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In vivo screening for the identification and characterization of prokaryotic, metabolite-responsive transcription factors

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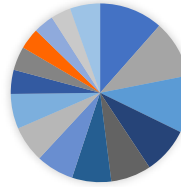
LRP TRANSCRIPTION FACTORS

- ✕ Repressors, activators or dual regulators
- ✕ Perform either specific or global regulation
- ✕ Typical ligands are **amino acids**, but recently also other ligands have been found
- ✕ Only a small amount of Lrp TFs is characterized

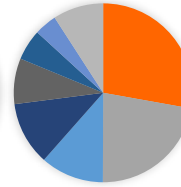
Huge variability, phylogenetically widespread with crucial physiological roles

➔ INTERESTING TARGETS FOR ENGINEERING

BACTERIA



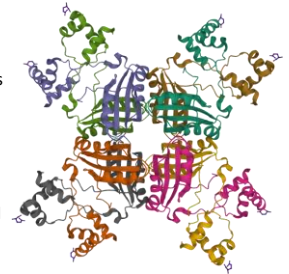
ARCHAEA



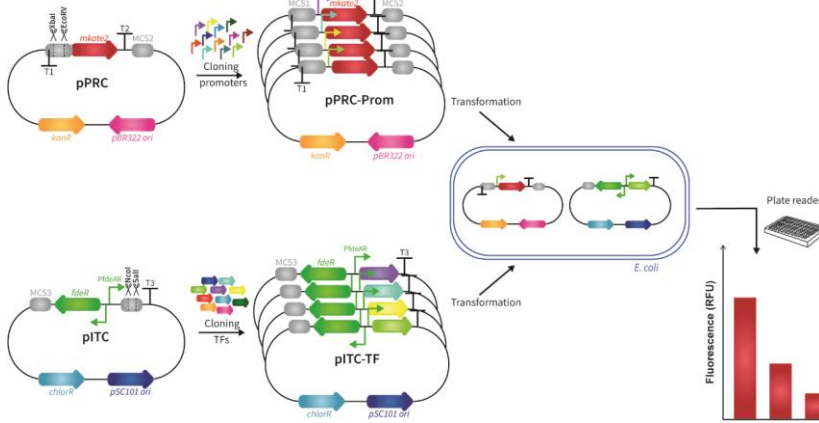
■ Lrp-type
■ Other TFs

Up: Distribution of TF families in a bacterial versus an archaeal genome

Right: Crystal structure of the Lrp-type TF Grp from *Sulfolobus tokodaii* (PDB: 2E7W), displaying an octameric structure with every subunit colored differently



WORKFLOW IN VIVO SCREENING

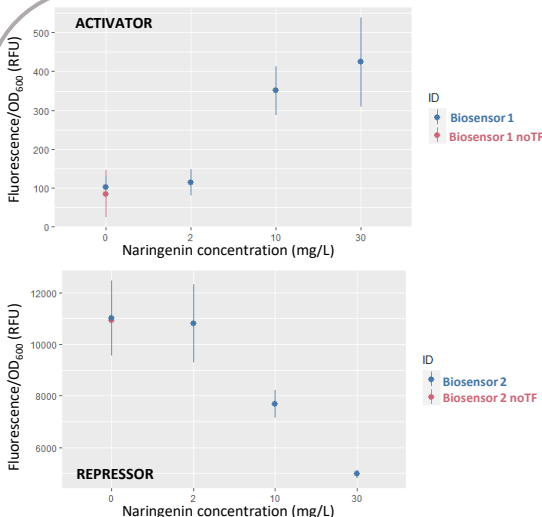


Centre for Synthetic Biology, Ugent



28 TFs were selected together with their promoters of interest. Cloning was performed in the **inducible TF construct** (pITC) and **promoter reporter construct** (pPRC), respectively, followed by a transformation in *E. coli*. Plate reader measurements were performed to determine OD and fluorescence levels.

RESULTS



Characteristics in *E. coli* out of 50 biosensors

Functional promoter	26
Regulatory mechanism	
▪ Repression	14
▪ Activation	6
AA response	7*

* 7 with clear response. Additional experiments are necessary to further examine the other biosensors.

- ✕ Both **archaeal** and **bacterial** TF-promoter pairs functioned well in *E. coli*
- ✕ TFs act as **repressor** or **activator**
- ✕ Different mechanisms for ligand interaction:
Co-repression
Co-activation
De-repression
De-activation
Ligand independent
- ✕ TFs with one or multiple ligands
➔ specific/global TFs

This screening method allows the characterization of a large set of unknown TFs of the Lrp family and their suitability to use in a functional biosensor in *E. coli*.