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Full Paper

Systematic promoter design for plasmid-encoded *S*-adenosylmethionine sensing systems

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Taro Watanabe[1,2], Yuki Kimura[1] and Daisuke Umeno[1]*

[1] Department of Applied Chemistry, Faculty of Science, and Engineering, Waseda University, 3-4-1 Ohkubo, Shinjuku-ku, Tokyo 169-8555, Japan

[2] Kirin Central Research Institute, Kirin Holdings Company, Limited, 2-26-1, Muraoka-Higashi, Fujisawa, Kanagawa 251-8555, Japan

(* corresponding author: umeno@waseda.jp)

Abstract

S-adenosylmethionine (SAM) is an important biomolecule that mainly acts as a methyl donor and plays many roles in a variety of biological functions. SAM is also required for the biosynthesis of valuable methylated compounds, but its supply is a bottleneck for these biosynthetic pathways. To overcome this bottleneck and to reconfigure SAM homeostasis, a high-throughput sensing system for changes in intracellular SAM availability is required. We constructed a plasmid that can detect the factors that can alter SAM availability using minimal components. It does so by placing a fluorescent protein under a promoter controlled by endogenous MetJ, a transcription factor that represses its own regulons upon binding with SAM. Next, to validate SAM-responsive behavior, we systematically reconstructed 10 synthetic promoters with different positions and with different number of metbox sites, sequences of MetJ binding. We found that a position between the −35 box and the −10 box was the most effective for repression and that this setup was suitable for detecting the genetic or environmental factors that can deplete and recover the intracellular SAM availability. Overall, the response patterns of the synthetic MetJ-regulated promoters characterized in this study may be useful for the development of further SAM biosensing systems.

Keywords: biosensor; promoter design; *S*-adenosylmethionine; transcription factor

Introduction

S-adenosylmethionine (SAM) is an essential biomolecule for all species, and various methylation reactions that involve SAM as a methyl donor are used to regulate physiological functions. In eukaryotes, epigenetic regulation of gene expression is controlled by the methylation of histones and DNA (Jones, 2012; Lee et al., 2005). In prokaryotes, DNA methylation is also used to control the cell cycle (Reisenauer et al., 1999), and this process involves distinguishing between self and nonself DNA (Tock and Dryden, 2005), as well as between nascent and template DNA strands during the DNA mismatch repair process (Putnam, 2021). In addition, the methylation of tRNA, rRNA, and translation machinery proteins is also essential for the maintenance of translational function and fidelity (Marbaniang and Vogel, 2016; Polevoda and Sherman, 2007). SAM is also involved in the regulation of receptor sensitivity (Bi and Sourjik, 2018) as well as the detoxification of toxic metal ions and metabolite analogs (Begum et al., 2022; Cai and Clarke, 1999). Finally, SAM is also widely used in the synthesis of bio-membrane lipids (Gibellini and Smith, 2010; Grogan and Cronan, 1997), biotin (Sirithanakorn and Cronan, 2021), polyamines (Sauter et al., 2013), quorum sensing molecules (Fast and Tipton, 2012), and signaling molecules (Chi et al., 2023). In these pathways, it acts as a donor of not only methyl groups, but also amine groups, aminopropyl groups, and an entire structural backbone. Thus, SAM has a very diverse set of functions.

Consequently, changes in intracellular SAM availability have a significant impact on the survival and physiological states of the organisms. SAM is biosynthesized by the reaction of L-methionine (L-Met) and ATP, and can be produced spontaneously under certain reaction conditions (Laurino and Tawfik, 2017). This fact recalls that SAM is a methylation source with a prebiotic origin. It has been also reported that at least five different SAM-binding proteins were present in the last universal common ancestor (Kozbial and Mushegian, 2005). Since it is deeply embedded within the physiological system, SAM is thought to be involved in various genetic and metabolic regulatory networks, and the mechanisms responsible for its homeostasis have not yet been fully elucidated. Indeed, many new regulatory mechanisms involving SAM have been recently discovered. These include a regulatory factor for L-Met biosynthesis in *Escherichia coli* (Switzer et al., 2018), the switching of energy metabolism via complex formation involving SAM synthetase and central metabolizing enzymes in budding yeast (Li et al., 2015), and the splicing regulation of SAM synthetase mRNA by methylation modification in *Caenorhabditis elegans* (Watabe et al., 2021). Understanding SAM homeostasis is also important for resolving bottlenecks in the biosynthetic processes of valuable secondary metabolites that involve methylation, including those of vanillin, methyl anthranilate, and ferulic acid (Liu et al., 2022; Luo et al., 2019; Zhou et al., 2022). Given the diversity of these functional roles, the search for unknown regulatory elements that monitor SAM homeostasis is an important research priority. To this end, the development of a high-throughput detection system for changes in intracellular SAM availability is essential.

To maintain SAM homeostasis, a feedback system must detect changes in SAM availability and restore it to the normal range. In *E. coli*, the transcription factor MetJ, a master regulator of SAM synthesis, plays these roles. For example, MetJ is a SAM-responsive repressor that negatively regulates the transcription of genes involved in the biosynthesis, regeneration, and precursor transport of SAM (Gal et al., 2002; Phillips et al., 1989). While MetJ contributes to maintain physiological SAM levels as a master regulator, it is itself a natural SAM sensing machinery. Thus, if employed effectively, it may be useful tool for the discovery of factors causing fluctuations in SAM availability.

