*Supplementary materials*

**Table S1.** The sequences and annotations of promoters used in this study.

| Promoter | Sequence | Source |
|---|---|---|
| cbp_2 | GAGTCGTGACTAAGAACGTCAAAGTAATTAACAATACAGCTATTTTTCTCATGCTTTT ACCCCTTTCATAAAATTTAATTTTATCGTTATCATAAAAAATTATAGACGTTATATTG CTTGCCGGGATATAGTGCTGGGCATTCGTTGGTGCAAAATGTTCGGAGTAAGGTGGA TATTGATTTGCATGTTGATCTATTGCATTGAAATGATTAGTTATCCGTAAATATTAATT AATCATATCATAAATTAATTATATCATAATTGTTTTGACGAATGAAGGTTTTTGGATA AATTATCAAGTAAAGGAACGCTAAAAATTTTGGCGTAAAATATCAAAATGACCAC**T TGAAT**TAATATGGTAAAGTAGA*TATAA*TATTTTGGTAAACATGCCTTCAGCAAGGTT AGATTAGCTGTTTCCGTATAAATTAACCGTATGGTAAAACGGCAGTCAGAAAAATA AGTCATAAGATTCCGTTATGAAAATATACTTCGGTAGTTAATAATAAGAGATATGAG GTAAGAGATACAAGATAAGAGATATAAGGTACGAATGTATAAGATGGTGCTTTTAG GCACACTAAATAAAAACAAATAAACGAAAATTTTA<u>AGGAGG</u>ACGAAAG | [1] |
| gapDH | TAATTACTGTATCTCTCTGGCA**TTGCCA**GGTTTTAATAAAGAT*TAAAAT*TATTGACTA GAAATAAAAAAATTGTCCATAATATTAATGGACAAAAAAACAAAGAATTACATCA AAGGAAGATAAAAATACTTTGTTAAAAAATTAATTATTTTTTATCTAAACTA**TTGAA A**ATGAAAATAAAATAATA*TAAAAT*GAATCATAGTGCAAGAGATACTTGCCAGAGGA TGAATATTTTACTGCATTCATGCTTTATGGCAGCTAATAGAGGCATTAAATTAAATTT TAATTTACAAT<u>AGGAGG</u>CGATATTAATG | [1] |
| gapDH/*tetO* | TAATTACTGTATCTCTCTGGCA**TTGCCA**actctatcattgatagag*TAAAAT*TATTGACTAGAA ATAAAAAAATTGTCCATAATATTAATGGACAAAAAAACAAAGAATTACATCAAAG GAAGATAAAAATACTTTGTTAAAAAATTAATTATTTTTTATCTAAACTA**TTGAAA**actc tatcattgatagag*TAAAAT*GAATCATAGTGCAAGAGATACTTGCCAGAGGATGAATATTTT ACTGCATTCATGCTTTATGGCAGCTAATAGAGGCATTAAATTAAATTTTAATTTACAA T<u>AGGAGG</u>CGATATTAATG | [1], This paper |
| eno | GGAAATATTAAAATGGAAATG**TTGAAA**AAATGTTTTAAGATGGGT*CATTAT*GGATA AAATATACTATGGTTTTGCAATAAATGCTTTCTATTAATTGGACTTTGTGGTAATATG GTAGAAGGATGCAGTGTTAATTTTTTAACATATAAAAATAAGCTATATGAAG<u>GGAG</u> AATGGAGA | [1] |
| 0815 | ATAATATATATCCGTATTTTACAATATCCATACGAATATGGATTATTTTATTTTGTTAT ATTTTTAACAAAAAAATTA**TTTAAA**CTTTTTCAATGGTGG*TATTAT*ATATATTGGTCA CAGTTTCAATGAATC AAAAAATAAA<u>GAGG</u>TGTTTAAT | [1] |
| 0966 | TTCTTACTACTCCCTTTGCATCTACAGACAAATTTTTCTCTTTAACTTTTCTTGTAACC GTATCCGCATACTTTTGTCATAAAGTAATTATAGATTATTATTACCGTCAAATCAAAT AATAAATTTTATATCGCCAATTCT**TTGCAT**CCGCTTTAAAGTCTGTGC*TATAAT*TATA TGGTTAAAATATCATAAAAAAAT AAAATAAAAGTTAAA<u>AGGA</u>ACTGATTTT | [1] |
| 2638 | GATAAACAAAGGACGGTTCAGGGCTTCTGCTCATCCTACTCTGCATTGTAAAAAGGT AGGATGAATTTTTATTTTTAATCTTATTGAAAAAAATTTTTGAAAATCGGTTTTA**TTA AAA**AAAGTGGGTATATT*TATAAT*AGTCAATTGATTGGTTAAAAAAATTTAAATAAG CAAACAGAATAATAACAAAA GTA<u>AGGAGG</u>AATTTGTT | [1] |
| 2926 | AAAATATACAAAGGTTTCTTGTGTTTTTAATACCGTTATGT*TAATATAAT*GTAATA*TAT ATTT*T*TATAAT*AATATGTATGAGAGATAGTGTTTTGCTATATTGCTATAAAGAATG<u>AGG AGG</u>GAACTAG | [1] |
| P<sub>xyl</sub>* | TTTGATATT<u>CCTCC</u>TT*AAAATA*ATATTGTAATACTTTTT**ACACAA**AAATAAAAGGTT ATTTTGCA**TTGACA**AAGATAATTAAATATTT*TATTAT*TAGTTCAtaagttagtttaatatactaa | [2] |

