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Authors: Víctor de Lorenzo

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EXECUTIVE SUMMARY

The *Standard European Vector Architecture* (SEVA) platform is a web-based resource and a material clone repository to assist the choice of optimal plasmid vectors for de-constructing and re-constructing complex prokaryotic phenotypes. The SEVA database (SEVA-DB) is a resource for implementation of a standard for physical assembly of vector plasmids and for their non-ambiguous nomenclature as well as the index for a repository of functional sequences and actual constructs available to the community. The database was designed to simplify the choice of a given vector for the sake of specific applications, in such a way the user can easily decide the best configuration of replication origins, antibiotic resistance and business segments. The SEVA-DB adopts simple design concepts imported from Systems Engineering into vector architecture and development to facilitate the swapping of functional modules and the extension genome engineering options to microorganisms beyond typical Laboratory strains. This platform has been implemented in the Molecular Environmental Microbiology Laboratory of the Centro Nacional de Biotecnología (CSIC) and its 3.0 updated version has been developed—among others—under the auspices of the BioRoBoost project. The updated version of the SEVA database can be found in: http://seva.cnb.csic.es/

Introduction: The SEVA standard

STRUCTURE BACKBONE MODULES FIND YOUR PLASMID THE SEVA COMMONWEALTH CONTACT & ORDER

Standard European Vector Architecture 3.0

Welcome to the CNB-hosted database and material repository of standard and modular plasmid vectors for (de/re) constructing complex bacterial phenotypes

The motivation for SEVA is the need of adopting a standard for engineering predictable and efficacious bacteria. The SEVA format is a good candidate to fill this gap: it enables the exchangeability of diverse elements (origins of replication, antibiotic selection markers) and the combination with many cargo molecules for varied end-applications. The **SEVA format** involves a set of rules for the physical assembly of the three basic components of plasmid vectors (origin of replication, selection marker and the business cargo) as well as a fixed nomenclature for the designation of the corresponding constructs. Each of the naturally-occurring sequences destined for the various constructs are minimized to the shortest DNA sequence that retains functionality. Furthermore, the sequences are erased of any of the following restriction sites: **HindIII, PstI, XbaI, BamHI, SmaI, KpnI, SacI, SalI, EcoRI, SfiI, SphI, AvrII, PshAI, SwaI, AscI, FseI, PacI, SpeI, SanDI** and **NotI**. Finally each of the modules is flanked by fixed, rare restriction sites: origin of replication by **FseI** and **AscI**; antibiotic marker by **SwaI** and **PshA**; and the cargo by **PacI** and **SpeI**.

Besides the standardized layout of constructs the SEVA format calls for the unequivocal designation for each of the vectors with the nomenclature explained in detail in that section and the code summarized in the plasmid list section. All vectors are called pSEVA followed by a digit cipher with four positions. The first one reflects the antibiotic resistance marker. The second position of the code is for the origin of replication. The third spot goes for the cargo module. In cases where there is a variant of the same cargo type we then add a capital letter to the numeral. And finally the fourth position is for the gadgets, which are designated by lower cases greek letters.

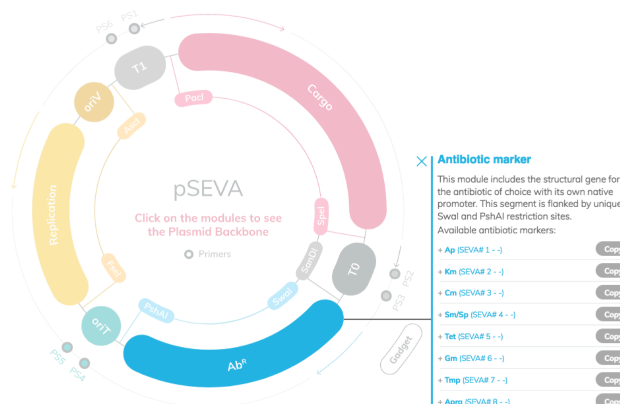
Plasmid already available in SEVA-DB and those that will enrich the collection in the future are distributed world-wide to researchers at any academic or non-profit laboratory. No MTAs will be requested prior to shipping the constructs to potential users of such categories. The SEVA-DB

will distribute petitions of a small number of clones at no cost for the user, but may charge handling, maintenance and shipping fees for large requests.

