Next-generation binary T-DNA vectors for (plant) synthetic biology

The CSIC and the Universitat Politècnica de València have developed an expression vector system that provides a flexible framework for plant biotechnology and synthetic biology applications. The system includes novel mini binary vectors with compatible origins. Vectors have been used successfully in plant transient and stable expression, CRISPR/Cas9-targeted genome mutagenesis, and for genetic circuit component and viral expression vector delivery.

Industrial partners from the agro-food or pharmaceutical industry are being sought to collaborate through a patent licence agreement.

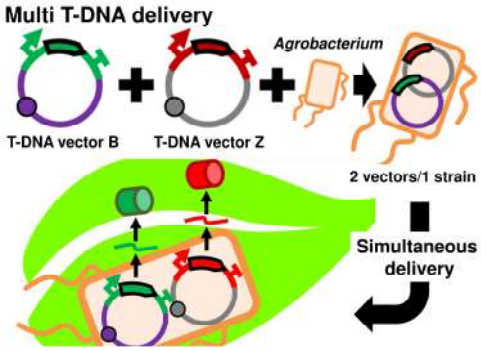
An offer for Patent Licensing

A mini binary vector system that allows high-throughput construct assembly and multiple T-DNA delivery

Plants are main food sources as well as expression platforms for the production of high-value compounds and pharmaceuticals. Multigene transfer is imperative in multiplex gene editing and to engineer complex traits, circuit designs, and metabolic pathways. To ease multigene transfer to plants, an expression system that relies on compatible binary T-DNA vectors was developed.

Viruses have significant economic impacts on many crops, but they are also a source of biotech tools. The vectors allowed one-step assembly of ssRNA, ss- and dsDNA virus clones that are suitable for agro-inoculation and provide the basis for engineering of new viral expression vectors and plant breeding tools.

The system might further be applied to transform of other eukaryotic cells, including fungi.



Compatible mini binary vectors are co-transformed into *Agrobacterium* cells. A two-vector/one-strain approach is used for simultaneous delivery and expression of multiple T-DNA cassettes in plants (adapted from *ACS Synthetic Biology* 2017;6(10):1962-1968).

Main innovations and advantages

- Minimalism, the reduced size (< 3.8 kb) of the vectors facilitates cloning and reverse genetic studies
- Flexibility, vectors autonomously replicate in *Escherichia coli* and *Agrobacterium*
- Stability, vectors include bacterial terminators that avoid transcriptional read-through to T-DNA cassettes and help to stabilize large/complex inserts
- Scalability, vectors are suitable for standard as well as high-throughput cloning methods (e.g., Golden Gate, GoldenBraid, and Gibson assembly)
- Standardization, vectors meet current plant synthetic biology standards and allow use of publically available DNA parts libraries
- Compatibility, vectors can be multiplexed with binary vectors commonly used by plant scientists for multi T-DNA delivery in a two-vector/one-strain expression approach

Patent Status

PCT patent application filed

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