A model for the evolution of prokaryotic DNA restriction-modification systems based upon the structural malleability of Type I restriction-modification enzymes.

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Supplementary Information

Table S1. Amino acid sequences for the HsdR, HsdS and HsdS used in this work.

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HsdR subunit (SauN315ORF189P 929 aa)
MAYQSEYALE NEMMNQLEQL GYERVTIRDN KQLLDNFRTI LNERHADKLE GNPLTDKEFQ RLLTMIDGKS IFESARILRD
KLPLRRDDES EIYLSFLDKK SWCKNKFQVT NQVSVEDTYK ARYDVTILIN GLPLVQVELK RRGIDINEAF NQVKRYRKQN
YTGLFRYIQM FIISNGVETR YFSNNDSELL KSHMFYWSDK QNNRINTLQS FAESFMRPCQ LAKMISRYMI INETDRILMA
MRPYQVYAVE ALIQQATETG NNGYVWHTTG SGKTLTSFKA SQILSQQDDI KKVIFLVDRK DLDSQTEEEF NKFAKGAVDK
TFNTSQLVRQ LNDKSLPLIV TTIQKMAKAI QGNAHLLEQY KTNKVVFIID ECHRSQFGDM HRLVKQHFKN AQYFGFTGTP
RFPENSSQDG RTTADIFGRC LHTYLIRDAI HDGNVLGFSV DYINTFKNKA LKAEDNSMVE AIDTEEVWLA DKRVELVTRH
IINNHDKYTR NRQYSSIFTV QSIHALIKYY ETFKRLNKKL EQPLTIAGIF TFKPNEDDRD GEVPYHSREK LEIMISDYNK
KFETNFSTDT TNEYFNHISK NVKKGVKDSK IDILIVVNMF LTGFDSKVLN TLYVDKNLMY HDLIQAYSRT NRVEKESKPF
GKIVNYRDLK KETDDALRVF SQTNDTDTIL MRSYEEYKKE FMDAYRELKM IVPTPHMVDD IQDEEELKRF VEAYRLLAKI
ILRLKAFDEF EFTIDEIGMD EQENEDYKSK YLAVYDQVKR ATAEKNKVSI LNDIDFEIEM MRNDTINVNY IMNILRQIDL
EDKAEQRRNQ EQIRRILDHA DDPTLRLKRD LIREFIDNVV PSLNKDDDID QEYVNFESIK KEAEFKGFAG ERSIDEQALK
TISNDYQYSG VVNPHHLKKM IGDLPLKEKR KARKAIESFV AETTEKYGV
HsdR subunit nucleotide sequence (SauN315ORF189P 2790 nt)
ATGGCATACCAAAGTGAATACGCATTAGAAAATGAAATGATGAATCAACTTGAACAATTGGGTTACGAAAGAGTAACGATACGTGATAATAA
GCAATTGCTTGATAATTTTAGAACGATTTTAAATGAGCGTCATGCGGACAAATTAGAAGGCAATCCCTTAACAGATAAAGAATTTCAACGTC
TGTTAACGATGATTGATGGGAAAAGTATTTTCGAGAGTGCCCGTATTTTACGTGATAAATTACCACTTAGACGTGATGATGAGTCTGAGATT
TATTTGTCGTTTTTAGATAAGAAAAGTTGGTGTAAAAATAAGTTTCAAGTGACGAATCAAGTATCTGTCGAGGATACATATAAAGCACGTTA
TGATGTAACGATATTAATCAACGGACTACCCCTTGTCCAAGTTGAATTGAAACGTCGAGGTATTGATATTAATGAGGCGTTTAACCAAGTAA
AACGTTACCGCAAACAAAATTACACAGGCTTATTCCGCTACATACAAATGTTTATCATTAGTAATGGTGTTGAAACGCGATACTTTTCTAAT
AATGATAGCGAACTATTGAAGAGTCACATGTTTTATTGGAGTGATAAACAGAATAACCGAATCAATCATTACAATCATTTGCTGAGTCGTT
TATGAGACCCTGTCAATTAGCTAAGATGATATCGCGCTATATGATTATTAATGAAACAGATAGAATACTGATGGCAATGCGTCCGTATCAAG
TGTATGCGGTAGAAGCACTTATTCAACAAGCGACTGAGACAGGGAATAATGGATATGTATGGCATACAACTGGAAGTGGTAAGACATTGACT
TCTTTTAAAGCGAGTCAGATTTATCACAGCAAGATGACATTAAGAAAGTTATCTTTTTGGTTGACCGTAAAGACTTGGATAGTCAAACAGA
AGAGGAATTTAATAAATTTGCTAAGGGTGCTGTAGACAAAACTTTTAATACCTCGCAACTGGTACGCCAACTAAATGATAAAAGTTTGCCAC
TTATTGTAACGACGATTCAAAAAATGGCTAAAGCGATTCAAGGGAATGCCCATTTATTAGAACAGTATAAAACGAATAAAGTTGTATTTATT
ATTGATGAGTGTCATCGCAGTCAATTTGGTGACATGCATCGTCTAGTTAAACAACATTTCAAAAATGCCCAATACTTTGGATTCACTGGTAC
GCCACGTTTTCCAGAAAATAGTAGTCAAGATGGTAGAACAACTGCAGATATTTTCGGTAGATGCTTACATACGTATTTAATTAGAGATGCCA
TTCATGATGGTAATGTACTTGGTTTCTCAGTTGACTATATTAATACTTTTAAAAATAAAGCTTTAAAAGCAGAAGATAACAGCATGGTTGAA
GCAATTGATACGGAAGAAGTATGGTTAGCGGATAAACGTGTGGAATTAGTAACACGACATATCATCAATAATCATGATAAATATACACGTAA
TCGTCAATATTCAAGTATATTTACAGTCCAAAGTATTCACGCGCTTATTAAATATTATGAGACATTTAAGCGACTTAACAAAAAGTTGGAAC
AACCGTTAACGATAGCTGGTATATTTACGTTTAAACCTAATGAAGATGATCGTGATGGTGAAGTGCCATATCATTCACGTGAAAAATTAGAG
ATAATGATTAGTGATTATAATAAAAAGTTCGAGACGAATTTTTCAACAGACACAACTAATGAGTAATTTTAATCATATTTCAAAAAAACGTTAA
AAAGGGCGTTAAAGATAGTAAAATTGATATCTTAATCGTTGTTAATATGTTCTTAACTGGTTTTGATAGTAAAGTACTGAACACTTTATATG
TTGATAAGAATTTAATGTATCATGATTTAATTCAAGCGTATTCACGTACAAATAGGGTTGAAAAGAATCAAAGCCATTTGGTAAAATTGTA
AACTATCGTGACTTGAAAAAAGAGACAGACGATGCACTGAGAGTATTCTCACAAACAAATGATACGGATACAATTTTAATGCGCAGTTATGA
AGAGTATAAAAAGAATTTATGGACGCTTATCGTGAGCTTAAAATGATTGTGCCGACACCACACATGGTTGATGACATTCAAGATGAAGAAG
AGCTAAAGCGCTTTGTTGAAGCTTATCGTTTATTAGCTAAAATAATATTACGTTTAAAAGCATTTGACGAGTTTGAGTTTACAATTGATGAA
ATTGGAATGGATGAACAAGAGAATGAAGACTATAAAAGTAAATATTTAGCTGTGTACGATCAAGTAAAAAGAGCGACGGCTGAGAAAAATAA
TTGATCTTGAAGACAAAGCGGAACAACGTCGTAACCAAGAACAAAATTAGACGCATTTTAGATCATGCAGATGATCCGACATTGAGGTTAAA
CGAGATCTAATTAGAGAATTCATCGACAATGTTGTACCTTCTTTAAATAAGGATGATGATATCGATCAAGAATATGTTAATTTCGAAAGTAT
TAAAAAAGAAGCGGAGTTCAAAGGATTTGCTGGAGAGAGATCTATCGATGAACAAGCCCTAAAAACAATTTCAAATGACTACCAGTATAGTG
GTGTTGTAAACCCACATCACCTTAAAAAAAATGATTGGTGATTTGCCATTGAAAGAAAAGCGTAAAGCAAGAAAGCCATTGAATCTTTCGTG
GCAGAAACAACTGAAAAATACGGTGTG
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HsdM (M.SauSTORF499P 518 aa)
MSITEKQRQQ QAELHKKLWS IANDLRGNMD ASEFRNYILG LIFYRFLSEK AEQEYADALS GEDITYQEAW ADEEYREDLK
