Crucial Role of the Chaperonin GroES/EL for Heterologous Production of the Soluble Methane Monooxygenase from *Methylomonas methanica* MC09

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**Plasmid construction**

Plasmids used in this study are listed in Table S1, shown as plasmids maps in Figure S1-4 and their sequences are presented at the end of this SI in Figure S18-22. The expression plasmid used for overproduction of the Strep-tagged sMMO hydroxylase from *Methylococcus methylans* MC09 was constructed as follows. Plasmid pSB_M1g-1-17 with an encoded GFP under control of the toluate inducible xyIS/Pm promotor variant ML1-17 served as the basis for the subsequent cloning steps. The gfp gene was equipped at the 5’ end with a Strep-tag II-encoding sequence via NEBuilder assembly and the oligo 207_StreptagII (see Table S2). From the resulting plasmid pLL210, the Spel restriction digested 7.8 kb fragment was assembled with the 7.3 kb fragment mmoXYBZDC_Orf1_mmoG fragment from *M. methanica*, which was amplified with the primers 255_Mm and 256_Mm (Table S2). This step yielded plasmid pLL319, which was transferred via electroporation into *E. coli* BL21.

For heterologous overproduction and subsequent purification, the mmoB gene from *Methylococcus methylans* MC09 was codon optimised for *E. coli* and inserted via the NdeI and BamHI restriction sites in the plasmid pET-16b. The 5’ end of mmoB was equipped with a 10x His-tag-encoding sequence resulting in the plasmid pZD01 (Figure S1). For co-synthesis of MmoZ, the mmoZ gene from *M. methylans* MC09 was codon optimised for *E. coli* and inserted via the SalI and BamHI restriction sites in the plasmid pETDuet-1. The 5’ end of mmoZ was equipped with a 6x His-tag-encoding sequence resulting in the plasmid pZD04 (Figure S2).
### Table S1: Plasmids used in this study

| Name            | Key feature                                                                 | Source                                      |
|-----------------|-----------------------------------------------------------------------------|---------------------------------------------|
| pSB_M1g-1-17    | xyIS/Ptg promotor variant ML1-17_RK2 replicon, gfpmut3, KanR                | Bakke et al. 2009[2]; Balzer et al. 2013[1]|
| pLL210          | pSB_M1g-1-17 with an encoded Strep-tag II in front of gfpmut3, KanR         | This study                                  |
| pLL319          | 7.3 kb mmoXYBZDC_Orf1_mmoG fragment from M. methanica in pLL210 with mmoX equipped at 5' with an encoded Strep-tag II, KanR | This study                                  |
| pZD01           | mmoB from M. methanica MC09 in pET-16b, AmpR                               | This study                                  |
| pZD02           | mmoC from M. methanica MC09 in pET-16b                                     | Lettau et al. 2021[3]                       |
| pZD04           | mmoZ from M. methanica MC09 in pETDuet, AmpR                              | This study                                  |
| pBB528 + pBB541 | groES/EL SpecR, ChlorR                                                     | De Marco 2007[4]                            |

### Table S1: Oligos used in this study

| Oligo   | sequence                                                                 |
|---------|--------------------------------------------------------------------------|
| 207_StreptagII | CTTTATCAACATGTACAATAATAATTTGAGTGCAATTATGCTAGCCTGGAGCGAAGTATTATGCTGGTACGAGGCA |
| 255_Mm   | CCCCCGCTTGGAAAAAGGCGCCACTATGCTGTAATGTGCAAGCCAC |
| 256_Mm   | CTTTACTTGGATGCTTTAAATCTCCTCAGTTTGGCAAGCC  |

### sMMO production and purification

*E. coli* BL21 with the plasmids pLL319 + pBB528 + pBB541 for the co-production of the chaperonin GroES/EL and additional pZD04 (MmoZ) were grown in rich Terrific Broth (TB) medium at 37 °C until OD600nm of 2 and were then induced with 2 mM toluat and 1 mM IPTG. The protein production phase was performed at 18 °C for 30 h. The harvested cells were resuspended in twice their volume of resuspension buffer (500 mM NaCl, 50 mM KPO4, pH 7.2 containing additional Protease Inhibitor (EDTA-free, Roche) and DNase I). After two passages through a chilled French press at a pressure of 6.2 MPa, the suspension was centrifuged at 100,000x g for 45 min. The soluble extract was applied to a 2 mL Strep-tag Superflow affinity chromatography column, washed with 6 mL of resuspension buffer with protease inhibitor and eluted with 12 mL of 5 mM desthiobiotin solution. The eluate was then concentrated in an Amicon Ultra-15 centrifugal cell (100K membrane; Amicon, Witten, Germany). To remove desthiobiotin after Strep-tag Superflow affinity chromatography, the elution buffer of MMOH was exchanged via an illustra NAP-10 column (GE Healthcare UK Limited, Buckinghamshire, UK) to 25 mM MOPS, 100 mM NaCl, 0.2 mM (NH4)2Fe(SO4)2, 1 mM TCEP, pH 7.2. Protein concentration was determined with BCA protein assay kit (Pierce, USA) as described previously.[5]