Accordingly, in this study we constructed plasmids containing promoters and reporter genes that are regulated by endogenous MetJ, and aimed to design biosensor systems capable of detecting fine changes in SAM availability. Although the gold standard in synthetic biology is the use of orthogonal motifs as biosensor components for the detection of intracellular metabolites that do not affect the metabolic network of the host cell (Bradley et al., 2016; Liu et al., 2018), those reporter systems involving intrinsic MetJ also have advantages. For example, their response sensitivity directly reflects the actual response of the metabolic network, and their designs minimize sensory components and metabolic burden. The characterization of sites critical for the interaction between MetJ and DNA and for the regulation of some MetJ-regulated genes has been performed previously via the of *lacZ* as a reporter gene under the MetJ promoter (He et al., 1992; LaMonte and Hughes, 2006). In this study, we reconstructed the synthetic MetJ-regulated promoter on a plasmid for monitoring and reporting changes in intracellular SAM availability (**Fig. 1**). We confirmed that the metbox, MetJ binding site, is most effective when placed between the −35 box and −10 box positions for detecting the genetic or environmental factors that can deplete and recover the intracellular SAM availability.

Materials and Methods

E. coli strains, media, and growth conditions. MG1655 and MG1655Δ*metJ*, a *metJ*-deficient mutant (lab collection), were used throughout this study. Plasmid cloning was performed using DH5α and Mach1 cells. All cells were incubated in LB medium (2.0% [w/v] LB lennox [Nacalai Tesque, Kyoto, Japan]) or LB agar medium (2.0% [w/v] LB and 2.0% [w/v] agar) unless otherwise noted. Antibiotics were used at the following concentrations: 30 μg/mL (chloramphenicol) and 30 μg/mL (streptomycin).

Plasmid construction. All plasmids and primers used in this study are listed in **Table S1** and **Table S2**. Plasmid construction was performed using an In-Fusion® HD Cloning Kit and an In-Fusion® Snap Assembly Master Mix (Takara Bio Inc., Shiga, Japan). Synthetic gene sequences that were used as PCR templates are listed in **Table S3**.

Functional analysis. Functional evaluations of synthetic MetJ-regulated promoters were performed using 96-well deep-well plates. Cells were incubated in LB + Glucose + Mg medium (2.0% [w/v] LB, 1 g/L D-glucose and 1 g/L $MgCl_2$ $6H_2O$) at 37°C and 1,000 rpm for at least 11.5 hours for preculturing. Then, the main culture was performed in M9-casamino acid medium (Na_2HPO_4 6 g/L, KH_2PO_4 3 g/L, NaCl 0.5 g/L, NH_4Cl 1 g/L, thiamine HCl 10 mg/L, $MgSO_4$ $7H_2O$ 0.5 g/L, D-glucose 3 g/L and casamino acid (Bacto™, Thermo Fisher Scientific) 5 g/L). After inoculation with 100-fold diluted preculture, cells were incubated at 37°C and 1,000 rpm. L-arabinose, an inducer of SAMase expression for pSAMase, and L-Met, a substrate for endogenous MetK, were added to the medium at the concentrations described in the figure captions. During sampling, the culture medium was first diluted 10-fold with a saline solution then cell density and fluorescence intensity were measured using a microplate reader (FilterMax F5, MolecularDevices) under the following conditions: sfGFP (485 nm excitation, 535 nm emission) and optical density (595 nm). Photographs were taken using an iPhone SE2 (Apple Inc.) with the iOS Camera app.

Results

Design of synthetic MetJ-regulated promoters

MetJ is known to control at least 12 regulons in *E. coli*. Moreover, the promoter and 5'-UTR sequences of the natural MetJ regulon contain two to five recognition sequences (metbox) in tandem (**Fig. S1**) (Gal et al., 2002; Marincs et al., 2006; Phillips et al., 1989; Thanbichler et al., 1999). The consensus metbox sequence was defined to be 5'-AGACGTCT-3' (He et al., 1996; Phillips et al., 1989) However, among the 12 regulons and the 50 annotated metbox sequences, only one on *metN* matched 100% with this consensus sequence. In addition, there is considerable variation in the copy number and position of the metbox. Given the remarkable level of sequence variation among MetJ regulons, we systematically tested 10 synthetic MetJ-regulated promoters based on the T5 promoter. The −35 box and −10 box sequences were the same in all promoters to minimize differences in promoter strength among them. It is known that the MetJ-SAM complex binds to the metbox as a homodimer, and it has been experimentally shown that two tandem metbox sequences are required for detectable repression (He et al., 1992; He et al., 2002). Therefore, we constructed three synthetic promoters with two consecutive consensus metbox sequences in the spacer ($MetP_{S2}$ in **Fig. 1**), proximal ($MetP_{P2}$), and distal positions ($MetP_{D2}$), respectively. Note that the subscript number indicates the number of metbox copies at each position (and that S, P, and D stand for Spacer, Proximal, and Distal locations, respectively). To determine the effect of the metbox copy number, we also constructed synthetic promoters with four-metbox copies ($MetP_{S2P4,}$ $MetP_{P4,}$ $MetP_{D4}$). In addition, we also adopted the natural sequence taken from the +2 to +29

sites of the *metF* promoter, which was annotated as containing three consecutive metbox sequences (Phillips et al., 1989). These operator sequences were then placed at the proximal or distal position of the T5 promoter or $MetP_{S2}$, yielding $MetP_{P3}$, $MetP_{D3}$, and $MetP_{S2P3}$. Finally, by placing *sfgfp* downstream of these promoters, changes in SAM concentration can be visualized by GFP expression (**Fig. 2**).

