Supplementary Data

An engineered small RNA-mediated genetic switch based on a ribozyme expression platform

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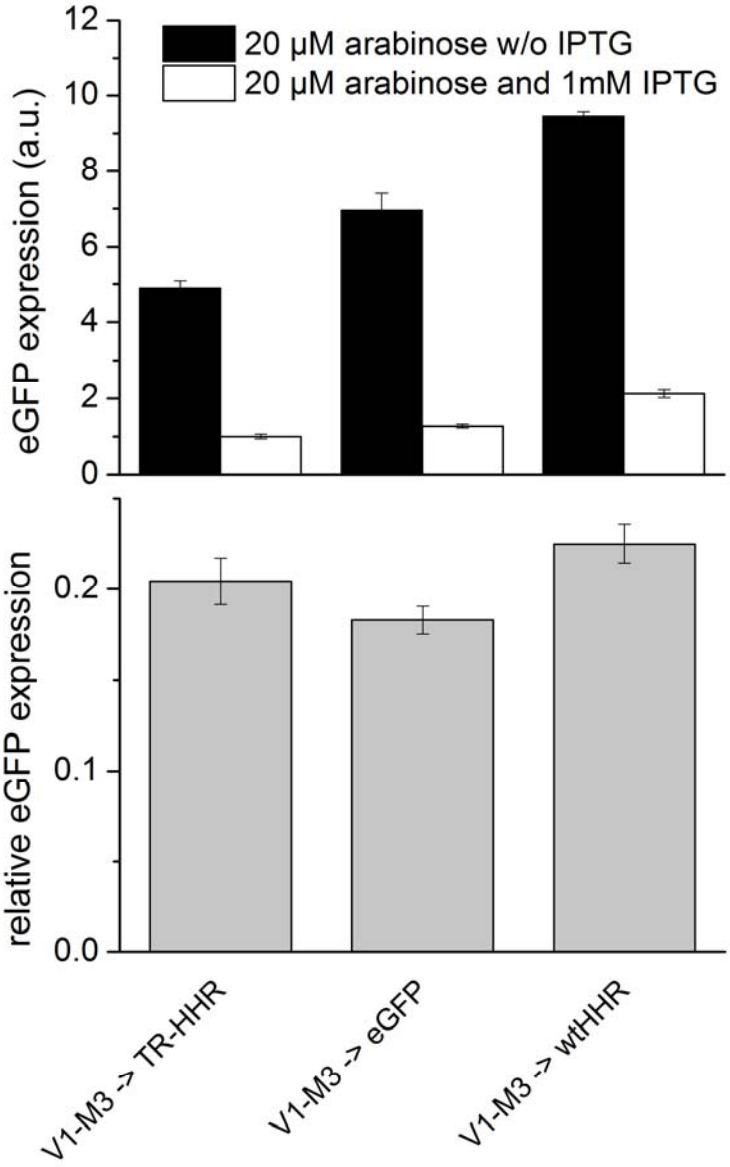
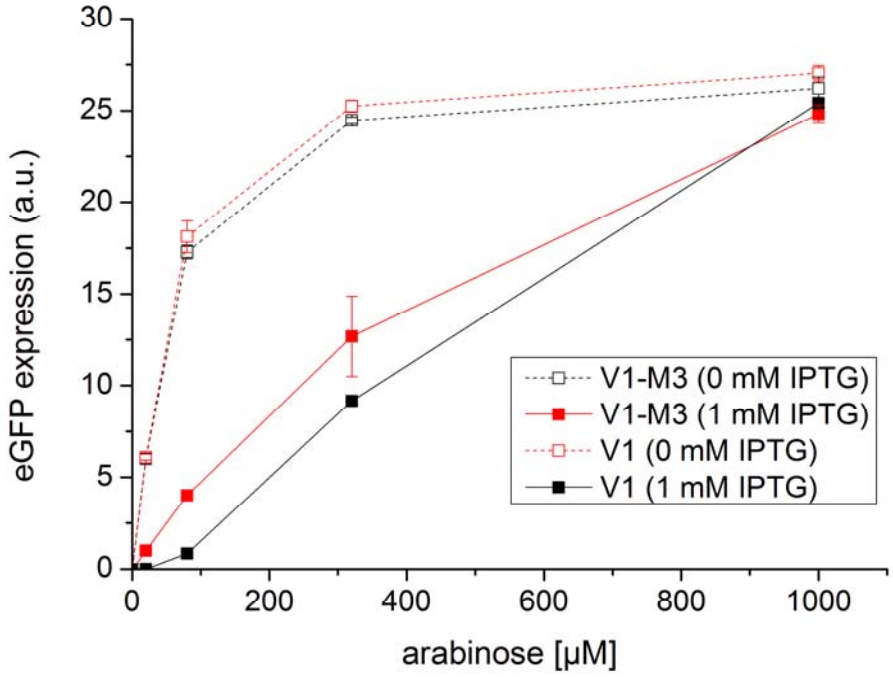
Content:

Supplementary Figure S1



Supplementary Figure S1. The effect of *ta*RNA V1-M3 on the eGFP mRNA under control of the TR-HHR was compared to the effect of *ta*RNA V1-M3 on the native eGFP mRNA and an eGFP mRNA controlled by a wt-HHR. Experiments were conducted with the *E. coli* Top10 strain which was cultivated in LB medium at 37°C. Top: Reporter gene expression of outgrown bacterial cultures induced with either 20µM arabinose without IPTG or 20 µM arabinose supplemented with 1mM IPTG were measured. Bottom: The relative eGFP expression levels of cultures induced with 20µM arabinose with 1mM IPTG to cultures supplemented with 20 µM arabinose without IPTG were determined. Errors bars represent the standard deviation of experiments performed in triplicates.

Supplementary Figure S2



Supplementary Figure S2. The influence of IPTG on eGFP expression of cultures expressing TR-HHR and either *ta*RNA V1-M3 or *ta*RNA V1 was measured. *E. coli* Top10 strain were used for the experiments and cultivated in LB medium at 37°C. Reporter gene expression levels of outgrown bacterial cultures induced with transcriptional inducers as indicated were determined. Errors bars represent the standard deviation of experiments performed in triplicates.

Supplementary Sequences

Sequences for the *ta*RNA constructs are shown. Hammerhead ribozyme for 5'-processing is marked in yellow, endonuclease sites used for molecular cloning procedures are in italic and changes in comparison to the *ta*RNA V1 are marked in red.

_ta_RNA V1

*GATCTGGTACC*CGGTACATCCAGCTGATGAGTCCCAAATAGGACGAAACGGTGAAAGCCGTCCT
GGATTCCACGGGTACCACCCAAATCCAGGAGGTCATTGGTAGTGGTGGTTAATGAAAATTAACTT
ACTACTACCATATATCTCTAGA*AAGCTT*CCTAGGCTTAAACGCCTGGGGTAATGACTCTCTAGCTT
G

_ta_RNA V1i

*GATCTGGTACC*CGGTACATCCAGCTGATGAGTCCCAAATAGGACGATACGGTGAAAGCCGTCCT
GGATTCCACGGGTACCACCCAAATCCAGGAGGTCATTGGTAGTGGTGGTTAATGAAAATTAACTT
ACTACTACCATATATCTCTAGA*AAGCTT*CCTAGGCTTAAACGCCTGGGGTAATGACTCTCTAGCTT
G

_ta_RNA V1-M1

*AGATCTGGTACC*CGGTACATCCAGCTGATGAGTCCCAAATAGGACGAAACGGTGAAAGCCGTCC
TGGATTCCACG*GGTACC*ACGGTTATCCAGGAGGTCATTGGTAGTGGTGGTTAATGAAAATTAACT
TACTACTACCATATATCTCTAGA*AAGCTT*CCTAGGCTTAAACGCCTGGGGTAATGACTCTCTAGCT
TG

_ta_RNA V1-M2

*AGATCTGGTACC*CGGTACATCCAGCTGATGAGTCCCAAATAGGACGAAACGGTGAAAGCCGTCC
TGGATTCCACG*GGTACC*TGGGTTTAGGTGGAGGTCATTGGTAGTGGTGGTTAATGAAAATTAACT
TACTACTACCATATATCTCTAGA*AAGCTT*CCTAGGCTTAAACGCCTGGGGTAATGACTCTCTAGCT
TG

