

Development of an epigenetic tetracycline sensor system based on DNA methylation

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Supplementary Information

Supplemental Tables

Supplemental Table 1: Statistical analysis of the readout of the tetracycline memory system used as whole-cell biosensor shown in Figure 6.

Supplemental Figures

Supplemental Figure 1: Sensitivity of DH5 α cells to tetracycline in liquid cultures.

Supplemental Figure 2: Investigation of the tetracycline sensor system at 28 °C with 10 μ M ZnSO $_4$.

Supplemental Figure 3: ON-state cell analysis of the tetracycline sensor system.

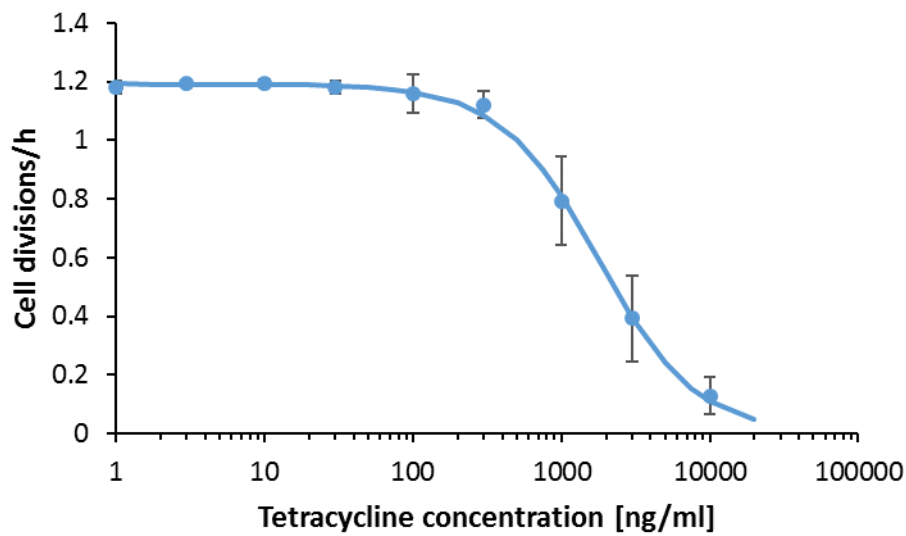
Supplemental Text

Supplemental Text 1: List of all plasmids used in this paper.

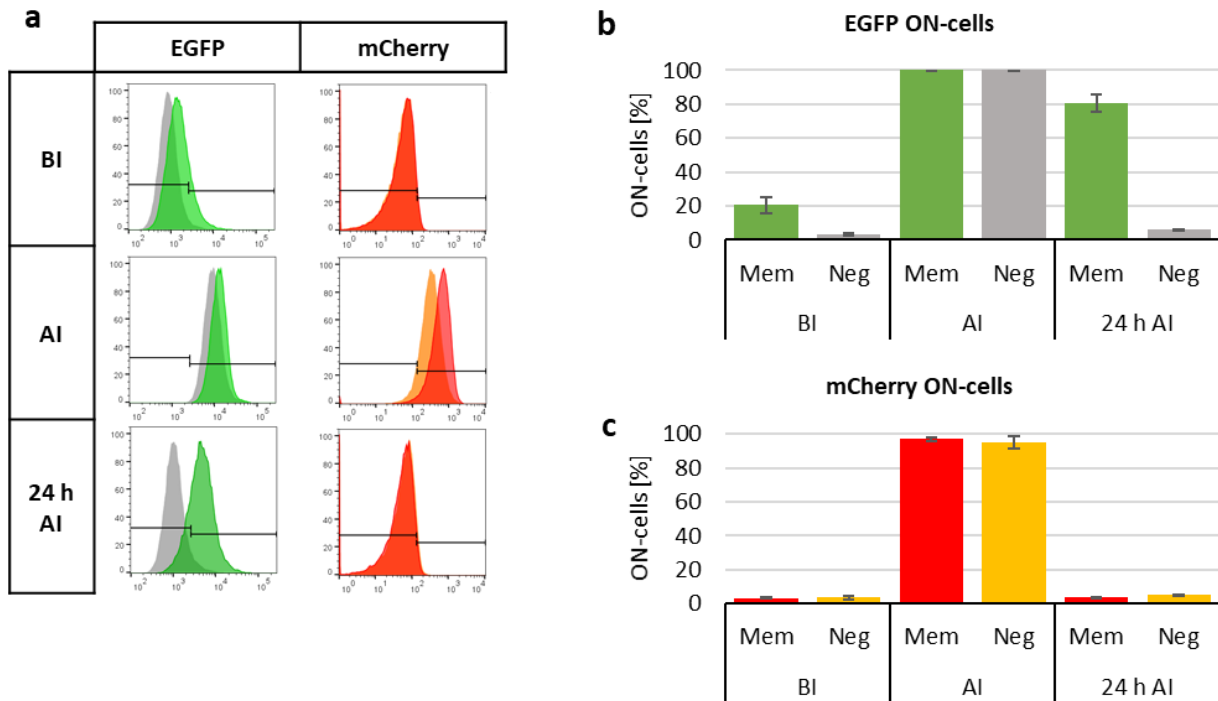
Supplemental Text 2: Tetracycline trigger plasmid annotated DNA sequence

