Development of high-throughput methods for heterologous expression in fungi

Hansen, Bjarne Gram; Hoof, Jakob Blæsbjerg; Nielsen, Michael Lynge; Patil, Kiran Raosaheb; Mortensen, Uffe Hasbro

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Development of high-throughput methods for heterologous expression in fungi

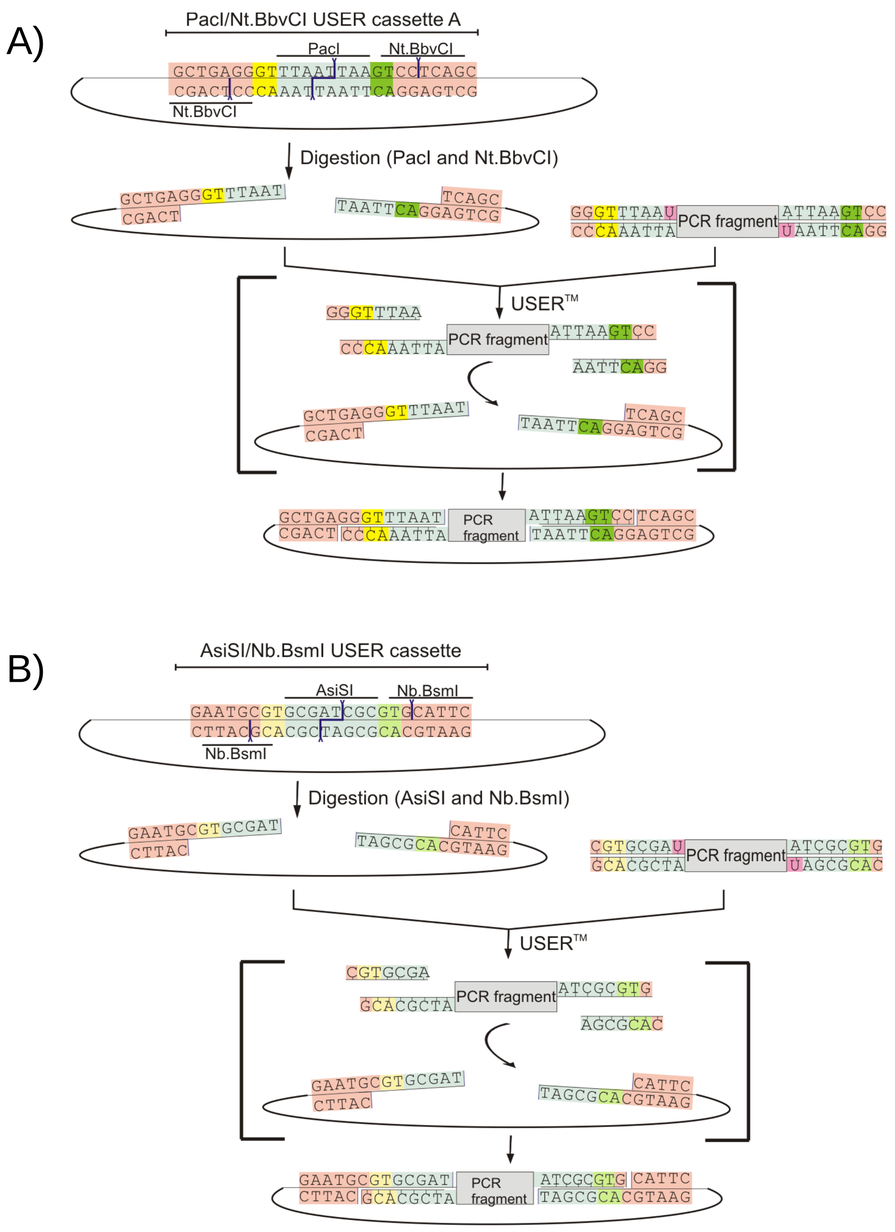
Bjarne G. Hansen, Jakob B. Nielsen, Michael L. Nielsen, Kiran R. Patil and Uffe H. Mortensen

Center for Microbial Biotechnology, DTU Systems Biology, Technical University of Denmark
Contact: bgha@bio.dtu.dk