We isolated about 0.83 ± 0.63 mg (average of total five purifications) homogenous MMOH from 1 g cell pellet (wet weight). In order to monitor the purification, samples of every purification step were analysed by SDS-PAGE (Figure S13). In the elution fraction, the pronounced bands were detected, which correspond to the three MMOH subunits calculated size of Strep-tagged-MmoX 62.0 kDa, MmoY 45.1 kDa, and MmoZ 18.9 kDa. A weak second band above the MmoZ band indicates the 6xHis-tagged MmoZ derivative (calculated size 20.5 kDa). Because of the already high purity of MMOH (> 95%), we decided to not proceed with further purification steps.
Spectroscopic measurements

Samples UV/visible spectra were recorded with a Varian Cary 60 (Agilent Technologies) at 20°C. The final working concentration of protein samples was around 45 mg/ml. Measurements were performed in 25 mM MOPS buffer with 100 mM NaCl at pH 7.2.

Electron Paramagnetic Resonance (EPR) spectroscopy was conducted on a Brucker EMX plus X-Band spectrometer equipped with an ER 4122 super-high Q resonator and an Oxford ESR900 helium flow cryostat. An Oxford ITC4 temperature controller was used for adjusting the temperature. The baseline was corrected by subtracting a reference spectrum of buffer solution recorded with the same experimental parameters. For subsequent corrections a spline function was used. Experimental parameters used were: microwave power 1 mW, microwave frequency 9.29 GHz, modulation amplitude 10 G, and modulation frequency 100 kHz. The isolated MMOH was analyzed at a concentration of 55 µM and a volume of 100 µL.

Optical emission spectroscopy

For the determination of iron in MMOH, metal analysis was performed using a Perkin-Elmer Optima 2100DV inductively coupled plasma-optical emission spectrometer (Perkin-Elmer, Fremont, CA, USA) following the protocol described previously[^6]. In short, 500 µL of protein samples were incubated overnight with equal amount of 65% nitric acid (Suprapur, Merck KGaA, Darmstadt, Germany) at 100 °C. Samples were filled up to 5 mL with water prior to ICP-OES analysis. Buffer samples without protein were treated the same way to check if footprint of metal is dissolved in the buffer. As reference, the multielement standard solution XVI (Merck) was used.

Activity measurements

The hydroxylation activity of MMOH was measured with nitrobenzene as the substrate under aerobic conditions (75 mM NaCl, 25 mM MOPS buffer, pH 8.0, 1 mM nitrobenzene). Proteins MMOH, MmoB and MmoC were added in the ratio H:B:C = 2:2:1. The reaction was started by adding 0.5 mM NADH. The production of p-nitrophenol was followed at 420 nm (VARIAN Cary 50 BIO UV-Visible Spectrometer, ε = 11.861 mM⁻¹ cm⁻¹ at pH 8.0). The production of p-nitrophenol is followed at 405 nm due to interferences with NADH at shorter wavelength.[^7] For the determination of reaction optima, first the optimum for salt concentration, and then the pH optimum were determined at 37 °C. Finally, the determination of the temperature optimum was performed. Salt concentrations were analysed in the range of 0-0.5 M NaCl (due to marine habitat from M. methylomonas), pH values ranged from 6.5-8.5, and temperature studies were performed from 10-42 °C. Furthermore, activity assays with addition of different catalase concentrations (0-8550 U/mg) were performed, because of the possibility of H₂O₂ production by MmoC, which could inhibit the sMMO activity. For the determination of optima conditions, no catalase was added. Each measurement was performed with technical triplicates but also with biological replicates.

For the exact calculation of the activity, the extinction coefficient of p-nitrophenol was determined additionally. Absorption of p-nitrophenol (0.1 mM in 25 mM MOPS buffer with 75 mM NaCl) was measured at different pH at 420 nm with three technical replicates. Law of Lambert-Beer was used to calculate the extinction coefficient. The extinction coefficients for pH 7.2 (used for purification) and pH 8.0 (used for activity measurements) were 9.164 and 11.861 mM⁻¹ cm⁻¹, respectively.
Figure S1: Plasmid map of pZD01 used for overproduction of MmoB from *M. methanica* MC09 (generated with SnapGene software, GSL Biotech, USA).

