Supplementary Materials

Dual control system - a novel scaffolding architecture of an inducible regulatory device for the precise regulation of gene expression

Horbal L., Luzhetskyy A.*

Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS) Helmholtz Center for Infectious Research (HZI), Department Microbial Natural Products, Actinobacteria Metabolic Engineering Group, Saarland University, Campus C2.3, 66123, Saarbrücken, Germany

*Corresponding author – Prof. Dr. Andriy Luzhetskyy

Materials and methods

1.1. Construction of cumate-inducible promoters with different strengths

To construct four different inducible promoters, the *cmt* operator (Horbal et al., 2014) was introduced downstream of the PA2, PA3, PA4 and PA8 semisynthetic promoters (Siegl et al., 2013) via PCR using four different reverse primers: CymRPA2operRev, CymRPA3operRev, CymRPA4operRev and CymRPA8operRev (Table 2). The forward primer in all four cases was the same – CymRForw (Table 2). As a result of PCR amplification, four 2.4 kb fragments were obtained and were digested with *Nhe*I/*Spe*I and cloned into the *Xba*I/*Spe*I hydrolyzed pGUS vector (Myronovkyi et al., 2011), yielding the plasmids pGCymRPA2, pGCymRPA3, pGCymRPA4 and pGCymRPA8 (Table 1); these plasmids contained a fusion of the *gusA* reporter gene with four promoters, PA2, PA3, PA4 and PA8, respectively, and the *cmt* operator.

1.2. Construction of synthetic gene circuits with three operons

A codon-optimized version of the *egfp* gene fused to the PA3 synthetic promoter and the *cmt* operator and the *mCherry* gene fused to the PA8 promoter and the *cmt* operator were synthesized by GenScript company (USA) as a single fragment and cloned into the *Eco*RV site of pJET1.2, yielding pJETEgfpMcherry. An *Eco*RV DNA fragment that contained both *egfp* and *mCherry* genes was retrieved from pJETEgfpMcherry and cloned into *Eco*RV-digested pGCymRP21, yielding pGCymPA3egfpA8RFP (Table 1).

To construct another gene circuit where the *gusA*, *egfp* and *mCherry* reporter genes were placed under the control of the PA2, PA8 and PA3 inducible promoters, respectively, the following steps were performed. First, the *mCherry* gene was

retrieved from pJETEgfpMcherry as a *Spe*I fragment and ligated into the *Nhe*I-hydrolyzed pJETEgfpMcherry plasmid, yielding pJETA3Mcherry (Table 1). The *egfp* gene was obtained as a *Nhe*I fragment from pJETEgfpMcherry and cloned into the *Spe*I-hydrolyzed pJETA3Mcherry, giving pJETA3McherryA8gfp (Table 1). Finally, the *Eco*RV fragment that contained both the *mCherry* and the *egfp* genes under the control of the PA3 and PA8 promoters, respectively, was cloned into *Eco*RV-digested pGCymRPA2, yielding pGCymRPA2A3RFPA8gfp (Table 1).

1.3. *Construction of dual control systems based on riboswitch*

A 1.9-kb fragment containing the *gusA* gene fused to the theophylline riboswitch (Rudolph et al., 2013) was amplified using the primers RiboswitchForward and RiboswitchRev (Table 2) from pGUST_ermE_E* (Table 1). The fragment was then digested with *Spe*I/*Eco*RV and cloned into respective sites of pGCymRP21 or pGUSRolPA3, yielding pGCymRibos or pGUSRolRibos (Table 1), respectively.

A *Bam*HI/*Eco*RV DNA fragment containing the *gusA* gene under the control of the CymR-based dual control system was retrieved from pGCymRRibos and cloned into respective sites of pKChyg (Myronovskyi et al., 2011), giving pKHygCymRibos (Table 1).

1.4. *Construction of a hyper-inducible riboswitch*

The pGCymRibos and pKCHygCymRibos plasmids were digested with *Swa*I and were self-ligated, yielding pGusP21Riboswitch and pKCHygP21Riboswitch (Table 1), respectively.

1.5. *Construction of dual control systems based on ribozyme*

A 1.954-kb fragment containing the *gusA* gene fused to the theophylline ribozyme (Rudolph et al., 2013) was amplified with PCR using the primers RibozymelinkerForw and RibozymelinkerRev (Table 2) from pGCymRP21 (Table 1). The fragment was digested with *Spe*I/*Eco*RV and cloned into respective sites of pGCymRP21, yielding pGCymRibozyme (Table 1), respectively.

1.6. *Construction of pTOShyg*

The apramycin resistance gene in the plasmid pTOS was replaced with the *hyg* cassette (pOJ10700) using the λRed recombination process (Gust et al., 2004) and HygRVSAmForw and HygRVSAmRev primers (Table 2). This generated the plasmid pTOShyg.