Supplemental Table 1: Statistical analysis of the readout of the tetracycline memory system used as whole-cell biosensor shown in Figure 6. The indicated p-values refer to the probability of an increase in the number of ON-state cells after induction when compared to the values before induction using a one-tailed two sample t-test (without assuming homogeneity of variance). NS, not significant (p-values larger than 0.05). AI, after induction.

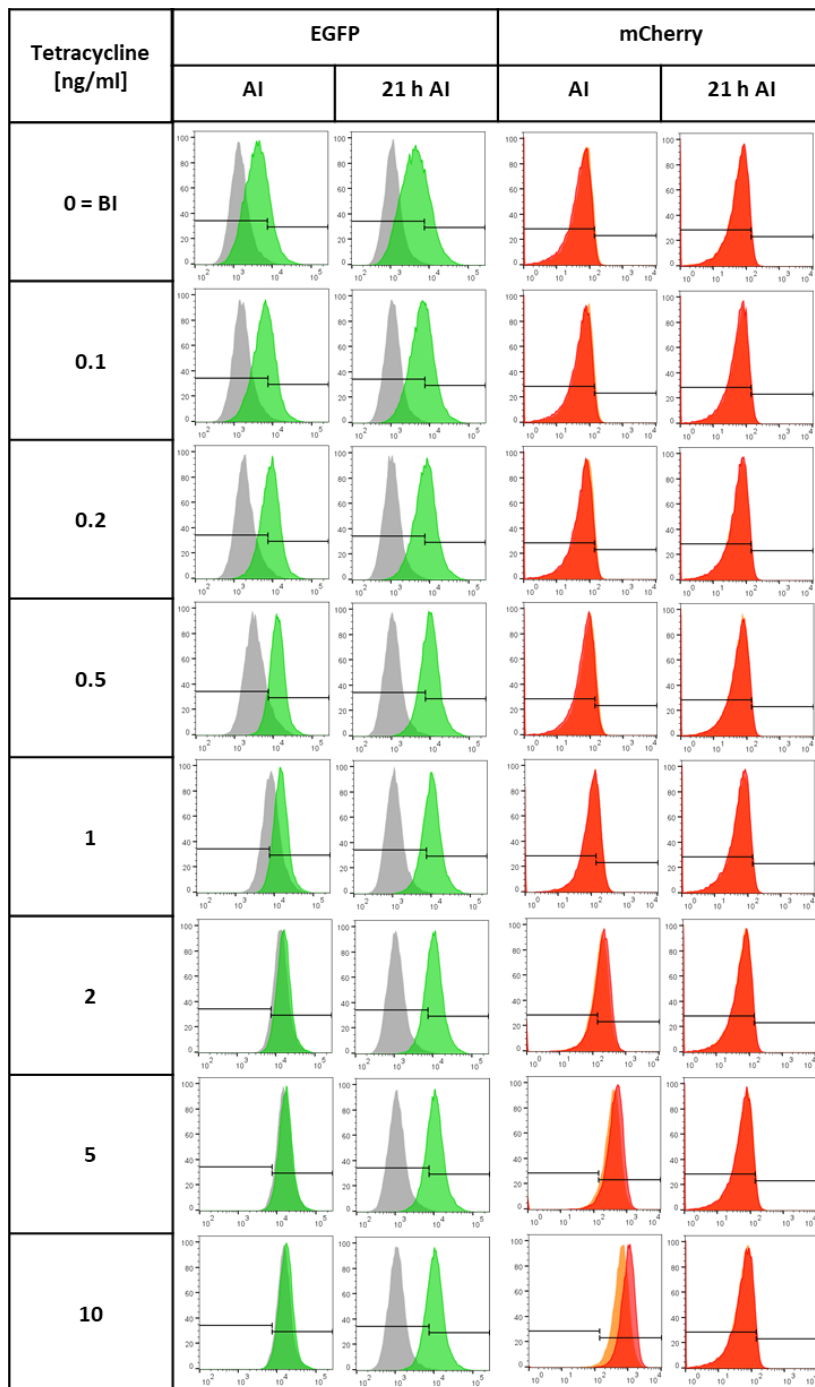
| Tetracycline memory system induced with tetracycline x ng/ml (AI) compared to before induction | p-value for fractions of ON-state cells measured with flow cytometry | |
|--|--|----------|
| | EGFP | mCherry |
| AI with 0.1 ng/ml | 2.13E-03 | NS |
| AI with 0.2 ng/ml | 2.07E-02 | 1.97E-02 |
| AI with 0.5 ng/ml | 7.24E-05 | 6.96E-03 |
| 21 h AI with 0.1 ng/ml | NS | NS |
| 21 h AI with 0.2 ng/ml | 4.73E-02 | NS |
| 21 h AI with 0.5 ng/ml | 7.96E-04 | NS |