AELIDQVGYF IEPQDLFSAM IREIETQDFD IEHLATAIRK VETSTLGEES ENDFIGLFSD MDLSSTRLGN NVKERTALIS
KVMVNLDDLP FVHSDMEIDM LGDAYEFLIG RFAATAGKKA GEFYTPQQVS KILAKIVTDG KDKLRHVYDP TCGSGSLLLR
VGKETQVYRY FGQERNNTTY NLARMNMLLH DVRYENFDIR NDDTLENPAF LGHTFDAVIA NPPYSAKWTA DSKFENDERF
SGYGKLAPKS KADFAFIQHM VHYLDDEGTM AVVLPHGVLF RGAAEGVIRR YLIEEKNYLE AVIGLPANIF YGTSIPTCIL
VFKKCRQDDD NVLFIDASND FEKGKNQNHL SDAQVERIID TYKRKETIDK YSYSATLQEI ADNDYNLNIP RYVDTFEEEA
PIDLDQVQQD LKNIDKEIAE VEQEINAYLK ELGVLKDE
HsdM nucleotide sequence (M.SauSTORF499P 1557 nt)
ATGTCTATTACTGAAAAACAACGTCAGCAACAAGCTGAATTACATAAAAAATTATGGTCGATTGCGAATGATTTAAGAGGGAATATGGATGC
GAGTGAATTCCGTAATTACATTTTAGGCTTTGATTTTCTATCGCTTTCATCTGAAAAAGCGGAACAAGAATACGCAGATGCCTTGTCAGGTG
AAGACATCACGTATCAAGAAGCATGGGCAGATGAAGAATACCGTGAAGACTTAAAAGCAGAATTAATTGATCAAGTTGGTTACTTTATTGAA
CCGCAAGATTTATTCAGCGCGATGATTCGTGAAATTGAAACGCAAGATTTCGATATCGAACATCTAGCGACGGCAATTCGTAAAGTTGAAAC
ATCAACATTAGGTGAAGAAAGTGAAAATGACTTTATCGGACTATTCAGCGATATGGATTTGAGTTCAACGCGACTAGGTAACAATGTCAAAG
AACGTACTGCGTTAATCTCTAAAGTCATGGTTAATCTTGACGACTTACCATTTGTTCACAGTGATATGGAAATTGATATGTTAGGTGATGCA
TACGAATTCCTTATCGGGCGCTTTGCGGCGACAGCGGGTAAAAAAGCTGGCGAGTTCTATACACCACAACAAGTATCTAAGATACTGGCGAA
GATTGTCACAGACGGTAAAGATAAATTACGTCATGTATATGACCCAACATGTGGTTCAGGTTCATTACTGTTACGTGTAGGTAAAGAAACGC
AAGTGTATCGTTATTTCGGTCAAGAACGTAACAATACCACATACAACTTAGCGCGCATGAACATGTTATTACATGATGTACGTTATGAAAAT
TTCGATATACGTAATGATGACACGTTGGAAAATCCAGCCTTTTTAGGCCATACATTTGATGCGGTTATTGCGAACCCACCATACAGTGCGAA
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ATGGACAGCAGATTCAAAATTTGAAAATGACGAACGATTCAGCGGATACGGCAAGCTTGCGCCAAAGTCTAAAGCAGACTTTGCCTTTATTC
AACACATGGTACATTACCTAGATGATGAAGGTACCATGGCAGTTGTACTACCGCATGGTGTCTTATTCCGTGGGGCTGCAGAAGGCGTCATT
CGTCGTTACTTAATTGAAGAAAAGAACTACCTAGAAGCCGTGATTGGCTTACCAGCGAATATTTTCTATGGGACAAGTATTCCAACATGTAT
TTTAGTATTTAAAAAATGTCGCCAACAAGACGACAACGTATTATTTATCGATGCATCCAATGATTTTGAAAAAGGAAAAAATCAAACCATT
TAAGCGACGCCCAAGTCGAACGCATTATAGACACATATAAGCGTAAAGAAACAATCGATAAATATAGCTACAGCGCGACATTACAAGAGATT
GCCGATAACGATTACAACTTAAACATTCCGAGATATGTCGATACATTCGAAGAAGAAGCGCCAATTGATTAGATCAAGTCCAACAAGATTT
GAAAAATATCGACAAAGAAATCGCAGAAGTTGAACAAGAAATCAATGCATACCTGAAAGAACTTGGGGTGTTGAAAGATGAG
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HsdS (S.SauSTORF499P with His tag recognising ACCNNNNNRTGA)
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MSNTQKKNVP ELRFPGFEGE WEEKKLGEFA GKVTQKNVDK KYIETLTNSA ELGIISQKDY FDKEISNIDN IKKYYVVEEN
DFVYNPRMSN YAPFGPVNRN KLGKKGVMSP LYTVFKIQNI DLNFIEFYFK SSKWYRFMAL NGDSGARADR FSIKDRTFME
MPLHIPCMDE QIKIGQFFSK LDRQIELEEQ KLELLQQQKK GYMQKIFSQE LRFKDENGKD YPEWEETTIK EIAQINTGKK
DTKDAITNGS YDFYVRSPIV YKINTFSYEG EAILTVGDGV GVGKVFHYVN GKFDYHQRVY KISDFKNYYG LLLFYYFSQN
FLKETKKYSA KTSVDSVRKD MIANMKVPRP IYIEQKKIGQ FIKRVDNKTK IQKQVIELLK QRKKSLLQKM FIPGGSHHHH HH
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HsdS nucleotide sequence (S.SauSTORF499P with His tag)
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ATGAGTAATACACAAAAGAAAAATGTGCCAGAGTTGAGATTCCCCGGGTTTGAAGGCGAATGGGAAGAGAAGAAGCTAGGTGAGTTTGCTGG
TAAAGTTACCCAAAAAAATGTTGATAAAAAATATATTGAGACATTAACTAATTCATCTGAGTTAGGTATCATATCTCAAAAGGATTATTTTG
ACAAAGAAATTTCGAATATAGATAATAATATTAAAAAGTACTATGTAGTTGAAGAGAATGATTTTGTTTATAACCCTAGAATGTCTAATTATGCT
CCATTTGGACCAGTAAATAGAAATAAGTTAGGGAAAAAAGGGGTCATGTCACCTCTTTATACTGTGTTTAAAATTCAAACATTGATTTAAA
CTTTATTGAGTTTTATTTTAAATCTTCAAAATGGTATAGATTTATGGCATTAAACGGTGATTCAGGTGCTCGAGCAGATAGGTTTTCTATTA
AAGATAGGACATTTATGGAAATGCCACTTCATATCCCATGTATGGATGAACAAATAAAAATCGGTCAGTTCTTCAGCAAACTTGACCGACAA
ATTGAATTAGAAGAACAAAAACTTGAATTACTTCAACAACAGAAAAAAGGCTATATGCAGAAAATCTTCTCGCAAGAATTGCGATTTAAAGA
TGAGAATGGTAAAGATTATCCGGAGTGGGAAGAAACTACTATAAAAGAAATTGCTCAAATTAACACTGGAAAGAAAGATACAAAAGATGCCA
TTACTAATGGGAGTTATGATTTTTACGTTAGATCTCCGATAGTTTATAAAATTAATACTTTTAGTTATGAAGGAGAGGCTATTTTAACTGTA
GGAGATGGAGTTGGCGTAGGTAAAGTTTTCCACTATGTAAATGGGAAATTTGATTATCATCAAAGAGTATACAAAATATCTGACTTTAAGAA
TTATTATGGATTATTGTTATTTTATTATTTTTCACAAAACTTTTTAAAAGAAACAAAGAAATATAGTGCGAAGACATCAGTTGATTCAGTTA
GAAAAGACATGATTGCTAATATGAAAGTACCGCGTCCTATTTATATAGAACAAAAAAAAATCGGTCAATTCATTAAAAGAGTAGACAACAAA
ACAAAAATTCAGAAACAAGTGATTGAATTACTTAAACAACGCAAAAAGTCATTACTTCAAAAGATGTTTATTCCCGGGGGATCCCATCATCA
TCATCATCATT
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Table S2. Oligonucleotide sequences for fusion of nuclease domain of *hsdR* to 5' end of *hsdM* and the amino acid sequences of the part of HsdR fused to HsdM. Nucleotide sequences in bold are part of the gene coding sequence.

