Novel nonspecific DNA adenine methyltransferases

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SUPPLEMENTARY DATA

Table 1S. Oligonucleotide primers used for cloning and mutagenesis. Introduced restriction enzyme sites are indicted in bold.

Name of the primer	Primer sequence (5' – 3')	Restriction site created or mutagenic substitution
HindNco	**CCATGG**CAAACAAAAAAACTTTTAAACAAGC	NcoI,
HindXho	**CTCGAG**GAATTTATAAATCATATTATCCTCATAAATTCC	XhoI
NmeNde	**CATATG**ATGCAAAAATACCACTCAACGGCC-	NdeI
NmeXho	**CTCGAG** ATCTATTTTATAAATCATATTGTCCTGATAC	XhoI
8Hf	GTTGGCGGTTTAGTCCGAATCAACTTC	
8Hr	ACCGTTTTATTGTTTACTTTCCG	
8HfNde	**CATATG**GCAAATCAAAACACCTTTAAACAAGC	NdeI
8HXho	**CTCGAG**GAATTTATAGACTAAATTGTCTTCATACGCCACTTG	XhoI
Hia5APPYf	cgCCACCTTACCTTTGCACCAAGCAG*	D194A
Hia5APPY	ccAATACAAACAACGCTTTCGGATCATTGC	D194A
NmeAPPf	gcgCCGCCTTATGTGTCCACTGCTCAGGG	D191A
NmeAPPr	cAATACCAGCAGAGTATTGGGGTTATGCTGATG	D191A
HinAPPf	gCCACCTTATTTATGCACTCGCCAAGAGAGTTAC	D194A
HinAPPr	cgCAAGTAATAGTAGCACCTTATCTTGATTTTG	D194A
Pvu1AT	GACTAGTAGATCTTAAGAAGGAGATATAACATGTCTGCAGCGAT	PvuI**
Pst1TGCA	CGCTGCAGACATGTTATATCTCCTTCTTAAGATCTACTAGTCTGCA	PstII**

* Lowercase letters represent nucleotides not complementary to the template

**The oligonucleotide duplex PstTGCA/Pvu1AT has sticky ends to create PstI and PvuI sites.

Table 2S. Oligonucleotide duplex DNAs used as substrates for *in vitro* methylation assays.

Duplex*	Length (bp)	Number of adenine residues	Sequence (5' – 3')
CA10	21	10	CACACACACACACACACACAC GTGTGTGTGTGTGTGTGTGTG
GA10	21	10	GAGAGAGAGAGAGAGAGAGAG CTCTCTCTCTCTCTCTCTCTC
TA50	50	50	ATATATATATATATATATATATATATATATATATATATATATATATATAT TATATATATATATATATATATATATATATATATATATATATATATATATA
AA21	21	21	AAAAAAAAAAAAAAAAAAAAA TTTTTTTTTTTTTTTTTTTTT
Cm2A1	21	3(1)**	CCCCCCCCCaAaCCCCCCCCC*** GGGGGGGGGTTTGGGGGGGGG
Cm2A3	23	5(3)**	CCCCCCCCCaAAAaCCCCCCCCC*** GGGGGGGGGTTTTTGGGGGGGGG

* To anneal duplexes, the complementary oligonucleotides were mixed, heated to 90°C for 5 min and slowly cooled to 25°C.

**() number of presumed substrate adenines in the duplex

***a - the marked adenine (lower case) is m^6A

Table 3S. Summary of the sensitivity of different restriction endonucleases to modifications introduced by the Nme1821 enzyme.

REase	Recognition site	Number of sites in pNmeET	Cleavage[a]
AdeI	CACNNNGTG	1	N
BglII	AGaTCT	1	N
Bsh1285I	CGRYCG R=A/G	5	N
Bsp119I	TTCGAA	0(2)[b]	N
Bsu15I	ATCGAT	2	N
CaiI	CAGNNNCTG	1	N
CseI	GACGC	12	N
CviQI	GTAC	6	N
DraI	TTTAAA	2	N
Eam1104I	GAAGAG	3	N
Eco147I	AGGCCT	0(1)[b]	N
Eco47III	AGCGCT	3	N
Eco91I	GGTNACC	1	N
Eco32I	GATATC	0(1)[b]	P, L
EcoRI	GAATTC	1	N
HindIII	AaGCTT	0(1)[b]	N
HinfI	GANTC	20	N
HphI	GGTGA/TCACC	17	N
LweI	GCATC	27	N
MboI*	GATC	23	N
MluI	ACGCGT	1	N
MlyI	GAGTC/GACTC	9	N
Mph1103I	ATGCAT	2	N
MunI	CAATTG	2	N
NdeI	CaTATG	1	N
NlaIII	CATG	26	N
PaeI	GCATGC	1	N
PfeI	GAWTC	11	N
PscI	ACATGT	1	N
PstI	CTGCAG	1	N
PvuII	CAGCTG	3	N
SchI	GAGTC/GACTC	9	N
XbaI	TCTGAG	1	N
XhoI	CTCGAG	1	N
XmiI	GTMKAC	3	N
BpiI	GAAGAC	5	P
AluI	AGCT	24	P,
HincII	GTYRAC	2	P, L
KspAI	GAATTC	1	P, L
SspI	aATATT	3	P
TaiI	ACGT	18	P, L
TasI	AATT	27	P
TaqI	TCGA	18	P, L
Tru1I	TTAA	28	P
VspI	ATTAAT	5	P,L
XapI	RAATTY	6	P

REase	Recognition site	Number of sites in pNmeET	Cleavage[a]
BcnI	CCSGG	13	Y
BglI	GGCN5GCC	2	Y
Bme1390I	CCNGG	23	Y
Bpu1102I	GCTNAGC	2	Y
Bsh1236I	CGCG	37	Y
Bsp143I*	GATC	23	Y
Bsp120I	GGGCCC	1	Y
Bsp68I	TCGCGA	1	Y
BspLI	GGNNCC	22	Y
Bst1107I	GTATAC	1	Y
BsuRI	GGCC	28	Y
Csp6I	GTAC	6	Y
DpnI*	Gm^6ATC	23	Y
Eco130I	CCWWGG	2	Y
Eco88I	CYCGRG	2	Y
EcoO109I	RGGNCCY	3	Y
FspBI	CTAG	8	Y
Hin1I	GRCGYC	5	Y
Hin6I	GCGC	53	Y
HpyF10VI	GCN7GC	46	Y
MbiI	GAGCGG/CCGCTC	4	Y
MspI	CCGG	32	Y
PvuI	CGATCG	1	Y
SmaI	CCCGGG	1	Y

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[a]**Abbreviations:** Y, complete cleavage; N, no digestion; P, partial digestion; L, plasmid linearized.
()b – number of recognition sites in plasmid pAltOKI

*tested in λ DNA dam- *in vitro* methylated with the Nme1821 enzyme.

Table 4S. Summary of the sensitivity of different restriction endonucleases to modifications introduced by the Hin1523 enzyme.

REase	Recognition site	Number of sites in pHinET	Cleavage[a]
AdeI	CACNNNGTG	1	N
Bsh1285I	CGRYCG R=A/G	4	N
Bsu15I	ATCGAT	2	N
CaiI	CAGNNNCTG	1	N
Eco147I	AGGCCT	0 (1)[b]	N
Eco32I	GATATC	0 (1)[b]	N
Mph1103I	ATGCAT	2	N
MunI	CAATTG	1	N
PaeI	GCATGC	1	N
PscI	ACATGT	1	N
PstI	CTGCAG	0 (2)[b]	N
XhoI	CTCGAG	1	N
XmiI	GTMKAC	1	N
TaiI	ACGT	16	P, L
AluI	AGCT	23	P
BpiI	GAAGAC	4	P
BglII	AGaTCT	1	P
CseI	GACGC	12	P
CviQI	GTAC	4	P,L
DpnI*	Gm^{6}ATC	23	Y
Eam1104I	GAAGAG	3	P
Eco47III	AGCGCT	3	P
Eco91I	GGTNACC	1	P
EcoRI	GAATTC	1	P
HindIII	AaGCTT	0 (1)[b]	P
HinfI	GANTC	19	P
HphI	GGTGA/TCACC	18	P
LweI	GCATC	23	P
MboI*	GATC	23	N
MluI	ACGCGT	1	P
MlyI	GAGTC/GACTC	10	P
NdeI	CaTATG	1	P
NlaIII	CATG	27	P
PfeI	GAWTC	9	N
PvuII	CAGCTG	3	P
SchI	GAGTC/GACTC	10	P
TaqI	TCGA	15	P, L
TasI	AATT	30	P
Tru1I	TTAA	32	P
VspI	ATTAAT	5	P
XapI	RAATTY	9	P
XbaI	TCTGAG	1	P
Bsp119I	TTCGAA	0 (1)[b]	P
HincII	GTYRAC	1 (GTTAAC)	P
KspAI	GAATTC	1	P
SspI	aATATT	3	P

REase	Recognition site	Number of sites in pHinET	Cleavage[a]
BcnI	CCSGG	12	Y
BglI	GGCN5GCC	1	Y
Bme1390I	CCNGG	21	Y
Bpu1102I	GCTNAGC	2	Y
Bsh1236I	CGCG	37	Y
Bsp143I*	GATC	23	Y
Bsp120I	GGGCCC	1	Y
Bsp68I	TCGCGA	1	Y
BspLI	GGNNCC	20	Y
Bst1107I	GTATAC	1	Y
BsuRI	GGCC	22	Y
Csp6I	GTAC	3	Y
DraI	TTTAAA	2	Y
Eco130I	CCWWGG	1	Y
Eco88I	CYCGRG	2	Y
EcoO109I	RGGNCCY	3	Y
FspBI	CTAG	6	Y
Hin1I	GRCGYC	5	Y
Hin6I	GCGC	32	Y
HpyF10VI	GCN7GC	41	Y
MbiI	GAGCGG/CCGCTC	4	Y
MspI	CCGG	29	Y
PvuI	CGATCG	1	Y

[a]**Abbreviations:** Y, complete cleavage; N, no digestion, P, partial digestion, L, plasmid linearized.
()b –number of recognition sites in plasmid pAltOKI
*tested in λ DNA dam- *in vitro* methylated with the Hin1523 enzyme.

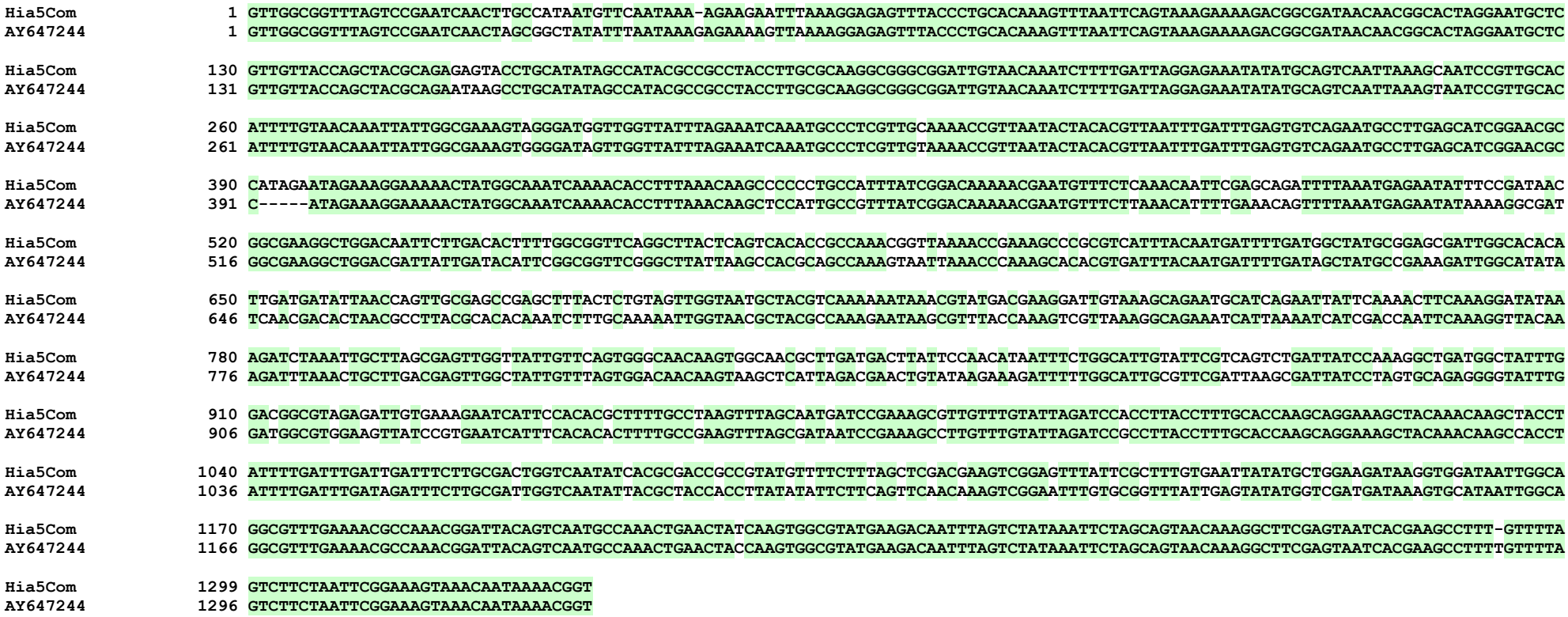

Figure 1S. DNA sequence comparison of a 1332-bp fragment of *H. influenzae* biotype aegyptius strain ATCC 11116 genomic DNA and the homologous segment of the *H. influenzae* biotype aegyptius strain 3031 genomic island (Acc. no. AY647244). Identical nucleotides are shaded green.

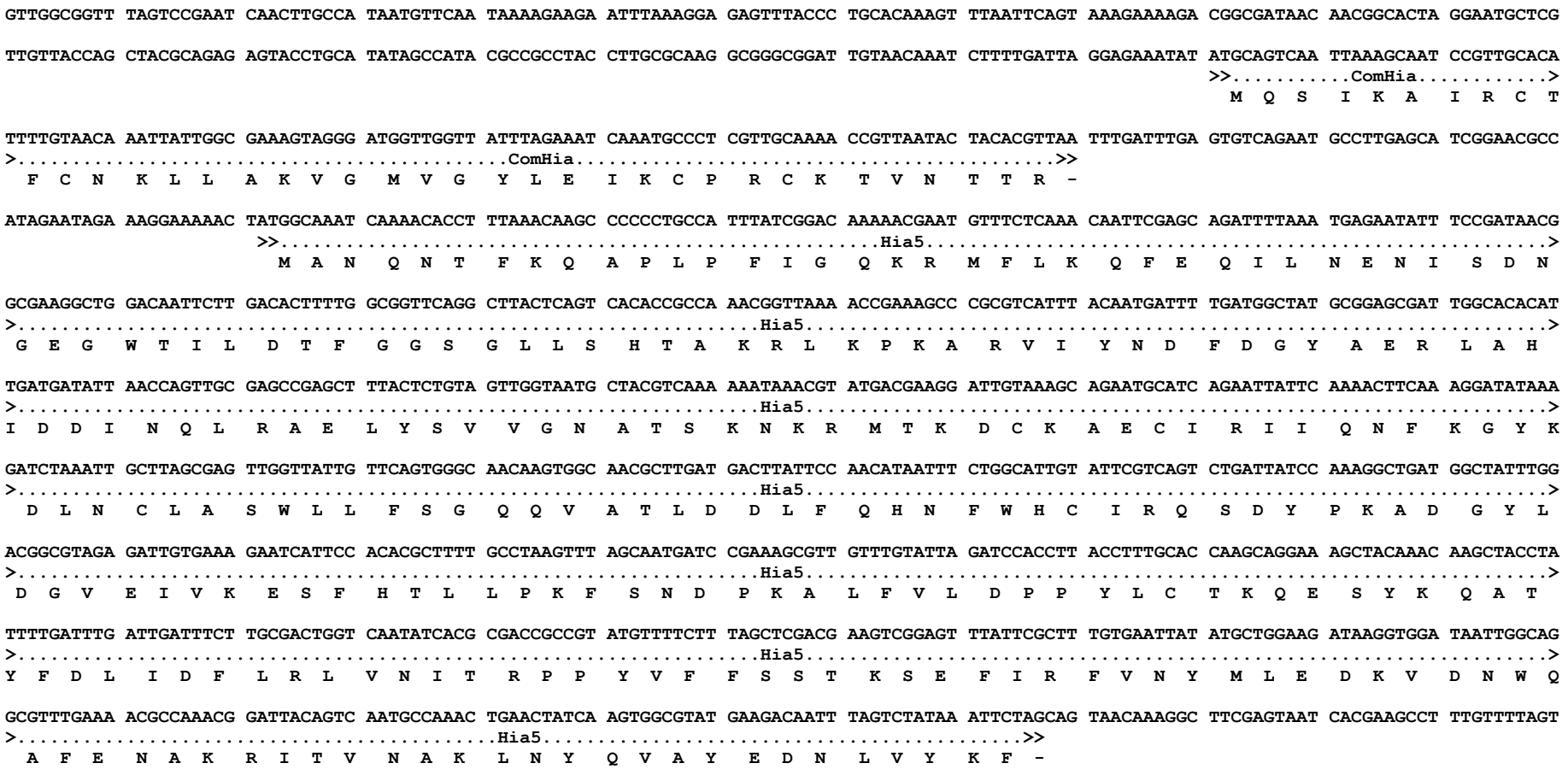

Figure 2S. Nucleotide sequence of a 1332-bp fragment of *H. influenzae* biotype aegyptius ATCC 11116 genomic DNA containing the *comHia* and *hia5* genes. The derived amino acid sequences of the ComHia and Hia5 proteins are shown below the DNA sequence.

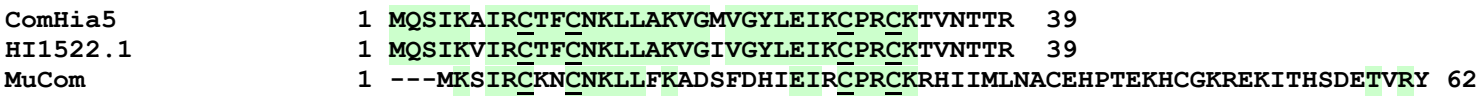

Figure 3S. Alignment of the putative Com-like amino acid sequence of *H. influenzae* biotype aegyptius ATCC 11116 with the predicted HI1522.1 Com-like protein of H. *influenzae* Rd and Mu Com. Conserved amino acid residues are shaded green. Four conserved cysteine residues that form the zinc finger are underlined.

