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Corrigendum

Characterization of the Type III restriction endonuclease PstII from *Providencia stuartii*

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Type III Restriction-Modification systems characteristically methylate a base in only one strand of their recognition sequence. In an earlier paper (1), we reported that PstII recognizes the asymmetric sequence 5′-CTGATG-3′ and methylates one of the adenines in the bottom strand of this sequence, forming 5′-Cm6ATCAG-3′. We have recently learned that this assignment is incorrect; PstII in fact methylates the adenine in the top strand, forming 5′-CTGm6ATG-3′. Prompted by data from Single Molecule Real Time (SMRT)-sequencing of the *Providencia stuartii* genome that unambiguously shows that PstII forms 5′-CTGm6ATG-3′ (Iain A. Murray, Geoffrey G. Wilson and Richard D. Morgan, personal communication), we revisited the methylation data (Figure 4 in (1)). The original synthetic DNA oligonucleotides used in our study were annealed and the radioactive methylation assay was repeated using a purified recombinant PstII preparation from 2005. The results obtained agree with the SMRT-sequencing data (Figure 1): synthetic pre-methylation of the adenine in the CTGATG-strand (168F/168RM3) reduced incorporation of radioactivity from S-[methyl-3H]-adenosyl-L-methionine approximately 100-fold compared to pre-methylation of either of the adenines in the CATCAG-strand (168FM1/168R and 168FM2/168R). The oligonucleotide numbering system (premethylated at position 1, 2 or 3) in the bar graph in Figure 4 of (1) is consistent with the naming of the original methylated DNA strands as listed in Figure 1a here. In reviewing our earlier experiment, we found the data were correct and that the error arose during manuscript preparation due to mislabelling of the adenines in the DNA cartoon of Figure 4 of (1). A corrected version of Figure 4 is given here (Figure 2). This error was then carried through to the text and to Figure 2A of (1), a corrected version of which is shown here (Figure 3).

In the text, the sentence on p4781 ‘When the **first** adenine on the **5′-CATCAG-3′** strand was pre-methylated (position 3)…’ should read ‘When the adenine on the **5′-CTGATG-3′** strand was pre-methylated (position 3)…’ i.e. ‘first’ should be removed and 5′-CATCAG-3′ replaced with 5′-CTGATG-3′.

These corrections do not change any of the other results or conclusions of the paper. The authors apologise for this error and for any confusion it has caused.

REFERENCES

1. Sears,A., Peakman,L.J., Wilson,G.G. and Szczelkun,M.D. (2005) Characterization of the type III restriction endonuclease PstII from Providencia stuartii. *Nucleic Acids Res.*, **33**, 4775–4787.

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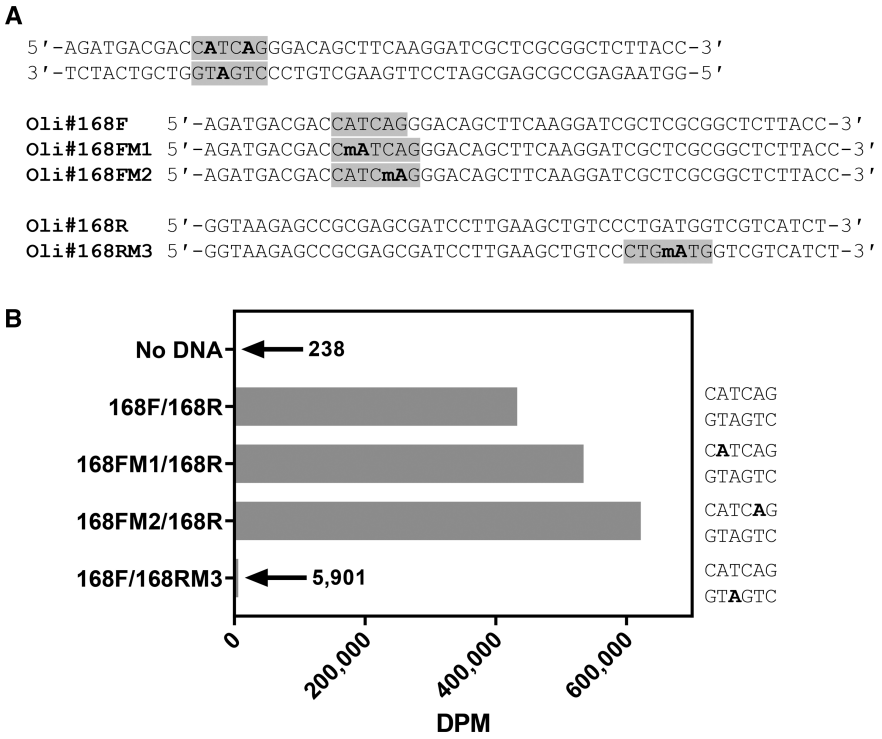