Fusion Name	PCR oligonucleotide name	Nucleotide Sequence
RM_EB_1 (not used)	Mu50nucendalpha-CC398-1TS-	5'**GCACTTATTCAACAAGCGACT**ATGTCTATTACTGAAAAACAACG 3'
	Mu50nucendalpha-CC398-1BS-	5'CGTTGTTTTTCAGTAATAGACAT**AGTCGCTTGTTGAATAAGTGC** 3'
RM_EB_2	Mu50nucendcoil-CC398-1TS	5'**CGACTGAGACAGGGAATAAT**ATGTCTATTACTGAAAAACAACG 3'
	Mu50nucendalpha-CC398-1BS	5'CGTTGTTTTTCAGTAATAGACAT**ATTATTCCCTGTCTCAGTCG** 3'
RM_EB_3	Mu50nuclease- CC398-1TS	5'**CATTTGCTGAGTCGTTTATGAGA**ATGTCTATTACTGAAAAACAACG 3'
	Mu50nuclease- CC398-1BS	5'CGTTGTTTTTCAGTAATAGACAT**TCTCATAAACGACTCAGCAAATG** 3'
RM_EB_4	RM_EB_7_TS	5'**CAGAATAACCGAATCAATACA**ATGTCTATTACTGAAAAACAACG 3'
	RM_EB_7_BS	5'CGTTGTTTTTCAGTAATAGACAT**TGTATTGATTCGGTTATTCTG** 3'
RM_EB_5	RM_EB_8_TS	5'**CAATCATTTGCTGAGTCGTTT**ATGTCTATTACTGAAAAACAACG 3'
	RM_EB_8_BS	5'CGTTGTTTTTCAGTAATAGACAT**AAACGACTCAGCAAATGATTG** 3'
RM_EB_6	RM_EB_9_TS	5'**CCCTGTCAATTAGCTAAGATGATA**ATGTCTATTACTGAAAAACAACG 3'
	RM_EB_9_BS	5'CGTTGTTTTTCAGTAATAGACAT**TATCATCTTAGCTAATTGACAGGG** 3'
RM_EB_7	RM_EB_10_TS	5'**CTGATGGCAATGCGTCCGTAT**ATGTCTATTACTGAAAAACAACG 3'
	RM_EB_10_BS	5'CGTTGTTTTTCAGTAATAGACAT**ATACGGACGCATTGCCATCAG** 3'
RM_EB_8	RM_EB_11_TS	5'**CCGCTACATACAAATGTTT**ATGTCTATTACTGAAAAACAACG 3'
	RM_EB_11_BS	5'CGTTGTTTTTCAGTAATAGACAT**AAACATTTGTATGTAGCGG** 3'
RM_EB_9	RM_EB_12_TS	5'**GGTGTTGAAACGCGATACTTTTCT**ATGTCTATTACTGAAAAACAACG 3'
	RM_EB_12_BS	5'CGTTGTTTTTCAGTAATAGACAT**AGAAAAGTATCGCGTTTCAACACC** 3'
RM_EB_10	RM_EB_13_TS	5'**GGCATACAACTGGAAGTGGT**ATGTCTATTACTGAAAAACAACG 3'
	RM_EB_13_BS	5'CGTTGTTTTTCAGTAATAGACAT**ACCACTTCCAGTTGTATGCC** 3'
RM_EB_11	RM_EB_14_TS	5'**GCGAGTCAGATTTTATCA**ATGTCTATTACTGAAAAACAACG 3'
	RM_EB_14_BS	5'CGTTGTTTTTCAGTAATAGACAT**TGATAAAATCTGACTCGC** 3'

Fusion Name	Amino acid sequence from HsdR
RM_EB_2	MAYQSEYALE NEMMNQLEQL GYERVTIRDN KQLLDNFRTI LNERHADKLE GNPLTDKEFQ RLLTMIDGKS IFESARILRD KLPLRRDDES EIYLSFLDKK SWCKNKFQVT NQVSVEDTYK ARYDVTILIN GLPLVQVELK RRGIDINEAF NQVKRYRKQN YTGLFRYIQM FIISNGVETR YFSNNDSELL KSHMFYWSDK QNNRINTLQS FAESFMRPCQ LAKMISRYMI INETDRILMA MRPYQVYAVE ALIQQATETG NN
RM_EB_3	MAYQSEYALE NEMMNQLEQL GYERVTIRDN KQLLDNFRTI LNERHADKLE GNPLTDKEFQ RLLTMIDGKS IFESARILRD KLPLRRDDES EIYLSFLDKK SWCKNKFQVT NQVSVEDTYK ARYDVTILIN GLPLVQVELK RRGIDINEAF NQVKRYRKQN YTGLFRYIQM FIISNGVETR YFSNNDSELL KSHMFYWSDK QNNRINTLQS FAESFMR
RM_EB_4	MAYQSEYALE NEMMNQLEQL GYERVTIRDN KQLLDNFRTI LNERHADKLE GNPLTDKEFQ RLLTMIDGKS IFESARILRD KLPLRRDDES EIYLSFLDKK SWCKNKFQVT NQVSVEDTYK ARYDVTILIN GLPLVQVELK RRGIDINEAF NQVKRYRKQN YTGLFRYIQM FIISNGVETR YFSNNDSELL KSHMFYWSDK QNNRINT
RM_EB_5	MAYQSEYALE NEMMNQLEQL GYERVTIRDN KQLLDNFRTI LNERHADKLE GNPLTDKEFQ RLLTMIDGKS IFESARILRD KLPLRRDDES EIYLSFLDKK SWCKNKFQVT NQVSVEDTYK ARYDVTILIN GLPLVQVELK RRGIDINEAF NQVKRYRKQN YTGLFRYIQM FIISNGVETR YFSNNDSELL KSHMFYWSDK QNNRINTLQS FAESF
RM_EB_6	MAYQSEYALE NEMMNQLEQL GYERVTIRDN KQLLDNFRTI LNERHADKLE GNPLTDKEFQ RLLTMIDGKS IFESARILRD KLPLRRDDES EIYLSFLDKK SWCKNKFQVT NQVSVEDTYK ARYDVTILIN GLPLVQVELK RRGIDINEAF NQVKRYRKQN YTGLFRYIQM FIISNGVETR YFSNNDSELL KSHMFYWSDK QNNRINTLQS FAESFMRPCQ LAKMI
RM_EB_7	MAYQSEYALE NEMMNQLEQL GYERVTIRDN KQLLDNFRTI LNERHADKLE GNPLTDKEFQ RLLTMIDGKS IFESARILRD KLPLRRDDES EIYLSFLDKK SWCKNKFQVT NQVSVEDTYK ARYDVTILIN GLPLVQVELK RRGIDINEAF NQVKRYRKQN YTGLFRYIQM FIISNGVETR YFSNNDSELL KSHMFYWSDK QNNRINTLQS FAESFMRPCQ LAKMISRYMI INETDRILMA MRP
RM_EB_8	MAYQSEYALE NEMMNQLEQL GYERVTIRDN KQLLDNFRTI LNERHADKLE GNPLTDKEFQ RLLTMIDGKS IFESARILRD KLPLRRDDES EIYLSFLDKK SWCKNKFQVT NQVSVEDTYK ARYDVTILIN GLPLVQVELK RRGIDINEAF NQVKRYRKQN YTGLFRYIQM F
RM_EB_9	MAYQSEYALE NEMMNQLEQL GYERVTIRDN KQLLDNFRTI LNERHADKLE GNPLTDKEFQ RLLTMIDGKS IFESARILRD KLPLRRDDES EIYLSFLDKK SWCKNKFQVT NQVSVEDTYK ARYDVTILIN GLPLVQVELK RRGIDINEAF NQVKRYRKQN YTGLFRYIQM FIISNGVETR YFS
RM_EB_10	MAYQSEYALE NEMMNQLEQL GYERVTIRDN KQLLDNFRTI LNERHADKLE GNPLTDKEFQ RLLTMIDGKS IFESARILRD KLPLRRDDES EIYLSFLDKK SWCKNKFQVT NQVSVEDTYK ARYDVTILIN GLPLVQVELK RRGIDINEAF NQVKRYRKQN YTGLFRYIQM FIISNGVETR YFSNNDSELL KSHMFYWSDK QNNRINTLQS FAESFMRPCQ LAKMISRYMI INETDRILMA MRPYQVYAVE ALIQQATETG NNGYVWHTTG SG
RM_EB_11	MAYQSEYALE NEMMNQLEQL GYERVTIRDN KQLLDNFRTI LNERHADKLE GNPLTDKEFQ RLLTMIDGKS IFESARILRD KLPLRRDDES EIYLSFLDKK SWCKNKFQVT NQVSVEDTYK ARYDVTILIN GLPLVQVELK RRGIDINEAF NQVKRYRKQN YTGLFRYIQM FIISNGVETR YFSNNDSELL KSHMFYWSDK QNNRINTLQS FAESFMRPCQ LAKMISRYMI INETDRILMA MRPYQVYAVE ALIQQATETG NNGYVWHTTG SGKTLTSFKA SQILS

