Introducing BglBrick gene assembly in lactic acid bacterium Lactococcus Lactis

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Lactic acid bacteria (LAB) as cell factories are important hosts for production and delivery of biomolecules of interest. As they have considerable biotechnological potential and increasing interest, novel tools for engineering of complex phenotypes are in demand. A lot of new DNA assembly methods have been developed in the last two decades to facilitate and advance the gene construction. One of the introduced assembly standards is BglBrick, which emerged from optimisation of BioBrick™ assembly. It is a method that consists of iterative DNA digestion and ligation using two different restriction enzymes that generate compatible cohesive ends. These can be ligated, thereby generating a scar sequence in DNA that cannot be digested with either of previously used enzymes. BglBrick assembly was so far limited to *Escherichia coli*; however, in this study it was introduced to a model LAB *Lactococcus lactis*. We constructed a new plasmid pNBBX, on the basis of pNZ8148, that employs BglII and BclI restriction enzymes, which produce GATC sticky ends. After ligation, a TGATCT scar sequence is formed between each of the two consecutive cassettes. Altogether, our plasmids encode NheI-BglII-gene-BclI-XhoI cassettes. We applied three model proteins to test their concomitant expression in *L. lactis*, namely near-infrared fluorescent protein (iRFP), NanoLuc™ luciferase and and affibody with the affinity for human epidermal growth factor receptor 2. We aim to clone all three model protein-encoding genes in pNBBX plasmid by using BglBrick assembly, resulting in 6 different theoretical assembly possibilities. We will determine the quantity of the three expressed model proteins obtained with plasmids thus assembled. The ultimate goal of the research is to develop an alternative gene assembly option for *L. lactis*, thereby facilitating multiple protein expression.