Supplemental Figure 1: Sensitivity of DH5 α cells to tetracycline in liquid cultures. Cell division rates were determined in liquid LB culture containing different concentrations of tetracycline during the exponential growth phase at 37 °C. Data are averages of three biological repeats, error bars indicate standard deviations. The line shows a fit to the Hill equation revealing a half-maximal inhibition of cell growth at 1760 ng/ml tetracycline under these conditions.



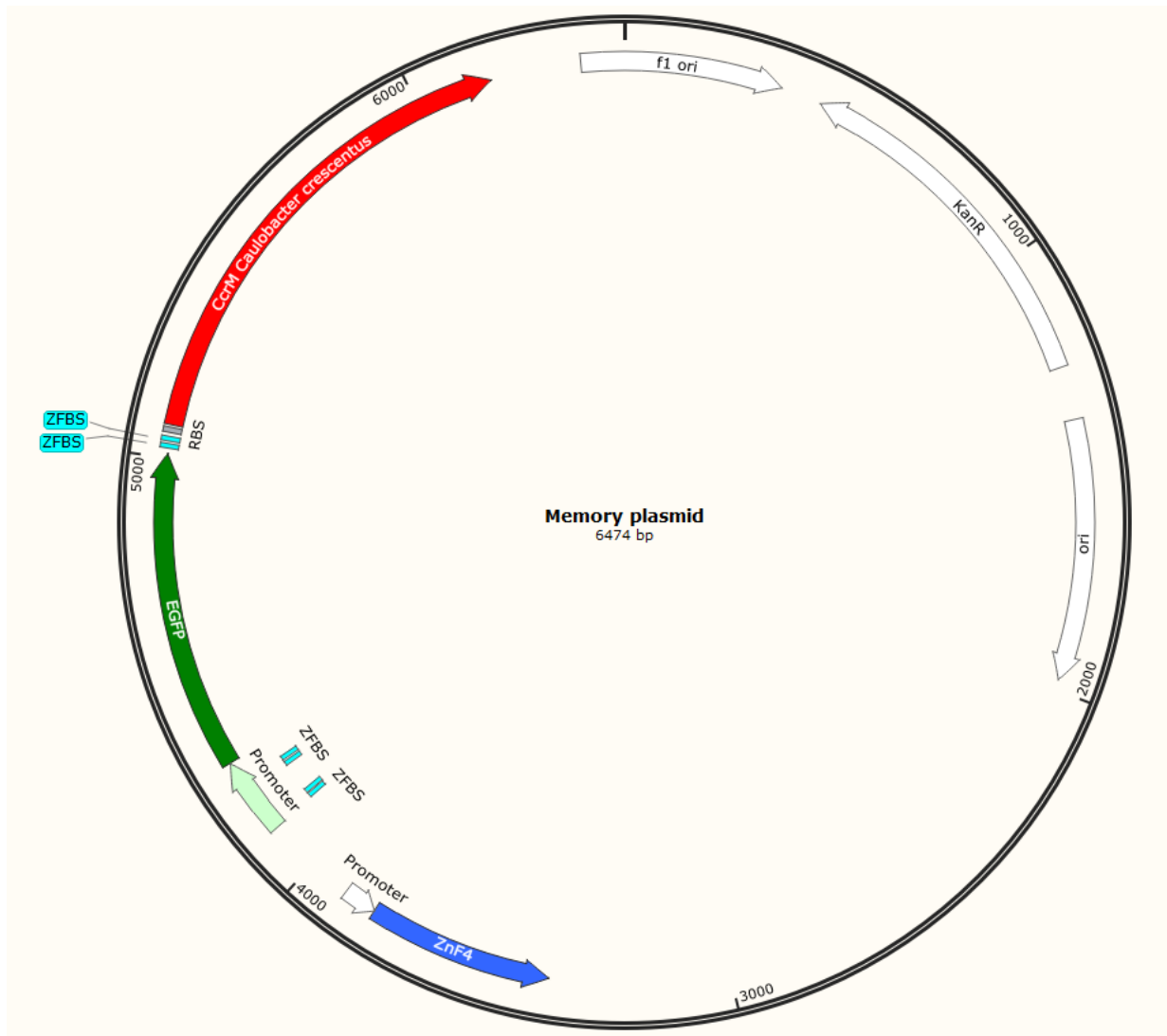
Supplemental Figure 2: Investigation of the tetracycline sensor system at 28 °C with 10 μ M ZnSO_4 . EGFP (green) and mCherry (red) expression was measured with flow cytometry of *E. coli* carrying TriTet and either the memory plasmid (Mem) or the negative control memory plasmid with an inactive CcrM mutant (Neg). Cells were cultured without tetracycline (before induction, BI) and with 10 ng/ml tetracycline (after induction, AI). The induced cultures were grown for further 24 h in a medium without tetracycline (24 h AI). a) Histograms of the single cell populations and the applied gates. b) and c) percentage of ON-state cells (ON-cells) for EGFP and mCherry. EGFP signal of the memory system = green bars, EGFP signal of the negative control = gray bars, mCherry signal of the memory system = red bars, mCherry signal of the negative control = orange bars. All averages are based on 3 biological replicates, error bars indicate the SD.