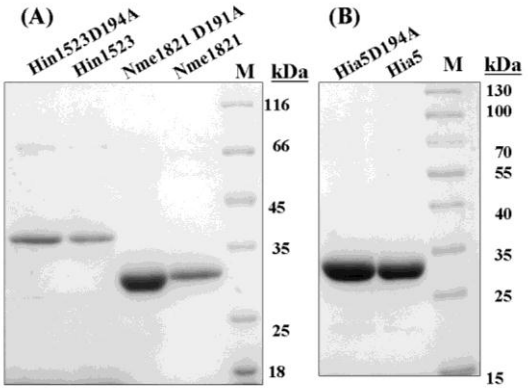

Figure 4S. Coomassie-stained SDS-12% PAGE gels of purified recombinant proteins: Hin1523, Nme1821 and their respective mutant variants Hin1523D194A and Nme1821D191A (A), and Hia5 and its mutant variant Hia5D194A (B). M – Molecular mass marker.

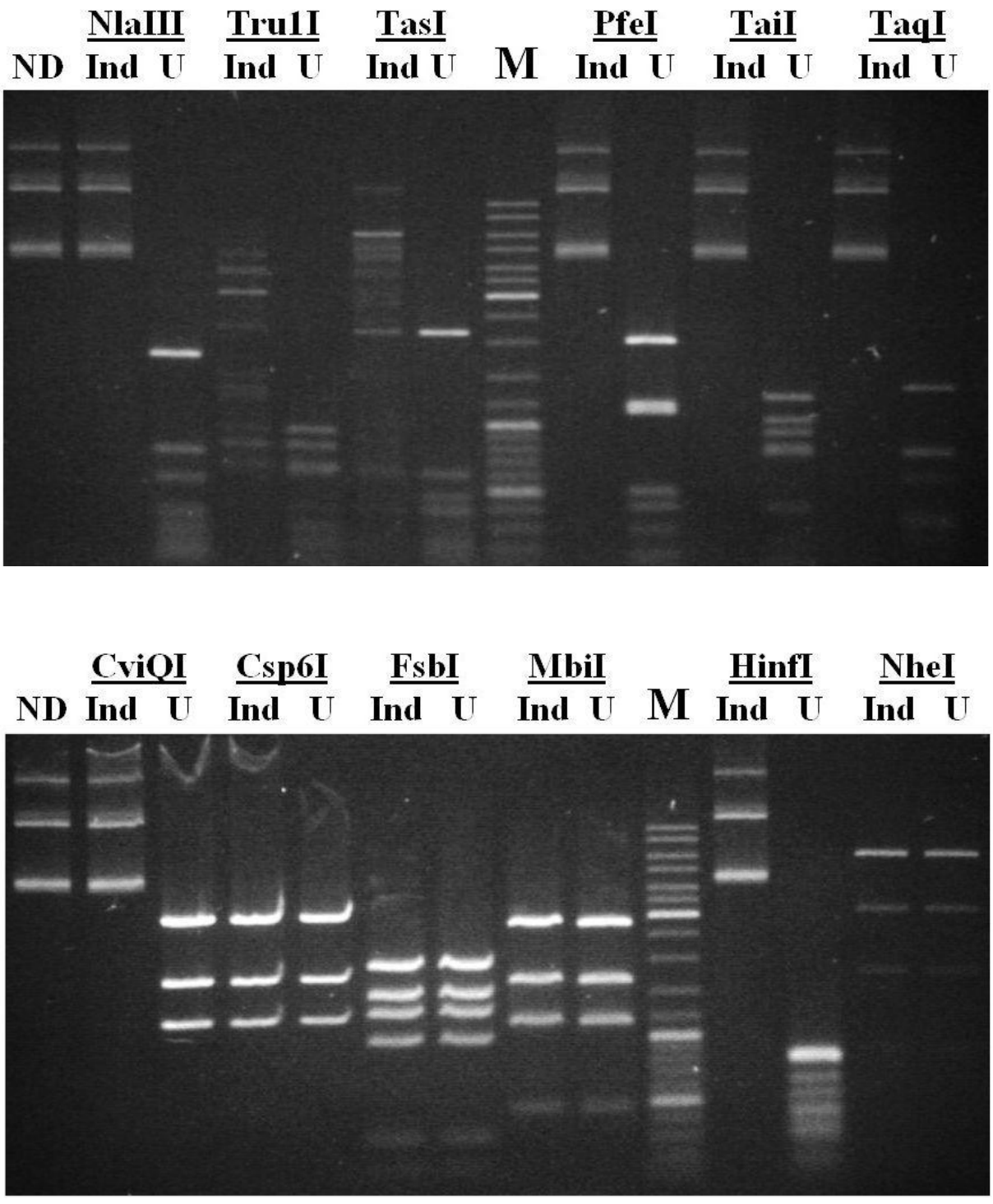

Figure 5S. Comparative restriction patterns of plasmid pHia5ET DNA prepared from *E. coli* ER2566 cells grown in the presence (Ind = pHia5ETi) or absence (U = pHia5ET) of inducer IPTG and cleaved