Introduction

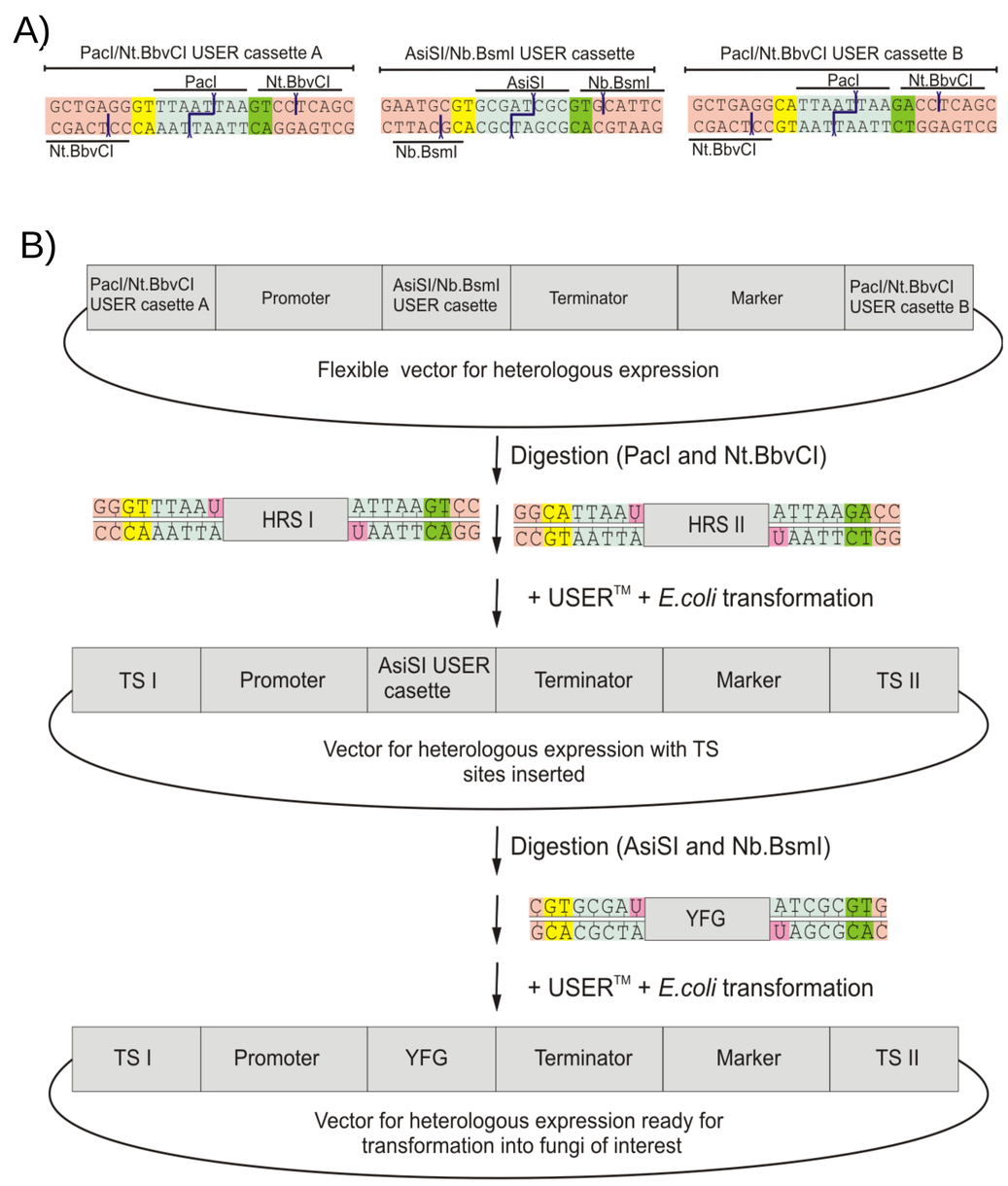
The rapid increase in the number of sequenced organisms has resulted in an explosion in the number of genes that are desired to heterologously express. However, heterologous expression requires the creation of DNA constructs which is the bottleneck in the construction of strains of interest in most projects. One promising technique to overcome this bottleneck is uracil-excision based cloning which was first described in the early 1990s. This technology has been available as a commercial kit for several years (USER™) however, the technology has remained largely unused. We have made several essential modifications to this technology which now allows simultaneous fusion and cloning of multiple PCR products independently of restriction sites. Here we present a flexible and fast approach to generate constructs for heterologous expression in fungi.

Efficient high-throughput cloning.



A PCR fragment amplified with PfuTurbo® Cx Hotstart DNA polymerase is mixed with the deoxyuridine-excising USER™(NEB) and the linearized vector, whereupon the hybridized product is ready to be transformed into *E.coli* without prior ligation. Directionality is preserved in the cloning event by two base difference in the overhangs (highlighted in yellow and green). A) USER compatible vector with PacI and Nt.BbvCI sites. B) USER compatible vector with AsiSI and Nb.BsmI sites.

Flexible vector for heterologous expression.in fungi of choice



A) The three USER casettes present in the vector in B). B) Generation of construct for heterologous expression. The vector is treated with PacI and Nt.BbvCI. PCR fragments of targeting sequence (TS) of choice ,is mixed with USER ™ and the vector fragments and transformed into *E.coli.* The resulting construct is treated with AsiSI and Nb.BsmI. PCR fragment of your favourite gene (YFG) is mixed with USER ™ and the linearized vector and transformed into *E.coli.*

Perspectives

The improved USER (uracil-specific excision reagent) technique provides the means of efficiently overcoming the bottleneck that cloning typically is in metabolic engineering projects. Furthermore, in addition to its role in creating constructs for heterologous expression the technology is highly suitable for high-throughput applications such as creating deletion and reporter/gene libraries. Based on the impact on the cloning output in our laboratories, we believe that this technique will be able to move molecular biology into an era where the cloning step occupies only a minor part of a metabolic engineering project.