

Supplementary Information

DNA methylases for site-selective inhibition of type IIS restriction enzyme activity

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Table S1 Methylases for cloning and expression

Methylase	Original source	Type	Protein Id	UniProtKB	EC number
M2. <i>Eco</i> 31I	<i>E. coli</i> RFL31	Type IIS DNA 5-cytosine methylase	AAM09639.1	Q8RNY6	2.1.1.37
M2. <i>Bsa</i> I	<i>B. stearothermophilus</i> 6-55	Type IIS DNA 5-cytosine methylase	AAR96017.1	Q6SPF5	2.1.1.37
M. <i>Osp</i> 807II	<i>Olsenella</i> sp. oral taxon 807 F0089	Type II N-6 adenine-specific DNA methylase	WP_083443746.1	Not found	2.1.1.72
M2. <i>Nme</i> MC58II	<i>N. meningitidis</i> B MC58 kan	Type IIS N-6 adenine-specific DNA methylase	AAF41140.1	Q9K090	2.1.1.72
M. <i>Sen</i> 0738I ^a	<i>S. enterica</i> subsp. <i>enterica</i> serovar Thompson CFSAN000738	Type I R-M system N-6 adenine-specific DNA methylase (M) subunit	APW12375.1	A0A3Y8CIH9	2.1.1.72
S. <i>Sen</i> 0738I ^a		Type I R-M system specificity (S) subunit	APW12376.1	A0A3Y6H358	-
M1. <i>Eco</i> 31I	<i>E. coli</i> RFL31	Type IIS N-6 adenine-specific DNA methylase	AAM09640.1	Q8RNY5	2.1.1.72
M. <i>Xmn</i> I	<i>X. manihotis</i> 7AS1	Type II N-6 adenine-specific DNA methylase	AAC44403.1	Q56788	2.1.1.72
M1. <i>Hpy</i> AII	<i>H. pylori</i> 26695	Type IIS N-6 adenine-specific DNA methylase	AAD08411.1	O25920	2.1.1.72
M2. <i>Hpy</i> AII	<i>H. pylori</i> 26695	Type IIS N-4 cytosine-specific DNA methylase	AAD08412.1	O25921	2.1.1.113
M1. <i>Mbo</i> II	<i>M. bovis</i> ATCC 10900	Type IIS N-6 adenine-specific DNA methylase	CAA40297.1	P23192	2.1.1.72

^aM.*Sen*0738I M subunit forms a complex with S.*Sen*0738I S subunit (M₂S) for methylation.

Table S2 Primers used in the PCR amplification of the methylases

Primer name	Sequence ^a	T _m (°C)
CO9301	5'-CTAGCC CATATG ATTCCAAACCACAAGG-3'	59
CO9302	5'-CTAGCC CATATG ACCAAGTCTGAAACATTC-3'	59
CO9303	5'-CTAG AAGCTT AAGCGACGGCTTAATGTTGC-3'	62
CO9304	5'-CTAG GGATCC TTAAAGCGACGGCTTAATG-3'	62
CO9305	5'-CTAGCC CATATG ATTCCCAACCACGTATC-3'	60
CO9306	5'-CTAGCC CATATG CTTATCATTAAACACGTGCC-3'	61
CO9307	5'-CTAG AAGCTT AATGATAGCTAAGGAGGACAGC-3'	61
CO9308	5'-CTAG GGATCC TTAAATGATAGCTAAGGAGGACAG-3'	61
CO9309	5'-CTAGCC CATATG GCAAAGAAGGACACAAAC-3'	61
CO9310	5'-CTAG CTCGAG TTCCCCTTCTTTAAACTCGTAAAC-3'	63
CO9311	5'-CTAG GGATCC TTATTCCTTCTTTAAACTCG-3'	61
CO9312	5'-CTAGCC CATATG ATCACAATCTCAAACGAAG-3'	59
CO9313	5'-CTAG AAGCTT TTTCGATGATACAGTAGCCAG-3'	60
CO9314	5'-CTAG GGATCC TTATTCGATGATACAGTAGCCAG-3'	62
CO9315	5'-CTAGCC CATATG TCAATCTCATCTGCTATTTAAAGC-3'	60
CO9316	5'-CTAG CTCGAG GTTCGCCTCTTTACCACTC-3'	64
CO9317	5'-CTAG CTCGAG TTAGTTCGCCTCTTTACCACTC-3'	64
CO9318	5'-CTAGCC CATATG GCGGTGGA AAAA ACTTATTGTAG-3'	62
CO9319	5'-CTAG CTCGAG GTTAATTGCGGCATCAGTCAGG-3'	66
CO9320	5'-CTAG GGATCC TTAGTTAATTGCGGCATCAGTCAG-3'	64
CO9323	5'-CTAGC GCTAGC ATGGAGGAGATCTTCTATATGAAAC-3'	63
CO9324	5'-CTAG GGATCC TTAATCGTAGTGCTGGAAGATG-3'	62
CO9325	5'-CTAGC CATGG AGGAGATCTTCTATATGAAAC-3'	59
CO9326	5'-CTAG AAGCTT ATCGTAGTGCTGGAAGATG-3'	60
CO9331	5'-CTAGC GCTAGC ATGCGTGATCTTGCTAGTACTTATCG-3'	66
CO9332	5'-CTAG AAGCTT TTACCCGGCGCGCAAACC-3'	67
CO9333	5'-CTAGC CATGGG TGATCTTGCTAGTACTTATCGTCGTG-3'	66
CO9334	5'-CTAG AAGCTT CCCGGCGCGCAAACCATC-3'	68
CO9339	5'-CTAGCC CATATG AACATTAATAAGGTGTTCTACC-3'	58
CO9341	5'-CTAG GCGGCCGC TTCAAAGTCAAATAAATTGCCC-3'	67
CO9342	5'-CTAGCC CATATG GTTAGAGAACATGTTAGAG-3'	57
CO9344	5'-CTAG AAGCTT GTTAATCTCCA ACTGGTTC-3'	59
CO9452	5'-CCTAGC CATATG AACATTAATAAGGTGTTCTACCAT-3'	59
CO9453	5'-CTAG GCGGCCGC TTATTCAAAGTCAAATAAATTGCCC-3'	67
CO9454	5'-CCTAGC CATATG GTTAGAGAACATGTTAGAGATC-3'	59
CO9455	5'-CTAG GCGGCCGC TTAGTTAATCTCCA ACTGGTTCAGC-3'	69
CO9384	5'-CTAGCC CATATG ATTCTGAATAAGATCTACATTGAG-3'	58
CO9385	5'-CTAG GGATCC TTATTCACACTCGTTATAGCG-3'	61
CO9386	5'-CTAG GCGGCCGC TTACACTCGTTATAGCG-3'	68

^aNucleotides in **bold** and underlined correspond to the restriction site of the enzyme.

Gene sequences of methylases

M2.Eco31I (POC1463-KPL01)

ATGACCAAGTCTGAAACATTCATGATTCCAAACCACAAGGCCGCCAAATTAAGTGAGCT
GGATATGATGATCGTTAACTCTGTCCCGCCTGGGGGAAACTGGAAGAATATTCCCTTGG
ATGTACCATCGAAACGTATCGAACAGATTTCGTGACAGCTATGCTCAAGGAAAAGGGTCG
CGCAGCACATACTACGGGCGTTTATTGCCCCGATATGCCAGCTTATACGATCAATACTTA
TTTCAATCGTCCTGGCAACGGATGCCACATTCACACTACGAGCAAGATCGCGTACTTTTCAC
AACGCGAAGCTGCACGTCTGCAGTCGTTCCCTGATGACTTTATCTTTTTTGGAGGTCAA
ACGGCGATTAAATACGCAAATCGGTAATGCCGTGCCTCCCTTTCTTGCGTTTCTTATTGC
AAAAGAAATTGAAAAAGCGATCGGTAATACCGGCTACTACATTGACTTATTTCAGTGGTG
CAGGCGGATTGGGGTTGGGCTTTAAGTGGGCGGGTGGACTCCATTGTTAGCTAATGAC
ATTGAGGAAAAGTACTTACAGACATACTCGAACAACGTACACAAAGAAGTTTTGTGCGG
AAGCATTTTCGGACAACGAACTTTTTCTAAGATCGCAGACAAGATTTCTGGCTTTAAGA
AATTATATTTTGATAAACAGCTGTGGATTCTGGGCGGGCCTCCGTGCCAGGGATTTAGC
ACGGCTGGCAACGCGCGTACAATGGACGACCCACGCAACAGTCTGTTTATGCACTACAA
GTCGCTGCTTAACGAGATTAAGCCGAATGGATTTCATTTTCGAGAACGTCGCCGGCCTGT
TGAACATGGAAAAAGGAAAGGTCTTTGAACGTGTTAAGGAGGAATTCTCGTCCACAATG
AAAACCATGAATGGTTGGATTTTAAATTCGGAACATTACGCAATTCCACAACGCCGTAA
GCGTGTAATTCTTGTGGGCAGCAATGATCCGCTGTTCTCGATCGAACCACCTCAGAAGC
TGACGGAAGATAAAGAGTCTTGGGTGTCAGTAAAAGATGCGTTATCTGACCTTCCCCCA
TTACAACACGGCGAGGATGGATCTGGTAAATACTATATCCACCACCCGGAATGATTA
CCAGTTGTTTATGCGTGGAACATTACACCCTCAGAGTATTATGAACGCAACATTAAGC
CGTCGCTTTAA

M2.Eco31I (POC1464-KPL02)

ATGACCAAGTCTGAAACATTCATGATTCCAAACCACAAGGCCGCCAAATTAAGTGAGCT
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ATGTACCATCGAAACGTATCGAACAGATTTCGTGACAGCTATGCTCAAGGAAAAGGGTCG
CGCAGCACATACTACGGGCGTTTATTGCCCCGATATGCCAGCTTATACGATCAATACTTA
TTTCAATCGTCCTGGCAACGGATGCCACATTCACACTACGAGCAAGATCGCGTACTTTTCAC
AACGCGAAGCTGCACGTCTGCAGTCGTTCCCTGATGACTTTATCTTTTTTGGAGGTCAA
ACGGCGATTAAATACGCAAATCGGTAATGCCGTGCCTCCCTTTCTTGCGTTTCTTATTGC
AAAAGAAATTGAAAAAGCGATCGGTAATACCGGCTACTACATTGACTTATTTCAGTGGTG
CAGGCGGATTGGGGTTGGGCTTTAAGTGGGCGGGTGGACTCCATTGTTAGCTAATGAC
ATTGAGGAAAAGTACTTACAGACATACTCGAACAACGTACACAAAGAAGTTTTGTGCGG
AAGCATTTTCGGACAACGAACTTTTTCTAAGATCGCAGACAAGATTTCTGGCTTTAAGA
AATTATATTTTGATAAACAGCTGTGGATTCTGGGCGGGCCTCCGTGCCAGGGATTTAGC
ACGGCTGGCAACGCGCGTACAATGGACGACCCACGCAACAGTCTGTTTATGCACTACAA
GTCGCTGCTTAACGAGATTAAGCCGAATGGATTTCATTTTCGAGAACGTCGCCGGCCTGT
TGAACATGGAAAAAGGAAAGGTCTTTGAACGTGTTAAGGAGGAATTCTCGTCCACAATG
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GCGTGTAATTCTTGTGGGCAGCAATGATCCGCTGTTCTCGATCGAACCACCTCAGAAGC
TGACGGAAGATAAAGAGTCTTGGGTGTCAGTAAAAGATGCGTTATCTGACCTTCCCCCA
TTACAACACGGCGAGGATGGATCTGGTAAATACTATATCCACCACCCGGAATGATTA

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CGTCGCTTTAA

M2.Eco31I_2 (POC1465-KPL03), truncated M2.Eco31I

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TTATTGCCCCGATATGCCAGCTTATACGATCAATACTTATTTCAATCGTCCTGGCAACGG
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AGTCGTTCCCTGATGACTTTTATCTTTTTTTGGAGGTCAAACGGCGATTAATACGCAAATC
GGTAATGCCGTGCCTCCCTTTCTTGCGTTTCTTATTGCAAAAGAAATTGAAAAAGCGAT
CGGTAATACCGGCTACTACATTGACTTATTCAGTGGTGCAGGCGGATTGGGGTTGGGCT
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ACATACTCGAACAACGTACACAAAGAAGTTTTGTGCGGAAGCATTTCGGACAACGAAAC
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TGTGGATTCTGGGCGGGCCTCCGTGCCAGGGATTTAGCACGGCTGGCAACGCGCGTACA
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GCCGAATGGATTCATTTTTCGAGAACGTGCGCGGCCTGTTGAACATGGAAAAAGGAAAGG
TCTTTGAACGTGTTAAGGAGGAATTCTCGTCCACAATGAAAACCATGAATGGTTGGATT
TTAAATTCGGAACATTACGCAATTCCACAACGCCGTAAGCGTGTAATTCTTGTGGGCAG
CAATGATCCGCTGTTCTCGATCGAACCACCTCAGAAGCTGACGGAAGATAAAGAGTCTT
GGGTGTCAGTAAAAGATGCGTTATCTGACCTTCCCCCATTACAACACGGCGAGGATGGA
TCTGGTAAATACTATATCCACCACCCGGAAAATGATTACCAGTTGTTTATGCGTGGA
CATTACACCCTCAGAGTATTATGAACGCAACATTAAGCCGTCGCTTTAA

M2.Eco31I_2 (POC1466-KPL04), truncated M2.Eco31I

ATGATTCCAAACCACAAGGCCGCCAAATTAAGTGAGCTGGATATGATGATCGTTAACTC
TGTCCTCGCCTGGGGGAAACTGGAAGAATATTCCTTGGATGTACCATCGAAACGTATCG
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TTATTGCCCCGATATGCCAGCTTATACGATCAATACTTATTTCAATCGTCCTGGCAACGG
ATGCCACATTCACTACGAGCAAGATCGCGTACTTTCACAACGCGAAGCTGCACGTCTGC
AGTCGTTCCCTGATGACTTTTATCTTTTTTTGGAGGTCAAACGGCGATTAATACGCAAATC
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GGGTGTCAGTAAAAGATGCGTTATCTGACCTTCCCCCATTACAACACGGCGAGGATGGA
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M2.BsaI (POC1467-KPL05)

ATGATTCCCAACCACGTATCTTCCAAGTTAAGTGAACCTTGACATGCTTATCATTTAAACA
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AGCAAATTCGTAAGTCTTATGCTGAGGGAAAAGGATCACGCTCAACTTACTATGGCCGC
CTGTTACCAGATATGCCAAGCTACACTATCAACACGTACTTCAACCGTCCAGGTAATGG
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AGTCTTTCCCTGACGATTTTCATCTTCTACGGTAGCAAGACGGCCATCAACAACCAGATC
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CATTCAAAGTGCTGATCGCAAAGAAGTCGAACCCGGACCGTCCATTGTTTGTCTTG
GGGGACCACCCTGCCAAGGGTTTTCGACTGCAGGAAAAAACGTTCAATCGTGGACGAG
CGCAACTGGCTGTTTCGAAAGTTACGTGAGTATCCTGAAAGAAGTCAAGCCAGACGGTTT
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TTAAATCGGAATTGTCTAAGACGGTGTGCAATCTTTTCGTATATAAACTTAATTCGGTG
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GAAGAACAGCGAGCCCCCTATCCCAATTACGTCTCTTAAAGGAGAAAAGACCCTGTTTG
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CCTAATGAGGACGGCTCTTGGAAGAACTACGTTTGTGAGCCACAAAATATTTATCAGTC
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TCATTTAA

M2.BsaI (POC1468-KPL06)

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CATTCAAAGTGCTGATCGCAAAGAAGTCGAACCCGGACCGTCCATTGTTTGTCTTG
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CGCAACTGGCTGTTTCGAAAGTTACGTGAGTATCCTGAAAGAAGTCAAGCCAGACGGTTT
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TCATTTAA