The effect of chromosomal metJ

First, we introduced the reporter plasmids into a *metJ* deletion strain (MG1655Δ*metJ*), and measured GFP fluorescence intensity (**Fig. 2B; closed bar**). Here, each of the promoters was free from repression by MetJ, and therefore exhibited the maximum level of GFP expression reachable upon full induction. Since the sequences of the −35 box and −10 box in these promoters and the reporter genes are the same among the ten samples tested, the variations in GFP expression come from the differences in translation initiation rates based on the sequence variation in 5'-UTR. Key factors affecting translation initiation rates are the global folding and unfolding of transcribed mRNAs, the regional folding and unfolding of nucleotides in the RBS region, and the interaction between the SD sequence and the ribosome (Reeve et al., 2014). Using RBS Calculator (version 2.1) (Espah et al., 2014), we found the variation in the relative values of translation initiation rates from 3,536 to 77,890 (**Fig. 2B**). This suggested that differences in 5'-UTR sequences due to mixing and matching the metbox sequences caused the variation in the maximum level of GFP expression.

Next, we introduced reporter plasmids into MG1655, a carrier of *metJ*, and measured its fluorescence intensity to evaluate the repression of transcription by MetJ. Significant reductions in GFP expression were observed for all promoters tested except the T5 promoter without a metbox sequence (**Fig. 2B**). This reduction in GFP expression suggested that MetJ-SAM complex was formed and bound to metbox sequences under the culture conditions in *E. coli*. The reported concentration of SAM in *E. coli* ranges from 28 to 180 μM during logarithmic growth and increases about ten-fold during the stationary phase (Bennett et al., 2009; Posnick and Samson, 1999). In contrast, the dissociation constant of MetJ for SAM measured ranges from 50 to 200 μM (Marti-Arbona et al., 2012; Parsons et al., 1995; Saint-Girons et al., 1986). Although we do not have an experimental data, MetJ is likely to be in the “repression mode” in the physiological state, if these values also apply to our strains.

The efficiency of repression varied depending on the position and number of metbox sequences. $MetP_{P2}$ and $MetP_{P3}$, whose metbox sites are located closer to the transcription start site, showed higher stringency than their counterparts, $MetP_{D2}$ and $MetP_{D3}$. It was shown for LacI and TetR that the repression strength decreases with the distance between the operator sequence and the transcription start site (Giordano et al., 1989; Karig et al., 2012). The dissociation constant between MetJ and the consensus metbox sequence is reported to be 4×10^{-9} M (He et al., 2002), which is similar to or lower than for LacI/lacO (10^{-9}–10^{-11} M)

(Forde et al., 2006) and TetR/tetO (10^{-10} M) (Kamionka et al., 2004). This finding is in line with the report that TetR failed to repress the promoter with TetO > 10 bases downstream of the transcription initiation site (Karig et al., 2012).

We found that the promoters with the metbox placed between the −35/−10 box positions exhibited a high repression capacity; these included $MetP_{S2}$, $MetP_{S2P2}$, $MetP_{S2P4}$, and $MetP_{S2P3}$. Placing the operator in this position has been reported to be most effective for TetR and LacI (Cox et al., 2007). Although subtle, the contribution of additional metbox copies placed downstream of the transcription start site was additional. The contribution was the highest for $MetP_{S2P4}$, which added four consensus metbox sequences, followed by $MetP_{S2P3}$, which added three P_{metF}-derived natural metbox sequences. In contrast, $MetP_{S2P2}$, which added only two consensus metbox sequences, did not show this increase in stringency. Effective repression by MetJ may require protein–protein interactions that occur between adjacent homodimers (He et al., 1992; He et al., 2002). Thus, the presence of three or more metbox copies in $MetP_{S2P4}$ or $MetP_{S2P3}$ may have led to binding stabilization via interactions among multiple MetJ homodimers, thereby leading to higher stringency.

Detection of decrease in SAM availability

We examined whether any of the synthetic MetJ-regulated promoters could respond to genetic factors known to alter SAM concentrations. For example, Posnick and Samson found that the expression of SAM lyase (SAMase) from the T3 phage can significantly reduce intracellular SAM in *E. coli* during the logarithmic growth phase to below the detection limit using high-performance liquid chromatography (Posnick and Samson, 1999). This enzyme has also been used to examine the response of native MetJ-regulated promoters to SAM depletion (LaMonte and Hughes, 2006). We constructed an L-arabinose inducible SAMase expression plasmid and introduced it into MG1655 cells harboring reporter plasmids depicted in **Fig. 1**. The resultant transformants were examined in their cellular GFP fluorescence in media containing different concentrations of L-arabinose (SAMase inducer) (**Fig. 3A**). Significant increase in GFP fluorescence was observed, and the degree of fluorescence increase corresponded with the concentrations of L-arabinose (**Fig. 3B**). Since the expression level of SAMase is controlled by the concentration of L-arabinose, it is expected that the intracellular SAM concentration should decrease with the increasing concentration of L-arabinose. Subsequently, MetJ-regulated promoters were turned “On”, leading to an increase in GFP expression with the concentration of L-arabinose. Note that all samples are identical except for the sequences neighboring the promoter.

$MetP_{S2P4}$, which showed the best signal-to-noise (S/N) ratio (**Fig. 2**), was extremely repressive under all conditions tested in **Fig. 3**. This behavior is termed "super-repression" (Reedstrom et al., 1996; Suckow et al., 1996; Zhou et al., 1995) apo-form of MetJ has at least a 100-fold weaker repression capacity than holo-form MetJ (Phillips and Phillips 1994). It is

possible that the apo-form of MetJ, with a reduced affinity to metbox, may repress Met_{PS2P4} due to the presence of six consensus metbox sequences, including those positioned in the most effective spacer position.

$MetP_{D3}$ and $MetP_{D4}$ showed large changes in their signal to SAMase expression levels. However, these promoters showed quite leaky expression even in SAMase-free conditions, and their performance was not high when measured by their S/N ratio. In contrast, $MetP_{S2}$ and $MetP_{S2P3}$ showed sufficient S/N ratio and stringency. Using these promoters, SAMase expression levels (i.e., the degree of decreasing SAM availability) was clearly eye-distinguishable.

Detection of recovery of SAM availability

The simplest way to increase the SAM supply in cells is to add L-Met, one of the direct biosynthetic substrates of SAM (**Fig. 4A**). Supplementation of L-Met is an established strategy for increasing the titer of methylation products by increased SAM availability (Kunjapur et al., 2016; Luo et al., 2019). Here, we investigated whether the increase in SAM supply following the addition of L-Met could be detected by synthetic MetJ-regulated promoters. Using MG1655 cells harboring reporter plasmids, different concentrations of L-Met were added to the culture medium and cellular GFP fluorescence intensity was then examined. However, no response to the addition of L-Met was observed in any of the strains tested (**Fig. 4B; left**). Most of the reporters are repressed in MG1655 (*metJ*$^+$ strain) (**Fig. 2B)**, and therefore MetJ should be in SAM-bound state in the culture condition adopted in this work. This observation is not contradicted to the reported values, the concentration of SAM in *E. coli* (Bennett et al., 2009; Posnick and Samson, 1999) and the dissociation constant of MetJ for SAM (Marti-Arbona et al., 2012; Parsons et al., 1995; Saint-Girons et al., 1986). Therefore, even if the SAM supply following with L-Met addition, it would be difficult to detect differences in repression strength induced by MetJ. Furthermore, the changing intracellular SAM availability may have been limited because the expression of MetK (SAM synthetase) and MetNIQ (L-Met transporter) were also repressed by MetJ.

Because of the leaky expression of SAMase even without inducer, the addition of L-Met alone caused decrease in GFP expression (**Fig. 4B; right**). Due to the activity of SAMase, the basal concentrations of SAM were considered to be maintained lower than that in the physiological state. Although L-Met is the direct precursor of proteins, and its supplementation could possibly cause the fluctuation in the translational capacity, we observed no detectable difference in cell growth profiles at all concentrations of L-Met tested (data not shown). Therefore, the observed L-Met-dependent decrease in GFP fluorescence intensity is likely to reflect the partial recovery of cellular availability of SAM, rather than other physiological factors such as changes in translational resources. L-met is the direct precursor of SAM and its addition has long been known to increase the methylation products in the cells (Kunjapur et al., 2016; Luo et al., 2019). LaMonte

and Hughes previously showed that natural MetJ-regulated promoters could respond to the supplementation of L-Met in M9 medium (LaMonte and Hughes, 2006). Even though we performed the supplementation of L-Met under SAMase leaky expression, for all tested synthetic promoters, the recovery of GFP fluorescence upon L-Met addition was only marginal, likely due to the high catalytic capacity of SAMase. We believe the feeding L-Met did increase the supply of SAM, but a significant fraction of this increased SAM was directly degraded by SAMase.

Discussion

To improve biomanufacturing processes, the availability of targeted precursors in strains is important. Therefore, it is important to construct a biosensor that detects its own supply. In this study, we aimed to construct a monitoring system using minimal genetic components that was capable of visualizing changes in SAM availability, the major source of methylation activity all across the domains of life. For this purpose, we constructed a plasmid-based SAM-sensing system that used chromosome-derived MetJ in *E. coli*. Although we were not able to detect an increase in cellular availability of SAM, we could visualize the decrease of SAM from the physiological conditions.

While the construction of biosensor systems on a multicopy reporter plasmid is advantageous for increasing signal gain, it can also significantly alter the effective concentration of intracellular MetJ due to the presence of multiple metbox sequences on multicopy plasmids. In the case of a constructing choline-responsive induction system using BetI, the endogenous expression of BetI was insufficient to stringently repress the corresponding promoters on plasmids, and we had to supplement endogenous expression with expression of *betI* present on the plasmid (Ike et al., 2015; Saeki et al., 2016). We found that this was not the case for MetJ; without additional expression of MetJ, plasmid-based synthetic MetJ-regulated promoters were well repressed. On the other hand, as long as we construct the SAM-sensing systems using the endogenous sensory component MetJ, it inevitably affects the behavior of the SAM homeostasis network. Probably, the system presented here would be most useful for screening for SAM consumers such as methyltransferase activity *in vivo* (**Fig. 3B**).