Supplemental Figure 3: ON-state cell analysis of the tetracycline sensor system. Exemplary histograms of the tetracycline memory system (Mem) or the negative control (Neg) of the EGFP (green or gray) and mCherry (red or orange) signals measured with flow cytometry. The gates used to distinguish between ON- and OFF-state cells are indicated. The fluorescence signal is plotted against the number of events and the histograms were normalized to the mode of the number of events. All events on the right side of the gate were defined as ON-state cells. The compiled data are shown in Figure 6.

Supplemental Text 1: List of all plasmids used in this paper.

Memory plasmid: The DNA sequence is given and annotated in Supplementary Note 1 of Maier et al. (2017). The plasmid map is provided below.



Negative control memory plasmid: The Negative control memory plasmid is identical to the Memory plasmid, except the D31A mutation in the *ccrM* gene, which leads to catalytic inactivation.

Arabinose trigger plasmid: The map of the arabinose trigger plasmid can be found in Maier et al. (2017) Supplementary Figure 7 B.

Tetracycline trigger plasmid: The plasmid map of the tetracycline trigger plasmid can be found in this work (Figure 4 B) and the annotated DNA sequence in Supplemental Text 2.

Supplemental Text 2: Tetracycline trigger plasmid annotated DNA sequence

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DEFINITION synthetic linear DNA
ACCESSION .
VERSION .
KEYWORDS TriTet_wRBS_paper (1 - 5864)
SOURCE synthetic DNA construct
ORGANISM synthetic DNA construct
REFERENCE 1 (bases 1 to 5864)
AUTHORS Jeltsch Group
TITLE Direct Submission
JOURNAL Exported Thursday, Jany 9, 2020 from SnapGene 3.3.3
<http://www.snapgene.com>

FEATURES Location/Qualifiers

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