M2.BsaI_2 (POC1469-KPL07), truncated M2.BsaI

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ACTTACCCCTTTTGTGCGCTAATGAGGACGGCTCTTGGAAGAAGTACGTTTGTGAGCCA
CAAAATATTTATCAGTCATTCATGCGCAAGAAGATCACAGCCCAGCAGTATATCGAGAT
GCTGTCCTCCTTAGCTATCATTTAA

M2.BsaI_2 (POC1470-KPL08), truncated M2.BsaI

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M.Osp807II (POC1471-KPL09)

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GATACAGTCTGGGAACCATTTGGAGGTCTGTGCTCGGCTAGTGTGGCATCGTCATTACT
TGGTCGCCGCAGTTACGCTGCAGAAATTGACGACACATTTTACAAGTTAGCAGCGGCCC
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GAATAA

M.Osp807II (POC1472-KPL10)

ATGGCAAAGAAGGACACAAACCTTAAGCTTTCCACCTTTATAAGGGTAACGTATCGGA
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CGGGCCTGTCCCTTTCAAAGTCGAATGAAGCGTGTGGTGTGAAGAACGCTGCGACCCGT
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GGCGGAGTACTGCATGCAATATGGTAAAAAACTTCTTGGCCCTATTTTCACTCTGGATG
GCAAACTATGATTTTCACTCATGATTGGGATAGTTTACGCACTACATGGAATCACCGC
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TGAACCAAAGCCCCCTGGACCTGATGCTTACTCAGGTAGCAGCAGCCAGTAATGTCGGC
GATACAGTCTGGGAACCATTTGGAGGTCTGTGCTCGGCTAGTGTGGCATCGTCATTACT
TGGTCGCCGCAGTTACGCTGCAGAAATTGACGACACATTTTACAAGTTAGCAGCGGCCC
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GAATAA

M2.NmeMC58II (POC1473-KPL11)

ATGATCACAATCTCAAACGAAGATAATATGATCCTGATGAGTCGCTATCCAGACAAGTA
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GAGATTATAAATTCAACATGAACGAATACAGCCAGTGGGATATTAAACCTGACCAGACC
TACTTCAACGAGTTATTTTCGCGTTAGCAAGAATCAGATTATTTGGGGGGGCAACTATTT
TGGAGAATTGTGGTTACGTAGCGAATATAATAAGGGCTTCATTATCTGGGACAAGAACC
AACCGGAAACCTTAAACAATTTTTTCGATGGCAGAAATGGCCTGGTCAAGTTTCGATCGC
CCATCGAAGATTTTTTCGCTTCAGTGTTTCGTAAAAACCGCAACAAGACACATCCGACGCA
GAAGCCAGTAGAGCTTTACCAATGGTTATTGAAGATGTACGCAAAACAGGGTGATAAAA
TTTTGGATACACACCTGGGATCTGGGACTTTAGCGATTGCATGTTGTATTGCCCAATTT
GATTTAACAGCGTGTGAGATTAACCTCTGATTATTACCAGCAGTCCATCGAGAAGATTAA
GAACAACCTGCCCGAAGCACGCATTTCCCTTTGGCCACCCTGGCTACTGTATCATCGAAT
AA

M2.NmeMC58II (POC1474-KPL12)

ATGATCACAATCTCAAACGAAGATAATATGATCCTGATGAGTCGCTATCCAGACAAGTA
CTTTGACCTGGCCATTGTTGACCCCCCTTATGGAATCTTGAACAAAACCTAAACGCGGTG
GAGATTATAAATTCAACATGAACGAATACAGCCAGTGGGATATTAAACCTGACCAGACC
TACTTCAACGAGTTATTTTCGCGTTAGCAAGAATCAGATTATTTGGGGGGGCAACTATTT
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AACCGGAAACCTTAAACAATTTTTTCGATGGCAGAAATGGCCTGGTCAAGTTTCGATCGC
CCATCGAAGATTTTTTCGCTTCAGTGTTTCGTAAAAACCGCAACAAGACACATCCGACGCA
GAAGCCAGTAGAGCTTTACCAATGGTTATTGAAGATGTACGCAAAACAGGGTGATAAAA
TTTTGGATACACACCTGGGATCTGGGACTTTAGCGATTGCATGTTGTATTGCCCAATTT
GATTTAACAGCGTGTGAGATTAACCTCTGATTATTACCAGCAGTCCATCGAGAAGATTAA
GAACAACCTGCCCGAAGCACGCATTTCCCTTTGGCCACCCTGGCTACTGTATCATCGAAT
AA

M.Sen0738I (POC1475-KPL13)

ATGTCAATCTCATCTGCTATTAAAAGCTTGCAAGACATCATGCGCAAAGATGCCGGTGT
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ATGCACAGGAACAGGCACTGGAGATTGAACAAGAAAAGTATCGTCTGCCTATGCCAGAA
CGCTATTTATGGCGCAATTGGGCCGCCGATAATGAAGGAATTACAGGGGATAAATTGTT
GGCGTTTCGTCAACGATGATCTTTTTTCCCACCTTGAAGGACTTGCCCGCCAGATCGACA
TCAACCCCCGCGGGTATGTAGTAAAGCAGGCTTTCTCCGACGCTTATAACTACATGAAA
AACGGGACGTTGCTGCGCCAAGTTATCAACAAGTTAAATGAGATTGACTTTACACGTGC
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GTAGACCCTAAACTGGGTGAGTCGATCATGGATCCGGCTTGTGGTACGGGGGGATTTTT
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TCTTGCAGAAACAAATTCATGGGGTCGAAAAGAAGCAATTACCACACTTGTGTGTACG
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TCGGGGGTACTGAGGAAGATGGCATTGAAAAGAACTTCCCCTCTGACATGCAGACACGT
GAGACGGCGGACTTATTTCTTCAGCTTATTATTGAGGTCTTAGCTAAGAACGGTCGTGC
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AGTTGCTGACCGAAGAGTGCAATTTGCACACAATTGTCCGTTTACCTAACGGCGTTTTT

AACCCCTATACGGGGATCAAACTAACCTGTTATTTTTTCACGAAAGGGCAGCCGACCAA
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CGCCCGTAACTTCAATCTTGATATTAAGAACCCCCACCAGGCAGAAaACCGTATCCCATG
ACCCAGACGAGCTGTTAGCACAAATACGCCAAACAGCAAGAGGCCATCCAACTCTTCGC
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M.Sen0738I (POC1476-KPL14)

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CGCTATTTATGGCGCAATTGGGCCGCCGATAATGAAGGAATTACAGGGGATAAATTGTT
GGCGTTCGTCAACGATGATCTTTTTTCCCACCTTGAAGGACTTGCCCGCCAGATCGACA
TCAACCCCCGCGGGTATGTAGTAAAGCAGGCTTTCTCCGACGCTTATAACTACATGAAA
AACGGGACGTTGCTGCGCCAAGTTATCAACAAGTTAAATGAGATTGACTTTACACGTGC
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GTAGACCCTAAACTGGGTGAGTCGATCATGGATCCGGCTTGTGGTACGGGGGGATTTTT
GGCATGCGCCTTCGACCACGTTAAAAACCATTATGCCCATACTGTGACTGACCATCAAA
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TAATAAACCACCTTTCATCGTGGGACGAGCAAATGGATGTTATCATCACTAACCCCCCAT
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S.Sen0738I (POC1477-KPL15)

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TCCTGCAACTGGCTGTCATGGGTAAGCTGGTGCCACAGGACCCAAACGATGAACCAGCA
TCTGAACTGCTGAAGCGTATTGCACAGGAAAAAGCACAGTTGGTAAAGGATGGAAAGAT
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CGGGTTGGGAATGGTGTTTATTTGAGGACGTCGTAGATATTCAATCGGGCATTACCAAG
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S.Sen0738I (POC1478-KPL16)

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GACAAGCCCGCTTCGGTGCTTTTAGAACGCATCGCGACAGAAAAAGCGGAGCTGGTGAA
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CTTCGACGTTTCATCGTTTGAAGTCCCATCAACTGATGGAGGGTGATATCCTTATCGCG
CGCTTACCGAACCCGATCGGACGCGCGTGCATCTTTCCTAAGCTTTCCCAAAGCGCTAT
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ACCACCTTGACGGACAGCCAGAATGCAGATGAGTTAGCTGAAAATTGGGCCCCGTATCAG
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ATTAATAA

M1.Eco31I (POC1479-KPL17)

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GCGATCCCTAGCGAAAAGATCTTACTGCAACACAGTGAGTTGAAACAGCTGGTAATTAG
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TTTACAACTTGACCTGAATGAGTTCAACAAATTGCTTAGTAGCTTCGACAAAATCGAG
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M1.Eco31I (POC1480-KPL18)

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GTTGCTGATCCCTTTTCAGGTGACGGTCGTCTGATCACTCTTTTGATCAAGCAGTGGAT
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CGATCGTCATGCCCCGCCAGCTTTTTTGCAGGACGAGCAATCCTATATTTTACGTGAGAAG
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M.XmnI (POC1481-KPL19)

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CACCTGTCCCGCCACAGTAGTTGGAGCACGAGTTTATGGCGCGATCGTGACTTTCACCT
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TCCCTACATCAGTGCTGGATGCTTCCGATGACTGGCGTGAGGTGCAGGAATTGCTGGTC
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GAACAAGTTAAGCGATGGTTTGCGCGCCGGGTAA

M.XmnI (POC1482-KPL20)

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CACCTGTCCCGCCACAGTAGTTGGAGCACGAGTTTATGGCGCGATCGTGACTTTCACCT
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M1.HpyAII (POC1483-KPL21)

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M1.*Hpy*AII (POC1484-KPL22)

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AACGTTGGTTCCCCAATCCCCAAGGGAAACTGTGCCTGGACGTGTGGGAAATTACTTCG
CAGCGCCACGTAGAGAAAGAAAAAGGAAAAATTCTGAAACCGAAACACCCATCGATCAA
ACCGAAGGCACTGATTGAGCGCATGATCAAGGCATCATCACACAAAAATGACCTTATCT
TGGACTTGTTTTCGGGCTCCGGGATGACTTCTTTAGTGGCAAAAAGCTTGGAGCGTAAT
TTTATCGGCTGTGAGTCCCACGCAGAGTATGTGCACGGATCACTGGAAATGTTCCGCTA
TAACGAGTGTGAATAA

M2.*Hpy*AII (POC1485-KPL23)

ATGAACATTAATAAGGTGTTCTACCATTTCATCGACAAATATGAACGAAGTACCCGATAA
TTCGGTTGATCTTATCATCACCTCGCCTCCTTATTTTAAATATCAAAGACTATGCCAAAA
ATGGCACACAAGACTTGCAACATTCTGCTCAGCATGTGGAAGATTTGGGTGCTTTAGAG
AAATACGAGGATTATCTGCTGGGTCTGTTGAAGGTTTGGTTGGAATGTTATCGTGCTCT
GAAGCCAAACGGTAAACTGTGCATCAACGTACCATTGATGCCTATGTTGAAGAAGGTAT
TGAATACGCATTATAATCGTCACATTTTCGACTTACACGCGGATATTCAACACTCAATT
CTTCATGATTTAAACAACATGTTGAAAAATAAGCCGAAGATGTTTTTGTGTTGGATGTGTA
TATTTGGAAGCGTGCCAATCCAACGAAACGCTTGATGTTTGGATCCTACCCGTACCCCC
GCAATTTTTTACGCACAGAATACTATTGAGTTCATCGGCGTTTTTCGTGAAGGATGGTAAA
CCAAAACAACCTACTGAAGAGCAAAAAGAGCAAAGTCAATTAACCCAAGAGGAATGGGT
AGAGTTCACTAAGCAAATCTGGGAGATTCTTATTCCTAATAAGAACGACATCGCTTTTG
GAAACACGCAGCCCTGATGCCCCGAGAACTGGCCCGCCGCTTGATTTCGTCTTTATTCT
TGTGTCGGCGACGTCTGCTTAGACCCATTTTCGGGCTCCGGCACGACGCTTCGCGAAGC
CAAGCTTCTTAAACGCAACTTCATTGGGTACGAGCTGTATGAAAACATAAACCGCTGA
TCGAGCAGAAATTGGGCAATTTATTTGACTTTGAATAA

M2.*Hpy*AII (POC1486-KPL24)

ATGAACATTAATAAGGTGTTCTACCATTTCATCGACAAATATGAACGAAGTACCCGATAA
TTCGGTTGATCTTATCATCACCTCGCCTCCTTATTTTAAATATCAAAGACTATGCCAAAA
ATGGCACACAAGACTTGCAACATTCTGCTCAGCATGTGGAAGATTTGGGTGCTTTAGAG
AAATACGAGGATTATCTGCTGGGTCTGTTGAAGGTTTGGTTGGAATGTTATCGTGCTCT
GAAGCCAAACGGTAAACTGTGCATCAACGTACCATTGATGCCTATGTTGAAGAAGGTAT
TGAATACGCATTATAATCGTCACATTTTCGACTTACACGCGGATATTCAACACTCAATT
CTTCATGATTTAAACAACATGTTGAAAAATAAGCCGAAGATGTTTTTGTGTTGGATGTGTA
TATTTGGAAGCGTGCCAATCCAACGAAACGCTTGATGTTTGGATCCTACCCGTACCCCC
GCAATTTTTTACGCACAGAATACTATTGAGTTCATCGGCGTTTTTCGTGAAGGATGGTAAA
CCAAAACAACCTACTGAAGAGCAAAAAGAGCAAAGTCAATTAACCCAAGAGGAATGGGT
AGAGTTCACTAAGCAAATCTGGGAGATTCTTATTCCTAATAAGAACGACATCGCTTTTG
GAAACACGCAGCCCTGATGCCCCGAGAACTGGCCCGCCGCTTGATTTCGTCTTTATTCT
TGTGTCGGCGACGTCTGCTTAGACCCATTTTCGGGCTCCGGCACGACGCTTCGCGAAGC

CAAGCTTCTTAAACGCAACTTCATTGGGTACGAGCTGTATGAAAACCTATAAACCGCTGA
TCGAGCAGAAATTGGGCAATTTATTTGACTTTGAATAA

M1.*Mbo*II (POC1487-KPL25)

ATGGTAGAGAACATGTTAGAGATCAATAAAATCCACCAAATGAATTGCTTCGATTTTTT
GGACCAGGTTGAGAACAAATCTGTTCAAGTTGGCGGTCATTGATCCTCCATATAACCTGA
GTAAGGCGGATTGGGATTCTTTTGATTACATAACGAGTTCCTGGCATTACGTACCGC
TGGATCGATAAGGTTCTGGACAAGTTGGACAAAGACGGGTCGCTTTACATCTTTAACAC
TCCGTTTAAATTGCGCTTTTATTTGCCAGTACCTTGTTAGTAAGGGAATGATCTTTCAA
ACTGGATTACGTGGGATAAACGTGACGGAATGGGGTCAGCGAAACGCCGTTTTAGCACA
GGCCAAGAGACTATCCTGTTCTTTAGCAAATCCAAAACCATAACATTTAACTACGATGA
GGTCCGCGTCCCTTACGAGTCAACCGACCGTATCAAGCACGCCAGTGAGAAGGGAATCC
TGAAAAACGGAAAACGTTGGTTCCCGAACCCAAACGGCCGTCTGTGCGGAGAGGTGTGG
CATTTCAAGTAGTCAGCGTCACAAGGAGAAGGTTAATGGTAAAACCGTTAACTTACGCA
CATTACCCCTAAGCCCCGTGACCTGATTGAACGTATCATTGCGGCCAGTTCTAATCCTA
ATGACCTTGTCTTAGACTGTTTCATGGGGTCTGGCACAACCTGCCATTGTAGCCAAGAAG
TTGGGGCGTAACTTCATCGGATGCGATATGAACGCAGAAATATGTAAATCAGGCAAATTT
TGTGCTGAACCAGTTGGAGATTAATAA