Table S3. Amino acid sequences for MS_fus, ½S and M½S_fus. The sequences derived from HsdS are underlined.

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MS_fus
MSITEKQRQQ QAELHKKLWS IANDLRGNMD ASEFRNYILG LIFYRFLSEK AEQEYADALS GEDITYQEAW ADEEYREDLK
AELIDQVGYF IEPQDLFSAM IREIETQDFD IEHLATAIRK VETSTLGEES ENDFIGLFSD MDLSSTRLGN NVKERTALIS
KVMVNLDDLP FVHSDMEIDM LGDAYEFLIG RFAATAGKKA GEFYTPQQVS KILAKIVTDG KDKLRHVYDP TCGSGSLLLR
VGKETQVYRY FGQERNNTTY NLARMNMLLH DVRYENFDIR NDDTLENPAF LGHTFDAVIA NPPYSAKWTA DSKFENDERF
SGYGKLAPKS KADFAFIQHM VHYLDDEGTM AVVLPHGVLF RGAAEGVIRR YLIEEKNYLE AVIGLPANIF YGTSIPTCIL
VFKKCRQQDD NVLFIDASND FEKGKNQNHL SDAQVERIID TYKRKETIDK YSYSATLQEI ADNDYNLNIP RYVDTFEEEA
PIDLDQVQQD LKNIDKEIAE VEQEINAYLK ELGVLKDEMS NTQKKNVPEL RFPGFEGEWE EKKLGEFAGK VTQKNVDKKY
IETLTNSAEL GIISQKDYFD KEISNIDNIK KYYVVEENDF VYNPRMSNYA PFGPVNRNKL GKKGVMSPLY TVFKIQNIDL
NFIEFYFKSS KWYRFMALNG DSGARADRFS IKDRTFMEMP LHIPCMDEQI KIGQFFSKLD RQIELEEQKL ELLQQQKKGY
MQKIFSQELR FKDENGKDYP EWEETTIKEI AQINTGKKDT KDAITNGSYD FYVRSPIVYK INTFSYEGEA ILTVGDGVGV
GKVFHYVNGK FDYHQRVYKI SDFKNYYGLL LFYYFSQNFL KETKKYSAKT SVDSVRKDMI ANMKVPRPIY IEQKKIGQFI
KRVDNKTKIQ KQVIELLKQR KKSLLQKMFI PGGSHHHHHH
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HsdM
MSITEKQRQQ QAELHKKLWS IANDLRGNMD ASEFRNYILG LIFYRFLSEK AEQEYADALS GEDITYQEAW ADEEYREDLK
AELIDQVGYF IEPQDLFSAM IREIETQDFD IEHLATAIRK VETSTLGEES ENDFIGLFSD MDLSSTRLGN NVKERTALIS
KVMVNLDDLP FVHSDMEIDM LGDAYEFLIG RFAATAGKKA GEFYTPQQVS KILAKIVTDG KDKLRHVYDP TCGSGSLLLR
VGKETQVYRY FGQERNNTTY NLARMNMLLH DVRYENFDIR NDDTLENPAF LGHTFDAVIA NPPYSAKWTA DSKFENDERF
SGYGKLAPKS KADFAFIQHM VHYLDDEGTM AVVLPHGVLF RGAAEGVIRR YLIEEKNYLE AVIGLPANIF YGTSIPTCIL
VFKKCRQQDD NVLFIDASND FEKGKNQNHL SDAQVERIID TYKRKETIDK YSYSATLQEI ADNDYNLNIP RYVDTFEEEA
PIDLDQVQQD LKNIDKEIAE VEQEINAYLK ELGVLKDE
½S
MS NTQKKNVPEL RFPGFEGEWE EKKLGEFAGK VTQKNVDKKY IETLTNSAEL GIISQKDYFD KEISNIDNIK KYYVVEENDF
VYNPRMSNYA PFGPVNRNKL GKKGVMSPLY TVFKIQNIDL NFIEFYFKSS KWYRFMALNG DSGARADRFS IKDRTFMEMP
LHIPCMDEQI KIGQFFSKLD RQIELEEQKL ELLQQQKKGY MQKIFSQELR FKDENGKGSH HHHHH
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M½S_fus
MSITEKQRQQ QAELHKKLWS IANDLRGNMD ASEFRNYILG LIFYRFLSEK AEQEYADALS GEDITYQEAW ADEEYREDLK
AELIDQVGYF IEPQDLFSAM IREIETQDFD IEHLATAIRK VETSTLGEES ENDFIGLFSD MDLSSTRLGN NVKERTALIS
KVMVNLDDLP FVHSDMEIDM LGDAYEFLIG RFAATAGKKA GEFYTPQQVS KILAKIVTDG KDKLRHVYDP TCGSGSLLLR
VGKETQVYRY FGQERNNTTY NLARMNMLLH DVRYENFDIR NDDTLENPAF LGHTFDAVIA NPPYSAKWTA DSKFENDERF
SGYGKLAPKS KADFAFIQHM VHYLDDEGTM AVVLPHGVLF RGAAEGVIRR YLIEEKNYLE AVIGLPANIF YGTSIPTCIL
VFKKCRQQDD NVLFIDASND FEKGKNQNHL SDAQVERIID TYKRKETIDK YSYSATLQEI ADNDYNLNIP RYVDTFEEEA
PIDLDQVQQD LKNIDKEIAE VEQEINAYLK ELGVLKDEMS NTQKKNVPEL RFPGFEGEWE EKKLGEFAGK VTQKNVDKKY
IETLTNSAEL GIISQKDYFD KEISNIDNIK KYYVVEENDF VYNPRMSNYA PFGPVNRNKL GKKGVMSPLY TVFKIQNIDL
NFIEFYFKSS KWYRFMALNG DSGARADRFS IKDRTFMEMP LHIPCMDEQI KIGQFFSKLD RQIELEEQKL ELLQQQKKGY
MQKIFSQELR FKDENGKDGS HHHHHH
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Table S4. Nucleotide sequences inserted into pUC19 to make the "E" series of plasmids for the DNA cleavage assay.

