ATG:biosynthetics
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ACDC-SD and ToggleTec vectors:
a new system for constructing modular multi-gene expression constructs

This newsletter describes how the assembly / disassembly cloning and substitution design (**ACDC-SD**) principle and a series of corresponding kits for different expression hosts will help you tackle such challenges in building and efficiently modifying such multi-gene expression assemblies effectively.

Organismal genes that encode **integrated protein machines** are frequently lumped into functional clusters, e.g. those involved in nonribosomal peptide synthesis (**NRPS**). The component proteins act in highly and tightly organized complexes to exert their physiological function.

The artificial integration of genetic function - such as production-optimized **educt–product** chains (e.g. in metabolic engineering) – at the functional level of biocatalysis is a challenge for synthetic biologists. The same holds true for probing and modifying structure-function relationships, e.g. when investigating intracellular or membrane-bound protein complexes that make up certain regulatory pathways.

ACDC-SD is fully compatible with and implemented in the **Toggle assembly system** (**ToggleTec vectors**). Any gene to be inserted into the vector thus needs to adhere to a limited number of design principles to exploit the full potential of the system (fig. 2).

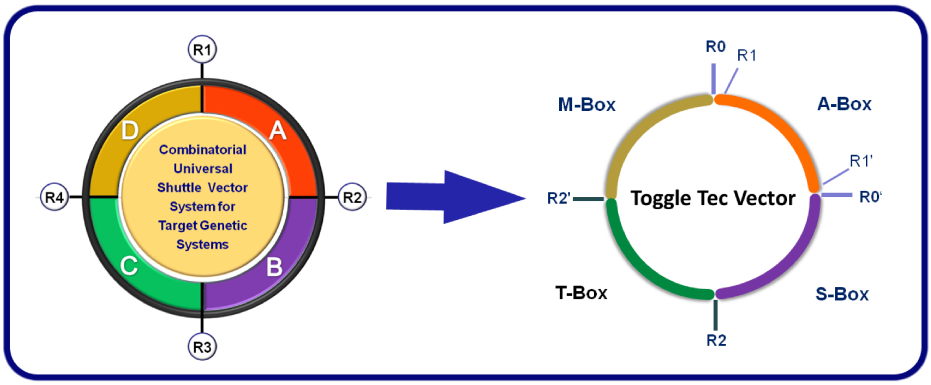

Figure 1: Basic layout of Toggle vectors
A-boxes host applications which will generally be gene cassettes or gene clusters, **S-boxes** are selectors, **T-boxes** are target functions, e.g. transposition sequences, and **M-boxes** host maintenance functions, i.e. origins of replication for the host organism, etc.

ATG offers ready-made systems for E.coli, insect and mammalian cells but can also assemble custom-made expression systems tailored to your host of interest.

Advantages of ATG's fully design-optimized ACDC-SD gene cassettes:

- Proven functional designs of gene clusters based on many years of expertise in **constructional synthetic biology**
- **Unified Design Concept** to realize genes, expression boxes/ cassettes, regulators etc.
- **Designs** incorporate a **de-assembly** option to regenerate individual expression boxes ? enables gene cluster revision and reorganization by **assembling** and/or **reusing** existing genetic elements

(fig. between sites X and Y)

- **Option for substitution cloning**: substitution of individual genetic elements or whole expression boxes in existing gene clusters (see fig. above)
- **Option for positional gene libraries** for the local evaluation of variants in a given gene-cluster construct

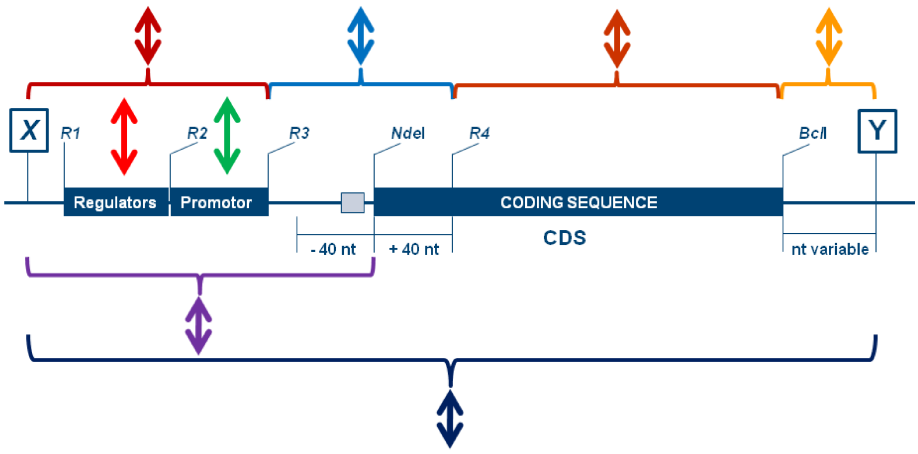

Figure 2: General lay-out of gene cassettes in the ACDC-SD. Functional and/or constructive elements such as regulators, promoters, leaders, coding sequence, terminators, etc. can be exchanged **individually** (colored double arrows and brackets at the top) or **en bloc** (dark blue double arrow and bracket at the bottom) by using design-inherent or specifically designed restriction sites.

ACDC-S design allows you to use standardized expression boxes where all genetic elements can be recycled and exchanged between synthetic biologists who use the same standards for different organisms. Swapping ACDC-S elements between gene clusters and researchers who work on the same project is easy.

All systems support a diversity of assembly strategies for the development of higher-order integrated genetic functionality, e.g.

- **Ligation strategies** such as **DONOR/ ACCEPTOR**-based methods with alternating selector toggle assembly
- **Exonuclease cloning (5' → 3' , 3' → 5')**
- **Recombination-mediated assembly, e.g. Cre/LoxP**

Classical multiple cloning sites can be introduced into the systems as well.

ACDS-S is suited for these **applications:**

- **multi-gene artificial biochemical pathway systems** – in vivo e.g.
  - *anabolic pathways* – biologically active compounds, fine chemicals, building blocks, biofuels etc.
  - *catabolic pathways* – biorefineries, bioremediation etc.
- **multi-gene hetero-protein-complex systems** – *in vivo*
- multiple in vitro bio-catalyst co-expression systems (*flexiBAC*)
- other: specific functionally targeted multi-genetic constructive designs – *in vivo*
- multiple-resistance mechanisms in plants etc.

Order your **ACDC-S** design for preassembled gene clusters of your choice or the special kits that allow you to assemble your gene clusters according to the **ACDC-S** design.

ATG is ***your*** **"Synthetic Biology"** system provider that provides fully integrated molecular service packages to help you improve the quality of your research and to accelerate your developments.

Shape the future world of molecular design … with ATG:biosynthetics!