Because MetJ represses its target promoters at physiological state in *E. coli*, our system was unable to detect increases in SAM availability (**Fig. 4B; left**). Mutating MetJ to reduce its affinity for SAM could allow direct detection of the increases beyond the physiological level. Alternatively, we demonstrated that we could detect the factor that can increase the SAM supply (L-Met addition) by transiently decreasing the basal SAM levels, due to leaky expression of SAMase as a SAM-consuming enzyme (**Fig. 4B; right**).

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Author Contribution

All three authors conceived the study and designed the experiments. T.W performed most of the experiments. Y.K. performed data analysis. T.W., Y.K., and D.U. wrote the manuscript.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

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Figure Legends

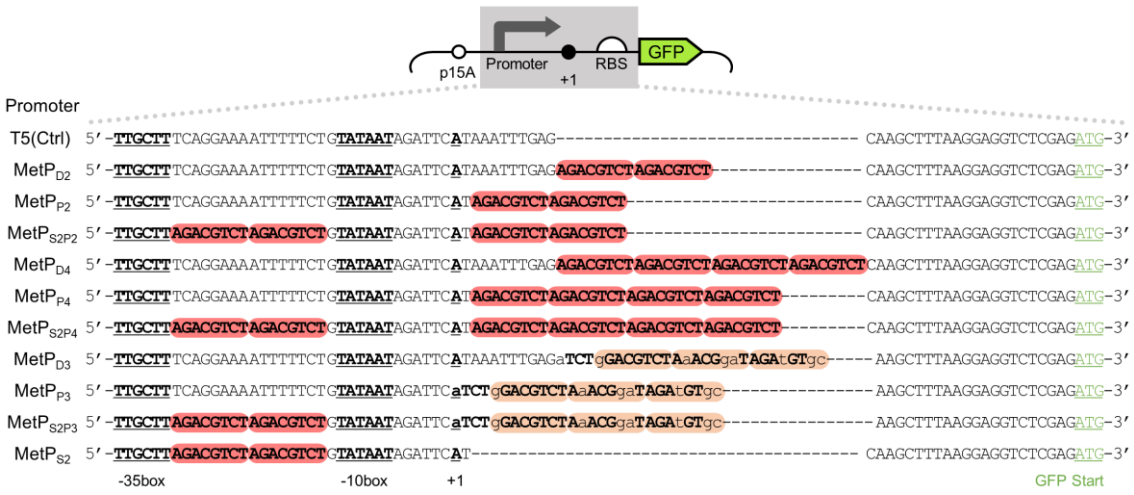
Fig. 1. Systematic design of synthetic MetJ-regulated promoters.
Sequences of promoters and 5'-UTRs used in this study are shown. Red boxes indicate a consensus metbox. Orange boxes indicate a native P_{metF} metbox. Upper case and bold letters in the metbox indicate residues that match the consensus metbox and lower case letters do not.

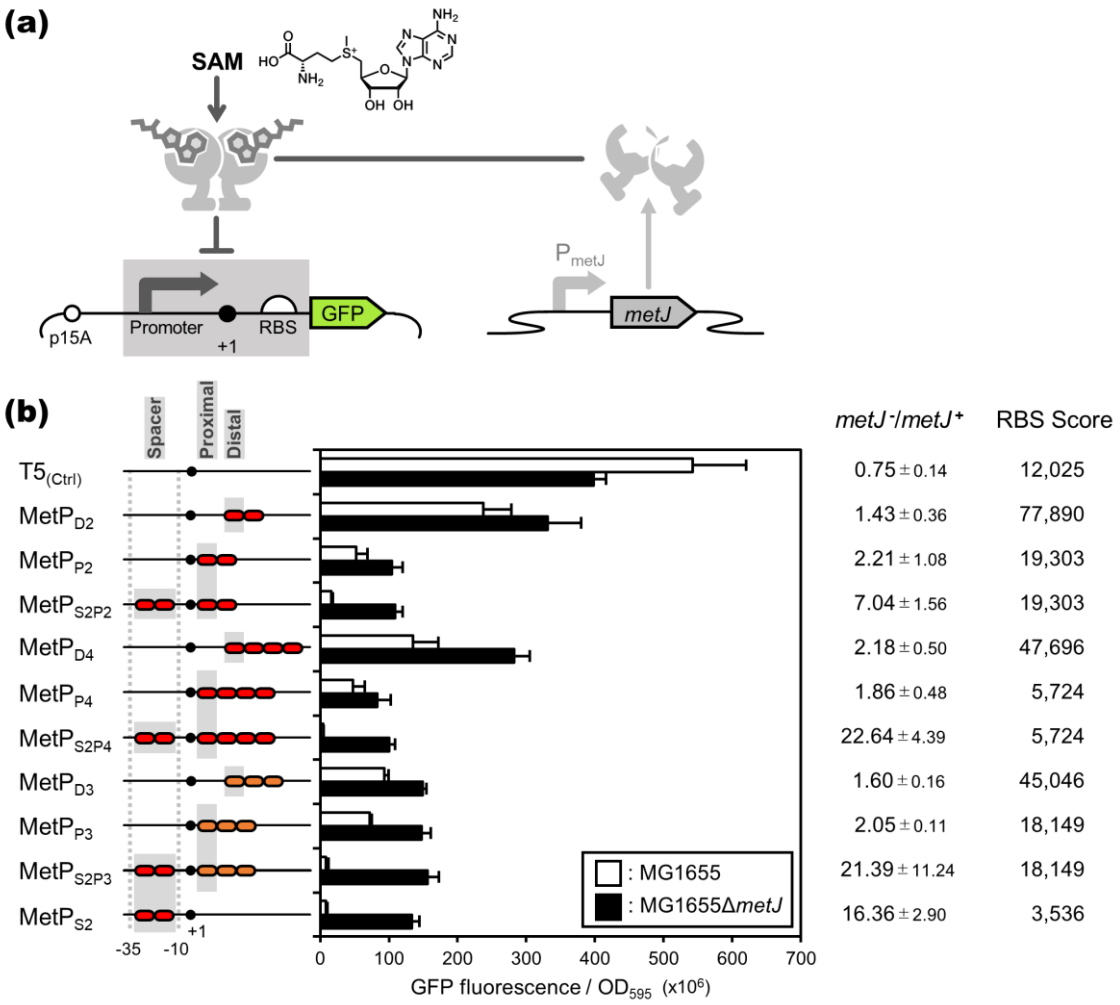
Fig. 2. Functional analysis of each synthetic MetJ-regulated promoter.
(a) SAM-sensing system using MetJ and a synthetic MetJ-regulated promoter. MetJ expressed from the chromosomal *metJ* binds to SAM and represses gene expression via binding to the metbox sequence. (b) Functional analysis of repression by MetJ. Cellular fluorescence of *E. coli* transformed with each reporter plasmid was compared with or without chromosomal *metJ*. Results are shown as the average of three replicates; error bars and plus–minus signs represent the standard deviation. $metJ^{-}/metJ^{+}$ was calculated by dividing the GFP fluorescence/OD_{595} values for MG1655Δ*metJ* by the values of MG1655. The RBS score was calculated with RBS Calculator version 2.1.