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CLONE E1
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CLONE E2
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CLONE E3
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CLONE E4
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CLONE E5
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CLONE E6
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CLONE E7
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CLONE E8
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CLONE E9
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CLONE E10
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CLONE E11
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CLONE E12
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CLONE E13
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CLONE E14
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CLONE E15
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CLONE E16
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CLONE E17
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CLONE E18
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CLONE E19
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CLONE E20
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ATACTCTGTATTGCAAATATGCGTCTATTAACTATTTGT
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Supplementary Figure S1. A PROMALS alignment of the amino acid sequences of the R subunit from *S. aureus* N315 with the R subunit of the Type IC enzyme, EcoR124I. The yellow shaded box indicates the motif essential for DNA cleavage in the nuclease domain. The green shaded boxes indicate the motifs in the motor domain essential for the ATPase-driven DNA translocation function associated with R subunits. The boxed sequences with the number of the RM_EB_X name underneath are the end points of the nuclease domain fragment fused to HsdM. "h" means alpha helix, "e" means beta strand, sequences in red or blue are the predicted or known secondary structures for the *S. aureus* sequence and the EcoR124I sequence respectively. The * indicates identical residues. (PROMALS: towards accurate multiple sequence alignments of distantly related proteins. Jimin Pei and Nick V. Grishin. *Bioinformatics* 2007; doi: 10.1093/bioinformatics/btm017).

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Conservation:                     *              ****  ** *   *   ***        * * * *
EcoR124I_GAANNNNNNRTCG_10    1  MTHQTHTIAESNNFIVLDKYIKAEPTGDSYQSESDLERELIQDLRNQGYEFISVKSQSAMLANVREQLQN  70
HsdR_subunit__SauN315ORF1    1  M------------------------AYQSEYALENEMMNQLEQLGYERVTIRDNKQLLDNFRTILNE      43
Consensus_ss:                                   eeeeeee     hhhhhhhhhhhhhh  ee    hhhhhhhhhhhhhh

Conservation:                       * *    * * *         *     *       ***     ***    ** *
EcoR124I_GAANNNNNNRTCG_10   71  -----LNGVVFNDSEWRRFTEQYLDNPSDGILDKTRKIHIDYICDFIFDDERLENIYLIDKKNLMRNKVQ  135
HsdR_subunit__SauN315ORF1   44  RHADKLEGNPLTDKEFQRLLTMIDG---KSIFESARILRDKL--PLRRDDESEIYLSFLDKKSWCKNKFQ  108
Consensus_ss:                   hhhhh       hhhhhhhhhhh     hhhhhhhhhhe eeeee  eeeeeeee        eee

Conservation:                      *         ****** ******* *** **   * *****   ** *        ** * *  * ***
EcoR124I_GAANNNNNNRTCG_10  136  IIQQFEQAGSHANRYDVTILVNGLPLVQIELKKRGVAIREAFNQIHRYSKESFNSENSLFKYLQLEVISN  205
HsdR_subunit__SauN315ORF1  109  VTNQVSVEDTYKARYDVTILINGLPLVQVELKRRGIDINEAFNQVKRYRKQN--YTGLFRYIQMEIISN   175
Consensus_ss:                   eeeeeeee       eeeeeee  eeeeeeee     hhhhhhhhhhhhh   hhh   eeeeeeee
                                                                                              8

Conservation:                   *  ****  * *      *   * *     *  *        *      *          * **
EcoR124I_GAANNNNNNRTCG_10  206  GTDTRYFANTTKRDKNSFDFTMNWAKSDNTLIKDLKDFTATQFQKHTLLNVLVNYSVFDSSQT-LLVMRE  274
HsdR_subunit__SauN315ORF1  176  GVETRYFSNNDSELLK--SHMFYWSDKQNNRINTLQSFAESFMRPCQLAKMISRYMIINETDRILMAMRF  243
Consensus_ss:                      eeee          hhhhhhhhh hhhhhhhhh hhh                        h
                                      9                      4    5 3       6                       7

Conservation:                   **  *  *               ** ****** *********        * * ** * *******
EcoR124I_GAANNNNNNRTCG_10  275  YQIAATERILWKIKSSFTAKNWSKPESGGYIWHTTGSGKTLTSFKAARLATELDFIDKVFFVVDRKDLDY  344
HsdR_subunit__SauN315ORF1  244  YQVYAVEALIQQATE---------TGNNGYVWHTTGSGRTLTSFKASQILSQQDDIKKVIFLVDRKDLDS  304
Consensus_ss:                   hhhhhhhhhhhhhhhhhhhhhh    eeeeee  hhhhhhhhhhhhhh    eeeee  hhhhh
                                      2              10             11

Conservation:                   **   *   *     *    **   * *     *******       *        ****  *****
EcoR124I_GAANNNNNNRTCG_10  345  QTMKEYQRFSPDSVNGSENTAGLKRNLDKDDNKIIVTTIQKLNNLMKAESDLPVY--NQQVVFIFDECHR  412
HsdR_subunit__SauN315ORF1  305  GTEEEFNKFAKGAVDKTFNTSQLVRQLNDKSLPLIVTTIQKMAKAIQGNAHLLEQYKTNKVVFIIDECHR  374
Consensus_ss:                   hhhhhhhhh    eeeee hhhhhhh   eeeehhhhhhhhhhhhhhhhh   eeeeee hhh

Conservation:                   ****     *  **   ******* ****    ***  *** ** *  *** *   ** * ***
EcoR124I_GAANNNNNNRTCG_10  413  SQFGDLAQKNLKKKFKRYYQFGFTGTPIFPENALG-SETTASVFGRELHSYVITDAIRDEKVLKFKVDYND  481
HsdR_subunit__SauN315ORF1  375  SQFGDMHRLVKQHFKNAQYFGFTGTPRFPENSSQDGRTTADIFGRCLHTYLIRDAIHDGNVLGFSVDYIN  444
Consensus_ss:                      hhhhhhhhhh   eeeeee   hhhhh   hhhh     hhhhhh       eeeeee

Conservation:                            *      *      *   *    *  * **    *               * *  *
EcoR124I_GAANNNNNNRTCG_10  482  VRPQFKSLETETDEKKLSAAENQQAFLHPMRIQEITQYILNNFRQKTHRTFPGSKGFNAMLAVSSVDAAK  551
HsdR_subunit__SauN315ORF1  445  TFKNKAL--KAEDNSMVEAIDTEEVWLADKRVELVTRHIINNHDKYTR-----NRQYSSIFTVQSIHALI  507
Consensus_ss:                      hhhhhhhhhhhhhhhhhhhhhhh  hhhhhhhhhhhhhhhhhhhhh   eeeee  hhhhh

Conservation:                        ** *****        ** ** *  **                    * *   *   **
EcoR124I_GAANNNNNNRTCG_10  552  AYYATFKRLQEEAANKSATYKPLRIATIFSFAANEEQNAIGEISDETFDTSAMDSSAKEFLDAAIREYNS  621
HsdR_subunit__SauN315ORF1  508  KYYETFKRLNKKLEQPL------TIAGIFTFKPNEDDRDGEVPY-----------HSREKLEIMISDYNK  560
Consensus_ss:                   hhhhhhhhhhhhhh     eeeeeee  hhhhhhhhhh        hhhhhhhhhhhhhhh

Conservation:                     * ******     *         **   ** **** *******   **** ***** ** * ** ***
EcoR124I_GAANNNNNNRTCG_10  622  HFKTNFSTD--SNGFQNYYRDLAQRVKNQDIDLLIVVGMFLTGFDAPTLNTLFVDKNLRYHGLMQAFSRT  689
HsdR_subunit__SauN315ORF1  561  KFETNFSTDTTNEYFNHISKNVKKGVKDSKIDILIVVNMFLTGFDSKVLNTLYVDKNLMYHDLIQAYSRT  630
Consensus_ss:                   hh            hhhhhhhhhh   eeeeeee hhh   eeeeee   hhhhhhhhhh

