

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

Bernauw, Amber; Bervoets, Indra; Peeters, Andries ; De Paepe, Brecht; De Mey, Marjan; Peeters, Eveline

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

Amber Joka Bernauw<sup>1,\*</sup>, Indra Bervoets<sup>1</sup>, Andries Peeters<sup>2</sup>, Brecht De Paepe<sup>2</sup>, Marjan De Mey<sup>2</sup>, Eveline Peeters<sup>1</sup>

1. Research group of Microbiology, Department of Bioengineering Sciences, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium  
2. Centre for Synthetic Biology, Department of Biotechnology, Ghent University, Coupure Links 653, B-9000 Gent, Belgium

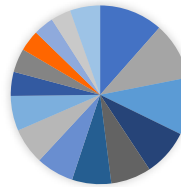
\* Amber.Joka.Bernauw@vub.be

## LRP TRANSCRIPTION FACTORS

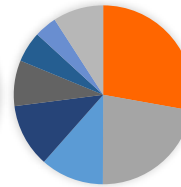
- ✎ Repressors, activators or dual regulators
  - ✎ Perform either specific or global regulation
  - ✎ Typical ligands are **amino acids**, but recently also other ligands have been found
  - ✎ Only a small amount of Lrp TFs is characterized
- Huge variability, phylogenetically widespread with crucial physiological roles**

➔ INTERESTING TARGETS FOR ENGINEERING

## BACTERIA



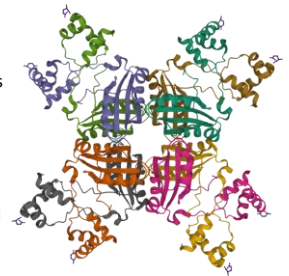
## ARCHAEA



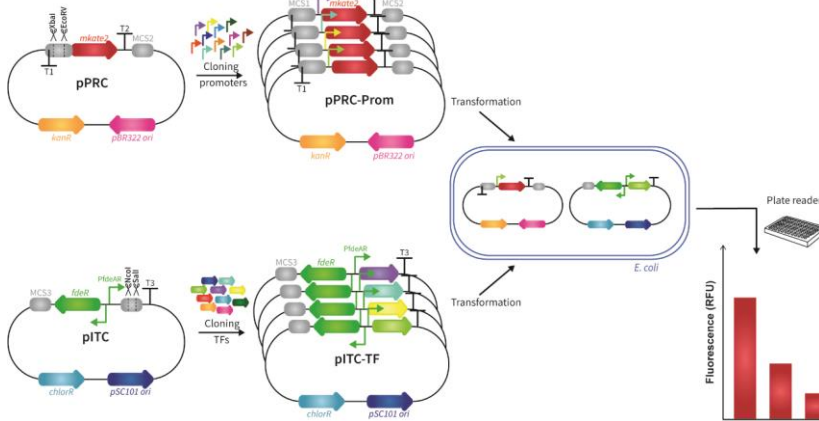
■ Lrp-type  
■ Other TFs

**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

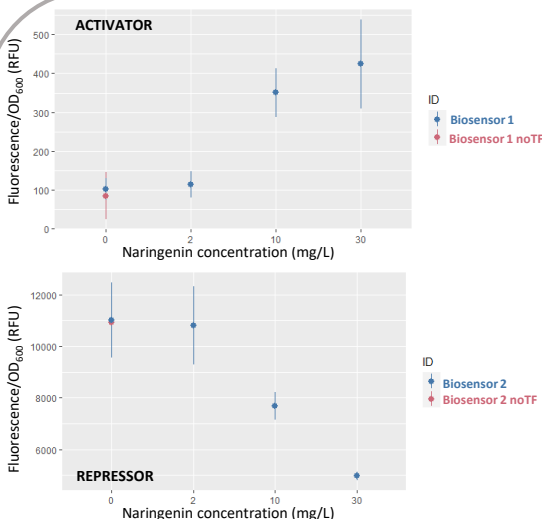


Centre for Synthetic Biology, Ugent



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	
▪ <b>Repression</b>	14
▪ <b>Activation</b>	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*.**