Development of high-throughput methods for heterologous expression in fungi

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

Publication date:
2009

Document Version
Publisher's PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):
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.

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.