Figure S2: Plasmid map of pZD04 used for overproduction of MmoZ from *M. methanica* MC09 (generated with SnapGene software, GSL Biotech, USA).
Figure S3: Plasmid map of pLL319 used for overproduction of the sMMO hydroxylase from *M. methanica* MC09 (generated with SnapGene software, GSL Biotech, USA).
Figure S4. Multiple Sequence alignment of MmoG with GroEL. Sequences of GroEL from Escherichia coli K12 (EcK12), Pseudomonas putida F1 (PpF1), Cupriavidus necator H16 (CnhH16), Thermus thermophilus HB8 (ThHB8) and Xanthomonas oryzae (Xo) and sequences of MmoG from methanotrophs Methylococcus capsulatus BATH (McBath), Methylothermus arcticus N1 (McN1), Methylococcus trichosporum OB3b (Mob3b), Methylocella silvestris BL1 (MsIBL1), Methylosinus sporium (Msp), Methylovulum miyakovense HT12 (MmHT12) and Mme from Methylocoronas methanica MC09 (MmcMC09) were shown.
Figure S5: Homology model of MmoG (pink) from *M. methanica* MC09 without lid calculated by using SWISS-MODEL [8] based on PDB: 6KFV (GroEL in yellow, homo-14-mer) with a model quality estimation (QMEANDisCo Global) of 0.70 ± 0.05. Left top view, middle side view, left single subunit.

Figure S6: Homology model of MmoG (orange) from *M. methanica* MC09 calculated by using SWISS-MODEL [8] based on PDB: 4V4O (GroEL in yellow and with its “lid” GroES and bound substrate in green) with a model quality estimation (QMEANDisCo Global) of 0.72 ± 0.05. Left top view, middle side view, right single subunit.
Figure S7 Comparison of MmoG (orange) based on PDB: 4V4O and eukaryotic chaperonin PDB: 4V81\(^\text{[9]}\) (green) with a build-in lid (red arrow).

| Protein         | Sequence                                                                 | Length |
|-----------------|---------------------------------------------------------------------------|--------|
| Mc Bath Orf1    | MTEQFPQQLREMIEQLDAISQLARKEGKLAASLGTGVRALKKEYWDHVTLPNEEWEEL               | 60     |
| Mj BL2 Orf2     | MKEDEQFPQQLREMIEQLDAISQLARKEGKLAASLGTGVRALKKEYWDHVTLPNEEWEEL             | 58     |
| Msp Orf1        | MGIDEQFPQQLREMIEQLDAISQLARKEGKLAASLGTGVRALKKEYWDHVTLPNEEWEEL             | 58     |
| Mm HT12 Orf1    | MNEADQEFLHDMVQLDOTTQLAIESQLMKTHIAGRVEELLEYWRLQHLSAEEVEEF                 | 58     |
| Mc Bath Orf1    | KRTMDFORELWNSRLRRARTSRNGEAYMRHLSAARKNEQS--------------------------------- | 106    |
| Mj BL2 Orf2     | KRYMWDNCKKLIWNRGSLRNSRRVAAAGAYMINSTQTAAPASAPAKARAPKFL                    | 99     |
| Msp Orf1        | RRTLDPOIRLLSVIRVERVNNAPRVAAGRSAMTLNASREDIDITAENK-------------------------| 116    |
| Mm HT12 Orf1    | KKRMDYIQIRILSRSKRAHTRVEVQFTLMKASPRLKIPFRS--------------------------------| 104    |
| Mc Bath Orf1    | KRMDHADORKLTIWLRSLRILHQSRAKAGRELKRNSID-----------------------------------| 97     |

Figure S8 Sequence alignment of MmoE (ORF1). Hypothetical proteins encoded within sMMO fragments of other methanotrophs *Methylococcus capsulatus* BATH (McBath), *Methylomicrobium japonense* NI (MjNI), *Methylocella silvestris* BL1 (MsiBL1), *Methylosinus sporium* (Msp), *Methylomonas miyakonense* HT12 (MmiHT12) and MmoE from *Methylomonas methanica* MC09 (MmMC09) were shown.
Figure S9: *In silico* docking experiments via Haddock 2.4\[10\] between AlphaFold2 predicted structures of GroEL (blue) and GroES (green, both left) from *M. methanica* MC09 and between MmoG (pink) and GroES (green, both middle) from *M. methanica* MC09. On the right side, an enlargement of single subunits are shown (GroES in green, GroEL in blue and MmoG in pink). The docking scores of the two structures (Haddock scoring) were -398.34 and -289.89 for MmoG/GroES and GroEL/GroES, respectively.

Figure S10: Structural changes between superimposed MmoG (orange) based in PDB: 4V4O with a lid (not shown, see S6) and based on PDB:6KFV) without lid (pink, see Fig S5) of single subunits. Left top view, right side view. Red arrow shows potential interface to lid, see also Fig. S7.
Figure S11: Predicted structure of MmoE (cyan, left and middle in different views, in green leucines, in orange lysines and arginines, which are typical for basic leucine zipper domains) based on RoseTTaFold\cite{11} in comparison to GroES (PDB: 4V4O, right in green).

Figure S12: Evaluation of sMMO production in E. coli BL21 with pBB528 + pBB541 (GroESL) + pLL319 (sMMO) by SDS-PAGE gel 20 µg total protein 1, 4, 7: 28.7 µg, 57.4 µg and 86.1 µg soluble extracts was added to each lane of a 4-15 % gradient gel, respectively; 2, 5, 8: 15 µl wash fraction after 10 column volumes; 3, 6, 9, 10, 11: 11 µg, 22 µg, 44 µg, 66 µg, 132 µg purified MMOH, respectively; M protein marker. Cultivation in TB, sMMO production was induced with 0.1 mM IPTG, 2mM toluate overnight at 18°C. Calculated sizes of subunits are indicated.
Figure S13: Purification of heterologous produced MMOH. Cell extract (CE), soluble extract (SE), one wash fraction (W), the elution fractions (E1, E2) and the buffer-exchanged and concentrated protein solution (UKP) were separated by SDS-PAGE and stained with Coomassie brilliant blue. A standard protein ladder (M) was used for size determination. For UKP and for CE/SE 2 µg and 20 µg (total protein) were applied, respectively. For W, E1 and E2 15 µl samples (max. pocket volume) were added.

Figure S14: Influence of sMMO activity and co-synthesising MmoZ on a separate plasmid. p-nitrophenol formation from nitrobenzene was measured for sMMO without and with additional MmoZ after different inducer concentrations of IPTG to produce the proteins. Measurements were performed at optimal condition (75 mM NaCl, 37 °C and pH 8.0). The means of three technical replicates and standard deviations are shown.
Figure S15: Adding catalase extends the period of sMMO activity. To lower the inactivation of sMMO activity due to H$_2$O$_2$ by MmoC, different catalase concentrations (0-8850 U/mg) were added to the nitrobenzene assay at optimal condition (75 mM NaCl, pH 8.0, 37 °C). MMOH was produced in E. coli BL21 containing the plasmids pLL319, pBB528, pBB541 and pZD04.

Figure S16: Iron reconstitution. To test an improvement of iron content of MMOH, a reconstitution experiment was performed. MMOH (47 µM, purified in the absence of (NH$_4$)$_2$Fe(SO$_4$)$_2$) was incubated for 30 min at 30 °C in iron reconstitution buffer (25 mM MOPS, 120 mM NaCl, 2 mM DTT, 5 v/v% glycerol, 0.5 mM (NH$_4$)$_2$Fe(SO$_4$)$_2$, pH 7.2) and then directly used for the nitrobenzene assay (25 mM MOPS, 75 mM NaCl, pH 8.0, 30 °C). Control is without addition of iron. The means of three technical replicates and standard deviations are shown.
Figure S17 UV-Vis spectrum of MMOH. UV-visible absorption spectrum of purified MMOH. MMOH was stored and measured in 25 mM MOPS, pH 7.2 with 100 mM NaCl.

Figure S18: Homology model of MMOH including subunit MmoX (green), MmoY (grey) and MmoZ (pale red) from M. methanica MC09 calculated by using SWISS-MODEL[8] based on PDB: 1MTY (in cyan, homodimer), 1XMH, 7M8Q with a model quality estimation (QMEANDisCo Global) of 0.87 ± 0.05, 0.80 ± 0.07, 0.83 ± 0.07, for MmoX, MmoY, MmoZ, respectively. Brown spheres are iron atoms.
Figure S19. Sequence alignment of MmoX. Sequences from *Methylococcus capsulatus* BATH (McBath), *Methylomonas* BATH (MjNI), *Methylosinus trichosporium* OB3b (MtOB3b), *Methylocella silvestris* BL1 (MsiBL1), *Methylosinus sporium* (Msp), *Methylomonas* BATH (MmMC09), *Methylomonas* BATH (MmHT12) and *Methylomonas methanica* MC09 (MmMC09) were shown. Black framed amino acids (AA) represent conserved glutamates and histidines coordinating the diiron active site. Conserved glutamates and aspartates at the surface are indicated in orange. According to the homology model (Figure S18), proposed additional negative charged amino acids on the surface of MmeMC09 MmoX are shown in red.
Y. According to the homology model (Figure \text{McBath}, \text{MjNI}, \text{MtOB3b}, \text{McIB1L}, \text{Msp}, \text{MsiT12}, \text{MmeC09}), proposed additional negative charged amino acids on the surface are indicated in red. According to the homology model (Figure \text{S20}), proposed additional negative charged amino acids on the surface of \text{MmeC09} \text{MmOY} are shown in red.
Figure S21: Sequence alignment of MmoZ. Sequences from *Methylococcus capsulatus* BATH (McBath), *Methylomicrobium japonense* NI (MjNI), *Methylosinus trichosporium* OB3b (MtOB3b), *Methylocella silvestris* BL1 (MsBL1), *Methylosinus sporium* (Msp), *Methyllovulum miyakonense* HT12 (MmHT12) and *Methylomonas methanica* MC09 (MmMC09) were shown. Conserved glutamates and aspartates at the surface are indicated in orange. According to the homology model (Figure S18), proposed additional negative charged amino acids on the surface of MmMC09 MmoZ are shown in red.
Figure S22. EPR spectrum of as isolated MMOH from *Methylomonas methanica* MC09 recorded at 10 K with a microwave power of 1 mW. The spectrum shows only a small contribution of a rhombic signal ($g_x = 1.95, g_y = 1.87, g_z = 1.77$), which is characteristic for the mixed-valence state of catalytic diiron center in MMOH. Therefore the active site is presumably mostly in the diferred configuration. Sharp signals in the region of $g = 2.0$ are related to very weak artefacts of the cavity.

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Figure S18: Plasmid sequence of pZD01 (mmoB)

