Seminar Speaker Series

in the framework of Interreg V-A project CAPSID

presents

Dr. Jana Neuhold

Protein Technologies Facility, VBCF, AT

GoldenBac:
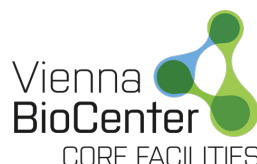## A simple, highly efficient, and widely applicable system for construction of multi-gene expression

29. 04. 2021 at 14:00

Online virtual talk via Zoom

Join this talk here

Organized by:

Biomedicínske Centrum
Slovenská Akadémia Vied

Vienna
BioCenter
CORE FACILITIES

Dr. Jana Neuhold

Protein Technologies Facility, VBCF, AT

ANNOTATION

Recombinant protein production and purification of large protein complexes in eukaryotes requires efficient methods to generate multi-gene expression constructs, where each individual gene is under the control of its own promoter and terminator. Current methods are based either on serial rounds of combination of several vectors containing loxP sites via the Cre-lox technology, or on multiple rounds of gene combination via PCR or other methods. These methods are multi-step, have lower efficiencies than single gene cloning, and may require laborious processes to verify that all genes of interest are present in the final product. Here, we describe a rapid and simple Golden Gate-based system for the generation of multi-gene expression constructs compatible with baculovirus expression vector systems (BEVS) using either Tn7 transposition or KO1629-based homologous recombination, which we refer to as "GoldenBac".

TAKE HOME MESSAGE

This robust, single-step cloning system provides an easy-to-use method for generation of multi-gene expression constructs for both transposition and homologous recombination-based baculovirus systems, making this technology available across all laboratories using baculovirus expression systems. This highly efficient and simple method allows for rapid incorporation of multi-gene expression cloning into the standardized service portfolio of protein production facilities and can also easily be adopted by any laboratory for routine generation of multi-gene baculovirus constructs.

REFERENCES

[1] Neuhold J, Radakovics K, Lehner A, Weissmann F, Garcia MQ, Romero MC, Berrow NS, Stolt-Bergner P. GoldenBac: a simple, highly efficient, and widely applicable system for construction of multi-gene expression vectors for use with the baculovirus expression vector system. **BMC Biotechnol.** 2020 May 12;20(1):26.

[2] Smakowska-Luzan E, Mott GA, Parys K, Stegmann M, Howton TC, Layeghifard M, Neuhold J, Lehner A, Kong J, Grünwald K, Weinberger N, Satbhai SB, Mayer D, Busch W, Madalinski M, Stolt-Bergner P, et al. An extracellular network of Arabidopsis leucine-rich repeat receptor kinases. **Nature**. 2018 Jan 18;553(7688):342-346.

[3] Mott GA, Smakowska-Luzan E, Pasha A, Parys K, Howton TC, Neuhold J, Lehner A, Grünwald K, Stolt-Bergner P, et al. Map of physical interactions between extracellular domains of Arabidopsis leucine-rich repeat receptor kinases. **Sci Data.** 2019 Feb 26;6:190025.

Organized by: