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

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

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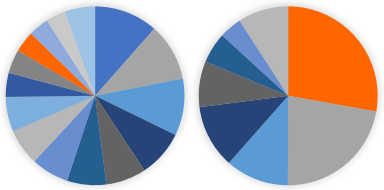
LRP TRANSCRIPTION FACTORS

- 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

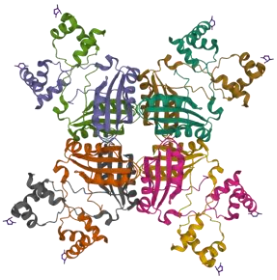
Huge variability, phylogenetically widespread with crucial physiological roles

→ **INTERESTING TARGETS FOR ENGINEERING**

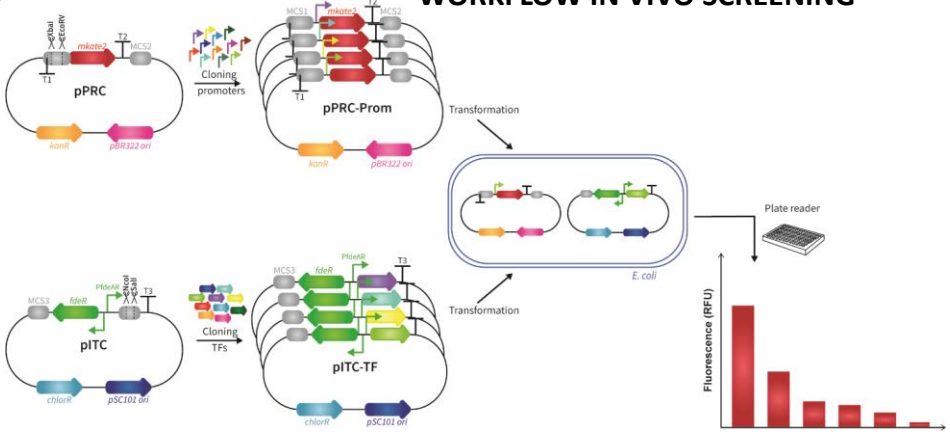


Up: Distribution of TF families in a bacterial versus an archaeal genome

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



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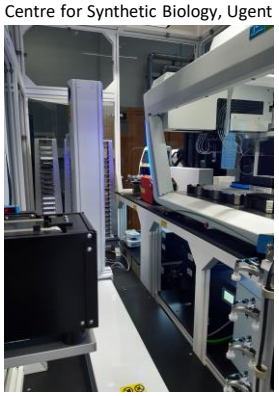
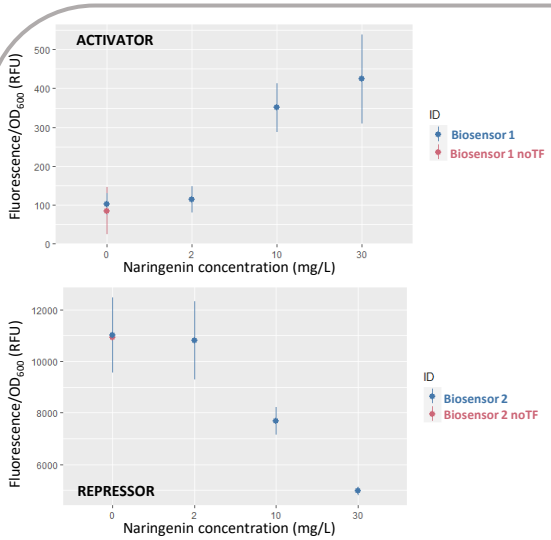


28 TFs were selected together with their promoters of interest. Cloning was performed in the **inducible TF construct** (pITC) and **promoter reporter construct** (pPRC), respectively, followed by a transformation in *E. coli*. Plate reader measurements were performed to determine OD and fluorescence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