TTCTTGAGACGAAAGGGCCTGCTGATACCCATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTGAAAG
TAAAGATGCTGAAGATCAGTTGGCTACAGAGTGGTTACATCGAAGCTGGATCTCAACACGCTGTAAGATCTCT
TGAAAGTTTTCGCCGCCGAAAGCATTTTTTTCAATGAGACCATTTTTAAATGCTGATGTCGGCAGGTTATATT
CCCGTGTGGACGCCCGGGAAGACGCGAATACTCGTGCACCGCCTACACTTTCTCAGATGAGATATCTA
ACCAGTACAGAAGATCTTATGCGGATGCATTACGATAGAAGATATAGCTGATGCGTCACCTTACCTGAC
CGGCAAGAAATTAAGTACTGGAGGAGAGGAGCGATTGAAAAAAAGGATAG
ATCCGCTCAAGAACAACACTCCGAGCGATGCTGATGCTCGTCAGGGGCTCCGGCAGAGGACGAGG
TTCTTGAGACGAAAGGGCCTGCTGATACCCATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACG
TAAAGATGCTGAAGATCAGTTGGCTACAGAGTGGTTACATCGAAGCTGGATCTCAACACGCTGTAAGATCTCT
TGAAAGTTTTCGCCGCCGAAAGCATTTTTTTCAATGAGACCATTTTTAAATGCTGATGTCGGCAGGTTATATT
CCCGTGTGGACGCCCGGGAAGACGCGAATACTCGTGCACCGCCTACACTTTCTCAGATGAGATATCTA
ACCAGTACAGAAGATCTTATGCGGATGCATTACGATAGAAGATATAGCTGATGCGTCACCTTACCTGAC
CGGCAAGAAATTAAGTACTGGAGGAGAGGAGCGATTGAAAAAAAGGATAG
ATCCGCTCAAGAACAACACTCCGAGCGATGCTGATGCTCGTCAGGGGCTCCGGCAGAGGACGAGG
TTCTTGAGACGAAAGGGCCTGCTGATACCCATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACG
TAAAGATGCTGAAGATCAGTTGGCTACAGAGTGGTTACATCGAAGCTGGATCTCAACACGCTGTAAGATCTCT
TGAAAGTTTTCGCCGCCGAAAGCATTTTTTTCAATGAGACCATTTTTAAATGCTGATGTCGGCAGGTTATATT
CCCGTGTGGACGCCCGGGAAGACGCGAATACTCGTGCACCGCCTACACTTTCTCAGATGAGATATCTA
ACCAGTACAGAAGATCTTATGCGGATGCATTACGATAGAAGATATAGCTGATGCGTCACCTTACCTGAC
CGGCAAGAAATTAAGTACTGGAGGAGAGGAGCGATTGAAAAAAAGGATAG
ATCCGCTCAAGAACAACACTCCGAGCGATGCTGATGCTCGTCAGGGGCTCCGGCAGAGGACGAGG
TTCTTGAGACGAAAGGGCCTGCTGATACCCATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACG
TAAAGATGCTGAAGATCAGTTGGCTACAGAGTGGTTACATCGAAGCTGGATCTCAACACGCTGTAAGATCTCT
TGAAAGTTTTCGCCGCCGAAAGCATTTTTTTCAATGAGACCATTTTTAAATGCTGATGTCGGCAGGTTATATT
CCCGTGTGGACGCCCGGGAAGACGCGAATACTCGTGCACCGCCTACACTTTCTCAGATGAGATATCTA
ACCAGTACAGAAGATCTTATGCGGATGCATTACGATAGAAGATATAGCTGATGCGTCACCTTACCTGAC
CGGCAAGAAATTAAGTACTGGAGGAGAGGAGCGATTGAAAAAAAGGATAG
ATCCGCTCAAGAACAACACTCCGAGCGATGCTGATGCTCGTCAGGGGCTCCGGCAGAGGACGAGG
TTCTTGAGACGAAAGGGCCTGCTGATACCCATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACG
TAAAGATGCTGAAGATCAGTTGGCTACAGAGTGGTTACATCGAAGCTGGATCTCAACACGCTGTAAGATCTCT
TGAAAGTTTTCGCCGCCGAAAGCATTTTTTTCAATGAGACCATTTTTAAATGCTGATGTCGGCAGGTTATATT
CCCGTGTGGACGCCCGGGAAGACGCGAATACTCGTGCACCGCCTACACTTTCTCAGATGAGATATCTA
ACCAGTACAGAAGATCTTATGCGGATGCATTACGATAGAAGATATAGCTGATGCGTCACCTTACCTGAC
CGGCAAGAAATTAAGTACTGGAGGAGAGGAGCGATTGAAAAAAAGGATAG
ATCCGCTCAAGAACAACACTCCGAGCGATGCTGATGCTCGTCAGGGGCTCCGGCAGAGGACGAGG
TTCTTGAGACGAAAGGGCCTGCTGATACCCATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACG
TAAAGATGCTGAAGATCAGTTGGCTACAGAGTGGTTACATCGAAGCTGGATCTCAACACGCTGTAAGATCTCT
TGAAAGTTTTCGCCGCCGAAAGCATTTTTTTCAATGAGACCATTTTTAAATGCTGATGTCGGCAGGTTATATT
CCCGTGTGGACGCCCGGGAAGACGCGAATACTCGTGCACCGCCTACACTTTCTCAGATGAGATATCTA
ACCAGTACAGAAGATCTTATGCGGATGCATTACGATAGAAGATATAGCTGATGCGTCACCTTACCTGAC
CGGCAAGAAATTAAGTACTGGAGGAGAGGAGCGATTGAAAAAAAGGATAG
ATCCGCTCAAGAACAACACTCCGAGCGATGCTGATGCTCGTCAGGGGCTCCGGCAGAGGACGAGG
TTCTTGAGACGAAAGGGCCTGCTGATACCCATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACG
TAAAGATGCTGAAGATCAGTTGGCTACAGAGTGGTTACATCGAAGCTGGATCTCAACACGCTGTAAGATCTCT
TGAAAGTTTTCGCCGCCGAAAGCATTTTTTTCAATGAGACCATTTTTAAATGCTGATGTCGGCAGGTTATATT
CCCGTGTGGACGCCCGGGAAGACGCGAATACTCGTGCACCGCCTACACTTTCTCAGATGAGATATCTA
ACCAGTACAGAAGATCTTATGCGGATGCATTACGATAGAAGATATAGCTGATGCGTCACCTTACCTGAC
CGGCAAGAAATTAAGTACTGGAGGAGAGGAGCGATTGAAAAAAAGGATAG
ATCCGCTCAAGAACAACACTCCGAGCGATGCTGATGCTCGTCAGGGGCTCCGGCAGAGGACGAGG
TTCTTGAGACGAAAGGGCCTGCTGATACCCATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACG
TAAAGATGCTGAAGATCAGTTGGCTACAGAGTGGTTACATCGAAGCTGGATCTCAACACGCTGTAAGATCTCT
TGAAAGTTTTCGCCGCCGAAAGCATTTTTTTCAATGAGACCATTTTTAAATGCTGATGTCGGCAGGTTATATT
CCCGTGTGGACGCCCGGGAAGACGCGAATACTCGTGCACCGCCTACACTTTCTCAGATGAGATATCTA
ACCAGTACAGAAGATCTTATGCGGATGCATTACGATAGAAGATATAGCTGATGCGTCACCTTACCTGAC
CGGCAAGAAATTAAGTACTGGAGGAGAGGAGCGATTGAAAAAAAGGATAG
ATCCGCTCAAGAACAACACTCCGAGCGATGCTGATGCTCGTCAGGGGCTCCGGCAGAGGACGAGG
TTCTTGAGACGAAAGGGCCTGCTGATACCCATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACG
TAAAGATGCTGAAGATCAGTTGGCTACAGAGTGGTTACATCGAAGCTGGATCTCAACACGCTGTAAGATCTCT
TGAAAGTTTTCGCCGCCGAAAGCATTTTTTTCAATGAGACCATTTTTAAATGCTGATGTCGGCAGGTTATATT
CCCGTGTGGACGCCCGGGAAGACGCGAATACTCGTGCACCGCCTACACTTTCTCAGATGAGATATCTA
ACCAGTACAGAAGATCTTATGCGGATGCATTACGATAGAAGATATAGCTGATGCGTCACCTTACCTGAC
CGGCAAGAAATTAAGTACTGGAGGAGAGGAGCGATTGAAAAAAAGGATAG
ATCCGCTCAAGAACAACACTCCGAGCGATGCTGATGCTCGTCAGGGGCTCCGGCAGAGGACGAGG
TTCTTGAGACGAAAGGGCCTGCTGATACCCATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACG
TAAAGATGCTGAAGATCAGTTGGCTACAGAGTGGTTACATCGAAGCTGGATCTCAACACGCTGTAAGATCTCT
TGAAAGTTTTCGCCGCCGAAAGCATTTTTTTCAATGAGACCATTTTTAAATGCTGATGTCGGCAGGTTATATT
CCCGTGTGGACGCCCGGGAAGACGCGAATACTCGTGCACCGCCTACACTTTCTCAGATGAGATATCTA
ACCAGTACAGAAGATCTTATGCGGATGCATTACGATAGAAGATATAGCTGATGCGTCACCTTACCTGAC
CGGCAAGAAATTAAGTACTGGAGGAGAGGAGCGATTGAAAAAAAGGATAG
ATCCGCTCAAGAACAACACTCCGAGCGATGCTGATGCTCGTCAGGGGCTCCGGCAGAGGACGAGG
TTCTTGAGACGAAAGGGCCTGCTGATACCCATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACG
Figure S19: Plasmid sequence of pZD04 (mmoZ)