M1.*Mbo*II (POC1488-KPL26)

ATGGTAGAGAACATGTTAGAGATCAATAAAATCCACCAAATGAATTGCTTCGATTTTTT
GGACCAGGTTGAGAACAAATCTGTTCAAGTTGGCGGTCATTGATCCTCCATATAACCTGA
GTAAGGCGGATTGGGATTCTTTTGATTACATAACGAGTTCCTGGCATTACGTACCGC
TGGATCGATAAGGTTCTGGACAAGTTGGACAAAGACGGGTCGCTTTACATCTTTAACAC
TCCGTTTAAATTGCGCTTTTATTTGCCAGTACCTTGTTAGTAAGGGAATGATCTTTCAA
ACTGGATTACGTGGGATAAACGTGACGGAATGGGGTCAGCGAAACGCCGTTTTAGCACA
GGCCAAGAGACTATCCTGTTCTTTAGCAAATCCAAAACCATAACATTTAACTACGATGA
GGTCCGCGTCCCTTACGAGTCAACCGACCGTATCAAGCACGCCAGTGAGAAGGGAATCC
TGAAAAACGGAAAACGTTGGTTCCCGAACCCAAACGGCCGTCTGTGCGGAGAGGTGTGG
CATTTCAAGTAGTCAGCGTCACAAGGAGAAGGTTAATGGTAAAACCGTTAACTTACGCA
CATTACCCCTAAGCCCCGTGACCTGATTGAACGTATCATTGCGGCCAGTTCTAATCCTA
ATGACCTTGTCTTAGACTGTTTCATGGGGTCTGGCACAACCTGCCATTGTAGCCAAGAAG
TTGGGGCGTAACTTCATCGGATGCGATATGAACGCAGAAATATGTAAATCAGGCAAATTT
TGTGCTGAACCAGTTGGAGATTAATAA

Protein sequences of methylases with His6-tag

M2.Eco31I (POC1463-KPL01), N-terminal His6-tag

MGSSHHHHHHSSGLVPRGSHMTKSETFMI PNHKA AKLSELDMMI VNSVPPGGNWKNIPL
DVPSKRIEQIRDSYAQGKGS RSTYYGRLLPDMPAYTINTYFNRPGNGCHIHYEQDRVLS
QREAA RLQSFPDDFIFFGGQT AINTQIGNAVPPFLAFLIAKEIEKAIGNTGYYIDLFSG
AGGLGLGFKWAGWTPLLANDIEEKYLQTY SNNVHKEVLCGSISDNETFSKIADKISGFK
KLYFDKQLWILGGPPCQGFSTAGNARTMDDPRNSLFMHYKSLLNEIKPNGFIFENVAGL
LNMEKGKVFERVKEEFSSTMKT MNGWILNSEHYAIPQRRKR VILVGSNDPLFSIEPPQK
LTEDKESWVSVKDALS DLPPLQHGEDGSGKYYIHHPENDYQLFMRGNITPSEYYERNIK
PSL

M2.Eco31I (POC1464-KPL02), C-terminal His6-tag

MTKSETFMI PNHKA AKLSELDMMI VNSVPPGGNWKNIPLDVPSKRIEQIRDSYAQGKGS
RSTYYGRLLPDMPAYTINTYFNRPGNGCHIHYEQDRVLSQREAA RLQSFPDDFIFFGGQ
TAINTQIGNAVPPFLAFLIAKEIEKAIGNTGYYIDLFSGAGGLGLGFKWAGWTPLLAND
IEEKYLQTY SNNVHKEVLCGSISDNETFSKIADKISGFKKLYFDKQLWILGGPPCQGF
TAGNARTMDDPRNSLFMHYKSLLNEIKPNGFIFENVAGLLNMEKGKVFERVKEEFSSTM
KTMNGWILNSEHYAIPQRRKR VILVGSNDPLFSIEPPQKLTEDKESWVSVKDALS DLP
LQHGEDGSGKYYIHHPENDYQLFMRGNITPSEYYERNIKPSLKLAAALEHHHHHH

M2.Eco31I_2 (POC1465-KPL03), truncated M2.Eco31I, N-terminal His6-tag

MGSSHHHHHHSSGLVPRGSHMI PNHKA AKLSELDMMI VNSVPPGGNWKNIPLDVPSKRI
EQIRDSYAQGKGS RSTYYGRLLPDMPAYTINTYFNRPGNGCHIHYEQDRVLSQREAA RL
QSFPDDFIFFGGQT AINTQIGNAVPPFLAFLIAKEIEKAIGNTGYYIDLFSGAGGLGLG
FKWAGWTPLLANDIEEKYLQTY SNNVHKEVLCGSISDNETFSKIADKISGFKKLYFDKQ
LWILGGPPCQGFSTAGNARTMDDPRNSLFMHYKSLLNEIKPNGFIFENVAGLLNMEKGK
VFERVKEEFSSTMKT MNGWILNSEHYAIPQRRKR VILVGSNDPLFSIEPPQKLTEDKES
WVSVKDALS DLPPLQHGEDGSGKYYIHHPENDYQLFMRGNITPSEYYERNIKPSL

M2.Eco31I_2 (POC1466-KPL04), truncated M2.Eco31, C-terminal His6-tag

MIPNHKA AKLSELDMMI VNSVPPGGNWKNIPLDVPSKRIEQIRDSYAQGKGS RSTYYGR
LLPDMPAYTINTYFNRPGNGCHIHYEQDRVLSQREAA RLQSFPDDFIFFGGQT AINTQI
GNAVPPFLAFLIAKEIEKAIGNTGYYIDLFSGAGGLGLGFKWAGWTPLLANDIEEKYLQ
TYSNNVHKEVLCGSISDNETFSKIADKISGFKKLYFDKQLWILGGPPCQGFSTAGNART
MDDPRNSLFMHYKSLLNEIKPNGFIFENVAGLLNMEKGKVFERVKEEFSSTMKT MNGWI
LNSEHYAIPQRRKR VILVGSNDPLFSIEPPQKLTEDKESWVSVKDALS DLPPLQHGEDG
SGKYYIHHPENDYQLFMRGNITPSEYYERNIKPSLKLAAALEHHHHHH

M2.BsaI (POC1467-KPL05), N-terminal His6-tag

MGSSHHHHHHSSGLVPRGSHMI PNHVSSKLSELDMLI IKHVPPGGNWKDIPEWVPSKRL
EQIRKSYAEGKGS RSTYYGRLLPDMPSTINTYFNRPGNGCHIHYEQDRTLSQREAA RL
QSFPDDFI FYGSKTAINNQIGNAVPPLLAYQIAKAFPFGQFVDLFSGAGGLSLGFLWA
GWKPIIANDIDKWALTTYMNNIHNEVVLGDIRDEKVSETIIQKCLIAKKS NPDRPLFVL

GGPPCQGFSTAGKKRSIVDERNWLFESEYVSILKEVKPDGFIFENVGTGLLSMEKGAFEM
VKSELSKTVSNLFVYKLNSVDYGVPPQRRNRVVIIGDSTGTKNSEPPPIPTSLKGKTLF
DALSSAISVKEALSDLPLLSPNEDGSWKNYVCEPQNIYQSFMRKKITAQQYIEMLSSLA
II

M2.BsaI (POC1468-KPL06), C-terminal His6-tag

MIPNHVSSKLSELDMLIIKHVPPGGNWKDIPWVPSKRLEQIRKSYAEGKGSRSSTYYGR
LLPDMPSYTINTYFNRPGNGCHIHYEQDRTLSQREAAARLQSFDDFIYFGSKTAINNQI
GNAVPPLLAYQIAKAFPFKGQFVDLFSGAGGLSLGFLWAGWKPIIANDIDKWALTTYMN
NIHNEVVLGDIRDEKVSSETIIQKCLIAKKSNDPDRPLFVLGGPPCQGFSTAGKKRSIVDE
RNWLFESEYVSILKEVKPDGFIFENVGTGLLSMEKGAFEMVKSELSKTVSNLFVYKLNSV
DYGVPPQRRNRVVIIGDSTGTKNSEPPPIPTSLKGKTLFDALSSAISVKEALSDLPLLS
PNEDGSWKNYVCEPQNIYQSFMRKKITAQQYIEMLSSLAIIKLAAALEHHHHHH

M2.BsaI_2 (POC1469-KPL07), truncated M2.BsaI, N-terminal His6-tag

MGSSHHHHHHSSGLVPRGSHMLIIKHVPPGGNWKDIPWVPSKRLEQIRKSYAEGKGS
STYYGRLLPDMPSYTINTYFNRPGNGCHIHYEQDRTLSQREAAARLQSFDDFIYFGSKT
AINNQIGNAVPPLLAYQIAKAFPFKGQFVDLFSGAGGLSLGFLWAGWKPIIANDIDKWA
LTTYMNNIHNEVVLGDIRDEKVSSETIIQKCLIAKKSNDPDRPLFVLGGPPCQGFSTAGKK
RSIVDERNWLFESEYVSILKEVKPDGFIFENVGTGLLSMEKGAFEMVKSELSKTVSNLFV
YKLNSVDYGVPPQRRNRVVIIGDSTGTKNSEPPPIPTSLKGKTLFDALSSAISVKEALS
DLPLLSPNEDGSWKNYVCEPQNIYQSFMRKKITAQQYIEMLSSLAII

M2.BsaI_2 (POC1470-KPL03), truncated M2.BsaI, C-terminal His6-tag

MLIIKHVPPGGNWKDIPWVPSKRLEQIRKSYAEGKGSRSSTYYGRLLPDMPSYTINTYF
NRPGNGCHIHYEQDRTLSQREAAARLQSFDDFIYFGSKTAINNQIGNAVPPLLAYQIAK
AFPFKGQFVDLFSGAGGLSLGFLWAGWKPIIANDIDKWALTTYMNNIHNEVVLGDIRDE
KVSSETIIQKCLIAKKSNDPDRPLFVLGGPPCQGFSTAGKKRSIVDERNWLFESEYVSILKE
VKPDGFIFENVGTGLLSMEKGAFEMVKSELSKTVSNLFVYKLNSVDYGVPPQRRNRVVI
IGDSTGTKNSEPPPIPTSLKGKTLFDALSSAISVKEALSDLPLLSPNEDGSWKNYVCEP
QNIYQSFMRKKITAQQYIEMLSSLAIIKLAAALEHHHHHH

M.Osp807II (POC1471-KPL09), N-terminal His6-tag

MGSSHHHHHHSSGLVPRGSHMAKKDTNLKLSHLYKGNVSEVYGRWPSPDLIVSDGAYGV
RGFRGDTVDAAGLTDWYKPHVLAWAKAAKPSTSLWFWNTEVGWATVHPLLLSTGWEYVQ
LAVWDKGLAHIAGNVNGKTIRQLPVVTEVAALYRRTVYLETGDGLTLNAKSWLRAEWR
SGLSLSKSNEACGVKNAATRKYLTADWLWYWP PGDAVQKMAEYCMQYGGKTSWPYFSLD
GKTMISAHDWDSLRTTNHRNGVTNVWSRPLADSERLKGTMERSAPRTYKPTKQSAAH
LNQKPLDLMLTQVAAASNVDGTVWEPFGGLCSASVASSLLGRRSYAAEIDDTFYKLAAA
RLNEANAYFESNGVYEFKEGE

M.Osp807II (POC1472-KPL10), C-terminal His6-tag

MAKKDTNLKLSHLYKGNVSEVYGRWPSPDLIVSDGAYGV RGFRGDTVDAAGLTDWYKPH
VLAWAKAAKPSTSLWFWNTEVGWATVHPLLLSTGWEYVQLAVWDKGLAHIAGNVNGKTI

RQLPVVTEVAALYRRTVYLETGDGLTLNAKSWLRAEWRRSGLSLSKSNEACGVKNAATR
KYLTADWLWYWP PGDAVQKMAEYCMQYGKKT SWPYFSLDGKTMISAHDWDSLRTTWNHR
NGVTNVWSRPPLADSERLKGT MERSAPRTYKPTKQSA AHLNQKPLDLMLTQVAAASNVG
DTVWEPFGGLCSASVASSLLGRRSYAAEIDDTFYKLAAARLNEANAYFESNGVYEFKEG
ELEHHHHHH

M2.NmeMC58II (POC1473-KPL11), N-terminal His6-tag

MGSSHHHHHHSSGLVPRGSHMITISNEDNMILMSRYPD KYFDLAIVDPPYGILNKTKRG
GDYKFN MNEYSQWDIKPDQTYFNELFRVSKNQI IWGGNYFGELWLRSEYNKGFI IWDKN
QPETLNNFSMAEMAWSSFD RPSKIFRFSVRKNRNKTHPTQKPVELYQWLLKMYAKQGDK
ILDTHLGSGTLAIACCIAQFDLTACEINSDYYQQSIEKIKNNLPEARISFGHPGYCII E

M2.NmeMC58II (POC1474-KPL12), C-terminal His6-tag

MITISNEDNMILMSRYPD KYFDLAIVDPPYGILNKTKRGGDYKFN MNEYSQWDIKPDQ
TYFNELFRVSKNQI IWGGNYFGELWLRSEYNKGFI IWDKNQPETLNNFSMAEMAWSSFD
RPSKIFRFSVRKNRNKTHPTQKPVELYQWLLKMYAKQGDKILDTHLGSGTLAIACCIAQF
DLTACEINSDYYQQSIEKIKNNLPEARISFGHPGYCII EKLA AALEHHHHHH

M.Sen0738I (POC1475-KPL13), N-terminal His6-tag

MGSSHHHHHHSSGLVPRGSHMSISSAIKSLQDIMRKDAGVDGDAQRLGQLSWLLFLKIF
DAQEQALEIEQEKYRLPMPERYLWRNWAADNEGITGDKLLAFVNDDLFP TLKDLPAQID
INPRGYVVKQAFSDAYNYMKN GTLLRQVINKLNEIDFTRASERHLFGDIYEQILRDLQA
AGNAGEFYTPRAVTRFMVERVDPKLGESIMDPACGTGGFLACAFDHVKNHYAHTVTDHQ
ILQKQIHGVEKKQLPHLLCTTNMLLHGIEVPVQIRHDNTLNKPLSSWDEQMDV IITNPP
FGGTEEDGIEKNFPSDMQTR ETADLFLQLIEVLAKNGRAAVVLPDGT LFGEGVKT KIK
KLLTEECNLHTIVRLPNGVFNPYTGIKTNLLFFTKGQPTKEIWFYEHYPYPAGVKNYSKT
KPMKFEEFQAEIDWWGNEADGFASRVENEQAWKVSIDEVIARNFNLDIKNPHQAETVSH
DPDELLAQYAKQQEAIQT LRHQLRDILGTALSGKEAN

M.Sen0738I (POC1476-KPL14), C-terminal His6-tag

MSISSAIKSLQDIMRKDAGVDGDAQRLGQLSWLLFLKIFDAQEQALEIEQEKYRLPMPE
RYLWRNWAADNEGITGDKLLAFVNDDLFP TLKDLPAQIDINPRGYVVKQAFSDAYNYM
KN GTLLRQVINKLNEIDFTRASERHLFGDIYEQILRDLQAAGNAGEFYTPRAVTRFMVER
VDPKLGESIMDPACGTGGFLACAFDHVKNHYAHTVTDHQILQKQIHGVEKKQLPHLLCT
TNMLLHGIEVPVQIRHDNTLNKPLSSWDEQMDV IITNPPFGGTEEDGIEKNFPSDMQTR
ETADLFLQLIEVLAKNGRAAVVLPDGT LFGEGVKT KIKKLLTEECNLHTIVRLPNGVF
NPYTGIKTNLLFFTKGQPTKEIWFYEHYPYPAGVKNYSKTKPMKFEEFQAEIDWWGNEAD
GFASRVENEQAWKVSIDEVIARNFNLDIKNPHQAETVSHDPDELLAQYAKQQEAIQT LR
HQLRDILGTALSGKEANLEHHHHHH