Conservation:                   **       * ** ** ***   * **    *   * ** **   * **
EcoR124I_GAANNNNNNRTCG_10  690  NRIYDATKTFGNIVTFRDLERSTIDAITLFGDKNTKNVVLEKSYTEYMEGFTDAATGEAKRGFMTVVSEL  759
HsdR_subunit__SauN315ORF1  631  NRVEKESKPFGKIVNYRDLKKETDDALRVFSQTNDTDTIIMRSYEEYKKEFMDAYRE---------LKMI  691
Consensus_ss:                             eeeeee   hhhhhhhhhh    hhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhh

Conservation:                         *   * ** **  ***       * ***         * *
EcoR124I_GAANNNNNNRTCG_10  760  EQRFPDPTSIESEKEKKDFVKLFGEYLRAENILQNYDEFATLKALQQIDLSDPVAVEKFKAEHYVDDEKF  829
HsdR_subunit__SauN315ORF1  692  VPTPHMVDDIQDEEEELKRFVEAYRLLAKIILRLKAFDEFEFTIDEIGMDEQENED--------------  746
Consensus_ss:                   hhhhhhhh   hhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhh
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Conservation:                                      *  *  *     *        *          *  **      **  **
EcoR124I_GAANNNNNNRTCG_10      830  AELQTIRLPADRKIQDYRSAYNDIRDWQRREKEAEKKEKSTTDWDDVVFEVDLLKSQEINLDYILGLIFE  899
HsdR_subunit__SauN315ORF1      747  ----------------YKSKYLAVYDQVK---RATAEKNKVSILNDIDFEIEMMRNDTINVNYIMNILRQ  797
Consensus_ss:                      hhhhhh     hhhhhhhhhhhhhhhhhhhhhhhhhhh hhhhhhhhhhhhhhhhhhh hhhhhhhhhh

Conservation:                          *        *  *  *         *  *  *    **        *        *       *         *
EcoR124I_GAANNNNNNRTCG_10      900  HNRQNKGKGEMIEEVKRLIRS---SLGNRAKEGLVVDFIQQTNLDDLPDKASIIDAFFTFAQREQQREAE  966
HsdR_subunit__SauN315ORF1      798  IDLEDKAEQRRNQEQIRRILDHADDPTLRLKRDLIREFIDNVVP-SLNKDDDIDQEYVNFESIKKEAEFK  866
Consensus_ss:                      hhh  hhhhhhhhhhhhhhh  hhhhhhhhhhhhhhhh    hhhhhhhhhhhhhhhhhhhh

Conservation:                          *     *  *  *                    **          *
EcoR124I_GAANNNNNNRTCG_10      967  ALIKEENLNEDAAKRYIRTSLKREYATENGTELNETLPKLSPLNPQYKTKKQAVFRKSSRLLRSLKA 1033
HsdR_subunit__SauN315ORF1      867  GFAGERSIDEQALKTISNDYQYSGVVNP---HHLKKMIGDLPLKEK-RKARKAIESFVAETTEKYGV  929
Consensus_ss:                      hhhhhh  hhhhhhhhhhhhh hhhhhhhhhhhhhhhh    hhhhhhhhhhhhhhhhhhhhhh
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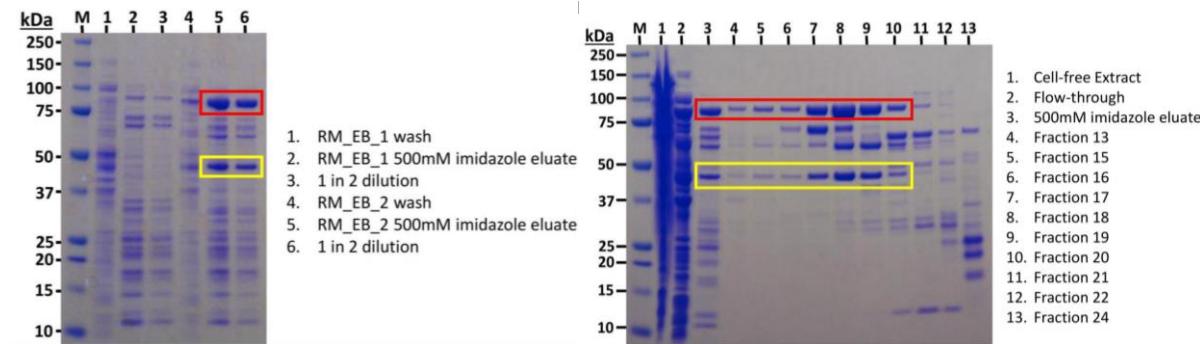

