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Tracking Stress Adaptation in Bacteria using a "Library of Global Regulators of Gene Expression"

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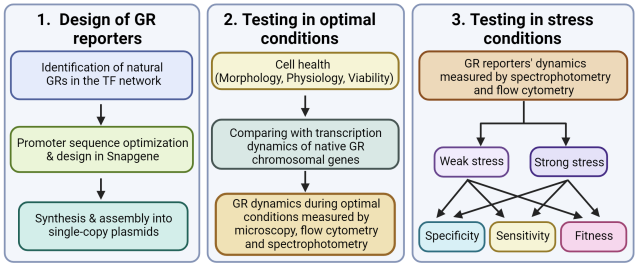
Introduction

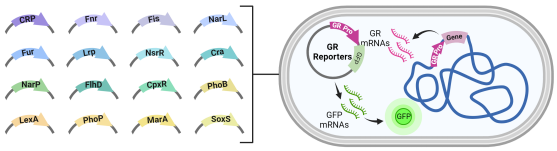
- A few transcription factors regulate one-third of all genes of *E. coli*, allowing the bacterium to quickly adapt to environmental changes, conserve energy, and perform essential life functions.
- Tracking their numbers over time should provide new insight on how they influence the activation of specific cellular programs under specific stress.
- We developed a library of fluorescent transcriptional reporters for these global regulators (GRs) and tested their sensitivity and specificity in stress conditions.

Workflow



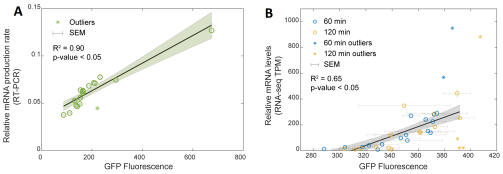
Results & Conclusions

1. Assembly of GR reporters in Single-copy plasmids



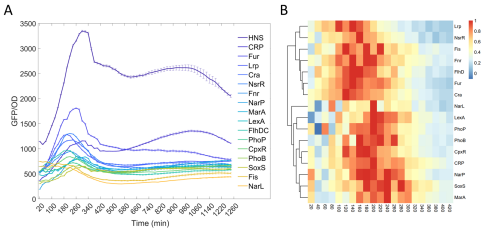
- Each of the 16 constructs harbors a single-copy plasmid coding for a fast-maturing, non-toxic green fluorescent protein (GFPmut3) fused to the full-length copy of the native promoter.

2. Comparing the plasmid activity with chromosomal GR genes



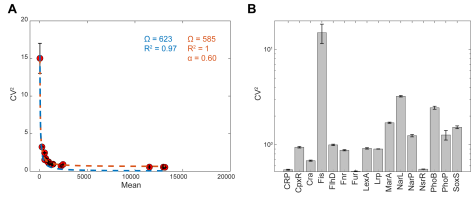
- We found a perfect correlation between the average mRNA levels of the natural GR genes (measured by RT-PCR and RNAseq) with the average GFP levels from almost all the GR reporter plasmids.

3. Monitoring GR expression pattern during exponential and stationary growth conditions



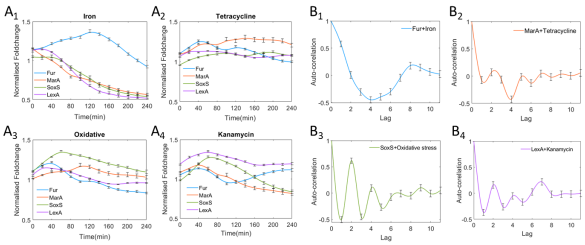
- We monitored the GRs' transcription activity in exponential and stationary growth phases and calculated the relative change in their corresponding promoter activity. We found two distinct dynamical behaviors.

4. Comparing single-cell variability in between GRs



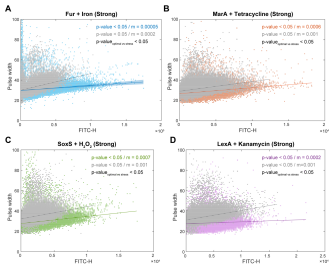
- The reporters have inherited specific features of the dynamics of transcription of the natural promoters, controlling not only the mean rate of production, but also the single-cell variability in the RNA production dynamics.

5. Testing specificity and sensitivity to weak stress conditions



- We found the plasmids to be sensitive and specific to individual stresses (A_1-A_4) without affecting their growth.
- We also observed the effects of auto-regulation in GRs during these stresses (B_1-B_4).

6. Efficiency of GR reporters during strong stress conditions



- We studied the behavior of the reporters under 'strong' stresses when the growth rate differs from the optimal condition.
- There is a positive, statistically significant correlation between the reporters' signal and the pulse width.
- However, the fluorescence levels are much stronger under stresses.

Example future applications