S.Sen0738I (POC1477-KPL15), N-terminal His6-tag

MGSSHHHHHHSSGLVPRGSHMAVEKLIVDHIDTWT TALQTRSTAGRGS SGKIDLYGIKK
LRELILELAVRGKLPQDPNDKPASVLLER IATEKAELVKQGKIKKQKPLPEISEEEKP
FELPAGWEWARLNELAPMGIIDGDWIESKDQDPSGAYRLIQLADVGVGDFDKDS DRYIN

TSTFHRLNCHQLMEGDILIRLPNPIGRACIFPKLSQSAITVVDIATMRPSGNYSAEYI
ISAINSLTFRQQVESFGKGATRFRIATGHLKTLTLLPIPPVQEQYSIFKKIKELMSLCDQ
LEQYSLTSLDAHQQQLVETLLTTLTDSQNADELAENWARISEHFDTLFTTEVSIDALKQT
ILQLAVMGKLVLPQDPNDEPASELLKRIAQEKAQLVKDGKMKKQKPLPPISDEEEKPFELP
SGWEWCLFEDVVDIQSGITKGRNLANRKLISIPYLRVANVQRGYLDLSEVKEIDIPEEE
KDKYHVIKGDLLITEGGDWDTVGRTTVWCHDWYIANQNHVFKGRIIGQDIDPYWLETYM
NSPYARDYFASASKQTTNLASINKTQLRGCPVAIPPSSEA EKIMLKLNDNFNELCEKLKL
QIQSAQQQTQLHLADALTDAAIN

S.Sen0738I (POC1478-KPL16), C-terminal His6-tag

MAVEKLIVDHIDTWTALQTRSTAGRGSSGKIDLYGIKKLRELILELAVRGKLVLPQDPN
DKPASVLLERATEKAELVKQGKIKKQKPLPEISEEEKPFELPAGWEWARLNELAPMGI
IDGDWIESKDQDPGAYRLIQLADVGVGDFKDKSDRYINTSTFHRLNCHQLMEGDILIA
RLPNPIGRACIFPKLSQSAITVVDIATMRPSGNYSAEYIISAINSLTFRQQVESFGKGA
TRFRIATGHLKTLTLLPIPPVQEQYSIFKKIKELMSLCDQLEQYSLTSLDAHQQQLVETLL
TTLTDSQNADELAENWARISEHFDTLFTTEVSIDALKQTILQLAVMGKLVLPQDPNDEPA
SELLKRIAQEKAQLVKDGKMKKQKPLPPISDEEEKPFELPSGWEWCLFEDVVDIQSGITK
GRNLANRKLISIPYLRVANVQRGYLDLSEVKEIDIPEEEKDKYHVIKGDLLITEGGDWD
TVGRTTVWCHDWYIANQNHVFKGRIIGQDIDPYWLETYMNSPYARDYFASASKQTTNLA
SINKTQLRGCPVAIPPSSEA EKIMLKLNDNFNELCEKLKLQIQSAQQQTQLHLADALTDAA
INLEHHHHHH

M1.Eco31I (POC1479-KPL17), N-terminal His6-tag

MGSSHHHHHHSSGLVPRGSHMASMEEIFYMKHIHLINSLSLDETTFKFTKKATGKYITDP
KIALLMIEKLLPLINSCDKKSYNVADPFSGDGRITLLIKQWMINGFPDVEWNVYLFDI
ENTGLTYAKNALSELKLAGANINITIKNSDVFEFKKYVDYFDCVITNPPWENIKPDSR
ELDFFEPSMKSMYIDSLREFDDYLSRVLPYSQPKRKFAWGNTLSRVGAELSLEICNKN
GLVAIVMPASFFADEQSYILREKFFNSGRIDCINYYPAEAKLFGGADVSSCSFIFNKGE
SLNDNIQLSVYDKNLNIKSLGFFDLSSIDSQYLSIPVSQGVHAVHLLRKLQEGYPTWGS
LEKNGEIWAGREIDETGSSDWTQKSGGGLLFIKGKMIGRYNFHNEKSLRITKKIDKVL
SNSNFVRIAWRDISRPSQKRMIATIIPPNSLAGNSLGVVYYKSGSQDSLFSLLGIINSL
CFEFQLRSFLATGHVSLSALRKTAIPSEKILLQHSELKQLVISCIEGCCDAELKIEAYV
AKNIYKLDLNEFNKLLSSFDKIELAEKESLLRIFQHYD

M1.Eco31I (POC1480-KPL18), C-terminal His6-tag

MEEIFYMKHIHLINSLSLDETTFKFTKKATGKYITDPKIALLMIEKLLPLINSCDKKSYN
VADPFSGDGRITLLIKQWMINGFPDVEWNVYLFDIENTGLTYAKNALSELKLAGANIN
ITIKNSDVFEFKKYVDYFDCVITNPPWENIKPDSREL DFFEPSMKSMYIDSLREFDDY
LSRVLPYSQPKRKFAWGNTLSRVGAELSLEICNKNGLVAIVMPASFFADEQSYILREK
FFNSGRIDCINYYPAEAKLFGGADVSSCSFIFNKGESLNDNIQLSVYDKNLNIKSLGFF
DLSSIDSQYLSIPVSQGVHAVHLLRKLQEGYPTWGSLEKNGEIWAGREIDETGSSDWTQ
KSGGGLLFIKGKMIGRYNFHNEKSLRITKKIDKVLSNSNFVRIAWRDISRPSQKRMIAT
IIPPNSLAGNSLGVVYYKSGSQDSLFSLLGIINSLCFEFQLRSFLATGHVSLSALRK

AIPSEKILLQHSELKQLVISCIEGCCDAELKIEAYVAKNIYKLDLNEFNKLLSSFDKIE
LAEKESLLRIFQHYDKLAAALEHHHHHH

M.*Xmn*I (POC1481-KPL19), N-terminal His6-tag

MGSSHHHHHHSSGLVPRGSHMASMRDLASTYRRAHNTMRNLDGLQPQEAFEELLKFLFL
KQMSEEDGIAPTTGSAIRKRFAFHLSRHSSWSTSLWRDRDFHLSDQCLEQLNALFSGIN
FTQIDYDIRSAALREFLTPEVRKGLGIFLTPDEVVREVVSFVDPSSAKCLDPACGSGT
FLIEVIKKWRKENAQKISVWGADKNPRMLLIGELNLGHFPGLTFNRALMDSLVEPGKRH
SKPWCRYGYFDFILTNPPFGVTVEASGAAYSGYDIAFTANGEPRARQSSEWLFVEQSLR
WLKPGGTLAVVLPRSVLTNPSSAYERSLLAKLGYLKAVIQLPPETFLVTGAQTNTVVAF
IEKYASDKDREKKVGVVQATLSNVGYDSTGRPRQGSQLDGLAKHMRDPKGSPCDYVSVL
APQKADRTFEALSAKSASKRRSNLGVPLGLDLASEIRTGRTPPRAAYSENGGLFLIKVGN
LTGSGINWIARDNFI DTSTMTSRKLGGIQLVKNGDIVLTSSAHSPVYIAKKVDIITKV
PEWVGGRASFVGEVMLVRPKQERIAPMLLLAYLRQPTVASQIQEMVRGQTAHLHADDLA
ALMVPTSVLDASDDWREVQELLVEEVALNDRLEIARLQQSIANKLSDGLRAG

M.*Xmn*I (POC1482-KPL20), C-terminal His6-tag

MRDLASTYRRAHNTMRNLDGLQPQEAFEELLKFLFLKQMSEEDGIAPTTGSAIRKRFAF
HLSRHSSWSTSLWRDRDFHLSDQCLEQLNALFSGINFTQIDYDIRSAALREFLTPEVRK
GLGIFLTPDEVVREVVSFVDPSSAKCLDPACGSGTFLIEVIKKWRKENAQKISVWGAD
KNPRMLLIGELNLGHFPGLTFNRALMDSLVEPGKRH SKPWCRYGYFDFILTNPPFGVT
EASGAAYSGYDIAFTANGEPRARQSSEWLFVEQSLRWLKPGGTLAVVLPRSVLTNPSSA
YERSLLAKLGYLKAVIQLPPETFLVTGAQTNTVVAFIEKYASDKDREKKVGVVQATLSN
VGYDSTGRPRQGSQLDGLAKHMRDPKGSPCDYVSVLAPQKADRTFEALSAKSASKRRSN
LGVPLGLDLASEIRTGRTPPRAAYSENGGLFLIKVGNLTGSGINWIARDNFI DTSTMTS
RKLGGIQLVKNGDIVLTSSAHSPVYIAKKVDIITKVPEWVGGRASFVGEVMLVRPKQER
IAPMLLLLAYLRQPTVASQIQEMVRGQTAHLHADDLAALMVPTSVLDASDDWREVQELLV
EEVALNDRLEIARLQQSIANKLSDGLRAGKLAAALEHHHHHH

M1.*Hpy*AI (POC1483-KPL21), N-terminal His6-tag

MGSSHHHHHHSSGLVPRGSHMILNKIYIEDVFTFLDKLEDKSVDLAIIDPPYNLKIASH
DSFKNDEEFLTF SYAWIDKM LPKLKDTGSFYIFNTPFNCALFLAYLHHKKVHFLNFITW
VKKDG FANAKKRYNHAQESILFY SMHKKNYTFNADEIRIAYESAERIKHAQSKGILKNN
KRWFPNPKGKLCLDVWEITSQRHVEKEKGKILKPKHPSIKPKALIERMIKASSHKNDLI
LDLFSGSGMTSLVAKSLERNFIGCESHA EYVHGSLEMF RYNECE

M1.*Hpy*AI (POC1484-KPL22), C-terminal His6-tag

MILNKIYIEDVFTFLDKLEDKSVDLAIIDPPYNLKIASHWDSFKNDEEFLTF SYAWIDKM
LPKLKDTGSFYIFNTPFNCALFLAYLHHKKVHFLNFITWVKKDG FANAKKRYNHAQESI
LFY SMHKKNYTFNADEIRIAYESAERIKHAQSKGILKNNKRWFPNPKGKLCLDVWEITS
QRHVEKEKGKILKPKHPSIKPKALIERMIKASSHKNDLILDLFSGSGMTSLVAKSLERN
FIGCESHA EYVHGSLEMF RYNECEAAALEHHHHHH

M2.*Hpy*AI (POC1485-KPL23), N-terminal His6-tag

MGSSHHHHHHSSGLVPRGSHMNINKVIFYHSSSTNMNEVPDNSVDLIITSPPYFNIKDYAK
NGTQDLQHS AQHVEDLGALEKYEDYLLGLLK VWLECYRALKPNGKLCINVPLMPMLKKV
LNTHYNRHIFDLHADIQHSILHDLNNMLKNKPKMFLLDVYIWKRANPTKRLMFGSYPPY
RNFYAQNTIEFIGVFVKDGKPKQPTTEEQKEQS QLTQE EWVEFTKQIWEIPIPNKNDIAF
GKHAALMPAELARRLIRLYSCVGDVVLDPFSGSGTTLREAKLLKRNFIFYELYENYKPL
IEQKLGNLFD FE

M2.*Hpy*AI (POC1486-KPL24), C-terminal His6-tag

MNINKVIFYHSSSTNMNEVPDNSVDLIITSPPYFNIKDYAKNGTQDLQHS AQHVEDLGALE
KYEDYLLGLLK VWLECYRALKPNGKLCINVPLMPMLKKV LNTHYNRHIFDLHADIQHSI
LHDLNNMLKNKPKMFLLDVYIWKRANPTKRLMFGSYPPYPRNFYAQNTIEFIGVFVKDGK
PKQPTTEEQKEQS QLTQE EWVEFTKQIWEIPIPNKNDIAFGKHAALMPAELARRLIRLYS
CVGDVVLDPFSGSGTTLREAKLLKRNFIFYELYENYKPLIEQKLGNLFD FEAAALEHHH
HHH

M1.*Mbo*II (POC1487-KPL25), N-terminal His6-tag

MGSSHHHHHHSSGLVPRGSHMVENMLEINKIHQMNCDFDLQVENKSVQLAVIDPPYNL
SKADWDSFDSHNEFLAFTYRWIDKVLDKLDKDGSLYIFNTPFNCAFICQYLVS KGMI FQ
NWITWDKRDGMGSAKRRFSTGQETILFFSKSKNHTFNYDEV RVPYESTDRIKHASEKGI
LKNGKRWFPNPNGRLCGEVWHFSSQRHKEKVNGKTVKLTHITPKPRDLIERIIRASSNP
NDLVLD CFMGS GTTAIVAKKLGRNF IGCDMNAEYVNQANFVLNQLEIN

M1.*Mbo*II (POC1488-KPL26), C-terminal His6-tag

MVENMLEINKIHQMNCDFDLQVENKSVQLAVIDPPYNLSKADWDSFDSHNEFLAFTYR
WIDKVLDKLDKDGSLYIFNTPFNCAFICQYLVS KGMI FQNWITWDKRDGMGSAKRRFST
GQETILFFSKSKNHTFNYDEV RVPYESTDRIKHASEKGI LKNGKRWFPNPNGRLCGEVW
HFSSQRHKEKVNGKTVKLTHITPKPRDLIERIIRASSNPNDLVLD CFMGS GTTAIVAKK
LGRNF IGCDMNAEYVNQANFVLNQLEINKLAAALEHHHHHH

a**Non-switchable methylase: M2.Eco31I****BsaI recognition sequence**

5'... GGTCTC(N)₁▼ ... 3'
 3'... CCAGAG(N)₅▲ ... 5'

BsaI-associated methylase recognition sequence

5'... GGTCTC ... 3'
 3'... CCAGAG ... 5'

Methylated product

5'... GGTCTC(N)₁✗ ... 3'
 3'... CCAGAG(N)₅✗ ... 5'

b**Switch methylase: M.Osp807II****BsaI recognition sequence**

5'... GGTCTC(N)₁▼ ... 3'
 3'... CCAGAG(N)₅▲ ... 5'

BsaI-associated methylase recognition sequence

5'... GACNNNGTC ... 3'
 3'... CTGNNNCAG ... 5'

Methylated product

5'... GACNNGGTCTC(N)₁✗ ... 3'
 3'... CTGNNCCAGAG(N)₅✗ ... 5'

Methylation-switchable restriction site of M.Osp807II

5'... GACTTGGTCTC(N)₁✗ ... 3'
 3'... CTGAACCAGAG(N)₅✗ ... 5'

Always-cutable restriction site of M.Osp807II

5'... CTTCAGGTCTC(N)₁▼ ... 3'
 3'... GAAGTCCAGAG(N)₅▲ ... 5'

Fig. S1 (a) Representation of a non-switchable methylase, and **(b)** a switch methylase with their methylation-switchable and always-cutable restriction sites. The type IIS recognition sequence is highlighted in light blue, the type IIS restriction site is marked with ▼▲, the methylase recognition sequence is in red, the methylated base is in bold and underlined, altered bases to prevent methylase activity are in pink and the blocked restriction site is marked with a red x