with selected REases. Digest mixtures were electrophoresed on 0.8% agarose gels and stained with ethidium bromide. M – GeneRuler 100-10,000 bp size marker.

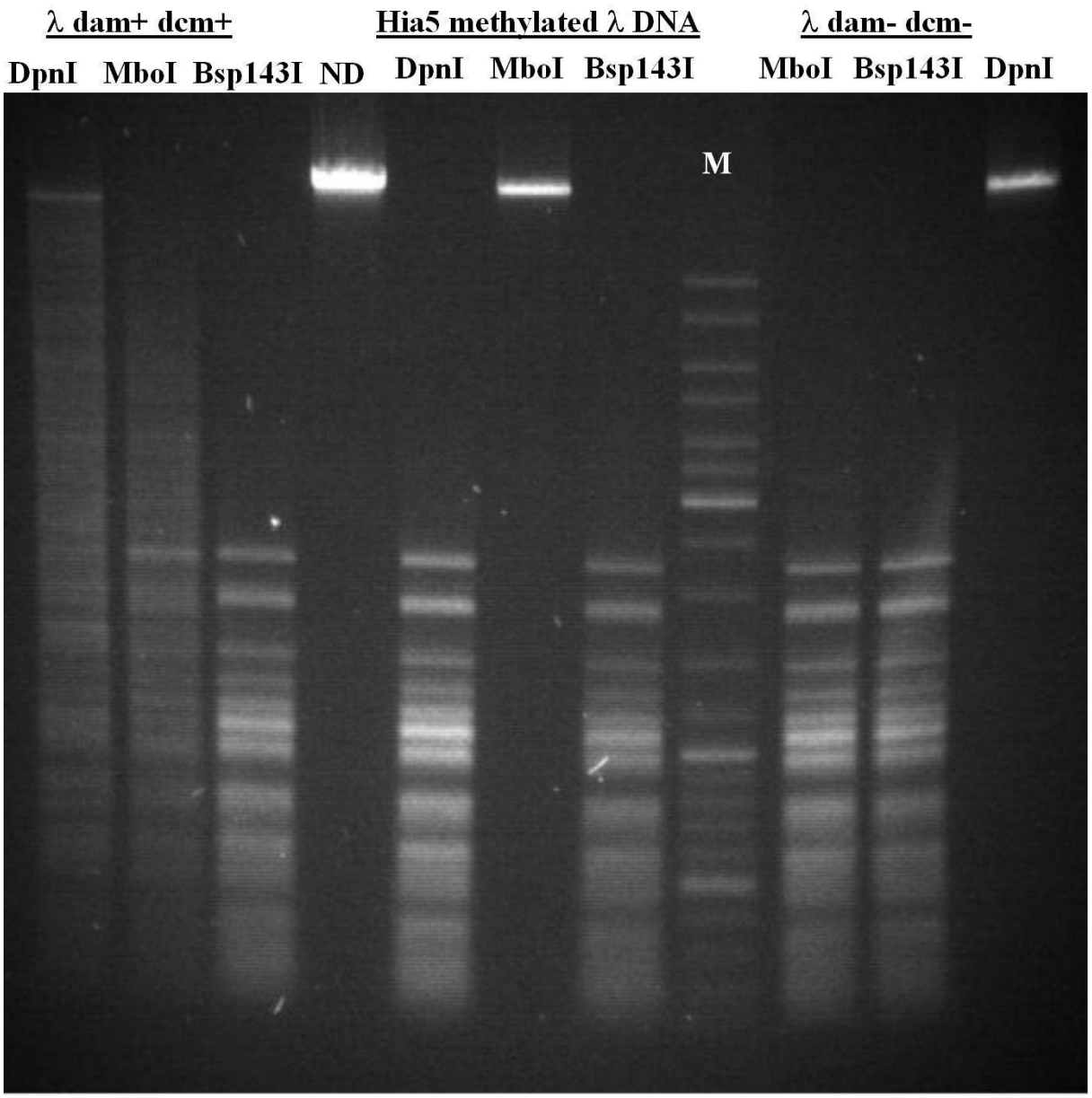

Figure 6S. Comparative restriction patterns of λ DNA dam+ dcm+, λ DNA dam- dcm- (controls) and Hia5 *in vitro*-methylated λ DNA cleaved with REases MboI, DpnI and Bsp143I. Digest mixtures were electrophoresed on 0.8% agarose gels and stained with ethidium bromide. ND- undigested λ DNA. M – GeneRuler 100-10,000 bp size marker.