caaaaATAAAGCAAGTAAAATATACCTAAAATATAAAAAAATTAGGATAGGAAAA
CGATAGTTATGAAGTGGCATTCA<u>AGGAGG</u>GAT GCAT

Predicted features are marked as follows: ribosome binding sites are underlined, -35 boxes are bolded, -10 boxes are italicized and bolded, and operators tetO1 (in gapDH 2tetO1) and xylO (in $P_{xyl}$) sequences are in lowercase.

*$P_{xyl}$ contains two sets of -35/-10/RBS. The first RBS, -10, and -35 sequences are for the transcription of the *xylR* gene downstream of this set. The second RBS, -10, and -35 sequences are for the gene of interest, i.e., the gene whose transcription will be controlled by the promoter.

**Table S2.** List of primers used in this study. All primers were ordered from IDT.

| Oligonucleotide | Sequence (5' - 3') | Purpose |
|---|---|---|
| adhB BglIII for | TAATCGAGATCTATGAAAGGTTTTGCAATGC | Amplification of *adhB** |
| adhB SalI rev | TAATCGGTCGACTTATGCTAATATTACAACAGGTTTG | |
| Bgal BglII for | TAATCGAGATCTATGAATGTGTTATCCTCAATTTG | Amplification of *lacZ** |
| Bgal SalI rev | TAATCGGTCGACCTAAACCTTCCCGGCTTC | |
| PgapDH PspOMI for | TAATACGGGCCCTAATTACTGTATCTCTCTGGC | Amplification of $P_{gapDH}$* |
| PgapDH BamHI rev | TAATACGGATCCTAATATCGCCTCCTATTGTAAATTAAA | |
| P2638 PspOMI for | TAATCGGGGCCCGATAAACAAAGGACGGTTC | Amplification of $P_{2638}$* |
| P2638 BamHI rev | TAATCGGGATCCAACAAATTCCTCCTTACTTTTG | |
| P2926 PspOMI for | TAATCGGGGCCCAAAATATACAAAGGTTTCTTGTG | Amplification of $P_{2926}$* |
| P2926 BamHI rev | ATAATCGGATCCAAAATATACAAAGGTTTCTTGTG | |
| Pcbp_2 PspOMI for | TAATCGGGGCCCGAGTCGTGACTAAGAACG | Amplification of $P_{cbp\_2}$* |
| Pcbp_2 BamHI rev | TAATCGGGATCCCTTTCGTCCTCCTTAAAATTTTC | |
| Peno PspOMI for | TAATCGGGGCCCGGAAATATTAAAATGGAAATGTTG | Amplification of $P_{eno}$* |
| Peno BamHI rev | TAATCGGGATCCTCTCCATTCTCCCTTCATATAG | |
| P0966 PspOMI for | TAATCGGGGCCCTTCTTACTACTCCCTTTGC | Amplification of $P_{0966}$* |
| P0966 BamHI rev | TAATCGGGATCCAAAATCAGTTCCTTTTAACTTTTATT | |
| P0815 PspOMI for | TAATCGGGGCCCATAATATATATCCGTATTTTACAATATCC | Amplification of $P_{0815}$* |
| P0815 BamHI rev | TAATCGGGATCCATTAAACACCTCTTTATTTTTTGATTC | |
| PgapDH BamHI for | AATCAGGATCCGGCCCTAATTACTGTATCTCTCTG | Amplification of gapDH-lacZ module to add to pUC19 |
| PgapDH BglII for | AATCAAGATCTGGCCCTAATTACTGTATCTCTCTGGC | Amplification of $P_{gapDH/tetO}$-*lacZ* module to add to pMTL86251 downstream of $P_{eno}$-tetR |
| TetO1_1 for | CTATTGAAAACTCTATCATTGATAGAGTAAAATGAATC-ATAGTGC | Add *tetO1* to region between 2nd -35 and -10 in $P_{gapDH}$ |
| TetO1_1 rev | ACTCTATCAATGATAGAGTTTTCAATAGTTTAGATAAAAAT | |
| TetO1_2 for | TCCCTATCAGTGATAGATAAAATTATTGACTAGAAATAAAAAATTGTCC | Add *tetO1* to region between 1st -35 and -10 in $P_{gapDH}$ |
| TetO1_2 rev | TCAATAATTTTACTCTATCAATGATAGAGTTGGCAATGCCAGAGAGATAC | |
| Eno rev EcoRI | ATTCGAATTCTCTCCATTCTCCCTTCATATAG | Amplification of $P_{eno}$ to clone in front of *tetR* |
| Eno for BamHI | ATAATCGGATCCGGAAATATTAAAATGGAAATGTTGAA | |
| tetR for EcoRI | ACTCTGAATTCATGTCTAGATTAGATAAAAGTAAAGTG | Amplification of *tetR* from *E. coli* to clone behind $P_{eno}$ |
| tetR rev PspOMI | ATTTGGGCCCAACTCGACATCTTGGTTAC | |

*Products were amplified from the appropriate pDGO plasmids [1] (**Table S3**).