PCR amplification of methylases

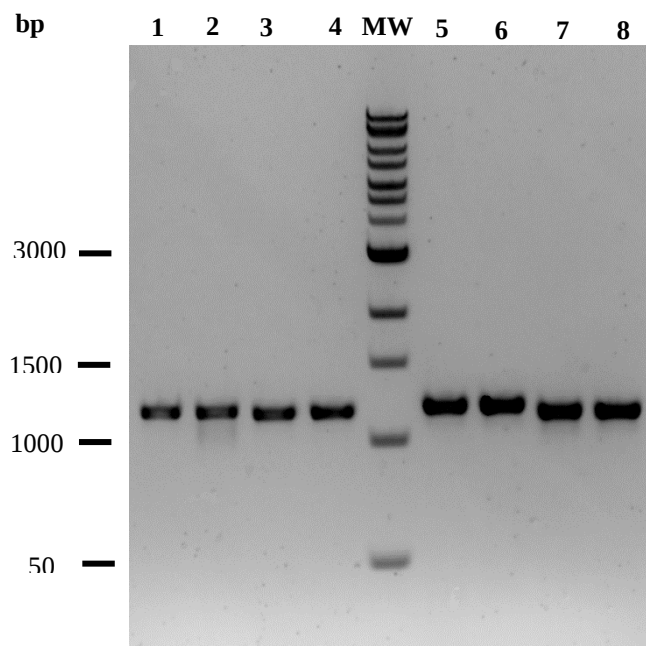


Fig. S2 Agarose gel electrophoresis (1%) showing the PCR products of M2.*Eco31I* and M2.*BsaI* and their truncated versions. Lanes: 1, KPL01; 2, KLP02; 3, KPL04; 4, KPL04; 5, KPL05; 6, KLP06; 7, KPL07; and 8, KPL08. MW, molecular weight marker (Quick-Load® 1 kb Extend DNA Ladder, New England Biolabs, N3239S, 0.5 to 48.5 kb)

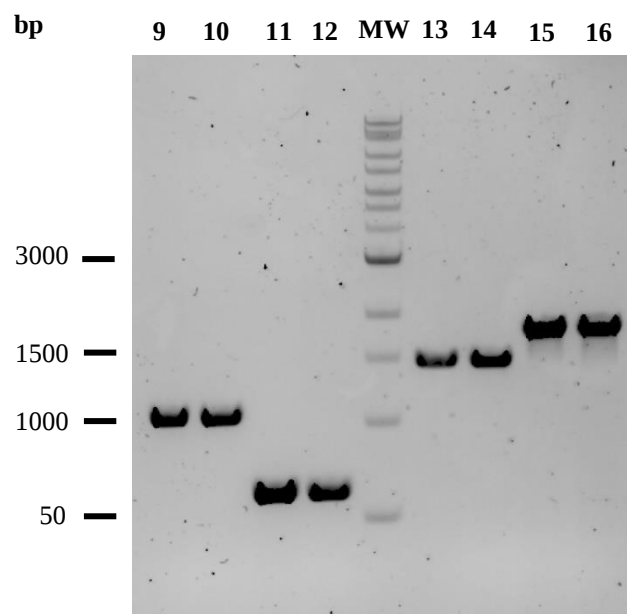


Fig. S3 Agarose gel electrophoresis (1%) showing the PCR products of M.*Osp807II*, M2.*NmeMC58II*, M.*Sen0738I*, and S.*Sen0738I*. Lanes: 9, KPL09; 10, KLP10; 11, KPL11; 12, KPL12; 13, KPL13; 14, KLP14; 15, KPL15; and 16, KPL16. MW, molecular weight marker (Quick-Load® 1 kb Extend DNA Ladder, New England Biolabs, N3239S, 0.5 to 48.5 kb)

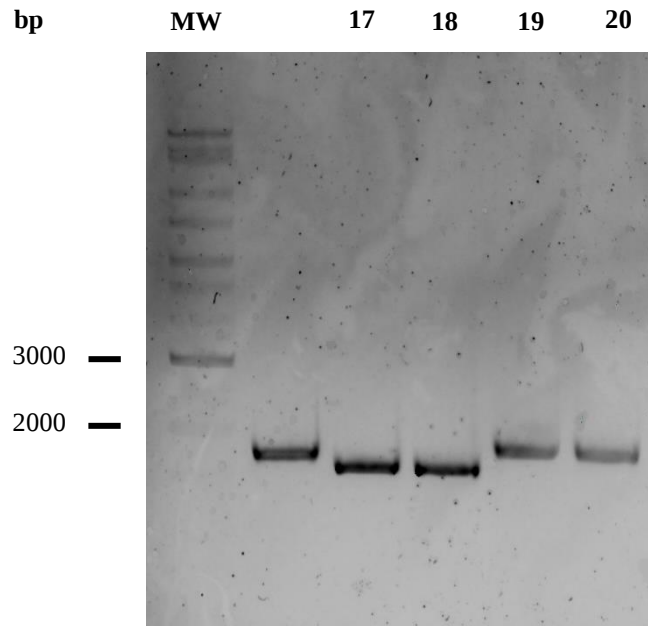


Fig. S4 Agarose gel electrophoresis (1%) showing the PCR products of M1.*Eco31I* and M.*XmnI*. Lanes: 17, KPL17; 18, KLP18; 19, KPL19; and 20, KPL20. MW, molecular weight marker (Quick-Load® 1 kb Extend DNA Ladder, New England Biolabs, N3239S, 0.5 to 48.5 kb)

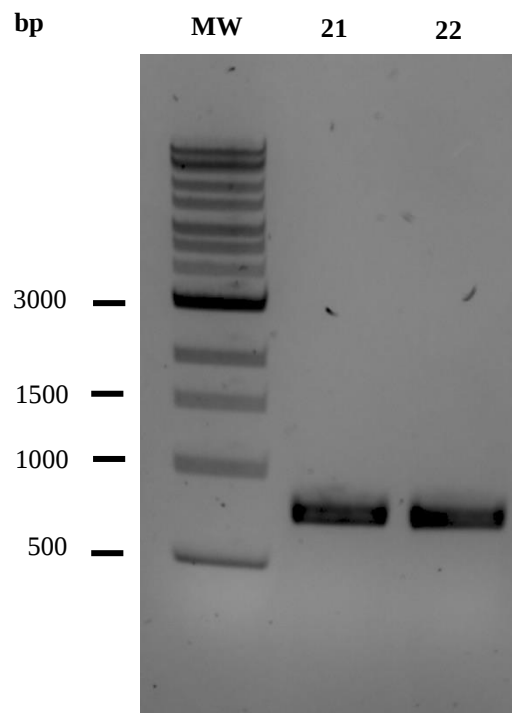


Fig. S5 Agarose gel electrophoresis (1%) showing the PCR products of M1.*HpyAII*. Lanes: 21, KPL21; and 22, KLP22. MW, molecular weight marker (Quick-Load® 1 kb Extend DNA Ladder, New England Biolabs, N3239S, 0.5 to 48.5 kb)

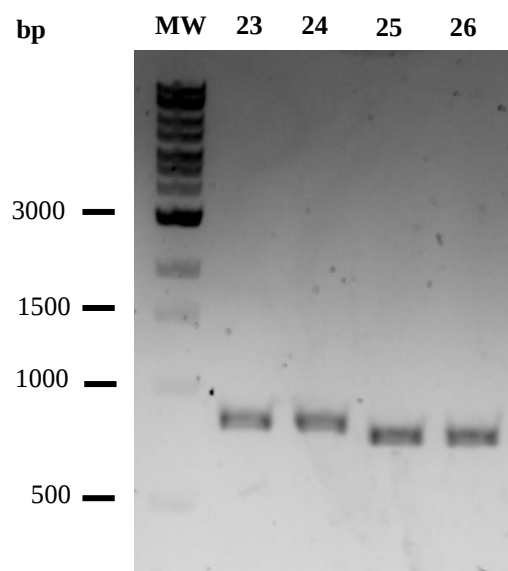


Fig. S6 Agarose gel electrophoresis (1%) showing the PCR products of M2.*Hpy*AI and M1.*Mbo*II. Lanes: 23, KPL23; 24, KLP24; 25, KPL25; and 26, KPL26. MW, molecular weight marker (Quick-Load® 1 kb Extend DNA Ladder, New England Biolabs, N3239S, 0.5 to 48.5 kb)

Protein expression of methylases

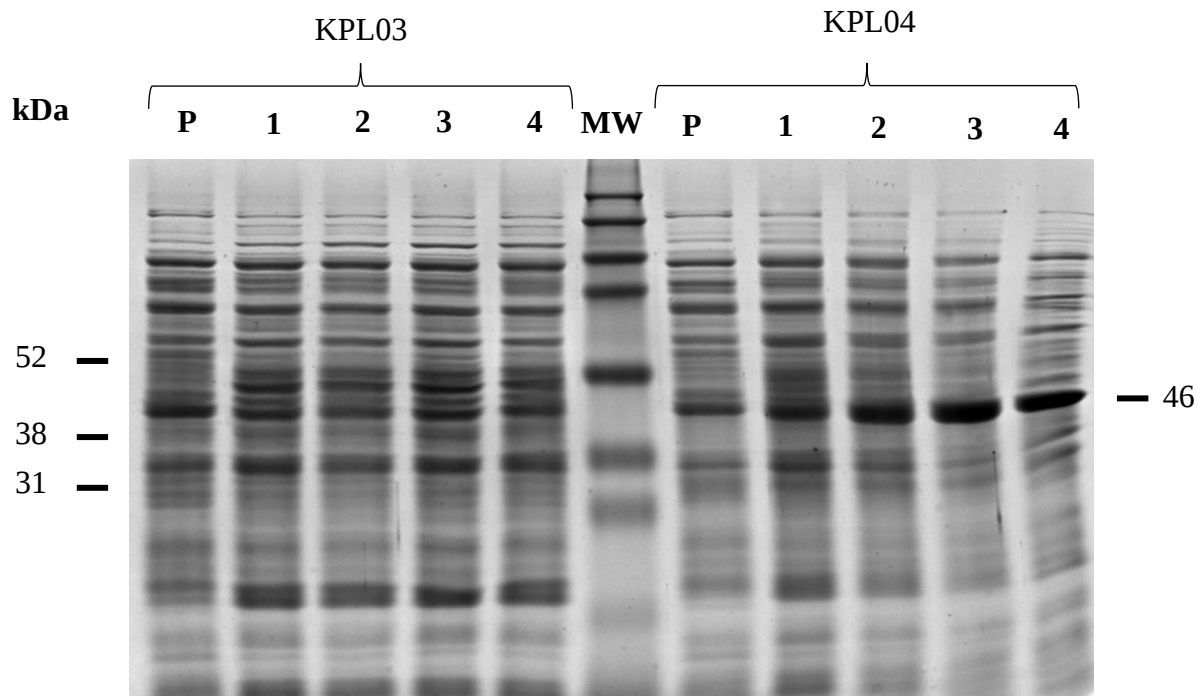


Fig. S7 SDS-PAGE showing the expression of M2.*Eco*31I_2 (KPL03 and KPL04) at different IPTG concentrations. Lanes: P, pre-induction; 1, 0.005 mM; 2, 0.05 mM; 3, 0.5 mM; and 4, 1 mM. MW, molecular weight marker (Amersham™ ECL™ Rainbow™ Marker - Full range, Full Range, Cytiva RPN800E, 12 to 225 kDa)

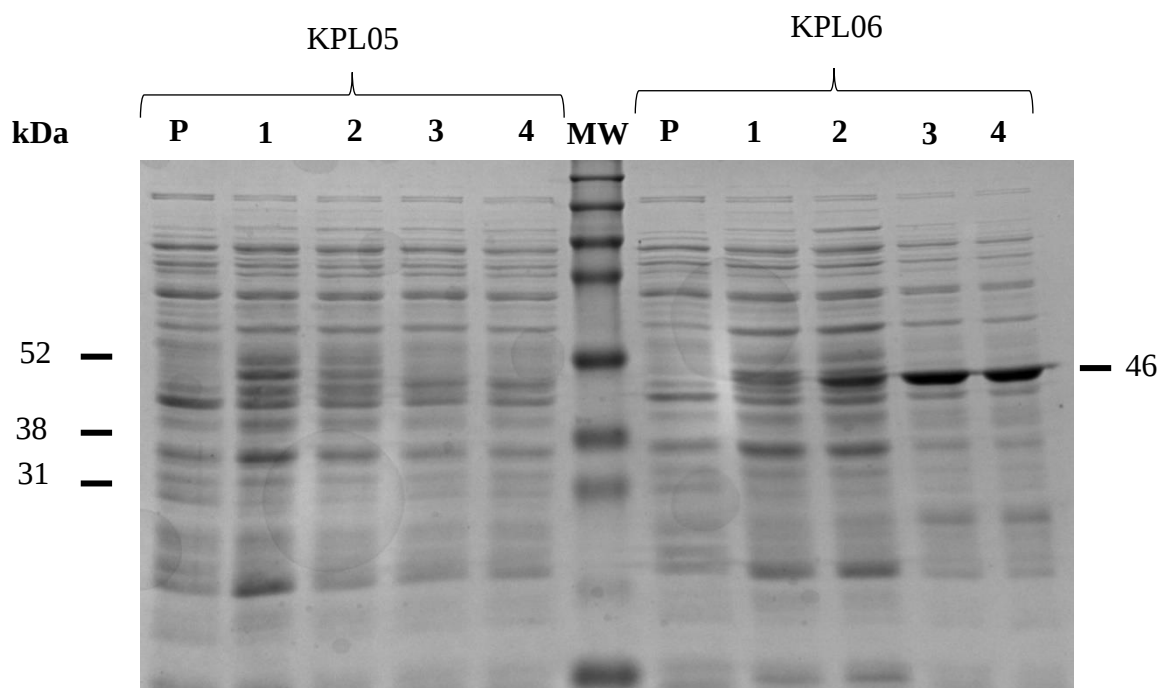


Fig. S8 SDS-PAGE showing the expression of M2.BsaI (KPL05 and KPL06) at different IPTG concentrations. Lanes: P, pre-induction; 1, 0.005 mM; 2, 0.05 mM; 3, 0.5 mM; and 4, 1 mM. MW, molecular weight marker (Amersham™ ECL™ Rainbow™ Marker - Full range, Full Range, Cytiva RPN800E, 12 to 225 kDa)

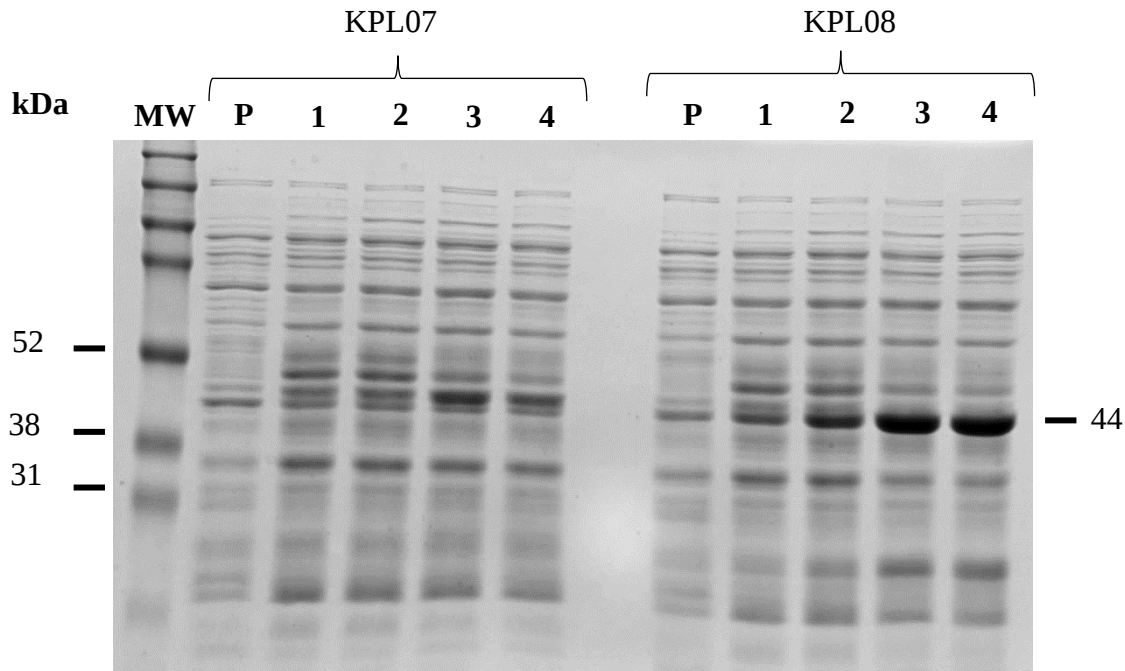


Fig. S9 SDS-PAGE showing the expression of M2.BsaI_2 (KPL07 and KPL08) at different IPTG concentrations. Lanes: P, pre-induction; 1, 0.005 mM; 2, 0.05 mM; 3, 0.5 mM; and 4, 1 mM. MW, molecular weight marker (Amersham™ ECL™ Rainbow™ Marker - Full range, Full Range, Cytiva RPN800E, 12 to 225 kDa)