Fig. 3. Response of each promoter to decrease in SAM availability.
(a) Detection of decrease in SAM availability by SAMase using MetJ. SAMase is induced by L-arabinose. SAMase degrades SAM to L-homoserine lactone (L-HL) and 5'-methyladenosine (5'-MTA). This decrease of SAM availability was detected by MetJ. (b) Functional analysis of the effect by decrease in SAM availability. *E. coli* harboring each reporter plasmid was transformed with pSAMase. We then evaluated differences in cellular fluorescence with and without SAMase expression and under various induction conditions. Results show the average of three replicates; error bars and plus–minus signs represent standard deviation. S/N was calculated by dividing the GFP fluorescence/OD_{595} values with pSAMase at 10 mM L-arabinose (SAMase inducer) condition by values without pSAMase. Photographs are culture medium of each transformants under blue light illumination. Note that the culture samples for $MetP_{S2P3}$, was photographed at two different location in the same plate, and their pictures were combined into one picture.

Fig. 4. Response of each promoter to increased SAM supply.

(a) Detection of increased SAM supply via L-Met addition using MetJ. The addition of L-Met, a substrate of SAM synthesis, increases intracellular SAM concentration via MetK expressed from the genomic *metK* gene. This increase is then detected by MetJ. (b) Functional analysis of the effect of increased SAM supply. *E. coli* harboring each reporter plasmid were used to evaluate differences in cellular fluorescence at various L-Met conditions with (right) or without SAMase expression (left). Results show the average of three replicates; error bars and plus–minus signs represent standard deviation. S/N was calculated by dividing the GFP fluorescence/OD_{595} values with no addition of L-Met by values at 2 g/L L-Met.


p15A
Promoter
+1
RBS
GFP
Promoter
T5(Ctrl) 5′-TTGCTTTCAGGAAAATTTTTCTGTATAATAGATTCATAAATTTGAG---------- CAAGCTTTAAGGAGGTCTCGAGATG-3′
MetP$_{D2}$ 5′-TTGCTTTCAGGAAAATTTTTCTGTATAATAGATTCATAAATTTGAGAGACGTCTAGACGTCT---------- CAAGCTTTAAGGAGGTCTCGAGATG-3′
MetP$_{P2}$ 5′-TTGCTTTCAGGAAAATTTTTCTGTATAATAGATTCATAGACGTCTAGACGTCT---------- CAAGCTTTAAGGAGGTCTCGAGATG-3′
MetP$_{S2P2}$ 5′-TTGCTTAGACGTCTAGACGTCTGTATAATAGATTCATAGACGTCTAGACGTCT---------- CAAGCTTTAAGGAGGTCTCGAGATG-3′
MetP$_{D4}$ 5′-TTGCTTTCAGGAAAATTTTTCTGTATAATAGATTCATAAATTTGAGAGACGTCTAGACGTCTAGACGTCTAGACGTCTCAAGCTTTAAGGAGGTCTCGAGATG-3′
MetP$_{P4}$ 5′-TTGCTTTCAGGAAAATTTTTCTGTATAATAGATTCATAGACGTCTAGACGTCTAGACGTCTAGACGTCT---------CAAGCTTTAAGGAGGTCTCGAGATG-3′
MetP$_{S2P4}$ 5′-TTGCTTAGACGTCTAGACGTCTGTATAATAGATTCATAGACGTCTAGACGTCTAGACGTCTAGACGTCT---------CAAGCTTTAAGGAGGTCTCGAGATG-3′
MetP$_{D3}$ 5′-TTGCTTTCAGGAAAATTTTTCTGTATAATAGATTCATAAATTTGAGaTCTgGACGTCTAaACGgaTAGAtGTgc-----AAGCTTTAAGGAGGTCTCGAGATG-3′
MetP$_{P3}$ 5′-TTGCTTTCAGGAAAATTTTTCTGTATAATAGATTCaTCTgGACGTCTAaACGgaTAGAtGTgc----------AAGCTTTAAGGAGGTCTCGAGATG-3′
MetP$_{S2P3}$ 5′-TTGCTTAGACGTCTAGACGTCTGTATAATAGATTCaTCTgGACGTCTAaACGgaTAGAtGTgc----------AAGCTTTAAGGAGGTCTCGAGATG-3′
MetP$_{S2}$ 5′-TTGCTTAGACGTCTAGACGTCTGTATAATAGATTCAT---------- CAAGCTTTAAGGAGGTCTCGAGATG-3′
-35box
-10box
+1
GFP Start


