

Supplementary Figure S1.

SMRT methylome analysis results for six native bacterial strains that have 2-MTase Type I R-M systems. All six methylomes indicate a split recognition motif characteristic of Type I R-M systems, but in which one strand contains m4C modification and one strand m6A modification. Additional modification motifs identified for these strains are also shown.

DSM 2075: *Desulfarculus baarsii*

Reports for Job DSM2075									
SMRT Cells: 2 Movies: 2									
Motif	Modified Position	Modification Type	% Motifs Detected	# Of Motifs Detected	# Of Motifs In Genome	Mean Modification QV	Mean Motif Coverage	Partner Motif	
RGATCY	3	m6A	94.74	4,517	4,768	51.9	28.5	RGATCY	
GAAGNNNNNGGC	3	m6A	91.93	1,389	1,511	49.7	27.6	GCCNNNNNCTTC	
GCCNNNNNCTTC	2	m4C	68.83	1,040	1,511	41.9	30.9	GAAGNNNNNGGC	
AGAAAT	5	m6A	88.37	661	748	47.8	27.4		
CCGCGCC	4	m4C	63.60	4,137	6,505	40.8	29.7		
Not Clustered	0		0.01	888	7,296,419	34.2	32.0		

DSM5219: *Methanohalophilus mahii*

Reports for Job DSM_5219_3									
# of SMRT Cells: 3 # of Movies: 5									
Motif	Modified Position	Modification Type	% Motifs Detected	# Of Motifs Detected	# Of Motifs In Genome	Mean Modification QV	Mean Motif Coverage	Partner Motif	
CGANNNNNRGA	3	m6A	94.02	1,369	1,456	57.7	42.2	TCYNNNNNTCG	
TCYNNNNNTCG	2	m4C	72.46	1,055	1,456	46.6	42.3	CGANNNNNRGA	
CGADNNNNWAGCT	3	m6A	82.61	19	23	45.5	41.8		
AGCT	3	m4C	80.96	7,380	9,116	50.2	41.3	AGCT	
Not Clustered	0		0.02	931	4,012,797	34.8	45.7		

DSM 2380: *Pelobacter carbinolicus*

Reports for Job DSM_2380_4									
# of SMRT Cells: 4 # of Movies: 8									
Motif	Modified Position	Modification Type	% Motifs Detected	# Of Motifs Detected	# Of Motifs In Genome	Mean Modification QV	Mean Motif Coverage	Partner Motif	
GACGAG	5	m6A	80.96	1,033	1,276	46.4	29.3		
CGCANNNNNGGC	4	m6A	75.87	676	891	46.3	29.8	GCCNNNNNTGCG	
GCCNNNNNTGCG	2	m4C	52.53	468	891	40.8	31.1	CGCANNNNNGGC	
Not Clustered	0		0.02	1,292	7,328,728	34.0	35.8		

DSM12260: *Aminomonas paucivorans*

Reports for Job DSM12260_mods_6poorcels									
SMRT Cells: 6 Movies: 6									
Motif	Modified Position	Modification Type	% Motifs Detected	# Of Motifs Detected	# Of Motifs In Genome	Mean Modification QV	Mean Motif Coverage	Partner Motif	
CAGNNNNCTCG	2	m6A	30.07	138	459	42.4	18.4	CGAGNNNNCTCG	
CGAGNNNNCTCG	3	m6A	25.49	117	459	44.0	18.4	CAGNNNNCTCG	
GGAGNNNNNGGC	3	m6A	20.29	648	3,194	41.1	18.7		
Not Clustered	0		0.06	3,089	5,256,228	34.9	26.7		

DSM 2522: *Bacillus cellulosilyticus*

Reports for Job DSM_2522_3									
# of SMRT Cells: 3 # of Movies: 5									
Motif	Modified Position	Modification Type	% Motifs Detected	# Of Motifs Detected	# Of Motifs In Genome	Mean Modification QV	Mean Motif Coverage	Partner Motif	
GAGNNNNNGGG	2	m6A	97.47	732	751	61.0	37.2	CCCNNNNNCTC	
CCCNNNNNCTC	2	m4C	66.44	499	751	43.6	39.1	GAGNNNNNGGG	
RCDGCAGCNV	2	m4C	37.32	228	611	41.2	41.6		
Not Clustered	0		0.04	3,707	9,361,231	35.9	42.3		

DSM14238: *Aequorivita sublithicola*

Reports for Job DSM_14238_4									
# of SMRT Cells: 4 # of Movies: 8									
Motif	Modified Position	Modification Type	% Motifs Detected	# Of Motifs Detected	# Of Motifs In Genome	Mean Modification QV	Mean Motif Coverage	Partner Motif	
GAANNNNCTGGAG	13	m6A	100.00	40	40	61.9	51.3		
CGTRAC	5	m6A	99.79	1,433	1,436	74.4	52.1		
AGGAAG	5	m6A	99.79	2,344	2,349	78.0	51.4		
CCANNNNNTTC	3	m6A	99.39	2,286	2,300	75.5	52.9	GAANNNNNTGG	
GAANNNNNTGG	3	m6A	98.96	2,276	2,300	71.9	52.5	CCANNNNNTTC	
GGANNNNNGGC	3	m6A	96.37	770	799	70.9	53.4	GCCNNNNNTCC	
GCCNNNNNTCC	2	m4C	94.12	752	799	57.1	54.0	GGANNNNNGGC	
CATG	2	m6A	93.76	5,067	5,404	65.2	53.5	CATG	
Not Clustered	0		0.10	6,996	7,025,915	36.6	57.2		

Supplementary Figure S2.