1.7. *Construction of plasmids for the control of cre expression*

A 1.1-kb DNA fragment containing the *cre* gene fused to a riboswitch was amplified from pLACre (Table 1) using the primers CreCymRiboswForw and CreCymRiboswitRev (Table 2). The fragment was then digested with *Spe*I/*Eco*RV and cloned into respective sites of pKHygCymRibos, yielding pKHygCymRibosCre (Table 1).

A *Spe*I/*Eco*RV fragment containing the *cre* gene fused to a riboswitch was obtained from pKHygCymRibosCre and cloned into respective sites of pGCymRP21 or pGUSRolRPA3, giving pGCymRibosCre and pGRolRibosCre (Table 1).

A *Bam*HI/*Eco*RV fragment containing the *cre* gene fused to the cumate dual control system was obtained from pKHygCymRibosCre and cloned into the *Bgl*II/*Sna*BI site of the pTOShyg vector, yielding pTOShygCymRibosCre (Table 1).

A *Bam*HI/*Eco*RV fragment containing the *cre* gene fused to the resorcinol dual control system was obtained from pGRolRibosCre and cloned into the *Bgl*II/*Sna*BI sites of the pTOShyg vector, yielding pTOShygRolRibosCre (Table 1).

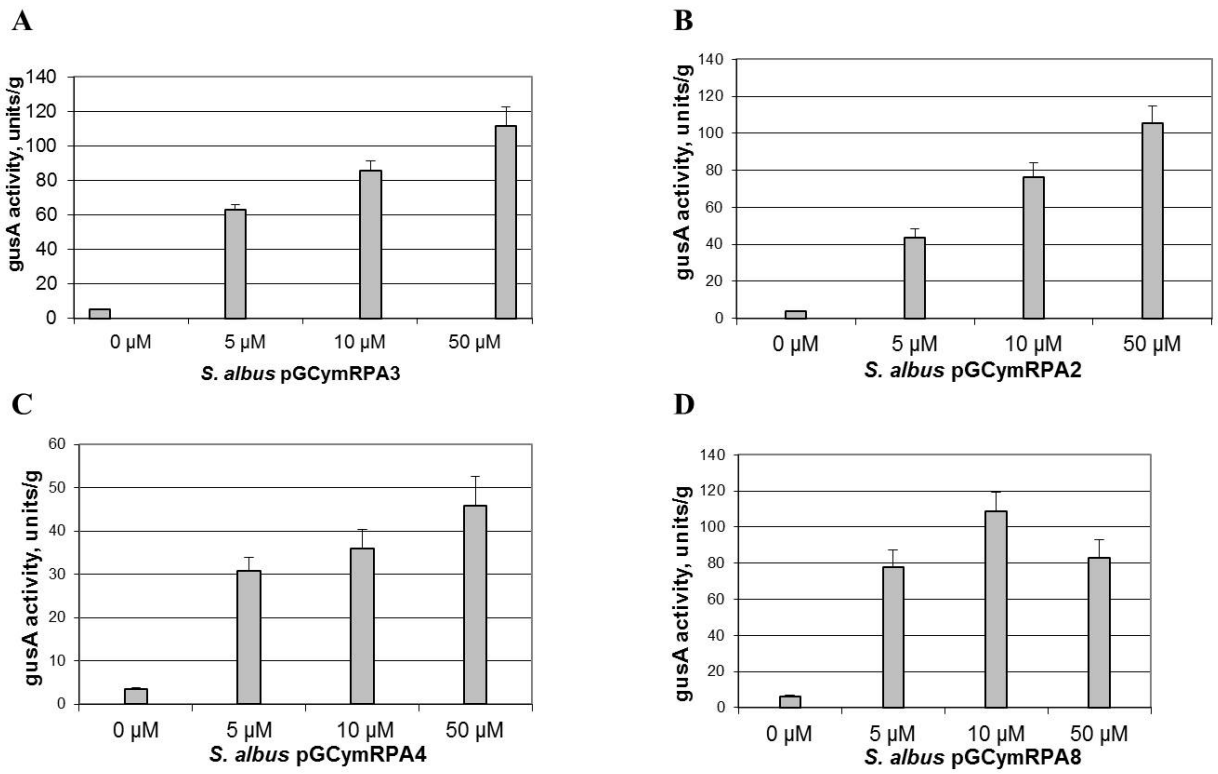

Figure S1. Glucuronidase activity in cell lysates of recombinant *Streptomyces albus* strains. (A) Activity in *S. albus* containing *gusA* under the control of the PA3-*cmt* inducible promoter. (B) Activity in *S. albus* containing *gusA* under the control of the PA2-*cmt* inducible promoter. (C) Activity in *S. albus* containing *gusA* under the control of the PA4-*cmt* inducible promoter. (D) Activity in *S. albus* containing *gusA* under the control of the PA8-*cmt* inducible promoter. The strains were grown in TSB medium for 2 days. Error bars indicate the standard deviations of triplicate experiments.