(a)
SAM
p15A
Promoter
+1
RBS
GFP
P metJ
metJ
(b)
Spacer
Proximal
Distal
T5(Ctrl)
MetPD2
MetPP2
MetPS2P2
MetPD4
MetPP4
MetPS2P4
MetPD3
MetPP3
MetPS2P3
MetPS2
+1
-35
-10
0
100
200
300
400
500
600
700
GFP fluorescence / OD595 (x10^6)
: MG1655
: MG1655ΔmetJ
metJ-/metJ+
RBS Score
0.75 ± 0.14
12,025
1.43 ± 0.36
77,890
2.21 ± 1.08
19,303
7.04 ± 1.56
19,303
2.18 ± 0.50
47,696
1.86 ± 0.48
5,724
22.64 ± 4.39
5,724
1.60 ± 0.16
45,046
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18,149
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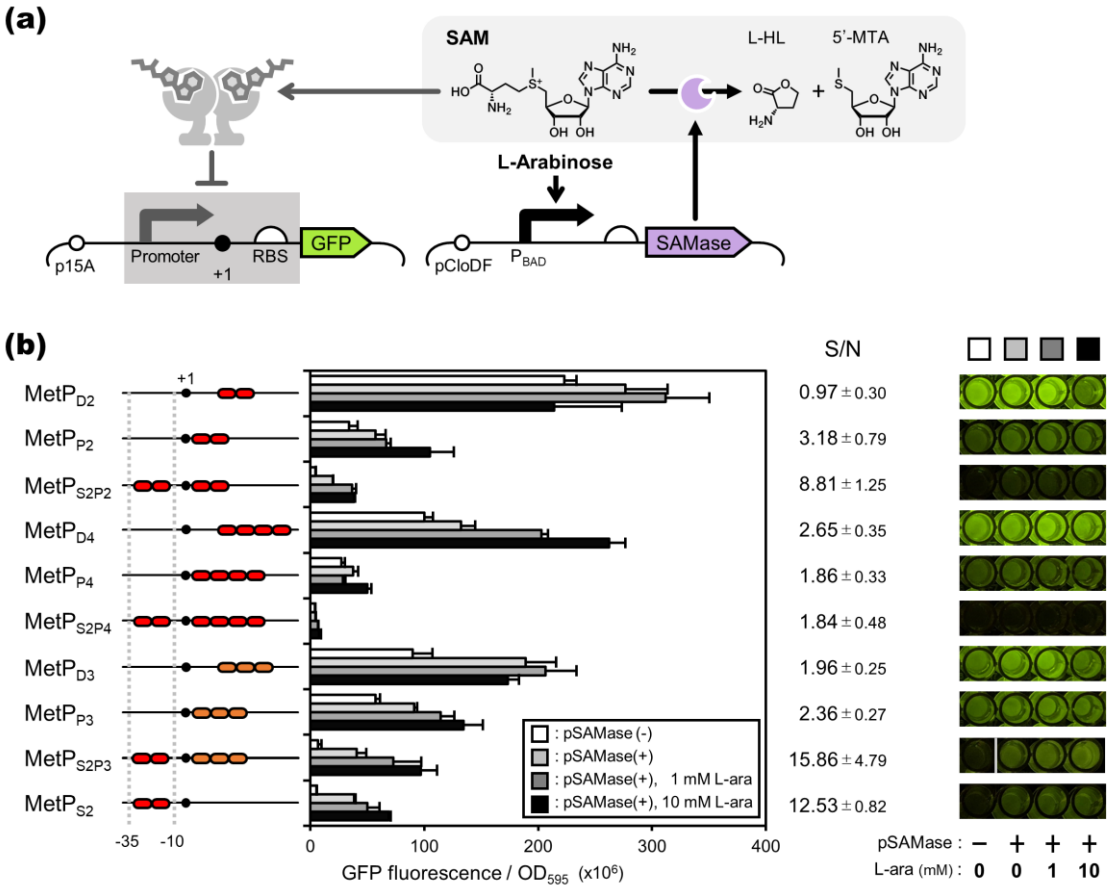
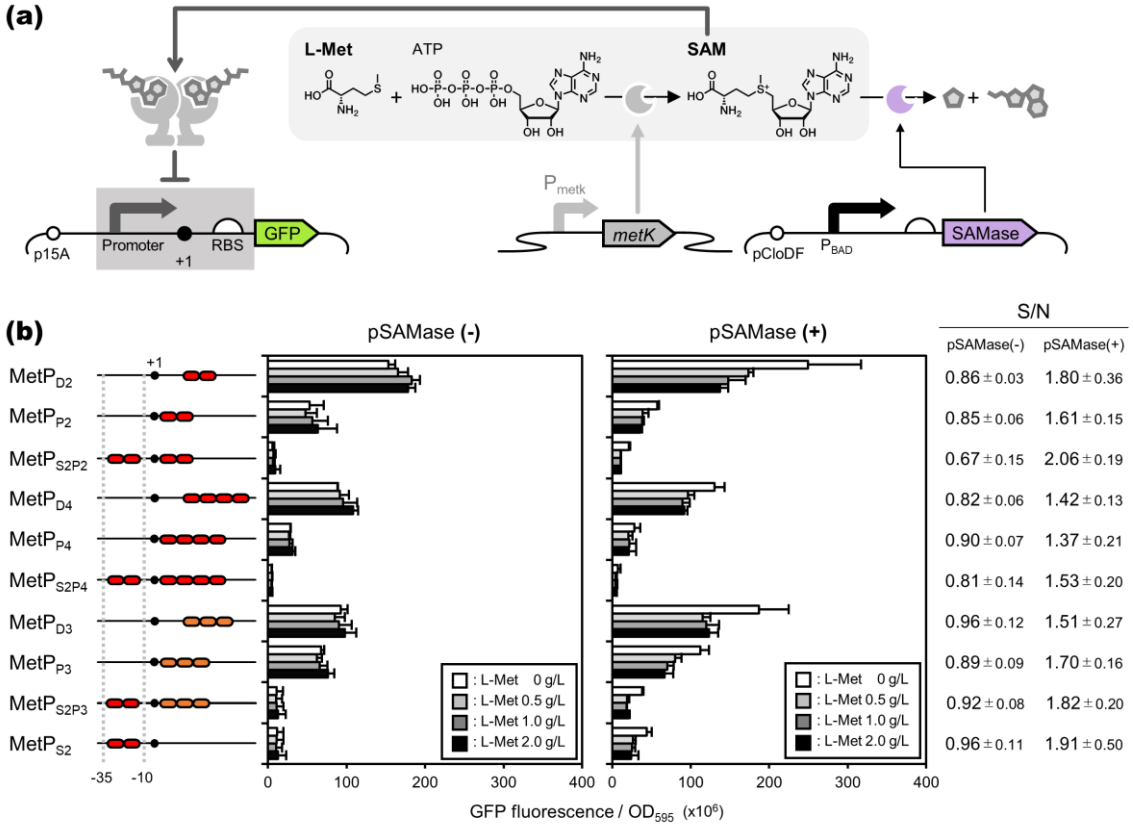
18,149
16.36 ± 2.90
3,536