**Table S3.** List of plasmids. All plasmids (with the exception of pDGO based plasmids) use pMTL86251 as the backbone.

| Plasmid | Description | Source |
|---|---|---|
| pMTL86251 | Modular shuttle vector for use in clostridial bacteria | [3] |
| pAL58 | Plasmid containing adhB reporter gene driven by gapDH promoter | This paper |
| pAL61 | Plasmid containing adhB reporter gene driven by 2926 promoter | This paper |
| pAL2B | Plasmid containing lacZ reporter gene driven by eno promoter | This paper |
| pAL60 | Plasmid containing lacZ reporter gene driven by 2638 promoter | This paper |
| pAL62 | Plasmid containing lacZ reporter gene driven by 2926 promoter | This paper |
| pAL64 | Plasmid containing lacZ reporter gene driven by cbp_2 promoter | This paper |
| pAL66 | Plasmid containing lacZ reporter gene driven by gapDH promoter | This paper |
| pAL731 | Plasmid containing lacZ reporter gene driven by 0966 promoter | This paper |
| pAL751 | Plasmid containing lacZ reporter gene driven by 0815 promoter | This paper |
| pAL88 | Plasmid containing *lacZ* reporter gene driven by $P_{gapDH/tetO}$ | This paper |
| pAL111 | Plasmid containing *lacZ* driven by $P_{gapDH/tetO}$ and *tetR* driven by $P_{eno}$ | This paper |
| pAL112 | Plasmid containing *lacZ* driven by $P_{gapDH}$ and *tetR* driven by $P_{eno}$ | This paper |
| pALX3 | Plasmid containing *lacZ* driven by $P_{xyl}$ promoter and *xylR* repressor gene | This paper |
| pDGO89 | Plasmid containing adhB reporter gene driven by 0815 promoter | Gift from Dan Olson [1] |
| pDGO95 | Plasmid containing *lacZ* reporter gene driven by gapDH promoter | Gift from Dan Olson [1] |
| pDGO102 | Plasmid containing *lacZ* reporter gene driven by eno promoter | Gift from Dan Olson [1] |
| pDGO105 | Plasmid containing *lacZ* reporter gene driven by 0966 promoter | Gift from Dan Olson [1] |
| pDGO106 | Plasmid containing *lacZ* reporter gene driven by 2638 promoter | Gift from Dan Olson [1] |
| pDGO108 | Plasmid containing *lacZ* reporter gene driven by 2926 promoter | Gift from Dan Olson [1] |
| pDGO117 | Plasmid containing *lacZ* reporter gene driven by cbp_2 promoter | Gift from Dan Olson [1] |

**Table S4.** Plasmids constructed for this study with promoter activity determined in *H. modesticaldum* and *E. coli*.

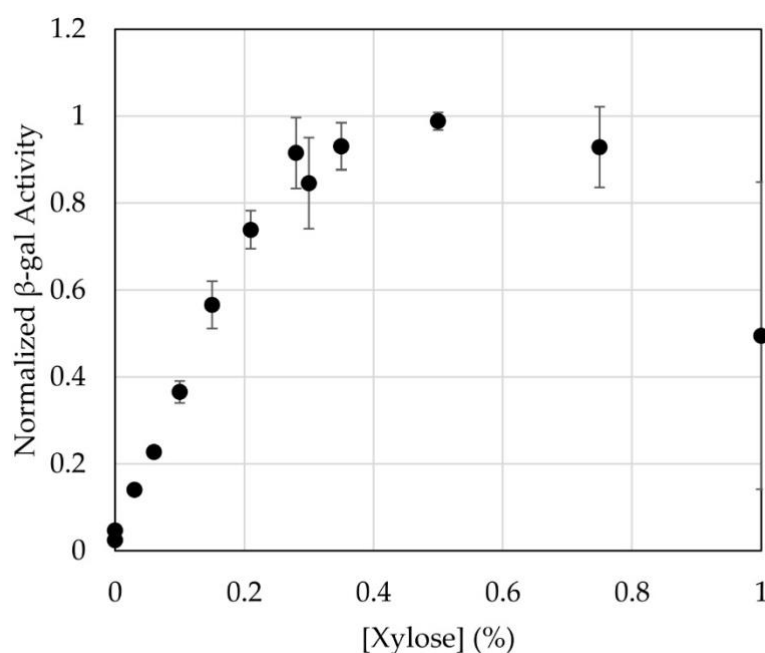| Plasmid | Promoter | Reporter gene | Detected activity in *H. modesticaldum* (U/mg protein) | Detected activity in *E. coli* (U/mg protein) |
|---|---|---|---|---|
| pAL2B | eno | *lacZ* | $0.60 \pm 0.019$ | $0.17 \pm 0.0050$ |
| pAL60 | 2638 | *lacZ* | $0.067 \pm 0.0042$ | $0.037 \pm 0.0028$ |
| pAL62 | 2926 | *lacZ* | $0.39 \pm 0.011$ | $0.39 \pm 0.011$ |
| pAL64 | cbp_2 | *lacZ* | $0.64 \pm 0.014$ | $0.39 \pm 0.011$ |
| pAL66 | gapDH | *lacZ* | $0.79 \pm 0.027$ | $0.25 \pm 0.0069$ |
| pAL731 | 0966 | *lacZ* | $0.11 \pm 0.0044$ | $0.11 \pm 0.0045$ |
| pAL751 | 0815 | *lacZ* | $0.10 \pm 0.0044$ | $0.013 \pm 0.00077$ |

