DIRECT DETECTION OF DNA METHYLATION AT CpA UNITS

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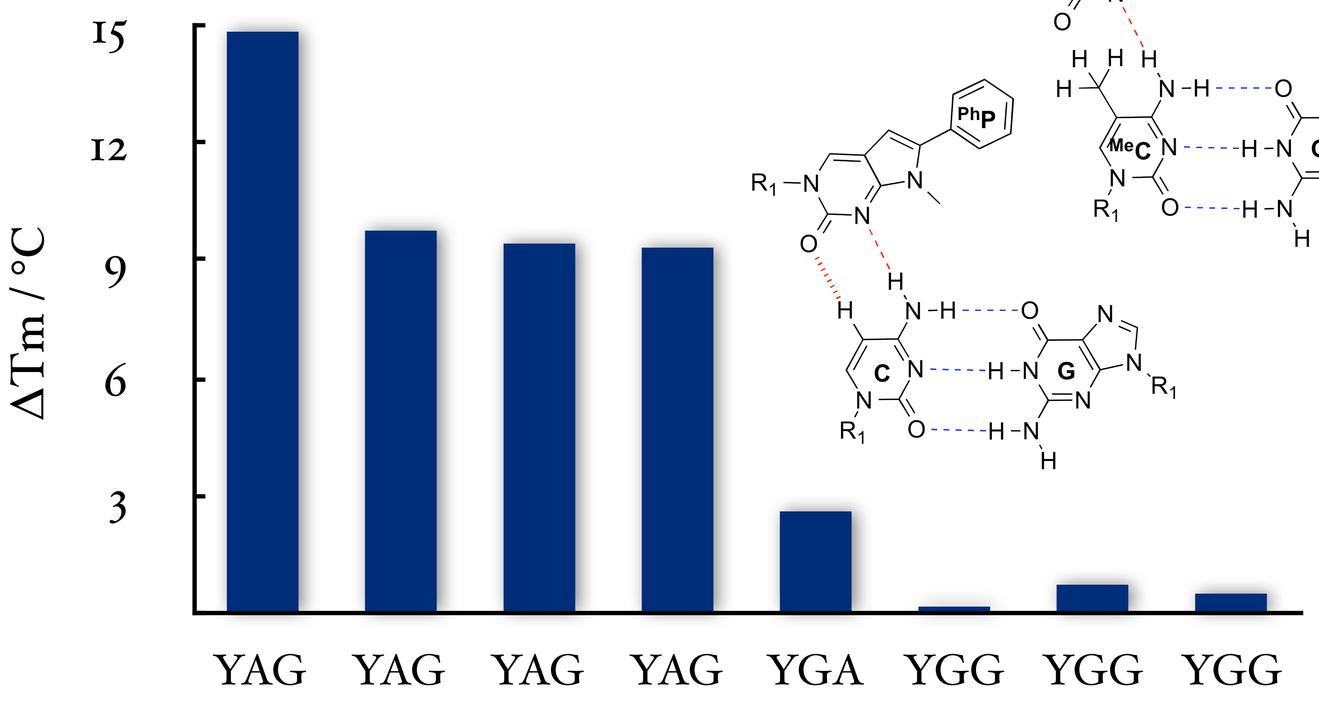
DNA **methylation patterns** are emerging as targets of great biological relevance. We have been working to develop a method to detect methylation patterns directly, without the need for bisulphite conversion or polymerase-aided replication.

PhP, a monomer first investigated for its ability to stabilise triplexes with cytosines in the purine strand (**CG inversions**) has been found to decrease stability of triplexes if the purine strand C is methylated.

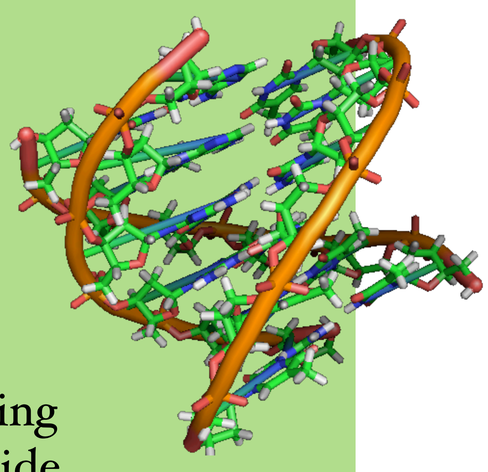
$$\Delta T_m = T_m(Y = C) - T_m(Y = {}^{Me}C)$$



While **CpA methylation** is easily detected by this method, it is not possible to distinguish between CpG and ^{Me}CpG. While the methylation frequency of CpG sites is usually the highest, CpA methylation is common in **embryonic stem cells** and in **plant cells**. In rice, the methylation frequency of CHG sites is 21%, while in maize it is as high as 74%

Triplex DNA

Triplex DNA is formed of **three strands of DNA**. Two strands form a normal duplex with Watson-Crick base pairing, with a third strand bound in the major groove *via* **Hoogsteen** base pairing. It is easiest to form a triplex where the third strand is a poly-pyrimidine binding to a stretch of purines in the DNA sequence. Binding a triplex-forming oligonucleotide does not require denaturation of the duplex and the **denaturation temperature** (T_m) of the triplex can be measured separately from the duplex denaturation.



In conclusion, we have developed a method capable of detecting **CpA methylation** in a purine stretch of a duplex. By using triplex forming oligonucleotides containing the monomer **PhP**, methylation at a CpA cytosine leads to a detectable change in **denaturation temperature**

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    5′- CTM CTX TMT MCC TMT MTC
5′- GGA GAG GAY AGA GGG AGA GAG GGC
3′- CCT CTC CTG TCT CCC TCT CTC CCG
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Example oligonucleotide set.
M = ^{Me}C, X = ^{Ph}P, Y = C or ^{Me}C

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