

51. An Innovative Cloning Platform for Pathway Engineering

Henrique C. DePaoli, Gerald A. Tuskan and Xiaohan Yang* (yangx@ornl.gov)

BioSciences Division, Oak Ridge National Laboratory, Oak Ridge, TN

<http://cambiodesign.org/>

Project Goals: Crassulacean acid metabolism (CAM) is a specialized mode of photosynthesis that features a temporal CO₂ pump with nocturnal CO₂ uptake, facilitates increased water-use efficiency (WUE), and enables CAM plants to inhabit water-limited environments such as semi-arid deserts or seasonally dry environments. CAM provides an excellent opportunity for engineering both enhanced WUE and photosynthetic performance into bioenergy crops. This project has two main goals: 1) to identify the CAM-associated genes using systems biology approaches and 2) to engineer CAM gene modules into C₃ species using synthetic biology approaches. The success of the project will allow biomass production on semi-arid, abandoned, marginal or degraded agricultural lands.

To establish synthetic biology capability for transferring CAM gene modules into C₃ species, we developed an innovative method for assembling DNA fragments in vitro. Several cloning strategies are available to support genetic engineering (DePaoli et al., 2014). However, the current availability of cloning methods imposes several limitations to seamless cloning of large and multi-gene constructs. Our technology, called “TNT-cloning”, assembles DNA parts in a simple, fast, efficient and flexible manner. Our system combines DNA fragments to be cloned in one single universal library, leaves no undesirable sequences behind, allows “one-pot” reaction with up to 3 fragments to be joined at once and automatically maintains the open reading frame (ORF) in-frame between genes of interest. By combining all cloning elements into one single universal library, the method allows a pre-determined assembly without the need of linkers/adaptors, resulting in a “scar-free” product. In addition to the cloning system, a new buffer, called “TNT-buffer”, was developed to allow quick and simultaneous digestion and ligation of DNA fragments, enhancing the efficiency several times compared with current commercial products. The vectors represent a binary platform, making the final gene construct reusable as well as immediately ready for plant transformation. Additionally, the set of plasmids support secondary and tertiary assembling in a loop format, with an exponential reduction of steps for assembling a large number of DNA parts. We demonstrated that the system is wholly functional by cloning, assembling and testing several fragments ranging from 30 bp to 12 kb.

Because this technique is compatible with isothermal (Gibson) assembly, virtually any fragment can be used as an element in the library and circularized without the need to carry the binary- backbone, expanding the technology’s use to other systems and making it of special interest for constructing new plasmids/circular molecules. This novel cloning platform will accelerate the creation of multiple gene constructs necessary for CAM engineering and will greatly support the construction and tuning of different pathways in a wide range of organisms.

Note: The TNT cloning system is protected under the invention disclosure 201403357, DOE S- 124,978 and is patent pending. The TNT-buffer formulation is protected under the invention disclosure 201403356, DOE S-124,977 and is patent pending.

References

1. DePaoli, H. C., Borland, A. M., Tuskan, G. A., Cushman, J. C., & Yang, X. (2014). Synthetic biology as it relates to CAM photosynthesis: challenges and opportunities. *Journal of Experimental Botany*, 65:3381-3393.

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