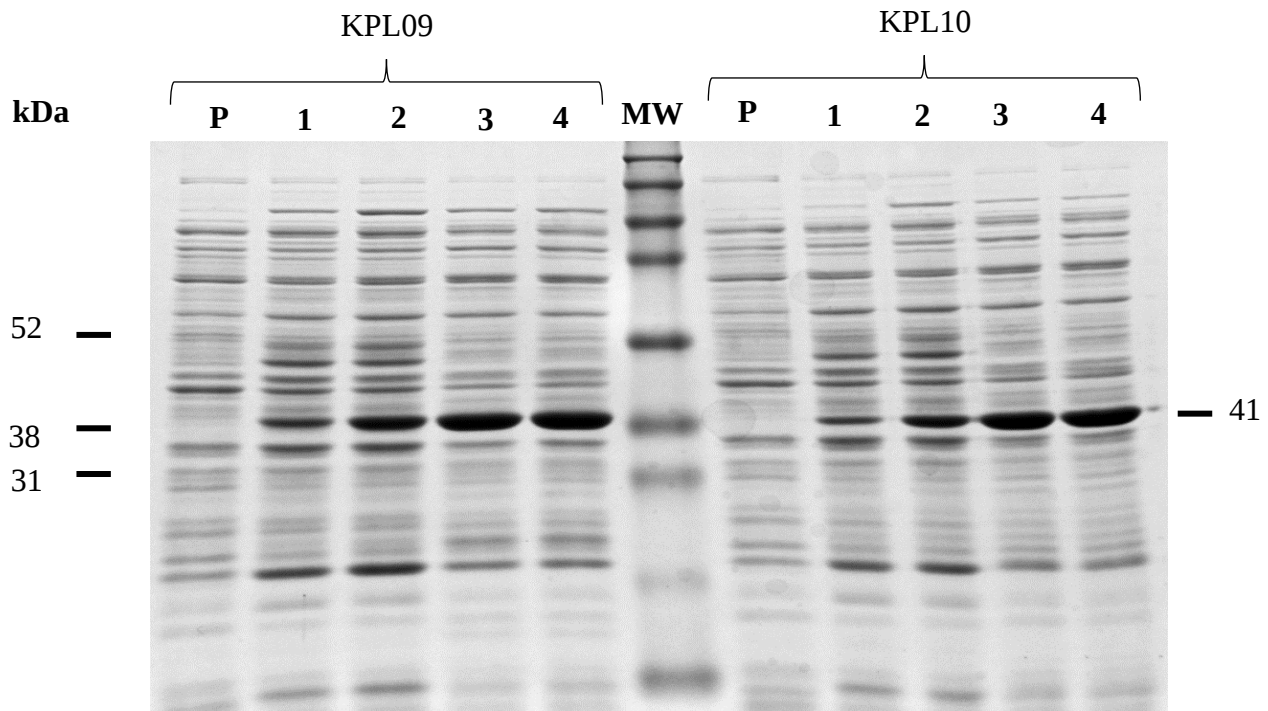


Fig. S10 SDS-PAGE showing the expression of *M.Osp807II* (KPL09 and KPL10) at different IPTG concentrations. Lanes: P, pre-induction; 1, 0.005 mM; 2, 0.05 mM; 3, 0.5 mM; and 4, 1 mM. MW, molecular weight marker (Amersham™ ECL™ Rainbow™ Marker - Full range, Full Range, Cytiva RPN800E, 12 to 225 kDa)

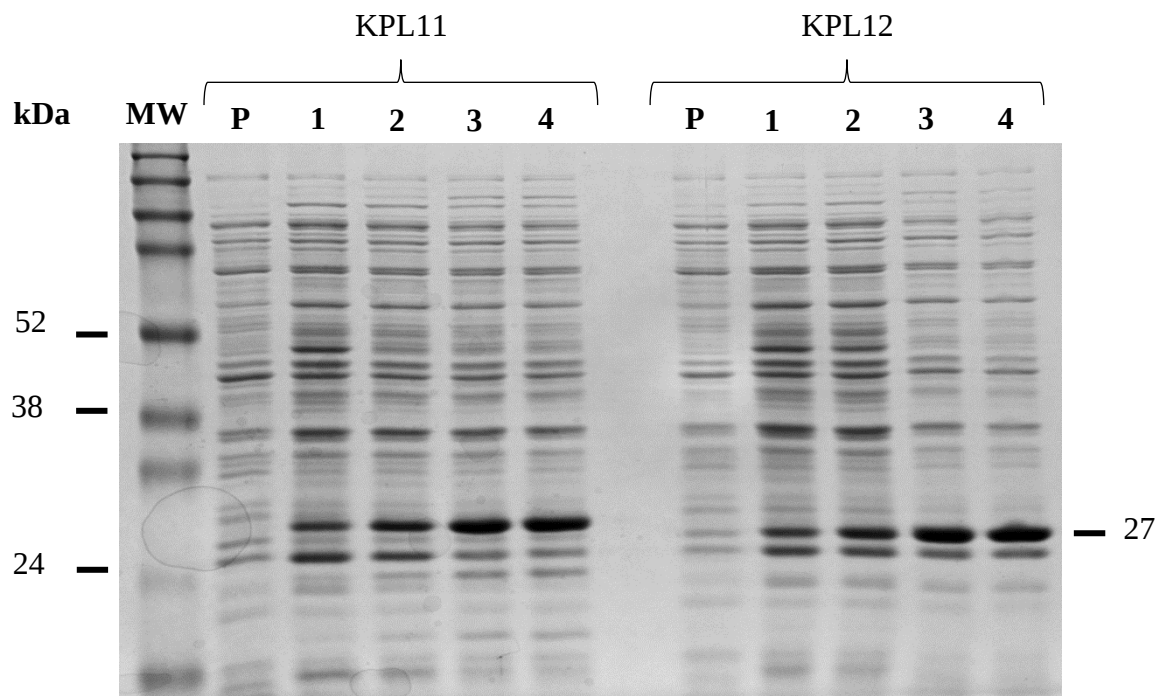


Fig. S11 SDS-PAGE showing the expression of *M2.NmeMC58II* (KPL11 and KPL12) at different IPTG concentrations. Lanes: P, pre-induction; 1, 0.005 mM; 2, 0.05 mM; 3, 0.5 mM; and 4, 1 mM. MW, molecular weight marker (Amersham™ ECL™ Rainbow™ Marker - Full range, Full Range, Cytiva RPN800E, 12 to 225 kDa)

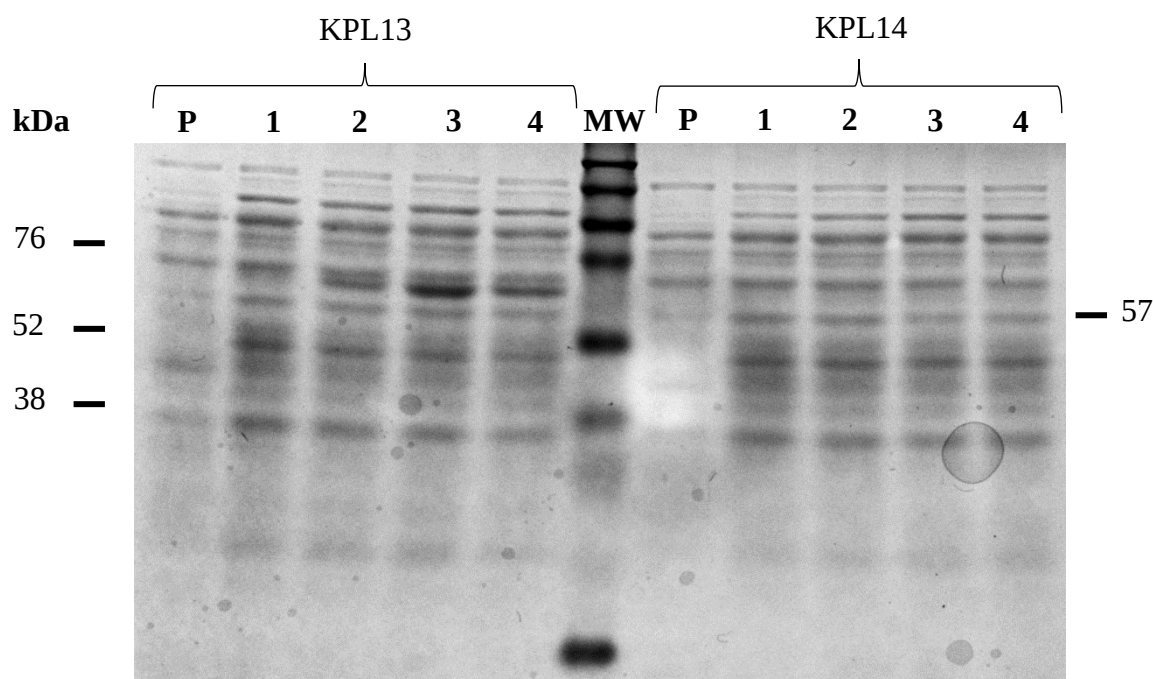


Fig. S12 SDS-PAGE showing the expression of *M.Sen0738I* (KPL13 and KPL14) at different IPTG concentrations. Lanes: P, pre-induction; 1, 0.005 mM; 2, 0.05 mM; 3, 0.5 mM; and 4, 1 mM. MW, molecular weight marker (Amersham™ ECL™ Rainbow™ Marker - Full range, Full Range, Cytiva RPN800E, 12 to 225 kDa)

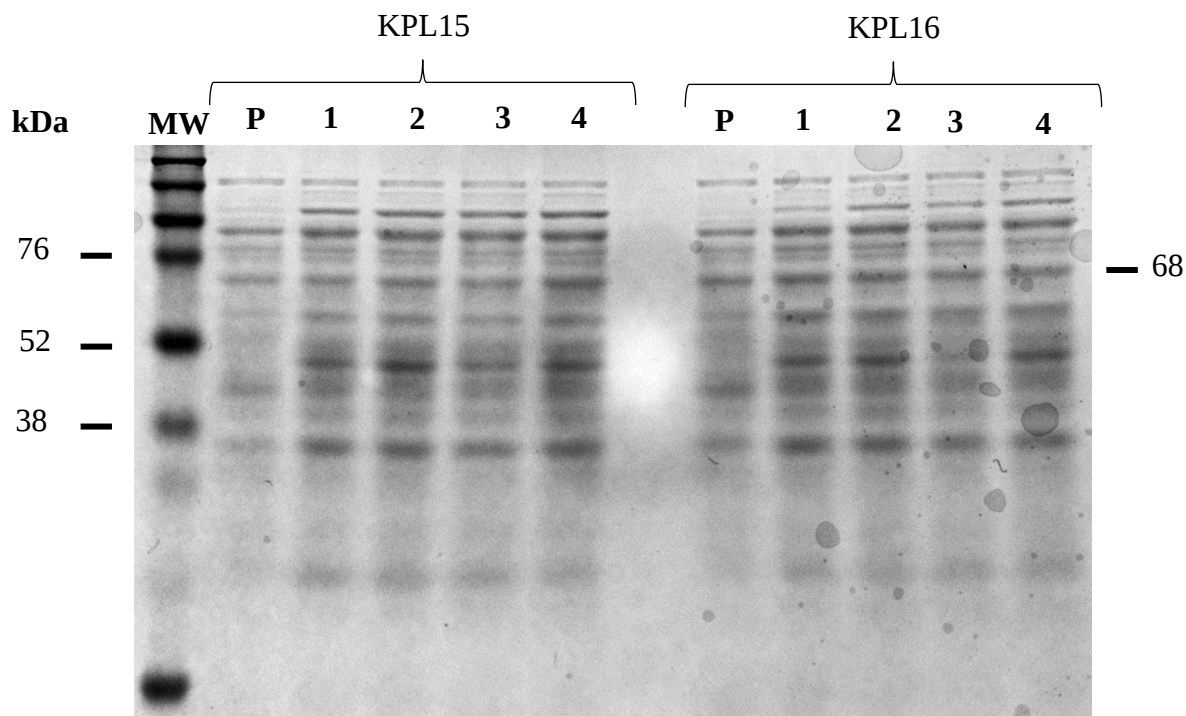


Fig. S13 SDS-PAGE showing the expression of *S.Sen0738I* (KPL15 and KPL16) at different IPTG concentrations. Lanes: P, pre-induction; 1, 0.005 mM; 2, 0.05 mM; 3, 0.5 mM; and 4, 1 mM. MW, molecular weight marker (Amersham™ ECL™ Rainbow™ Marker - Full range, Full Range, Cytiva RPN800E, 12 to 225 kDa)

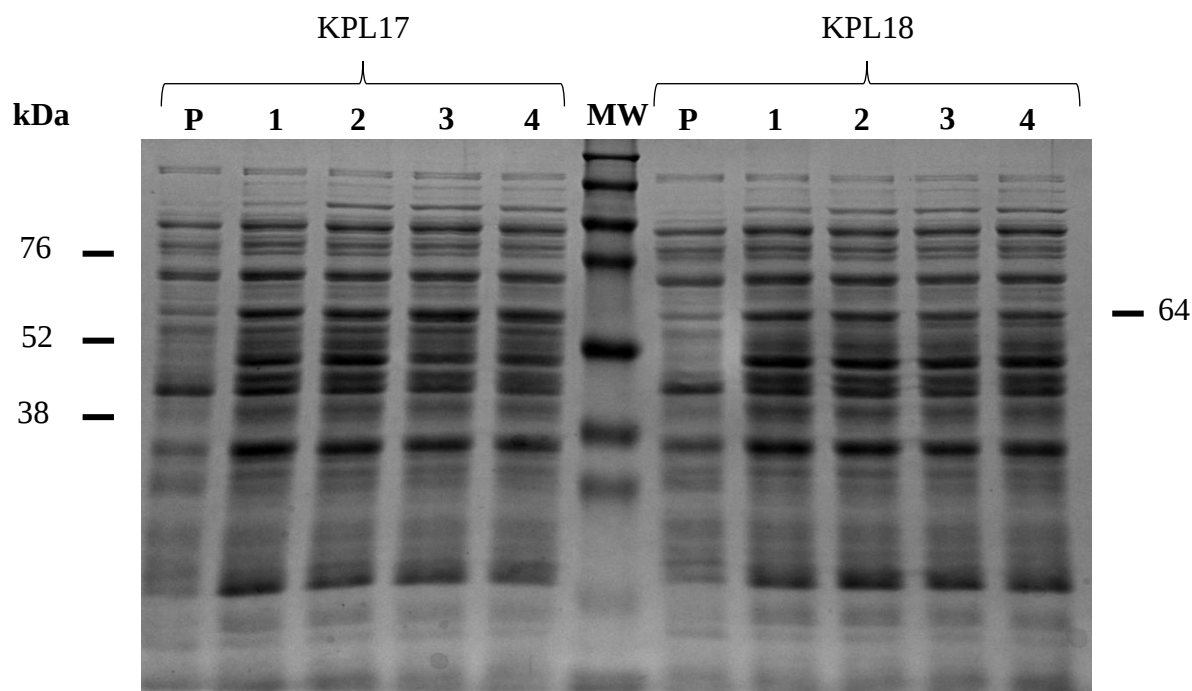


Fig. S14 SDS-PAGE showing the expression of M1.*Eco31I* (KPL17 and KPL18) at different IPTG concentrations. Lanes: P, pre-induction; 1, 0.005 mM; 2, 0.05 mM; 3, 0.5 mM; and 4, 1 mM. MW, molecular weight marker (Amersham™ ECL™ Rainbow™ Marker - Full range, Full Range, Cytiva RPN800E, 12 to 225 kDa)

