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and the 6-position of the pyrimidine to which the methyl group is to be transferred (13).

It has been shown directly that a cysteine is involved in the enzymatic reaction of the *HhaI* MTase (12). Within the bacteriophage MTases strong evidence exists that the variable segments of their sequences are responsible for their interaction with different DNA recognition sequences (14,15,16). A marginal similarity has been reported between some

m⁶A MTases and m⁵C MTases, and this led to speculations about the location of the SAM

binding site within the sequences (17).

Beginning with a set of twenty-seven DNA MTase sequences we have identified motifs

for their global alignment. Software has been developed that allows the detection of these

motifs and can assist in the semi-automatic alignment of the sequences. The motifs have

in each pair were shuffled and the similarity of these randomized sequences was scored

using FASTP. The mean and standard deviations of the scores from 150 such shuffles

were calculated. This mean was used as a baseline for the comparison of the two real

sequences. FASTP scores greater than three standard deviations above the mean were

considered significant (39,40,41).

Dot matrix plots were used to display amino acid sequence similarities. Eleven residue

long segments were compared by sliding two windows independently over the two

sequences. The similarity of the two segments was scored by using the metrics of DIAGON

Alignments

To produce a global alignment of the set of similar sequences we developed a new

procedure. A complete description of the algorithms used and their implementation will

were allowed and positions in which any amino acid was allowed. For example, the motif (P,T)XXXXXENV has two alternatives at the first position, any amino acid is allowed at the next five positions, and only single amino acids are allowed at the last three positions.

EcoRII

SinI

BsuRI

I

IV

Phi3T	28	L	I	G	F	S	E	I	D	K	Y	A	I	K	S	Y	C	A	H	55	G	D	I	S	K	123	K	D	E	R	G	T	L	F	F		
Rho11s	28	L	V	G	F	S	E	I	D	K	Y	A	I	K	S	Y	C	A	H	55	G	D	V	S	K	90	E	D	T	R	G	T	L	F	F		
SPR	28	L	V	G	F	S	E	I	D	K	Y	A	V	K	S	F	C	A	H	55	G	D	V	S	K	90	E	D	T	R	G	T	L	F	F		
HhaI	34	C	V	Y	S	N	E	W	D	K	Y	A	Q	E	V	Y	E	M	N	F	58	G	D	I	S	K	93	F	I	N	S	R	G	T	L	F	F



Figure 5. Sequence similarities within the variable regions of MTases recognizing GGCC. The variable region

from the end of block VIII to the beginning of block IX of *BsuRI* (vertical axes) is compared to the variable

regions of (a) *BspRI*, (b) *HaeIII* and (c) *Phi3T* (horizontal axes). The DIAGON program with a window length

of 11 and a threshold of 130 was used.

found that some enzymes that recognize the same sequence such as *BsuRI*, *BspRI* and

Block number	Motifs
I	DFF-G-GA-----G SL MG
IV	D---G-PCP-FS--G N Q W
VI	KP--FF-ENVKGF-A---G
	R LL P L S N S VV R M T
VI*	KP--FF-ENV-GF-A---G QT II N N K
	S VV M T
	ID HGLP K IC NS YNV Q VG
X	K---YKE-GNAI-I-A---A
	S RQ V V G
X*	YKE-GNAI-I-A---A QM SV P L F RQ V V G

Figure 6. Predictive sequence motifs of m⁵C MTases. The block number corresponds to the numbering of figure

4. At positions where more than one amino acid is acceptable the alternatives are listed. Dashes signify that any amino acid can occupy that position. Blocks VI* and X* are the modified motifs necessary to accommodate

the *Ngo*PII sequence.

sequence also contains three of the five motifs identified above (Figure 7a). The 'ENV' and 'Y ♦ GN' motifs (the abbreviations within the quotes denote the complete motifs derived

from blocks VI and X. ♦ marks a specified distance between two conserved amino acids)

