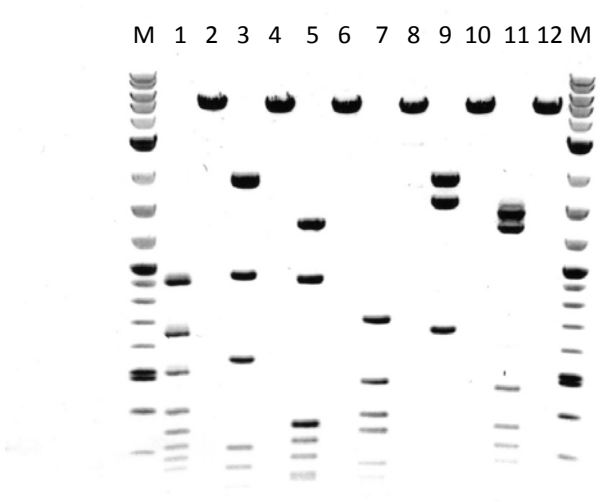
Supplementary Figure 1

Native mass spectrometry and SDS-PAGE analyses of purified M.EcoGII MTase

Supplementary Figure 2

M.EcoGII methylation of plasmid DNA *in vitro* inhibits cleavage by multiple restriction endonucleases (4-base cutters)



	Enzyme 1	Enzyme 2
Lanes 1, 2	BamHI (GGAT<u>C</u>C)	AluI (<u>A</u>GCT)
Lanes 3, 4	BamHI	MluCI (AATT)
Lanes 5, 6	BamHI	MseI (TTA<u>A</u>)
Lanes 7, 8	BamHI	NlaIII (C<u>A</u>TG)
Lanes 9,10	BamHI	RsaI (GT<u>A</u>C)
Lanes 11,12	BamHI	TaqI (TCG<u>A</u>)

Lanes 1, 3, 5, 7, 9 and 11: Unmodified pBR322 DNA
Lanes 2, 4, 6, 8, 10 and 12: M.EcoGII-modified pBR322 DNA

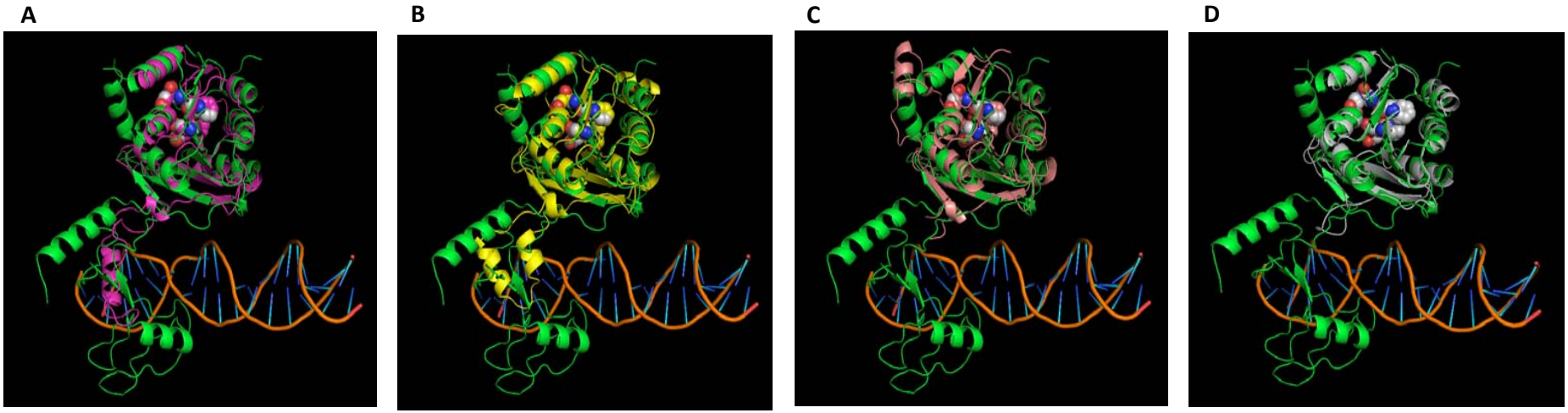
<u>A</u>, <u>C</u> = Sites of action of cognate MTases associated with each restriction endonuclease *in vivo* (where known).