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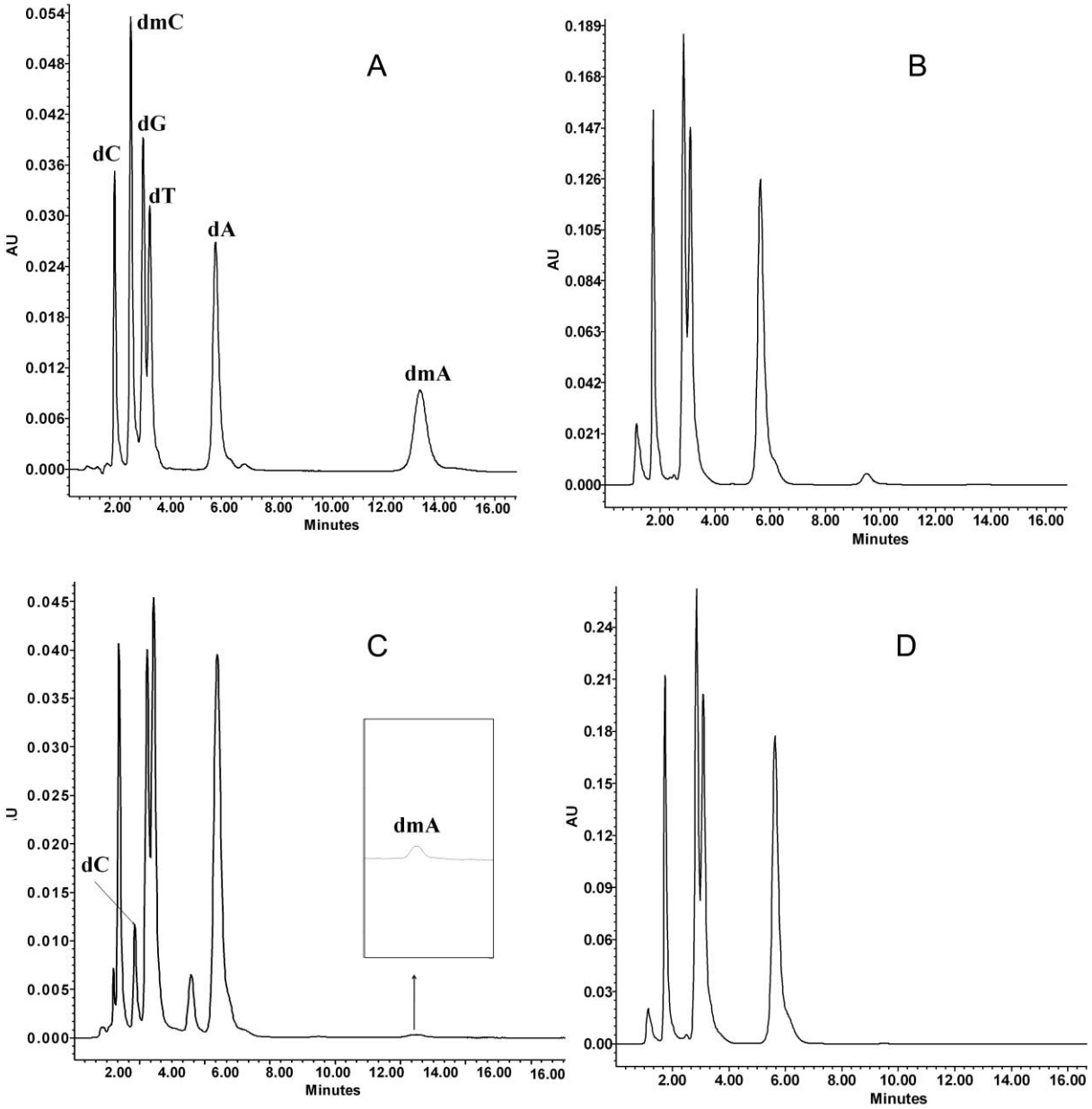

Figure 7S. HPLC profiles of deoxynucleosides in the standards mixture (A), and enzymatic digests of λ DNA dam+ dcm+ (B), the genomic DNA of *H. influenzae* biotype aegyptius ATCC 11116 (C) and λ DNA dam- dcm- (D).