GGGGGAATTGTGAGCGGATAAACATCTCCCCTCTAGAAATATTTGTATTAATCTTTAGGAGATATACCATGCTGCTCCTGAGATATCCGACCCGCAATGCTGGATGGCCAAAATTGCCACCCTGGATACAATGGCTAAAAGCCCATGCCTTCCTGACCGACTTCCGCGCCCGTCA
CATGAGTCCGTTCAAAACCGATTGGAGTCTGGAACTGGATGGCCTGTGGATTGAACTGAAAATTGAAGAAA
ACTGGCACTGCTGAAACATAAAGAATTCAATGATAGCCAGCTGCTGAATAATTGCAGCTGTGGTGCCGATGCA
CCAGCAGGTGGCCAATGCCGCAATTGCAAAAATGGAAGCCTGTGAAGATATGTATGAAGCAGAACGTATTCAT

GGGGGAATTGTGAGCGGATAAACATCTCCCCTCTAGAAATATTTGTATTAATCTTTAGGAGATATACCATGCTGCTCCTGAGATATCCGACCCGCAATGCTGGATGGCCAAAATTGCCACCCTGGATACAATGGCTAAAAGCCCATGCCTTCCTGACCGACTTCCGCGCCCGTCA
CATGAGTCCGTTCAAAACCGATTGGAGTCTGGAACTGGATGGCCTGTGGATTGAACTGAAAATTGAAGAAA
ACTGGCACTGCTGAAACATAAAGAATTCAATGATAGCCAGCTGCTGAATAATTGCAGCTGTGGTGCCGATGCA
CCAGCAGGTGGCCAATGCCGCAATTGCAAAAATGGAAGCCTGTGAAGATATGTATGAAGCAGAACGTATTCAT
Figure S20: Plasmid sequence of pLL210