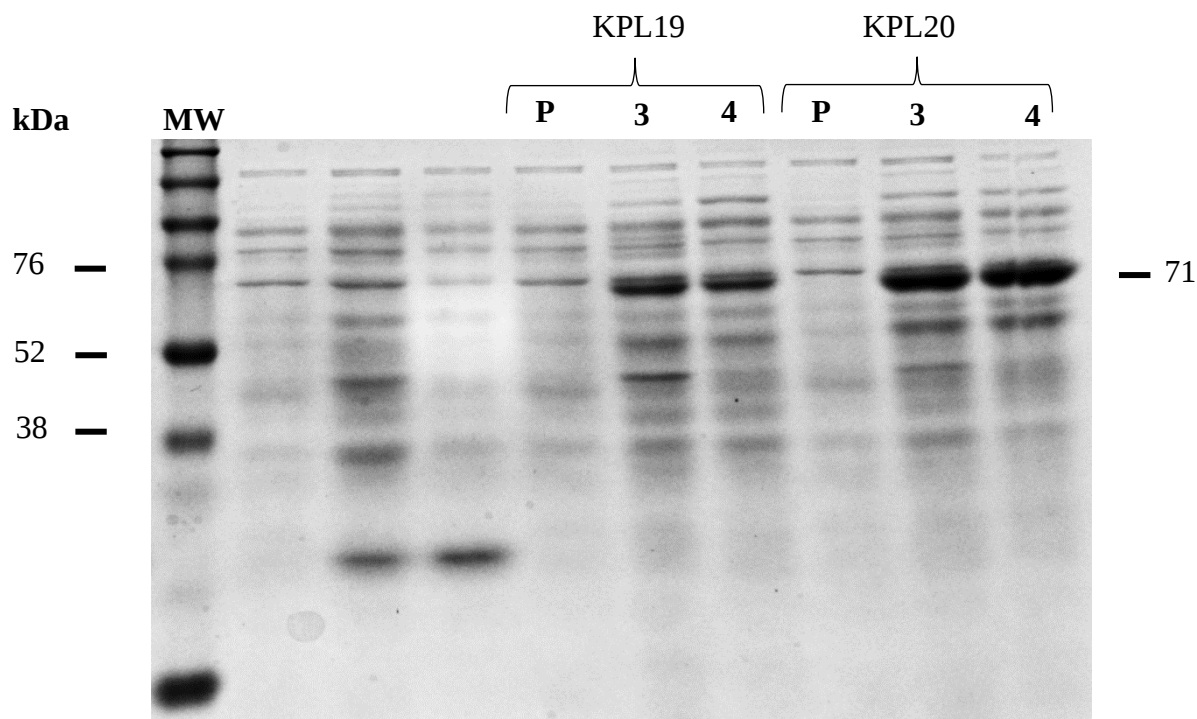


Fig. S15 SDS-PAGE showing the expression of M.*XmnI* (KPL19 and KPL20) at different IPTG concentrations. Lanes: P, pre-induction; 3, 0.5 mM; and 4, 1 mM. MW, molecular weight marker (Amersham™ ECL™ Rainbow™ Marker - Full range, Full Range, Cytiva RPN800E, 12 to 225 kDa)

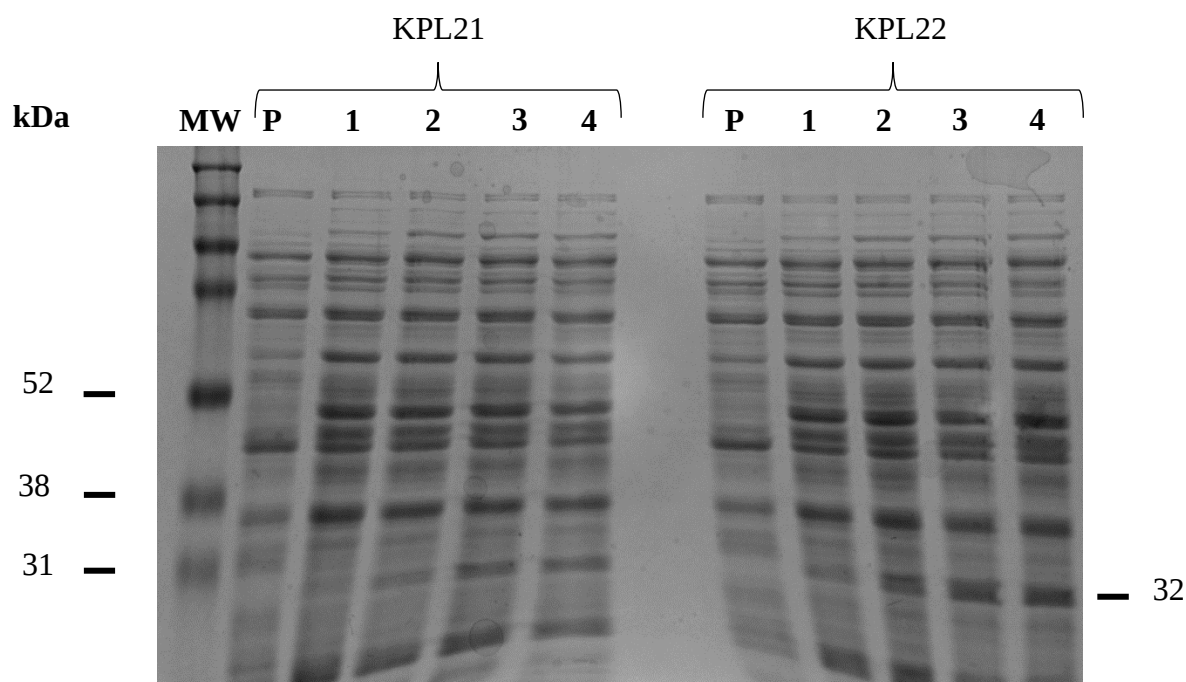


Fig. S16 SDS-PAGE showing the expression of M1.HpyAII (KPL21 and KPL22) at different IPTG concentrations. Lanes: P, pre-induction; 1, 0.005 mM; 2, 0.05 mM; 3, 0.5 mM; and 4, 1 mM. MW, molecular weight marker (Amersham™ ECL™ Rainbow™ Marker - Full range, Full Range, Cytiva RPN800E, 12 to 225 kDa)

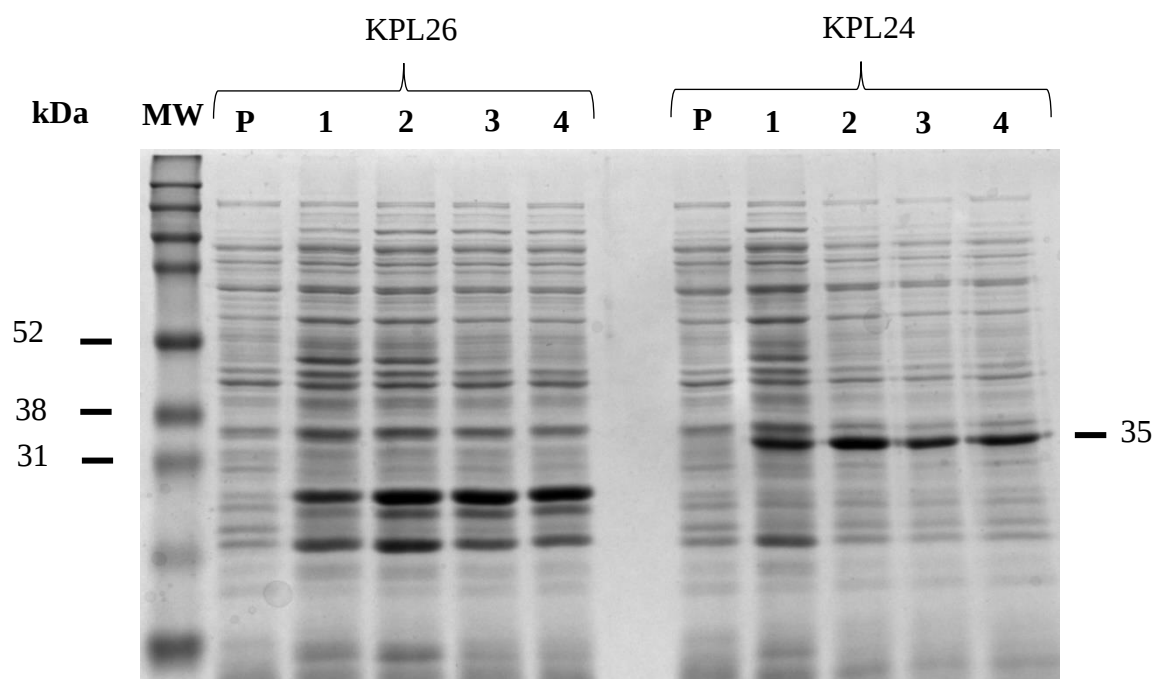


Fig. S17 SDS-PAGE showing the expression of M2.HpyAII (KPL24) and M1.MboII (KPL26) at different IPTG concentrations. Lanes: P, pre-induction; 1, 0.005 mM; 2, 0.05 mM; 3, 0.5 mM; and 4, 1 mM. MW, molecular weight marker (Amersham™ ECL™ Rainbow™ Marker - Full range, Full Range, Cytiva RPN800E, 12 to 225 kDa)

Analysis of DNA methylation

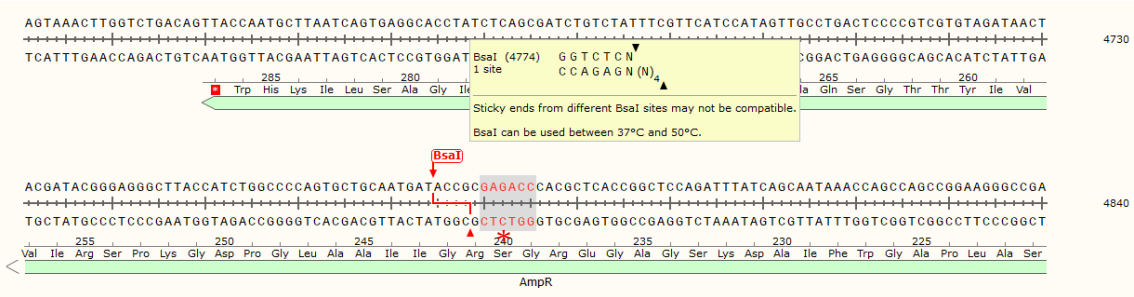


Fig. S18 pET15b fragment for testing the activity of M2.Eco31I, M1.Eco31I, and M2.BsaI BsaI-associated non-switchable methylases. The recognition sequence of the methylase is in red with the methylated base (C) for M2.Eco31I marked with a red asterisk (*). The BsaI recognition sequence is in the reverse direction

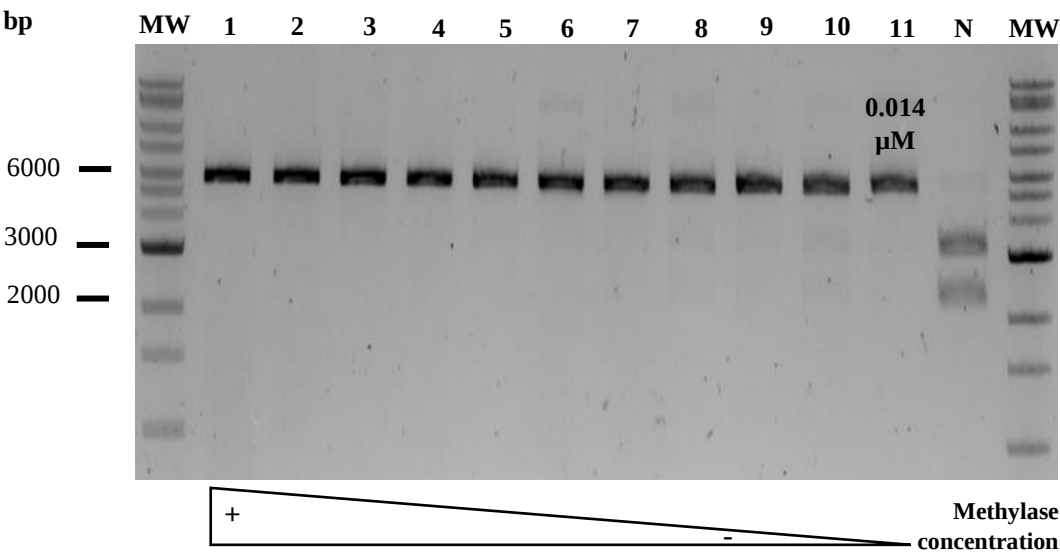


Fig. S19 Agarose gel electrophoresis (1%) showing the activity of M2.Eco31I_2 (KPL04) BsaI-associated non-switchable methylase on pET15b at different protein concentrations. Lanes: 1 to 11, 2-fold serial dilutions from 14 to 0.014 μM; and N, negative control (without methylase). MW, molecular weight marker (Quick-Load® 1 kb Extend DNA Ladder, New England Biolabs, N3239S, 0.5 to 48.5 kb)

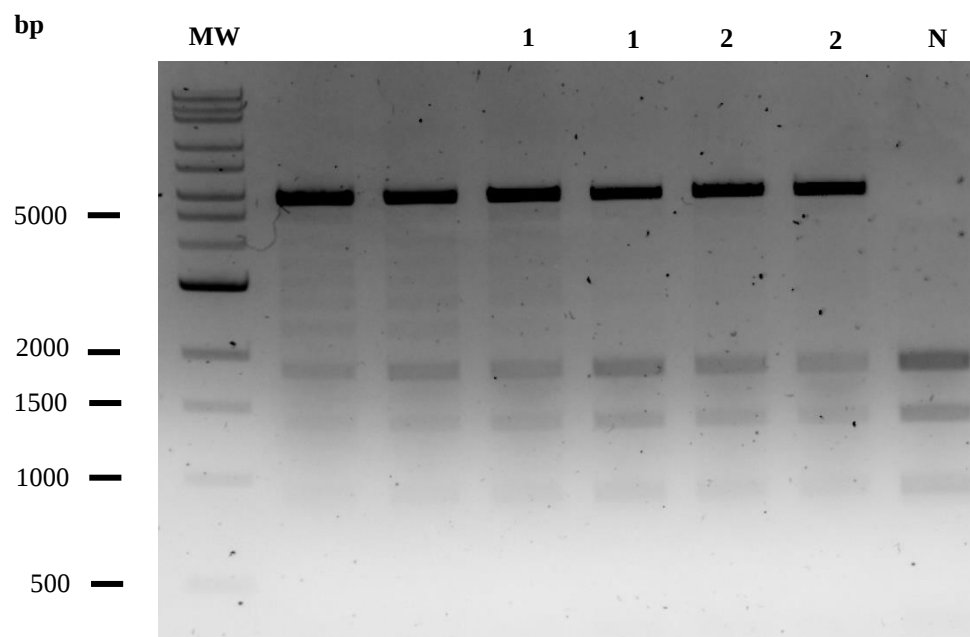


Fig. S22 Agarose gel electrophoresis (1%) showing the activity of M1.*HpyAII* (KPL21 and KPL22) *BpiI*-associated non-switchable methylase on pET15b. Lanes: 1, KPL21 (1.17 μ M); 2, KPL22 (1.96 μ M); and N, negative control (without methylase). MW, molecular weight marker (Quick-Load® 1 kb Extend DNA Ladder, New England Biolabs, N3239S, 0.5 to 48.5 kb). Bands less than 500 bp are not observed in the gel