(a)
SAM
L-HL
5'-MTA
L-Arabinose
p15A
Promoter
+1
RBS
GFP
pCloDF
P BAD
SAMase
(b)
S/N
MetP D2
MetP P2
MetP S2P2
MetP D4
MetP P4
MetP S2P4
MetP D3
MetP P3
MetP S2P3
MetP S2
0.97 ± 0.30
3.18 ± 0.79
8.81 ± 1.25
2.65 ± 0.35
1.86 ± 0.33
1.84 ± 0.48
1.96 ± 0.25
2.36 ± 0.27
15.86 ± 4.79
12.53 ± 0.82
-35
-10
0
100
200
300
400
GFP fluorescence / OD595 (x10^6)
: pSAMase (-)
: pSAMase(+)
: pSAMase(+), 1 mM L-ara
: pSAMase(+), 10 mM L-ara
pSAMase : – + + +
L-ara (mM) : 0 0 1 10


(a)
L-Met
ATP
SAM
P15A
Promoter
+1
RBS
GFP
P metk
metK
pCloDF
P BAD
SAMase
(b)
pSAMase (-)
pSAMase (+)
S/N
pSAMase(-)
pSAMase(+)
MetP D2 0.86 ± 0.03 1.80 ± 0.36
MetP P2 0.85 ± 0.06 1.61 ± 0.15
MetP S2P2 0.67 ± 0.15 2.06 ± 0.19
MetP D4 0.82 ± 0.06 1.42 ± 0.13
MetP P4 0.90 ± 0.07 1.37 ± 0.21
MetP S2P4 0.81 ± 0.14 1.53 ± 0.20
MetP D3 0.96 ± 0.12 1.51 ± 0.27
MetP P3 0.89 ± 0.09 1.70 ± 0.16
MetP S2P3 0.92 ± 0.08 1.82 ± 0.20
MetP S2 0.96 ± 0.11 1.91 ± 0.50
+1
-35
-10
: L-Met 0 g/L
: L-Met 0.5 g/L
: L-Met 1.0 g/L
: L-Met 2.0 g/L
0
100
200
300
400
GFP fluorescence / OD595 (x10^6)