SMRT methylome analysis results for each of the 2-MTase Type I systems identified in Figure S1, here expressed in the non-methylating *E. coli* host ER2796. The expression constructs contain the MTase only (ie, both MTase genes and the S (specificity) gene, but not the endonuclease gene). Differences in the "percent Motifs detected" reflect differing levels of coverage. The multiple, closely related putative motifs for DbaKI reflect both potential 'star activity' from expression in *E. coli*, and also over-calling by the SMRT analysis software.

DbaKI from DSM 2075: *Desulfarculus baarsii*

Reports for Job DbaKI M1M2S_pACYC_2796								
SMRT Cells: 1 Movies: 1								
Motif Summary								
Motifs	Modified Position	Modification Type	% Motifs Detected	# Of Motifs Detected	# Of Motifs In Genome	Mean Modification QV	Mean Motif Coverage	Partner Motif
GCCNNNNCTTC	2	m4C	97.74%	908	929	58.81	46.51	
GAAGNNNNNGDC	3	m6A	86.42%	1935	2239	65.51	43.46	
GAAGNNNNVAGC	3	m6A	51.72%	301	582	46.27	44.53	
GWAAGNNNNNGGC	4	m6A	40.29%	166	412	47.39	43.38	
GAAATNNNVGGC	3	m6A	30.48%	64	210	38.31	46.55	
GACNNNNCTTC	2	m6A	30.2%	183	606	50.23	46.61	
GAAGNNNNNGGAS	3	m6A	28.35%	55	194	41.84	47.15	
GCTNNNNHCTTCH	2	m4C	20.77%	76	366	43.17	46.97	
GCCNNNVCTTWC	2	m4C	17.39%	56	322	44.25	48.07	

Mma5219I from DSM5219: *Methanohalophilus mahii*

Reports for Job DSM5219_MScClone_vs_2796								
SMRT Cells: 1 Movies: 1								
Motif Summary								
Motif	Modified Position	Modification Type	% Motifs Detected	# Of Motifs Detected	# Of Motifs In Genome	Mean Modification QV	Mean Motif Coverage	Partner Motif
CGANNNNNRGA	3	m6A	73.43	2,957	4,027	43.8	25.3	TCYNNNNNTCG
TCYNNNNNTCG	2	unknown	33.00	1,329	4,027	37.7	27.1	CGANNNNNRGA
CGANNVNAAGCD	3	m6A	32.68	133	407	48.0	53.2	
Not Clustered	0		0.01	864	9,117,747	37.2	144.5	

PcaI from DSM 2380: *Pelobacter carbinolicus*

Reports for Job DSM2380_MScClone_vs_2796								
SMRT Cells: 1 Movies: 1								
Motif Summary								
Motif	Modified Position	Modification Type	% Motifs Detected	# Of Motifs Detected	# Of Motifs In Genome	Mean Modification QV	Mean Motif Coverage	Partner Motif
CGCANNNNNGGC	4	m6A	15.85	155	978	35.6	17.6	
Not Clustered	0		0.00	266	9,125,230	44.0	628.7	

Apa12260I from DSM12260: *Aminomonas paucivorans*

Reports for Job Apa12260_pACYC1_new_MODS								
SMRT Cells: 1 Movies: 1								
Motif Summary								
Motifs	Modified Position	Modification Type	% Motifs Detected	# Of Motifs Detected	# Of Motifs In Genome	Mean Modification QV	Mean Motif Coverage	Partner Motif
GGAGNNNNNGGC	3	m6A	100.0%	374	374	113.96	78.18	GCCNNNNCTCC
GCCNNNNCTCC	2	m4C	100.0%	374	374	84.89	79.31	GGAGNNNNNGGC

BceNI from DSM 2522: *Bacillus cellulosilyticus*

Reports for Job DSM2522_MScClone_vs_2796								
SMRT Cells: 1 Movies: 1								
Motif Summary								
Motif	Modified Position	Modification Type	% Motifs Detected	# Of Motifs Detected	# Of Motifs In Genome	Mean Modification QV	Mean Motif Coverage	Partner Motif
GAGNNNNNGG	2	m6A	47.19	411	871	39.2	17.8	
Not Clustered	0		0.00	316	9,125,337	33.8	26.3	

Asu14238II from DSM14238: *Aequorivita sublithincola*

Reports for Job DSM14238_MScClone_vs_2796								
SMRT Cells: 1 Movies: 1								
Motif Summary								
Motif	Modified Position	Modification Type	% Motifs Detected	# Of Motifs Detected	# Of Motifs In Genome	Mean Modification QV	Mean Motif Coverage	Partner Motif
GGANNNNNGGC	3	m6A	31.85	709	2,226	37.4	28.5	
Not Clustered	0		0.01	633	9,123,982	34.0	41.3	