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OLIGO-BASED GIBSON ASSEMBLY – A NEW WAY OF CREATING EXPRESSION VARIABILITY

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The advancements in the field of metabolic engineering and synthetic biology have allowed the rapid *de novo* construction of multi-enzyme heterologous pathways. However, in order to obtain an optimal flux through a pathway, the various regulatory elements (e.g. promoters, ribosome binding sites) need to be optimized. For example, the massive overexpression of a gene may result in metabolic burden, due to the withdrawal of NADH, ATP, and amino acids from the central metabolism required for the synthesis of the corresponding protein and the concurrent depletion of intermediates required for biomass synthesis or may lead to the accumulation of toxic intermediates due to an unbalanced pathway.

The current lack of in-depth knowledge on the various regulatory control levels renders combinatorial approaches popular for pathway optimization. In this context, methods to rapidly and efficiently create variability are crucial. To generate this variability, the promoter and the ribosome binding sites are typically randomized to modulate gene expression and to create gene expression libraries.

To date, a wide variety of methodologies have been proposed to introduce variability. Many make use of synthetic degenerated oligonucleotides which contain the promoter or RBS-sequences and which are linked to the gene of interest. However, the manner in which the degenerated oligonucleotides are linked to a certain gene may influence the final expression variability. In fact, the use of PCR may bias for the GC content of the oligonucleotide, which in turn may bias variability in the library.

In this study, a novel scarless method is proposed to rapidly introduce a library (of promoters) in front of a target gene. This is achieved by combining principles of *ligation chain reaction* (LCR) and Gibson assembly, called oligo-based Gibson assembly.

The library of promoters can be introduced by partially degenerated oligonucleotides which contain the promoter consensus region. As reporter protein, a fluorescent protein was used, since it facilitates further analysis. The obtained efficiency and generated variability were analyzed b y flow cytometry. In addition, the influence of PCR-induced sequence artifacts and bias was determined.

Subsequently, both a promoter library and a ribosome binding site library were introduced using this method. The obtained variability in gene expression was monitored. To this end, the variation in gene expression of the resulting cell population was analyzed using flow cytometry.

Thus, a rapid, efficient, and scarless method is proposed and validated to generate expression cassette libraries which uses oligo based Gibson assembly.