Figure 1. Repeat of the identification of the PstII methylation site. (**A**) Synthetic oligonucleotides (MWG Biotech) from the original study were annealed to generate 49bp dsDNA substrates with a PstII recognition site (grey). Pre-modified sequences (FM1, FM2, RM3) were generated by synthesizing oligonucleotides with an N6-methyl deoxyadenosine at the positions shown. (**B**) Scintillation counting of 49 bp dsDNA substrates following incubation with PstII and S-[methyl-3H]-adenosyl-L-methionine (3H-AdoMet). Methylation reactions contained 500 nM duplex oligonucleotide and 280 nM PstII in buffer NEB 4 (20 mM Tris-acetate, pH 7.9, 10 mM Mg acetate, 50 mM K acetate, 1 mM DTT) supplemented with 125 nM 3H-AdoMet and 0.02% (v/v) Triton-X100. Reactions were incubated for 3 hours at 37 C. The DNA was phenol/chloroform extracted and ethanol precipitated, and the extent of [methyl-3H] transfer quantified by scintillation counting.

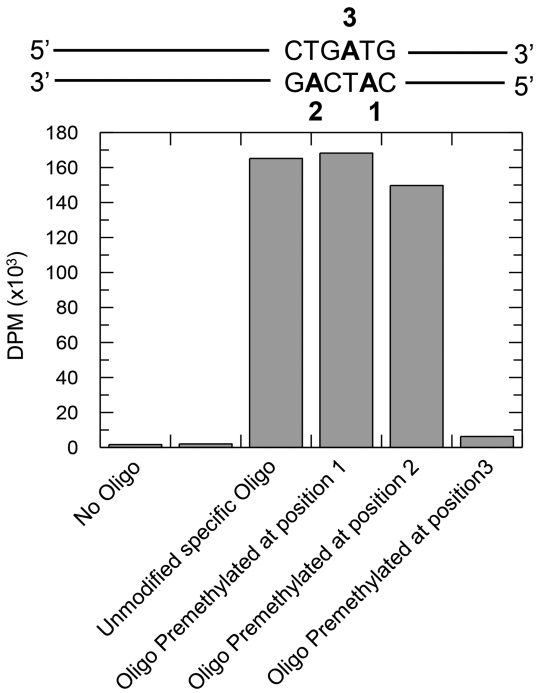

Figure 2. Corrected version of Figure 4 of (1). Identification of the PstII methylation site. (**A**) Oligonucleotides were synthesized and annealed to generate non-specific and specific DNA substrates. Pre-modified sequences were generated by synthesizing oligonucleotides with an N6 methyl deoxyadenosine at either position 1, 2 or 3. (**B**) Scintillation counting of oligonucleotide substrates following incubation with PstII and [^{3} H-methyl]-AdoMet. See main text for full details.

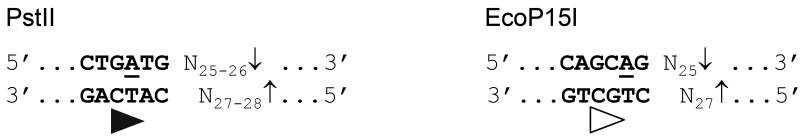

Figure 3. Comparison of the sequence-specificity and activities of PstII and EcoP15I.