Figure S2. Nickel affinity purification of the RM_EB_2 RM fusion protein (left panel). Size exclusion purification of the RM_EB_2 RM fusion protein collected from the nickel affinity column (right panel). The red rectangle highlights the RM fusion protein, whilst the yellow rectangle indicates the HsdS subunit. M indicates the marker lane.
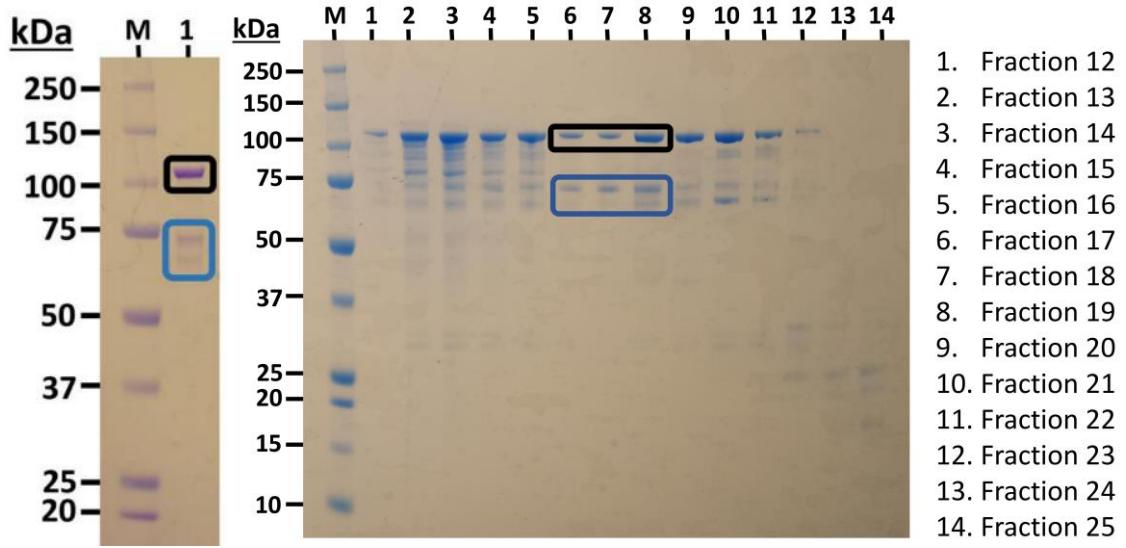
Figure S3. SDS-PAGE analysis of the purified MS$_{fus}$ protein (black box, left panel). The gel also shows a couple of contaminants (light blue box), the smaller of which was identified as a truncated form of the fusion. M is a marker lane. SDS-PAGE analysis of samples from the Sephacryl S200 size exclusion purification of the MS$_{fus}$ protein (right panel). The bands highlighted by the black box are the target protein, whilst the blue box highlights the co-eluting fragments. M – marker, lanes 1 to 14 are fractions 12 to 25 from the elution.
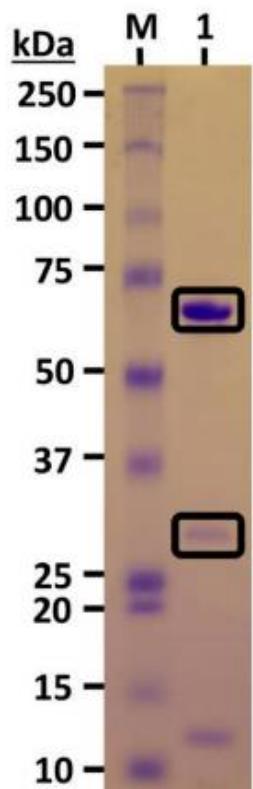
Figure S4. SDS-PAGE analysis of the purified M½S MTase. The black boxes highlight the HsdM subunit (upper) and the half HsdS protein (lower). M is a marker lane.
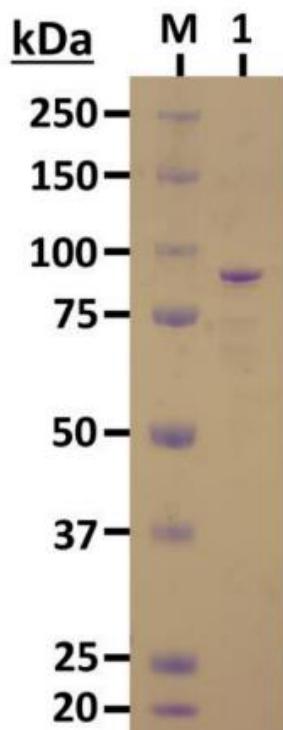
Figure S5. SDS-PAGE analysis of the purified M½S$_{fus}$ MTase fusion. M is a marker lane.
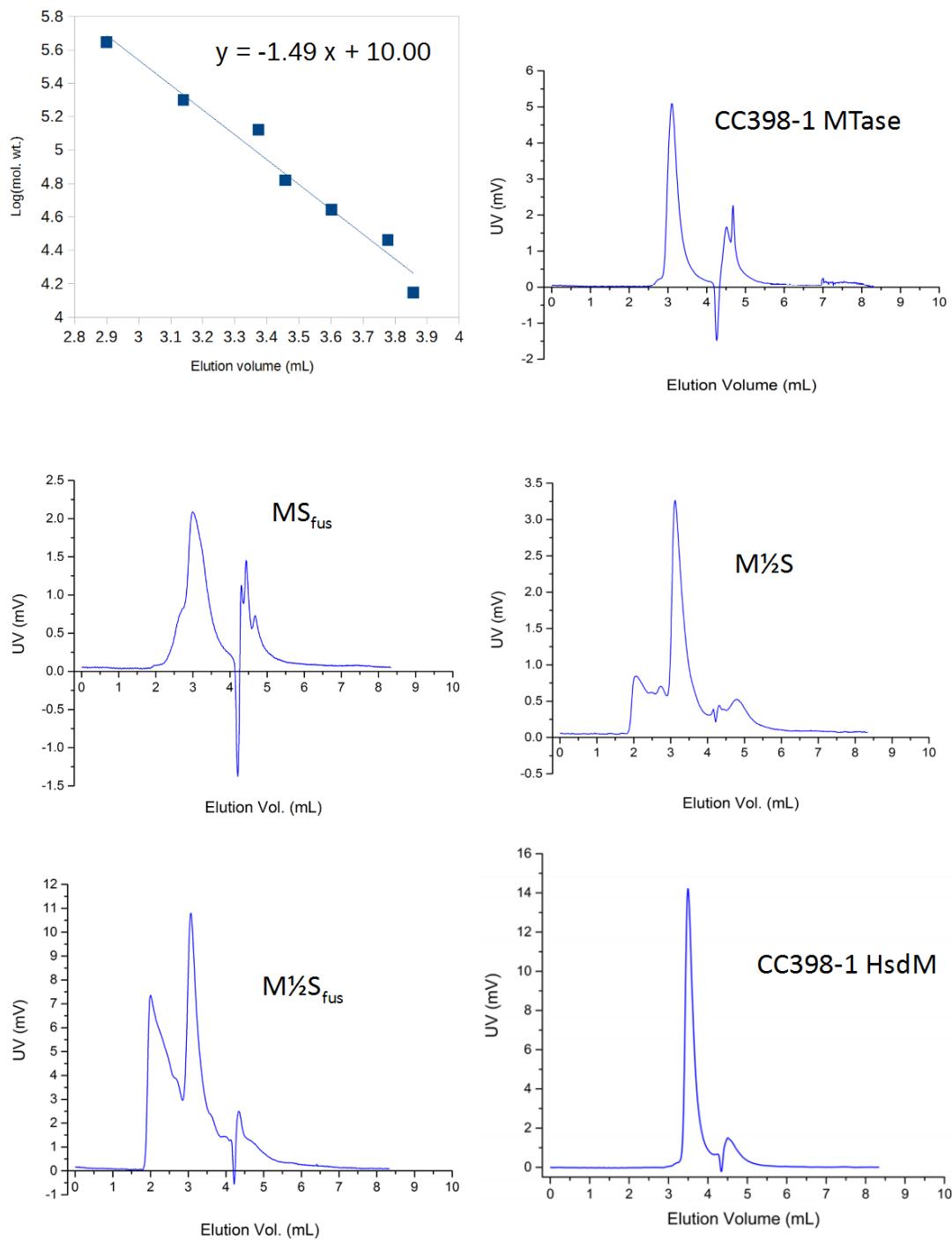
Figure S6. Analytical size exclusion analyses of wild type MTase (CC398-1), the MTase derivatives and the purified HsdM subunit from the CC398-1 RM system (CC398-1 HsdM). In the top left panel from left to right the calibration proteins are apoferritin, β-amylase, bovine serum albumin (dimer), bovine serum albumin (monomer), ovalbumin, carbonic anhydrase, α-lactalbumin. HPLC gel filtration used a Biosep-SEC-S3000 (30 cm x 0.46 cm) gel filtration column (Phenomenex); 50 μL samples in 20 mM Tris, 20 mM MES, 0.2 M NaCl, 10 mM MgCl$_2$, 7 mM β-mercaptoethanol, and 0.1 mM EDTA, pH 6.5, were injected onto the column. The flow rate was 0.5 mL/min, and detection was at 280 nm. The baseline oscillation at ~4.3 minutes in the elution profiles is due to the elution of the small amount of glycerol present in each injected protein sample. Material eluting prior to the main peak at ~3 minutes is due to protein aggregation in the injected sample.

Table S5. Molecular weights of main elution peaks from analytical size exclusion chromatography shown in Figure S6.

