4-3-2022

Investigating the role of oxidative stress response genes in protein secretion using a novel combinatorial Golden Gate based approach

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INVESTIGATING THE ROLE OF OXIDATIVE STRESS RESPONSE GENES IN PROTEIN SECRETION USING A NOVEL COMBINATORIAL GOLDEN GATE BASED APPROACH

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One of main challenges for the efficient production of secreted recombinant proteins by yeasts such as *Komagataella phaffii* (syn *Pichia pastoris*) is the redox stress generated by the oxidative folding of proteins in the Endoplasmic Reticulum (ER). In addition to ER resident proteins, many other cellular players are believed to be involved in this context. Genes responsible for the defence against oxidative stress might be essential for responding to the stress generated and maintaining an optimal redox state during protein folding and secretion. Rather than the effect of a single gene, it is likely that the combination of multiple genes is responsible for detoxifying ER generated redox stress. Therefore, the Golden Gate based Golden *Pi*CS cloning system (Prielhofer et al. 2017. BMC Syst Biol 11(1):123) presents itself as an amazing ally, as it is a very powerful, versatile and efficient tool, making combinations of multiple genes possible.

As a starting point, candidate genes were selected for the creation of a combinatorial plasmid library. The library was created by using complementary fusion sites, allowing genes to be cloned in different positions of the final expression vector. Thus, as result of a Golden Gate Assembly using all combinatorial plasmids, expression vectors bearing random combinations of the genes were generated. The library was evaluated in a trypsinogen secreting *K. phaffii*. The best combinations were identified and verified for the production of other secreted recombinant proteins.

Here, a novel application of the Golden PiCS toolbox is presented. This strategy can be applied in future studies that require versatile and efficient combination of genes.