The SEVA modules

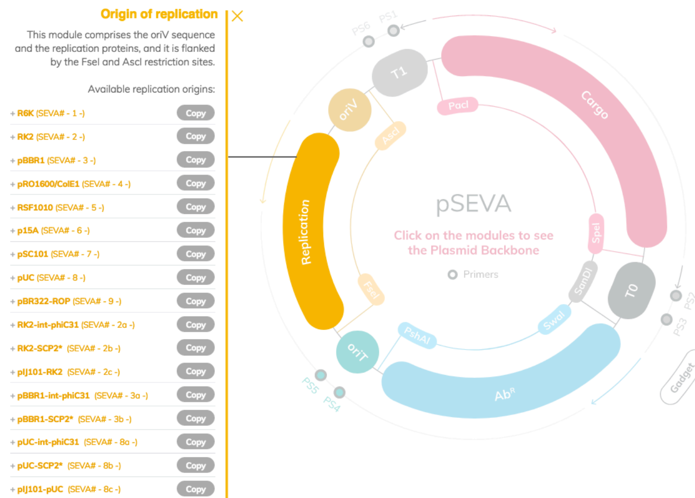
All SEVA plasmids have then very same organization. To standardize the SEVA modules, a number of criteria were followed. First, each of the naturally occurring sequences that were destined for the various constructs were minimized to the shortest DNA segments that retained full functionality. The sequences were then erased of any of the restriction sites that were present in the polylinker of the classical vector pUC18 and of SfiI, AvrII and NotI, but the encoded amino acids were retained by the mutated codons. The optimized fragments were produced by either site-directed mutagenesis of the DNA as required, or were entirely chemical synthesized. Each of the three fragments was then assembled in a shared frame that was composed of three connecting parts. The plasmid vectors are, therefore, composed of six functional modules and the location and orientation of the three connectors provide the backbone of reference for the standardized vector collection. The sequences that link the variable parts include the strong, *rho*-independent transcriptional terminators T_0 of phage lambda (103 bp) and T_1 (105 bp) of the *rrnB* operon of *E. coli*. These terminators function by avoiding any transcriptional read-through into adjacent sequences, which increases plasmid stability. The third connecting element comprises a 246-bp DNA segment from the conjugative, broad-host-range plasmid RP4 and is the plasmid's very efficient origin of transfer (*oriT*). This *oriT* allows for conjugative mobilization of pSEVA plasmids into organisms in which no alternative transformation protocols are available. Note that while *oriT* enables constructs to engage in conjugative gene transfer, its addition to SEVA vectors does not increase significantly chances of unintended uptake by naturally occurring microorganisms, which is largely caused by accidental DNA transformation. Because these three core sequences (T_0, T_1 and *oriT*) are shared by all plasmids of the series, specific primer pairs (PS1–PS6) were designed that hybridize to these regions and can be used to amplify any of the intervening segments.

The **first** variable part of the pSEVA constructs consists of the antibiotic selection marker. As shown in the figure below, the DNA segments that carry such markers include the structural gene for one antibiotic (Ab) resistance gene and its native promoter.



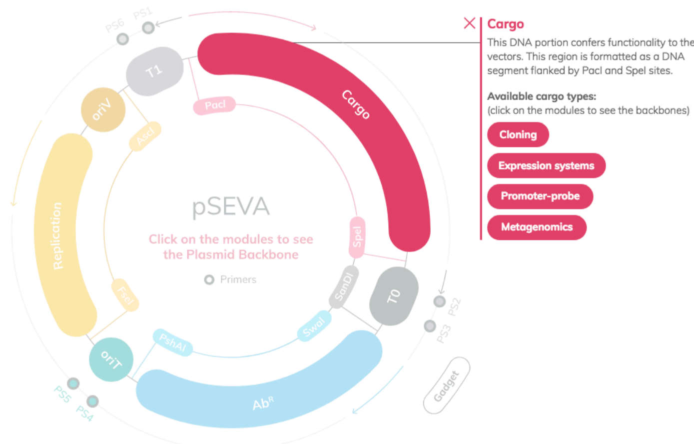
The unusual SwaI and PshAI restriction sites flank the Ab resistance unit and the expression of the Ab gene is oriented toward the *oriT*-connecting module. The size of the DNA segment for such an Ab resistance unit ranges from 0.8 to 1.3 kb, depending on the specific marker

The **second** exchangeable part of the SEVA vectors is a DNA segment that holds the **origin of replication** of the plasmid, which is produced and inserted in the plasmid frame as an AscI-FseI fragment.