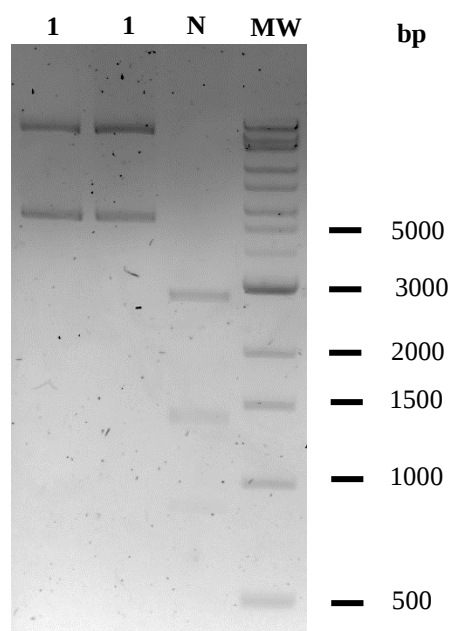


Fig. S23 Agarose gel electrophoresis (1%) showing the activity of M1.*MboII* (KPL26) *BpiI*-associated non-switchable methylase on pET15b. Lanes: 1, KPL26 (15 μ M); and N, negative control (without methylase). MW, molecular weight marker (Quick-Load® 1 kb Extend DNA Ladder, New England Biolabs, N3239S, 0.5 to 48.5 kb). The reactions were digested only with *BpiI*. The methylated plasmid exhibits a band corresponding to the undigested plasmid (5708 bp) and a band corresponding to nicked plasmid DNA. The non-methylated plasmid exhibits 5 bands (339, 374, 863, 1379, and 2753 bp) as a result of the digestion of *BpiI*. Bands less than 500 bp are not observed in the gel



Fig. S24 POC1399 for testing the activity of *M.Osp807II* *BsaI*-associated switch methylase. **(A)** *BsaI* sites at position 20 (methylation-switchable restriction site) where the recognition sequence of the methylase is in red and the methylated base (A: adenine) is marked with a red asterisk (*). **(B)** *BsaI* site at position 611 (always-cutable restriction site) where the recognition sequence of the methylase has been altered such that it is no longer recognised by the methylase and so is not functional (in magenta). The second and third *BsaI* recognition sequences are in the reverse direction



Fig. S25 POC1400 for testing the activity of *M2.NmeMC58II* *BpiI*-associated switch methylase. **(a)** *BpiI* sites at position 16 (methylation-switchable restriction site) where the recognition sequence of the methylase is in red and the methylated base (A: adenine) is marked with a red asterisk (*). **(b)** *BpiI* site at position 595 (always-cutable restriction site) where the recognition sequence of the methylase has been altered such that it is no longer recognised by the methylase and so is not functional (in magenta). The second and third *BpiI* recognition sequences are in the reverse direction

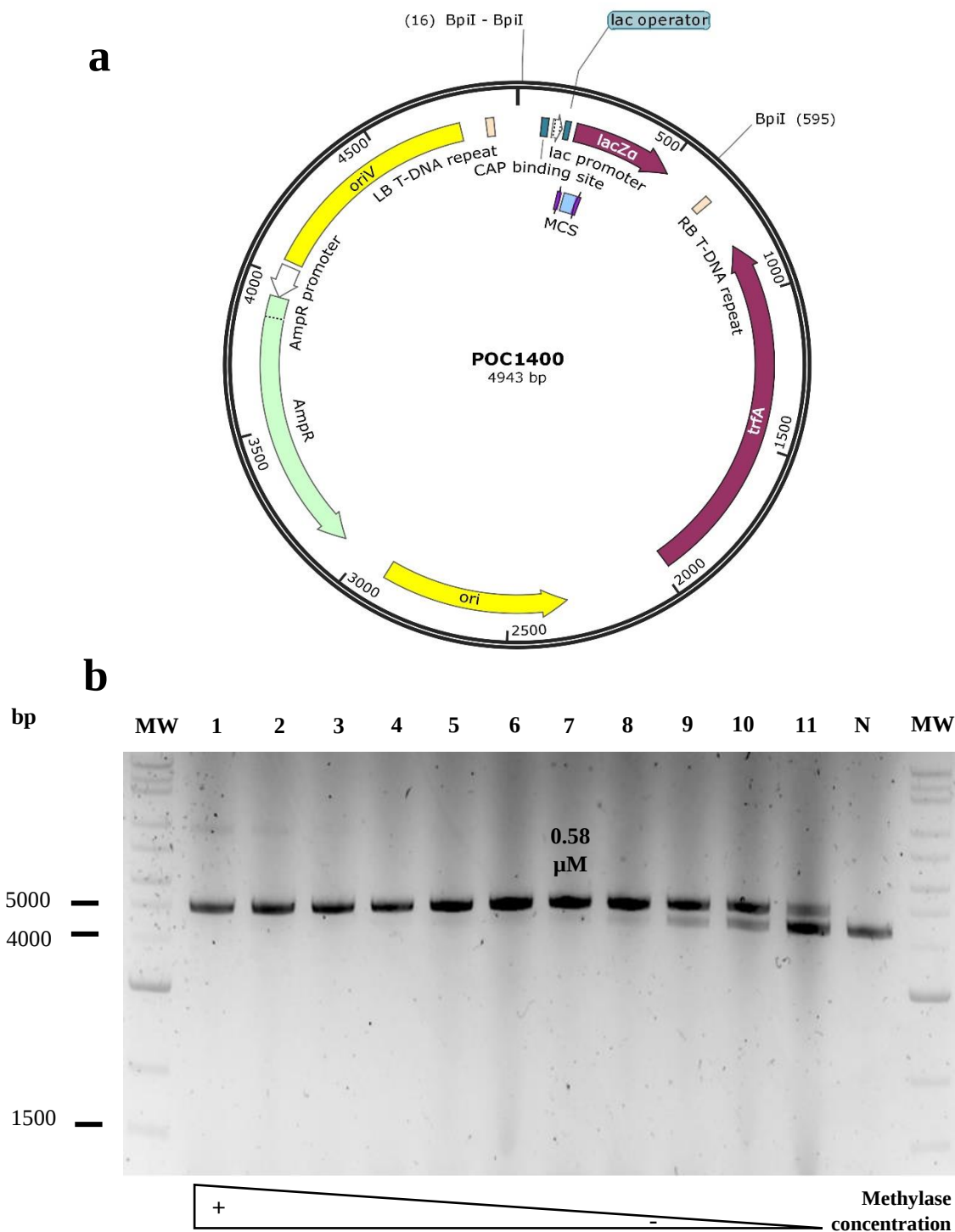


Fig. S26 Activity of *BpiI*-associated switch methylases on POC1400. **(a)** POC1400 showing the *BpiI* restriction sites for the activity test (Supplementary Figure S24). The methylated plasmid will exhibit a band on the gel (4943 bp) corresponding to the linearised plasmid digested by *BpiI* at position 595. The non-methylated plasmid will exhibit two bands (579 and 4364 bp) as a result of *BpiI* digestion at positions 16 and 595. **(b)** Agarose gel electrophoresis (1%) showing the activity of M2.NmeMC58II (KPL11) at different protein concentrations. Lanes: 1 to 11, 2-fold serial dilutions from 37.5 to 0.04 μ M; and N, negative control (without methylase). MW, molecular weight marker (Quick-Load® 1 kb Extend DNA Ladder, New England Biolabs, N3239S, 0.5 to 48.5 kb). Bands less than 1500 bp are not observed in the gel

a



b



Fig. S27 POC1401 for testing the activity of *M.XmnI* *LguI*-associated switch methylase. **(a)** *LguI* sites at position 19 (methylation-switchable restriction site) where the recognition sequence of the methylase is in red and the methylated base (A: adenine) is marked with a red asterisk (*). **(b)** *LguI* site at position 616 (always-cuttable restriction site) where the recognition sequence of the methylase has been altered such that it is no longer recognised by the methylase and so is not functional (in magenta). The second and third *LguI* recognition sequences are in the reverse direction

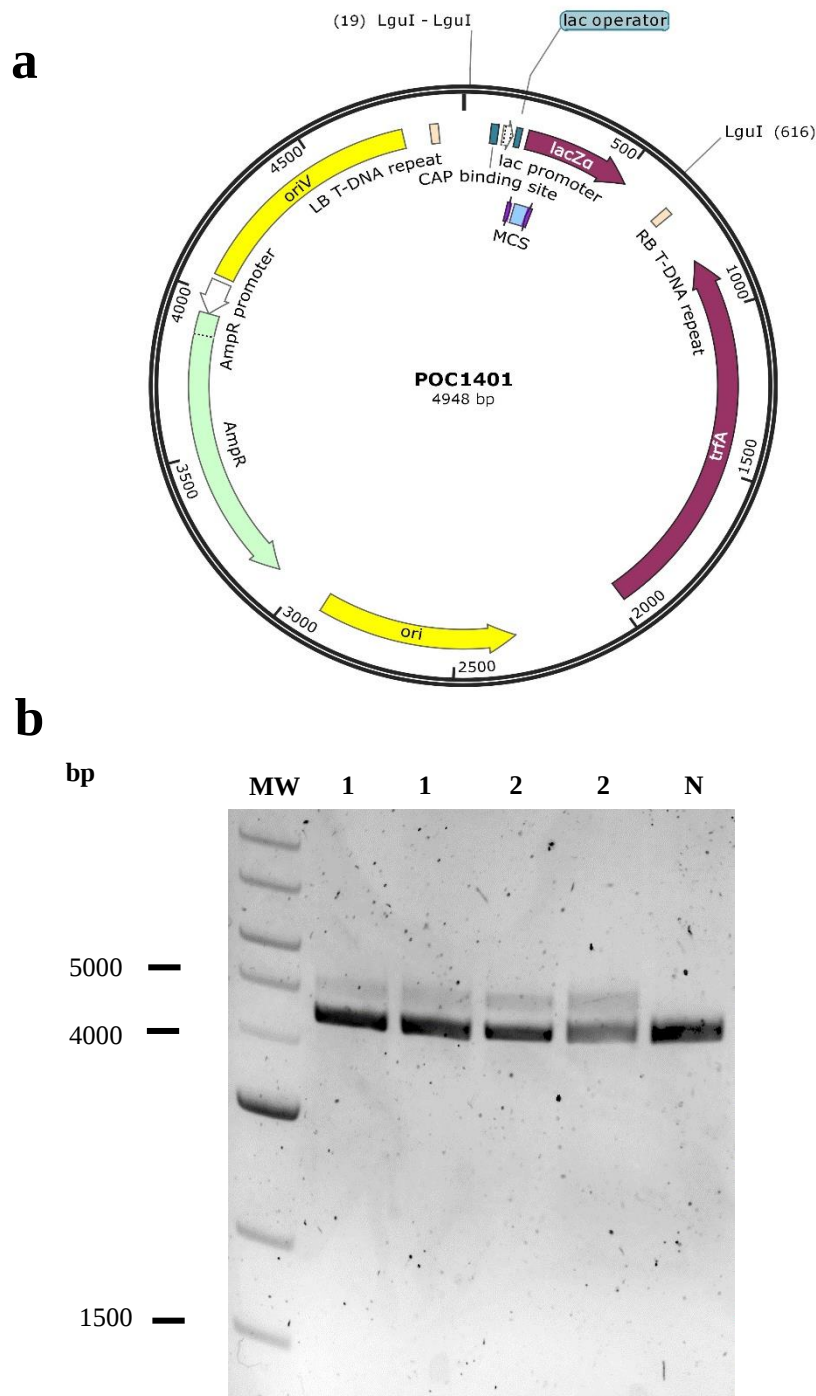


Fig. S28 Activity of *LguI*-associated switch methylases on POC1401. **(a)** POC1401 showing the *LguI* restriction sites for the activity test (Supplementary Figure S26). The methylated plasmid will exhibit a band on the gel (4948 bp) corresponding to the linearised plasmid digested by *LguI* at position 616. The non-methylated plasmid will exhibit two bands (597 and 4351 bp) as a result of *LguI* digestion at positions 19 and 616. **(b)** Agarose gel electrophoresis (1%) showing the activity of *M.XmnI* (KPL19 and KPL20). Lanes: 1, KLP19 at 0.94 μ M; 2, KPL20 at 2.2 μ M; and N, negative control (without methylase). MW, molecular weight marker (Quick-Load® 1 kb Extend DNA Ladder, New England Biolabs, N3239S, 0.5 to 48.5 kb). Bands less than 1500 bp are not observed in the gel

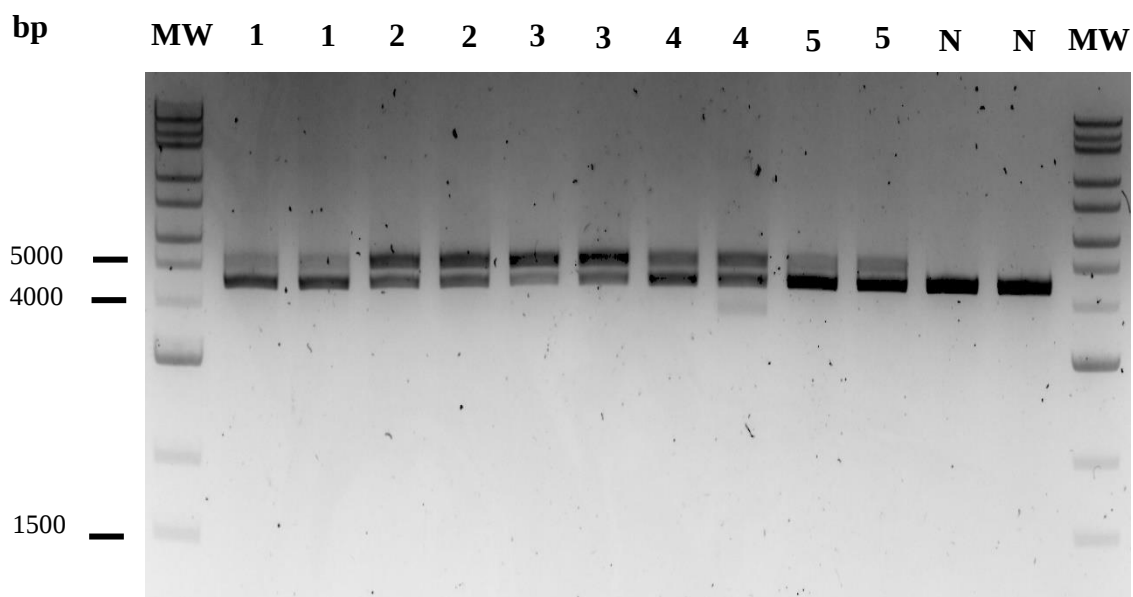


Fig. S29 Effect of pH on the activity of *M.XmnI* (KPL20) *LguI*-associated switch methylase on POC1401. The methylated plasmid exhibits a band on the gel (4948 bp) corresponding to the linearised plasmid digested by *LguI* at position 616. The non-methylated plasmid exhibits two bands (597 and 4351 bp) as a result of *LguI* digestion at positions 19 and 616. Lanes: 1, pH 6.9; 2, pH 7.5; 3, pH 7.9; 4, pH 8.4; 5, pH 8.8; and N, negative control (without methylase). MW, molecular weight marker (Quick-Load® 1 kb Extend DNA Ladder, New England Biolabs, N3239S, 0.5 to 48.5 kb). A concentration of 2.2 μ M of methylase was used. Bands less than 1500 bp are not observed in the gel