_ta_RNA V1-M3

AGATCTGGTACCCGGTACATCCAGCTGATGAGTCCCAAATAGGACGAAACGGTGAAAGCCGTCC
TGGATTCCACGGGTACCTGGGTTTAGGTCCTCCTCATTGGTAGTGGTGGTTAATGAAAATTAACTT
ACTACTACCATATATCTCTAGA*AAGCTT*CCTAGGCTTAAACGCCTGGGGTAATGACTCTCTAGCTT
G

_ta_RNA V1-M4

*AGATCTGGTACC*CGGTACATCCAGCTGATGAGTCCCAAATAGGACGAAACGGTGAAAGCCGTCC
TGGATTCCACG*GGTACC*ACCCAAATCCAGGAGGTCATTGGTAGTGGTGGTTAATGAAAATTAACT
TACTACTACCATATATCTCTTGGATAGA*AAGCTT*CCTAGGCTTAAACGCCTGGGGTAATGACTCTC
TAGCTTG

***ta*RNA V1-M5**

*AGATCTGGTACC*CGGTACATCCAGCTGATGAGTCCCAAATAGGACGAAACGGTGAAAGCCGTCC
TGGATTCCACG*GGTACC*ACCCAAATCCAGGAGGTCATTGGTAGTGGTGGTTAATGAAAATTAACT
TACTACTACCATATTAGAGAAGA*AAGCTT*CCTAGGCTTAAACGCCTGGGGTAATGACTCTCTAGCT
TG

***ta*RNA V1-M6**

*AGATCTGGTACC*CGGTACATCCAGCTGATGAGTCCCAAATAGGACGAAACGGTGAAAGCCGTCC
TGGATTCCACG*GGTACC*ACCCAAATCCAGGAGGTCATTGGTGGTTAATGAAAATTAACTTACTATA
TATCTCTAGA*AAGCTT*CCTAGGCTTAAACGCCTGGGGTAATGACTCTCTAGCTTG

***ta*RNA V1-M7**

*AGATCTGGTACC*CGGTACATCCAGCTGATGAGTCCCAAATAGGACGAAACGGTGAAAGCCGTCC
TGGATTCCACG*GGTACC*TACCTTTGTCTTCTTTAATACCCAAATCCAGGAGGTCATTGGTAG*AAGC
TT*CCTAGGCTTAAACGCCTGGGGTAATGACTCTCTAGCTTG

CrI

*AGATCT*GAAGAGAGGTATGACTGCCGCAATTTCAGCGCATAGGCGACGATCTCCAGGCCGTGGG
CTTAAAGCCTGGGGTAATGACTCTCTAGCTTG

Sequences for the reporter genes used in this study are shown. P_{Bad} promoters are underlined, hammerhead ribozymes are marked in yellow, translation start /end sites are written in bold letters and endonuclease sites used for molecular cloning procedures are in italic.

P_{Bad} - TR-HHR – eGFP – 3'UTR

GACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATACC*ACTAGT*GGACGTTTAGCTTTCTCCTTC
GGTACATCCAGCTGATGAGTCCCAAATAGGACGAAACCTCTTGGATTTGGGTATTAAAGAGGTCC
TGGATTCCACGAAGGAGATATACC**ATG…TAA**ATGCTCGAGGATCCGGCTGCTAACAAAGCCCGA
AAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATA*CCGCGG*

P_{Bad} - wt-HHR – eGFP – 3'UTR

GACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATACC*ACTAGT*GGACGTTTAGCTTTCTCCTTC
GGTACATCCAGCTGATGAGTCCCAAATAGGACGAAACGGTGAAAGCCGTCCTGGATTCCACGAA
GGAGATATACC**ATG…TAA**ATGCTCGAGGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGT
TGGCTGCTGCCACCGCTGAGCAATA*CCGCGG*

P_{Bad} - eGFP - 3'UTR

GACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATACC*ACTAGT*GGACGTTTAGCTTTAAGAAGG
AGATATACC**ATG...TAA**ATGCTCGAGGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTG
GCTGCTGCCACCGCTGAGCAATA*CCGCGG*