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Publication date:
2023

Document Version:
Submitted manuscript

High-throughput *in vivo* screening of novel prokaryotic, metabolite-responsive transcription factors for biosensor development

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In prokaryotes, transcription factors (TFs) are of uttermost importance for the regulation of gene expression. However, the majority is not characterized to date, which hampers both the understanding of fundamental processes and the development of biosensor applications. One way of analyzing TFs is through *in vivo* screening, enabling the study of TF-promoter interactions, ligand inducibility and specificity. A set of 30 prokaryotic TFs belonging to the Leucine-responsive Regulatory Protein (= Lrp) family, with respective promoters of interest, were selected for analysis as metabolite inducible systems. A reporter system was designed to use for cloning and expression of the TFs and promoters in *Escherichia coli*. By using an automized platform, fluorescence measurements were performed with strains containing each TF-promoter pair. While inducing the TF's expression at different levels, the functionality of the heterologous promoters in *E. coli* was determined, as well as the TF's regulation mechanism. Furthermore, experiments were performed in the presence of possible effector molecules, to learn more about the ligand specificity and sensitivity of each TF. The selection of TF-promoter pairs seemed to be a good representation of the Lrp family, since different regulatory mechanisms could be found, both ligand dependent and independent, and since some pairs were characterized by one specific amino acid as effector molecule, whereas others were sensitive to a whole range of amino acids. To conclude, this screening led to the initial characterization of numerous TFs and contributes to the general knowledge currently available about the Lrp family and the development of well-functioning biosensors.