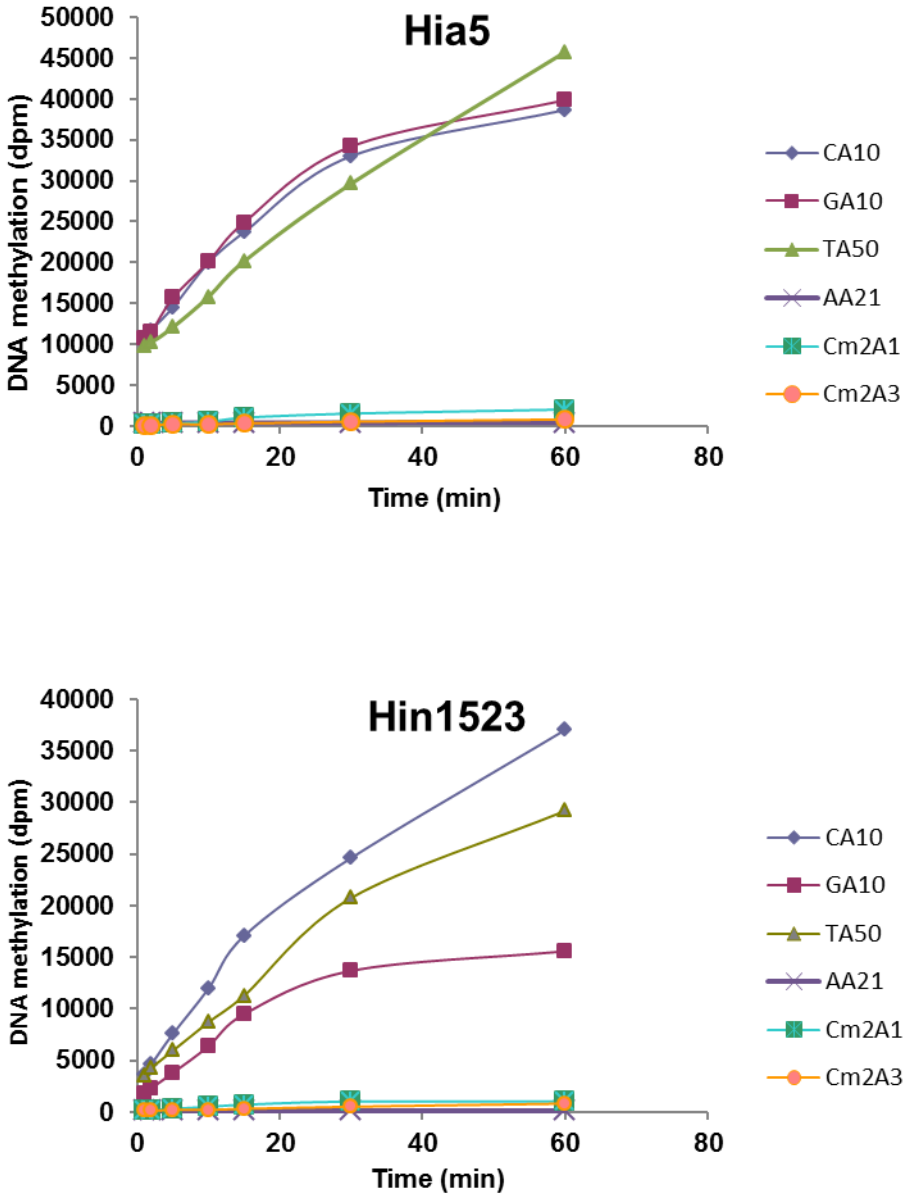

Figure 8S. Example of DNA methylation kinetics with the Hia5 or Hin1523 enzymes and synthetic oligonucleotides (**Table 2S**).

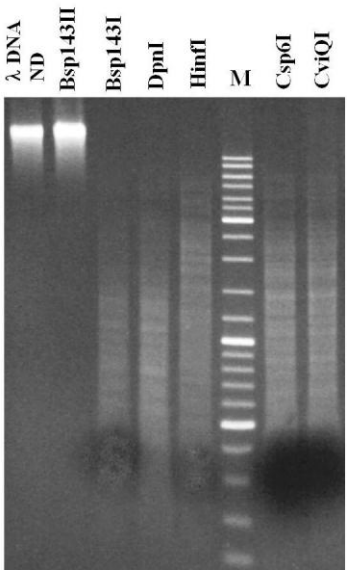

Figure 9S. Restriction patterns of *H. influenzae* biotype aegyptius ATCC 11116 genomic DNA cleaved with selected REases: Bsp143II (RGCGCY), Bsp143I (GATC), DpnI (Gm^6ATC), HinfI (GANTC), Csp6I (GTAC) and CviQI (GTAC). Digest mixtures were electrophoresed on 0.8% agarose gels and stained with ethidium bromide. ND- undigested λ DNA. M – GeneRuler 100-10,000 bp size marker.