The composition of such segment that endow replication is more complex than the one-gene counterparts for antibiotic selection because they include an *oriV* for replication initiation and may encode specific replication proteins and their corresponding promoters. Although the orientation of these sub-components may vary, the SEVA standard entails that the *oriV* sequence be proximal to the AscI end of the DNA fragment and that transcription of the replication protein(s), if any, points toward the FseI site of the *oriT* connector.The broad-host-range replication origins that are selected to this end have different sizes (1.6–3.7 kb) and endow the plasmids with different copy numbers, which are decided by the user.

Finally, the **third** variable element of the SEVA constructs is the DNA portion that bears the main functionality of the vector. We have designated this element as the **cargo module** and this module is always formatted as a PacI-SpeI fragment.



This region confers a specific purpose to the plasmid, whether for cloning, expression of heterologous genes, creating reporter gene fusions or for chromosomal integration. Note that

DNA fragments that are bordered by PacI-SpeI sites can also be cloned into the synthetic transposon vector of pBAM1 thus allowing for stable chromosomal integration of a DNA segment that is assembled in SEVA plasmids. The default SEVA cargo, which consists of a basic polylinker that is formed by the array of unique cloning sites of pUC18 SfiI/AvrII and NotI sites upstream of the EcoRI site and a second NotI site that is placed downstream of HindIII site. These flanking NotI sequences allow concatenation of cloned fragments (positioned between EcoRI and HindIII) into derived SEVA vectors where NotI is unique (see below). This format increases the number of fragments that can be assembled into a SEVA cargo.

Describing the SEVA collection with synthetic biology open language (SBOL)

The effort to standardize the physical and functional composition of the genetic tools that shape the SEVA collection would be in vain if we could not make the system compatible with former, current and future genetic engineering platforms. To this end, we pursue the representation of every significant functionality embedded in each of the vectors of the collection with the formalisms of the Synthetic Biology Open Language (SBOL, http://www.sbolstandard.org/). This is an open-source standard for *in silico* representation of genetic designs that, inter alia, allows exchanging designs, sending and receiving genetic circuits to and from biofabrication centers, facilitate their storage in repositories and embed genetic designs in publications.

For instance, if we take plasmid pSEVA111, potential user could, for example, open the file using SBOL Designer (http://clarkparsia.github.io/sbol/), a software tool for creating and visualizing designs, and simply replace the origin of replication (R6K in pSEVA111) with the pBBR1 sequence in order to build pSEVA131 following the same SBOL standard. The provided demo file (pSEVA111.xml) has been validated using the Java library libSBOLj (https://github.com/SynBioDex/libSBOLj) and can be imported by software tools as a correct SBOL construction.

Ongoing efforts are committed to develop a query-able database of SBOL files one for each SEVA vector plus isolated parts as antibiotic resistances or origins of replication. When designing a genetic circuit with a software tool, the user is able to import the carrier plasmid and embed the new design as the cargo segment. Therefore, the final sequence of the vector would be unequivocally annotated following standard descriptions (SBOL) and structures (SEVA) and ready to be shared or built. This would enable, for example, the synthesis of the newly defined cargo and the selection of a SEVA vector from the collection to integrate them both. As the SEVA vectors are widely used, the experimental information regarding their behavior under specific conditions is, at many cases, available and known. Taking advantage of that, we aim at enriching the SBOL files with important data about the 'context' of a vector so that the same user could choose between selecting either one plasmid or another depending on, for example, its copy number. This 'context' feature, which will complete the description of the system, is not included for now in the current SBOL, but the Developers Group are expected to release in the near future an updated version that fully supports extension.

Ongoing and future developments

The SEVA platform is a simple, robust and useful instrument to that end which has reportedly helped an active community of users in bringing Synthetic Biology to a suite of applications both in model bacteria (e.g. *E. coli*) and in industrially important cell factories (*Pseudomonas putida*). While the ultimate destiny of genetic engineering is complete synthesis of any DNA sequence of

interest (for which assembly vectors may not be necessary any longer), molecular tools for analysis and deployment of traits and genes of interest will still be required for a considerable period of time, in particular for addressing basic biological questions in non-model bacteria and for combining engineered properties with pre-existing biological qualities. As evidenced by the experience of the past fewyears, this is the area of action where the effort to maintain and further expand the SEVA platform deploys its full value. In the meantime, we will pursue the convergence of our platform with other vector standardization initiatives. Please check http://seva.cnb.csic.es/ for subsequent developments of the SEVA platform

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