M = 2-log Ladder 0.1-10.0 kb (NEB)

pBR322 plasmid DNA was prepared from *E. coli* ER2796 (a non-methylating strain lacking *dam*, *dcm* and *M.EcoKI* activities) and a sample of this DNA was subsequently methylated *in vitro* using purified M.EcoGII enzyme. Unmethylated and M.EcoGII-methylated DNA samples were each incubated with BamHI which is not inhibited by adenine methylation and another restriction enzyme that is known to be inhibited by this modification.

Supplementary Figure 3

Comparison of predicted M.EcoGII model structures derived from PHYRE2 threading analyses



The M.EcoGII model, determined by threading onto the EcoP15I modA structure with bound DNA, is shown in green in each panel. Panel A: comparison of EcoP15I model with M1.MboII model (magenta). Panel B: comparison of EcoP15I model with M.RsrI model (yellow). Panel C: comparison of EcoP15I model with M.PvuII model (wheat). Panel D: comparison of EcoP15I model with M1.HpyAVI model (grey). Note the significant differences in the predictions for the DNA recognition domain models (lower left) compared to the conservation of the MTase core in each case. In the case of the M.PvuII and M.HpyAVI templates no structural model was predicted for the DNA recognition domain (Panels C and D).

Supplementary Table I: M.EcoGII homologs

Source microorganism	% identity	(Pro)Phage	Reference
Escherichia coli O104:H4 C227-11 (M.EcoGII)	100	JQ182728	S1.
Escherichia coli O104:H4 C227-11 (M.EcoGI)	95	JQ086376	S1.
Escherichia coli (>700)	100-80	many	S2.
Escherichia coli O104:H4 11-4632 C3	97	AFVC01000043	S3.
Enterobacteria phage BP-4795	95	AJ556162	S4.
Escherichia coli DEC13E	95	AIHJ01000011	S5.
Shigella boydii Sb22	89	CP000036	S6.
Citrobacter rodentium ICC168	78	FN543502	S7.
Enterobacter species R4-368	78	CP00591	S8.
Kosakonia oryzae KO348	78	JZLI01000039	S9.
Cronobacter muytjensii ATCC 51329	78	CP012268	S10.
Enterobacter radicincitans DSM 16656	77	AKYD01000017	S11.

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SUPPLEMENTARY TABLE 2

LC-MS data for time course assay of M.EcoGII activity presented in Figure 2, panels C and D

Plasmid DNA	m6dA / (m6dA + dA)	Plasmid DNA	m6dA / (m6dA + dA)
pUC19 dam+ 01A	**1.9%**	pUC19 dam+ 07A	**71.0%**
pUC19 dam+ 01B	**2.0%**	pUC19 dam+ 07B	**71.2%**
pUC19 dam+ 02A	**2.1%**	pUC19 dam+ 08A	**71.0%**
pUC19 dam+ 02B	**2.1%**	pUC19 dam+ 08B	**71.2%**
pUC19 dam+ 03A	**51.4%**	pUC19 dam+ 09A	**79.2%**
pUC19 dam+ 03B	**52.2%**	pUC19 dam+ 09B	**79.2%**
pUC19 dam+ 04A	**52.3%**	pUC19 dam+ 10A	**77.8%**
pUC19 dam+ 04B	**52.4%**	pUC19 dam+ 10B	**77.7%**
pUC19 dam+ 05A	**65.5%**	pUC19 dam+ 11A	**84.5%**
pUC19 dam+ 05B	**65.5%**	pUC19 dam+ 11B	**84.7%**
pUC19 dam+ 06A	**66.1%**	pUC19 dam+ 12A	**85.1%**
pUC19 dam+ 06B	**66.2%**	pUC19 dam+ 12B	**85.2%**