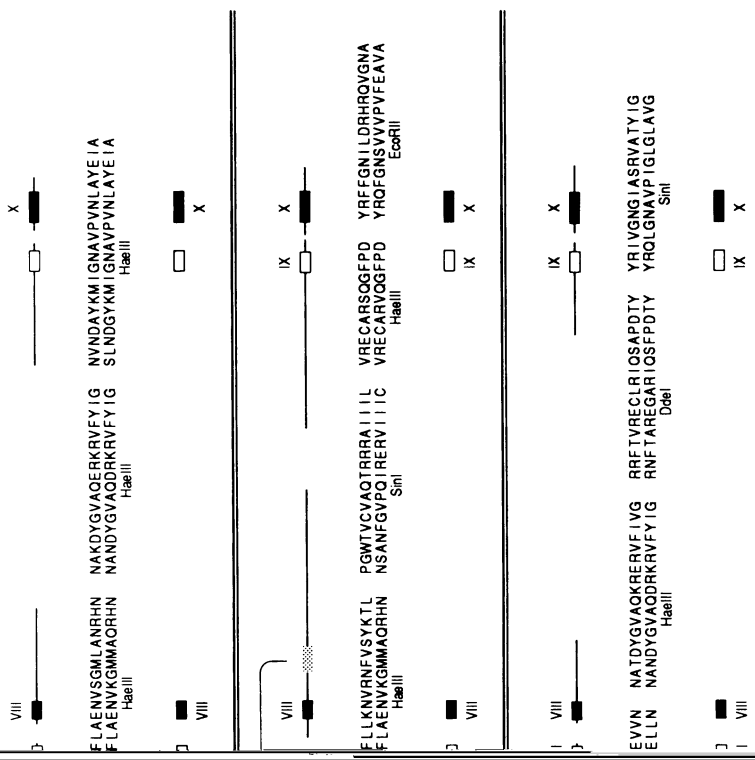
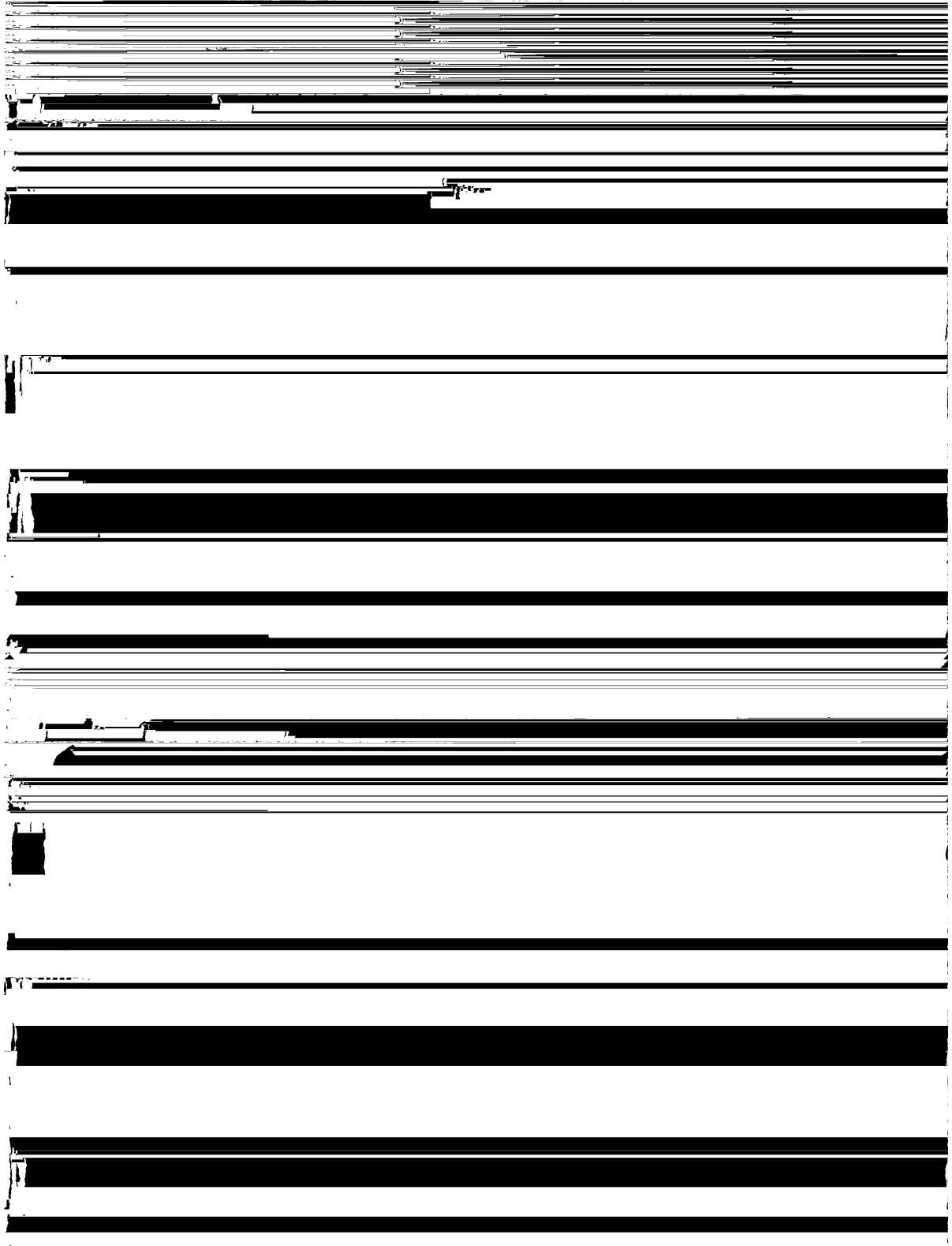


Figure 1. Schematic diagrams of the sequences of (a) the NgoPII MTase, (b) the murine 3488 (c), while the bottom lines show the generalized arrangements of the thirteen non blocks; the shaded box in (b) indicates the location of the segment containing the G-PC-S-G motif. The individual sequence segments of the numbered blocks are shown in the top lines. The order of the segments corresponds to the numbering of blocks in the bacterial



A second unidentified open reading frame was also found during the search with the motifs. This was present in the DNA fragment which contains the coding sequence for

present immediately upstream of the gene already characterized. The sequence is identical, at the nucleotide level, with that of the Phi3T secondary MTase for the length of the published sequence. This identity covers the variable regions between blocks VIII and IX

at the translated level in all reading frames would be a useful tool. It could be used to

highlight any regions of a newly determined sequence that should be checked carefully

for possible errors.

The putative MTases that we suggest are encoded by the *B. subtilis* phages Phi3T and Rho11s lie immediately upstream of the known multi-specific MTases of these two phages.

be done. Having more functional MTases on the same phage would be a simple way to

achieve protection against restriction enzymes with different specificity. The tandem configurations of two MTases could be precursors to MTases with multiple specificities.

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