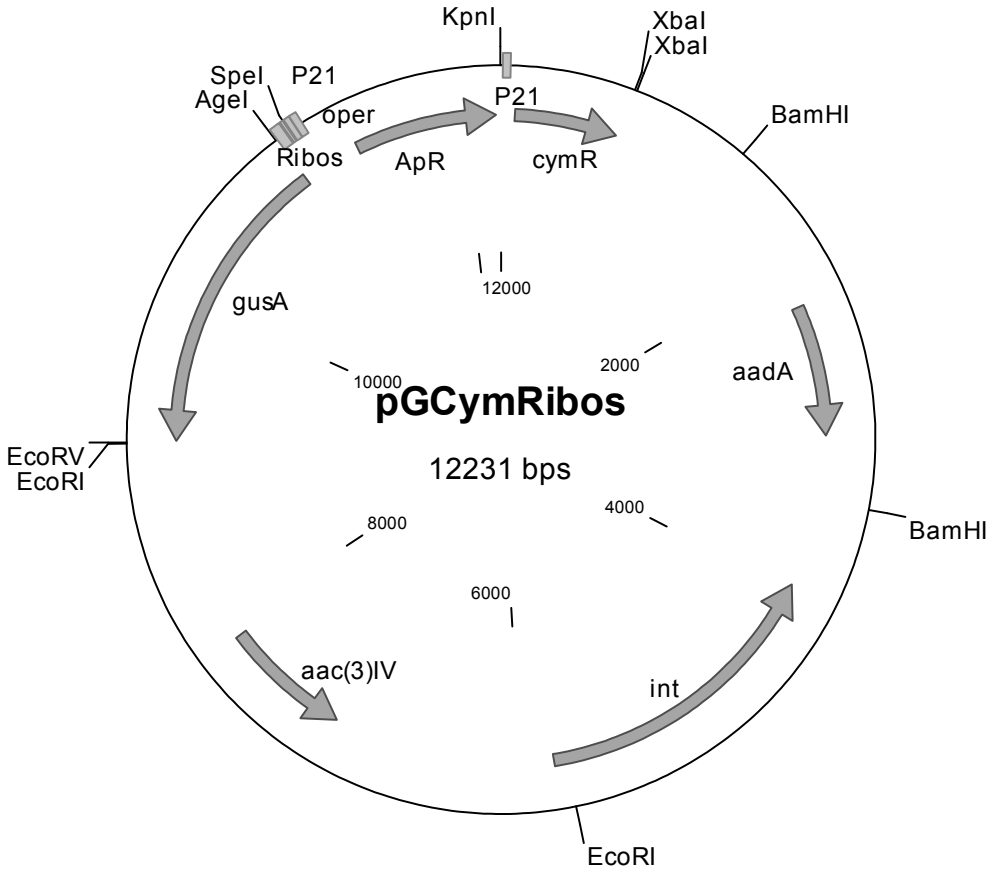

Figure S2. Map of the pGCymRRibos plasmid: *gusA*, reporter gene; P21, synthetic promoter; oper, *cmt* operator; Ribos, theophylline riboswitch; *cymR*, gene coding for the repressor; ApR, *aadA* and *aac(3)IV,* ampicillin, spectinomycin and apramycin resistance genes, respectively; *int*, integrase gene.

