

## Development of new USER-based cloning vectors for multiple genes expression in *Saccharomyces cerevisiae* - DTU Orbit (08/11/2017)

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*Saccharomyces cerevisiae* is one of the most widely used cell factory in industrial biotechnology and it is used for the production of fuels, chemicals, food ingredients, food and beverages, and pharmaceuticals. Such bioprocesses frequently require multiple rounds of metabolic engineering to obtain the production strain with the proper phenotype and product yield. However, the sequential number of metabolic engineering is time-consuming. Furthermore, the number of available selectable markers is also limiting the number of genetic modifications. To overcome these limitations, we have developed a new set of shuttle vectors for convenience of use for high-throughput cloning and selectable marker recycling. The new USER-based cloning vectors consist of a unique USER site and a CRE-loxP-mediated marker recycling system. The USER site allows insertion of genes of interest along with a bidirectional promoter of choice into the vector backbone with time- and cost-effective. The selectable marker cassette is flanked by loxP recognition sites for the CreA recombinase to allow reutilization of the same selectable marker. Furthermore, our USER vector set provides a choice of different selectable markers both auxotrophic and dominant markers for convenience of use. Our vector set also contains both integrating and multicopy vectors for stability of protein expression and high expression level. We will make the new vector system available to the yeast community and provide a comprehensive protocol for cloning in these vectors using USER cloning strategy.

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Authors: Kildegaard, K. R. (Intern), Jensen, N. B. (Intern), Maury, J. (Intern), Strucko, T. (Intern), Mortensen, U. H. (Ekstern), Förster, J. (Intern), Borodina, I. (Intern)

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