**Figure S1.** The normalized β-gal activity in *H. modesticaldum*. The activity of β-gal in *H. modesticaldum* transformed with pALX3 and grown with xylose at the indicated concentrations, normalized to the maximal activity obtained. The activity assays were performed on two separate days; therefore, data sets for each biological triplicate were scaled according to the activity at 0.35% and then normalized to the maximum activity. Points represent the average of normalizations, and error bars represent standard deviation.
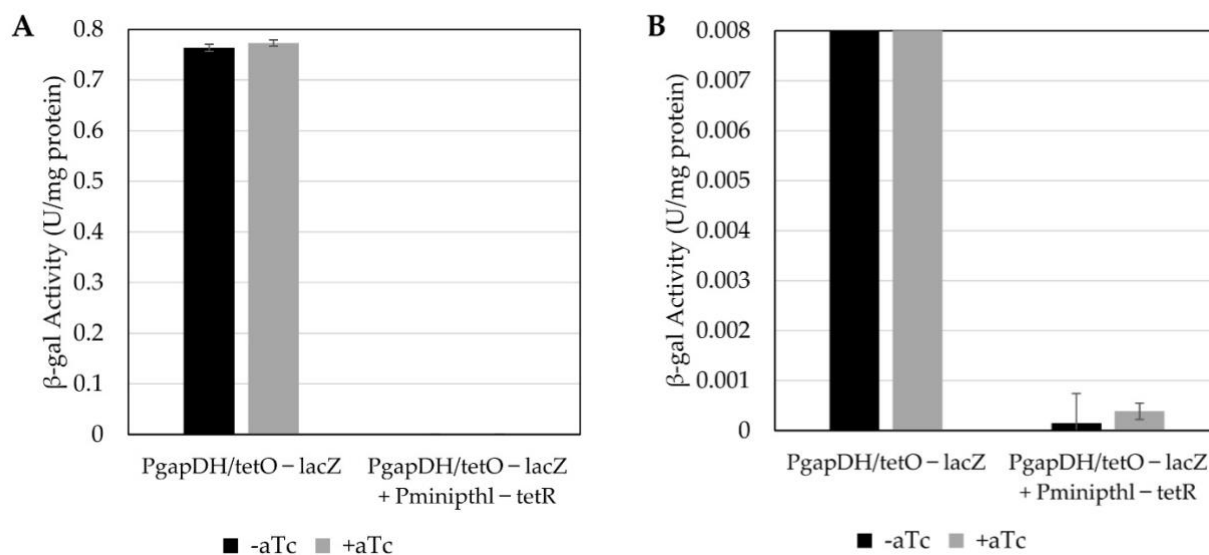


**Figure S2.** The activity of β-gal associated with different aTc plasmid constructs in *H. modesticaldum* with and without aTc inducer (200 μg L$^{-1}$). The constructs include P$_{gapDH/tetO}$ controlling *lacZ* (left), and a second construct including the *tetR* repressor controlled by P$_{minipthl}$ (right). Data represent the average rate of biological and technical triplicate; error bars represent the average standard deviation of each set of biological replicates. The ordinate scale is expanded 100-fold in plot **B** in order to see the activity of cells harboring the second construct.

## References

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2. Nariya, H.; Miyata, S.; Kuwahara, T.; Okabe, A. Development and Characterization of a Xylose-Inducible Gene Expression System for *Clostridium Perfringens* †.**2011**, *77*, 8439–8441, doi:10.1128/AEM.05668-11.
3. Heap, J.T.; Pennington, O.J.; Cartman, S.T.; Minton, N.P. A Modular System for Clostridium Shuttle Plasmids. *J. Microbiol. Methods* **2009**, *78*, 79–85, doi:10.1016/j.mimet.2009.05.004.