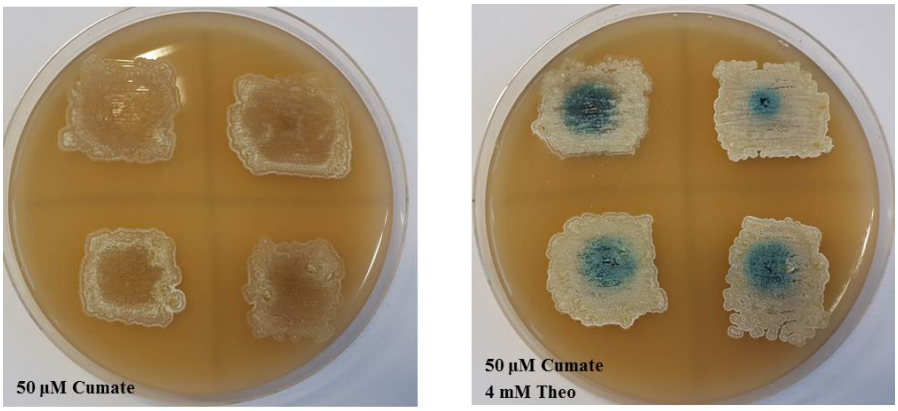

Figure S3. Colonies of *S. albus* pGCymRibos^{+} overlaid with X-Gluc solution growing in the presence or absence of inducers (cumate, theophylline) for 3 days. Blue halos are colored by 5, 5-dibromo-4, 4-dichloro-indigo, formed by the β-glucuronidase activity. The lawns of the strains that contained only the riboswitch grown on MS-agar without theophylline turned slightly blue within 2-4 hours after application of X-Gluc. In contrast, a lawn of *S. albus* and *S. lividans* containing the cumate-riboswitch dual control system, grown in the absence of inducers or in the presence of only cumate, did not turn blue even after several days after flooding with substrate. At the same time, lawns of the aforementioned strains grown in the presence of only theophylline or both inducers simultaneously turned dark blue in less than 30 minutes to 1 hour.

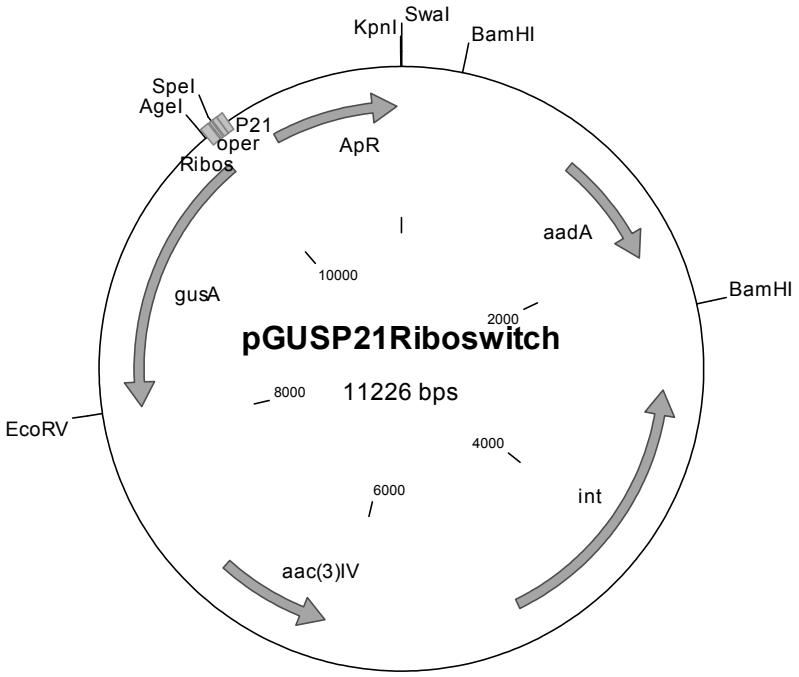

Figure S4. Map of the pGUSP21Riboswitch plasmid: *gusA*, reporter gene; P21, synthetic promoter; Ribos, theophylline riboswitch; oper, *cmt* operator; ApR, *aadA* and *aac(3)IV,* ampicillin, spectinomycin and apramycin resistance genes, respectively; *int*, integrase gene.

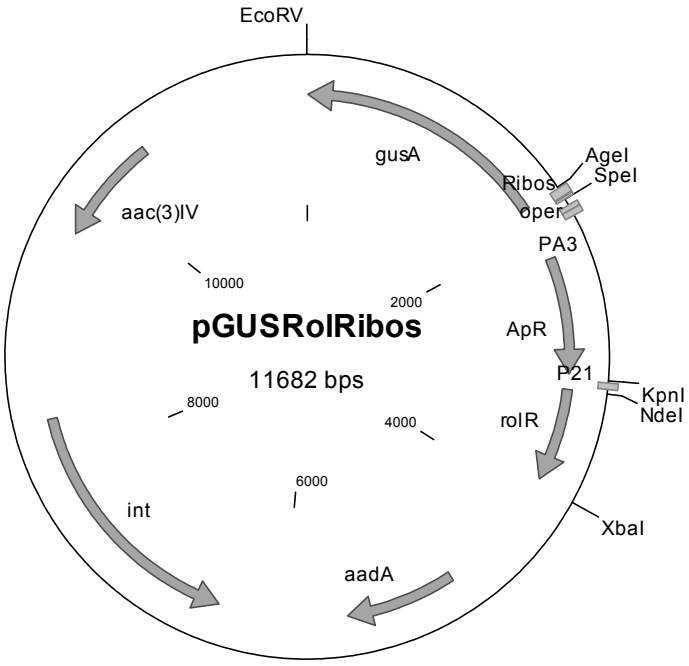

Figure S5. Map of the pGUSRolRibos plasmid: *gusA*, reporter gene; P21, synthetic promoter; Ribos, theophylline riboswitch; *oper*, *rolO* operator; *rolR*, gene coding for the repressor; ApR, *aadA* and *aac(3)IV,* ampicillin, spectinomycin and apramycin resistance genes, respectively; *int*, integrase gene.