TATGGCAGCTGGAGGAGCACGCAGCACCTGGCAATCCGAAAAGGCAGCCACTAGTTTAATTAATGGTGATGTTTTAAAGGTGAAGAACTTTTGACCTGGATGAGCATCAGGTTGCTGCTGCTAGGTTTTGAGTTTGAAGTTGTTCCCTTCTTTTCCCGTCAACGCTGGTTTGCCCCAGCAGGCGAAAATCCTGTTTGATGGTGGTTAAAGGCGGGATATAACATGAGCTGCTACGTGCAACGCGGACACCGCTCGGTAATGGCGCGCATTGCGCCCAGCGCCATCTGATCGTTGGCAACCAGCATCGCAGTGGGAACGGATGCCCTCATTCAGCATTTGCATGGTTTGTTGAAAACCGGACATGGCACTCCAGTCGCCCTCCCGTTCCGCTATCAGGTTGATCGGCGCGAGATTTAATCGCCGCGACAATTTGCGACGGCGCGTGCAGGGCCAGACTGGAGGTGGCAACGCCAATCAGCAACGACTGTTTGCCCGCCAGTTGTTGTGCCACGCGGTTGGGAATGTAAATTCAGCTCCGCCATCGCCGCTTCCACTTTTTCCCGCGTTTTCGCAGAAACGTGGCTGGCCTGGTTCACCACGCGGGAAACGGTCTGATAAGAGACACCGGCCATTACTCTGGCAGACATCGTATAACGTTACTGGTTTCACATTCACCACCCTGAATTGACTCTCCTCCGGGCGCTATCATGCCATACCGCGAAAGGTTTTGCGCCATTCGATGGTGTCCGGGATCTCGACGCTCTCCCTTATGCGACTCCTGCATTAAGGATCTCTAGCTAGAGTCAGCTTTATGCTTGTAACCGTTTTGTGAAAAAATTTTTAAAATAAAAAAGGGGACCTCTAGGGTCCCCAATTAATTAGTAATATAATCTTAAAGGTCATTCAAAGGTCATCCACCGGATCAGCTTAGTAAAGCCCTCGCTAGATTTTAATGCGGATGTTGCGATTACTTCGCCAACTATTGCGATAACAAGAAAAAGCCAGCCTTTCATGATATATCTCCCAATTTGTGTAGGGCTTATTATGCACGCTTAAAAATAATAAAAGCAGACTTGACCTGATAGTTTGGCTGTGAGCAATTATGTGCTTAGTGCATCTAACGCTTGAGTTAAGCCGCGCCGCGAAGCGGCGTCGGCTTGAACGAATTGTTAGACATTATTTGCC
Figure S21: Plasmid sequence of pLL319
Figure S22: Plasmid sequence of pZD02
CTGAGATATGGTGGTTGGAAGTCCGTATACCTATGATCGTATTAGCTTTAGTCCGGTTGGCATTAGCGTTGAA
GCAGAAATTGTGAACCTGGCCCCATTTTTTGATCTGGAATTTCCGGGCACCACCACCCACCGTAGTTATAG
CTGGAACAACTTACCCGCTGATATTACCCGCGCGTGGAACTGGAATTT
CTGATTCGTATTGTGGATAATGGCAAATTTTCTG
GTTGGCTGCAGAATCAGGCACATGTGGGTCAGAAAATTAATGTAAAGGTCCGAGTGGCATTTTTGGTCTGAA
AGAAAAATGCCTTTACCCCCGCCTATTTTTGTGCCCCGGACACCGCTGGCAACCTATTTCTGAGCATGGGAC
GTATGCGGAATGGGAAGAACCGCAGACTTATATCTATTTTGGGCTTAATACCGAAGCAAGTTTTCTA
TCGAGAAGACCTGAAACTCCTGAAAAATGGAATCCCAGAATCTGGCGACATTCGATTTTGAGAAAGCAGT
GATGATTGGAGTGGTGAAAAAGTAGTACGTGGTTGATGTGCTGCGATCTGCCAGGCGGTGCGGTTACC
CCGGATCTGTATCTGTGCAGTCCGGCCGTTATGGGTATGCTAGTATGCCGTTTGGCTGGCAACCGAGGATTG
CAGACAGAAGGGTTTTTCTGCGAACATTTGTCGATCTCCGAGGTGGCGGCTAAAGTACGGGGTTCTT
GAGGGGTTTTTGTGCTGAAAGGAGGACTATATCCGGATATCCCGCAAGGCGGCGAGGACTGCCGACAAC
AAGCCATAGCTACACGAGGCGGTGCGCGGGAGATGACGATGGGTGTTAGATTCCACGGTACGCTGACTGCGT
GAGCGATGAGCGTATTAGCTGAAAACTACGACTTACGCATGGCACATTTCCTGACCCGAAACAAAACG
TGAGAA