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-gen construct with BssHII-sites DNA-fragments can be sequence identically restored by cloning into pA0 vectors.

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

A

B

Ascl - A0 - MauBI Cycle 0 (cleavage Cycle 0 with MauBI for A1)
Ascl - A0 - ABM - A1 - MauBI Cycle 1 (cleavage Cycle 1 with MauBI for A2)
Ascl - A0 - ABM - A1 - ABM - A2 - MauBI Cycle 2 (cleavage Cycle 2 with MauBI for A3)
Ascl - A0 - ABM - A1 - ABM - A2 - ABM - A3 - MauBI Cycle 3 ditto
... etc
... CycleN
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 Ascl-AsiSI fragments are cloned into MauBI-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-BoXes 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 BssHII sites by destruction of an 8mer specificity. BssHII sites are intermediate located between molecular modules like A-Box-Elements. Gene clusters can be sequently de-assembled from existing constructs and recycled in other projects because the modules are separated systematically by A-Box constructional BssHII 8mer-core sites for recovery of e.g. A-Box Elements. For identical module recovery these can be cloned into variant pAD-vectors into Ascl and MauBI 8AD-sites.

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

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