Species	Assumed stoichiometry	Expected Mol. Wt. (kDa) for assumed stoichiometry	Measured Mol. Wt. (kDa)
MTase	M_2S_1	166	241
M½S	$(M½S)_2$	172	225
MS_{fus}	$(MS_{fus})_2$	213	325
$M½S_{fus}$	$(M½S_{fus})_2$	173	251
HsdM	$(HsdM)_1$	59	61
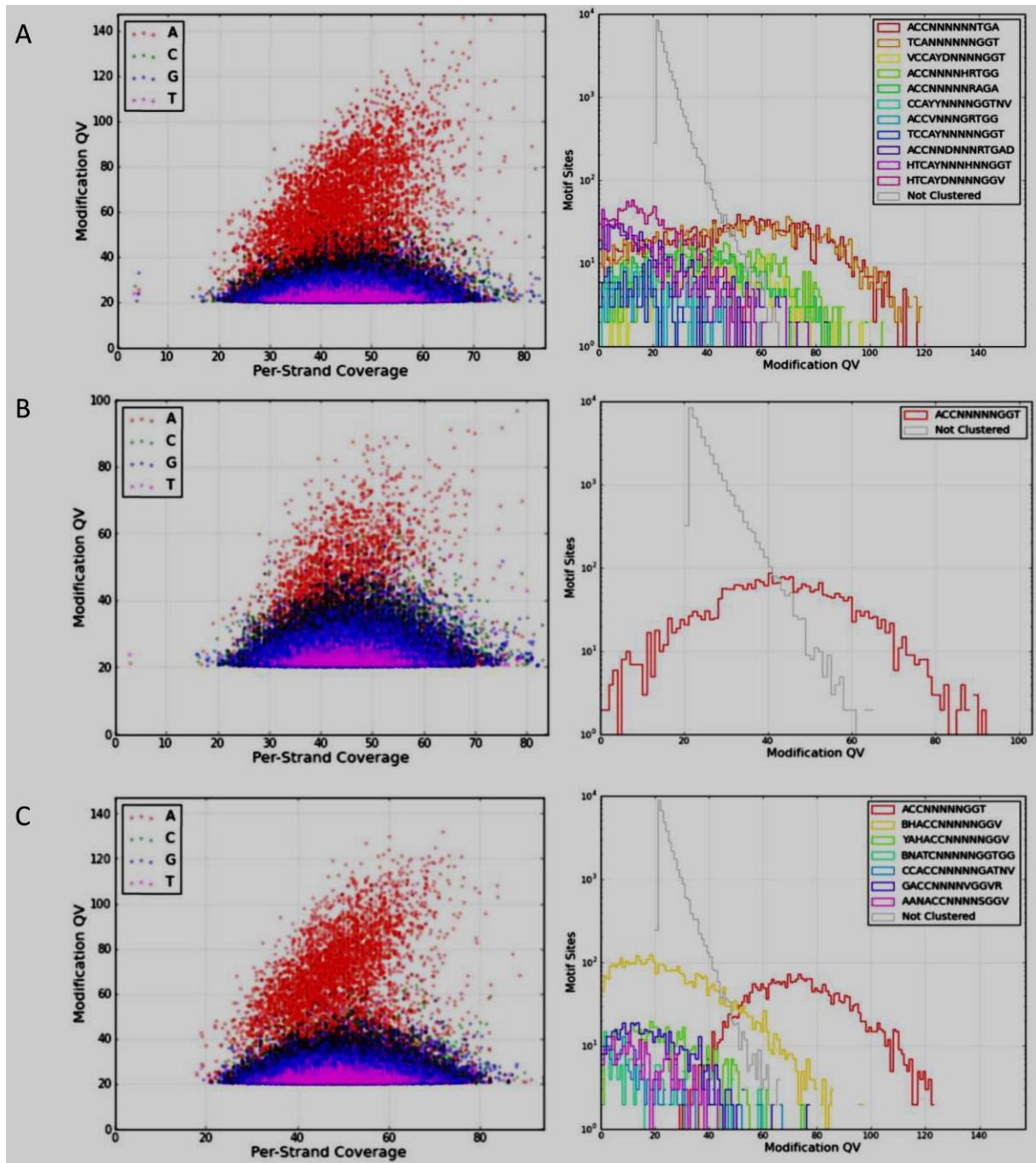
Figure S7. Results from SMRT Sequencing analysis of *E. coli* ER2796 genomic DNA, after modification by the MS$_{fus}$ MTase (A), M½S MTase (B), and the M½S$_{fus}$ MTase (C). Graphs on the left-hand side are scatter plots, which show the frequency of detection of specific base modification. For each of the MTases there is a higher frequency of points corresponding to N6-methylated adenine (red) with high quality value (QV). This indicates that the MTases are adenine MTases. The graphs on the right-hand side show the frequency of detection of methylated DNA sequence motifs. Again, in each case the red line shows the methylated motif detected at the highest frequency. In the case of the MS$_{fus}$ MTase (A), this is the wild-type motif, ACCN$_5$RTGA and its complement. For the other two MTases, M½S MTase (B) and the M½S$_{fus}$ MTase (C), this is the palindromic sequence, ACCN$_5$GGT.
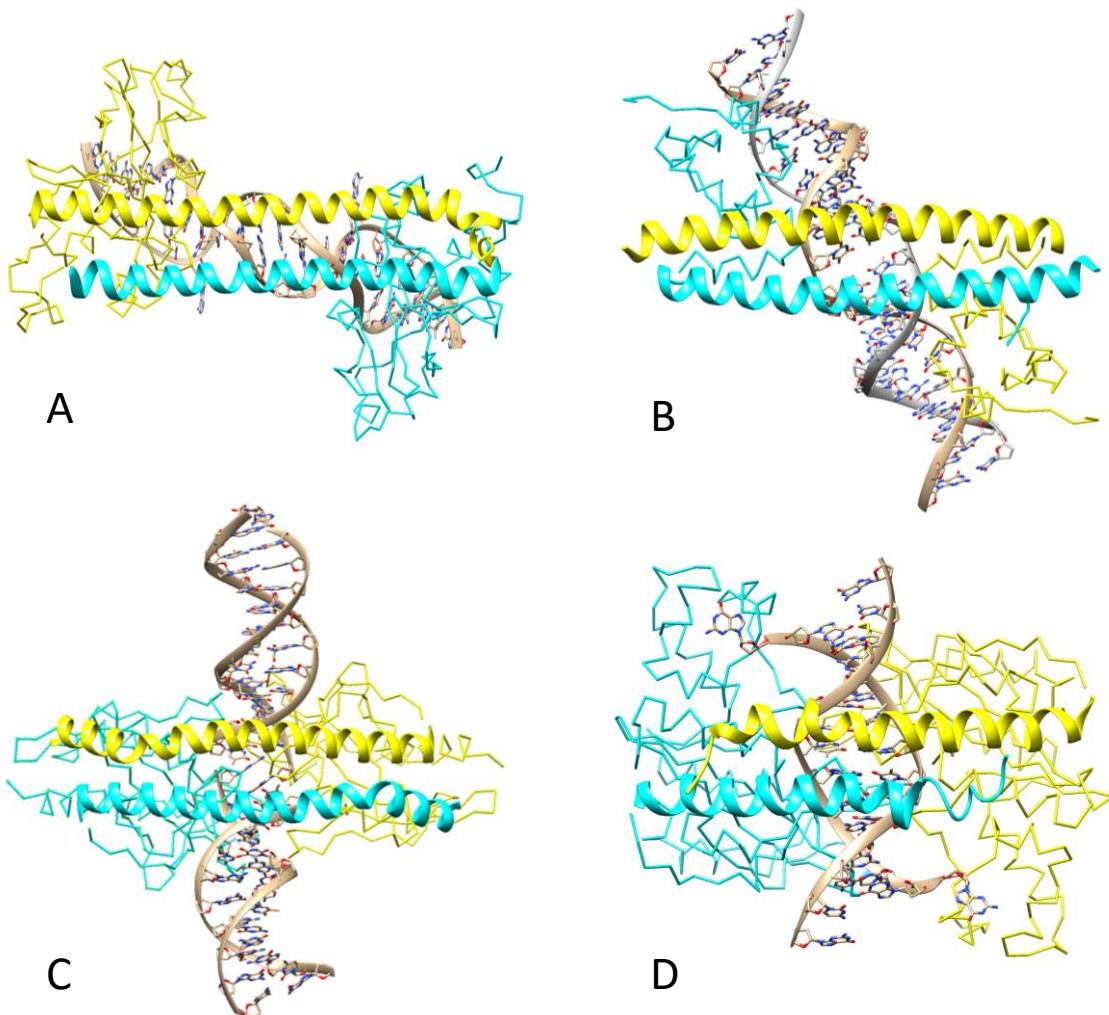
Supplementary figure S8. The angle between the two α helices (yellow and cyan, one from each protein subunit) and the helical axis of the bound DNA increases as the two parts of the target sequences (and the methylation/cleavage sites) become closer. (A) EcoKI HsdS shown bound to AACNNNNNNGTGC (PDB: 2Y7H, underlined bases are locations for methylation and 8 bp apart, amino acids 1-430 shown). (B) BmrR transcription factor bound to operator DNA containing the internal repeat TCCGGANNNNCCGGA (PDB:3D71, amino acids 1-117 shown, DNA sequences only 4 bp apart). (C) SwaI REase bound to DNA containing target ATTT^AAAT (PDB: 5TH3, blunt end cleavage marked by ^, palindromic target has no non-specific bp). (D) HincII REase bound to DNA containing target GTY^RAC (PDB: 2GIE, blunt end cleavage marked by ^, palindromic target has no non-specific bp).