5-methyl deoxycytosine (5-Me-dC) pairs with dG, and when substituted for dC in an oligonucleotide, increases the stability of the resulting duplex relative to the comparable unmodified form, raising the Tm by 1.3°C per 5-Me-dC residue added (1,2). 5-Me-dC thus can be used to improve the ability of an oligo to hybridize to its target. The presence of the hydrophobic 5-methyl group presumably acts to exclude water molecules from the duplex. 5-Me-dC’s is particularly useful in the following applications:

(a) **Strong-binding PCR primers**: 5-Me-dC-modified PCR primers have been shown to prime far better than their unmodified counterparts in PCR reactions, consistently yielding more product per cycle, permitting amplification at very high annealing temperatures (as high as 72°C), and interestingly, allowing excellent priming from within palindromic sequences (1). The improvement in priming efficiency could significantly reduce the number of amplification-related mutations in PCR products. 5-Me-dC primers also could be useful in several PCR applications, e.g., when short, specific primers are required, when only a limited quantity of template is available (e.g., ancient DNA), when DNA secondary structure in the primer binding site prevents binding of an unmodified primer, or when primer extension is blocked by downstream DNA secondary structure in the template.

(b) **Anti-sense**: Anti-sense oligonucleotides containing a CpG motif induce pro-inflammatory responses after *in vivo* administration to animals, including human, via activation of Toll-like receptor 9 (TLR9). Substitution of 5-Me-dC for dC in these motifs can prevent or sharply reduce these undesirable immune responses (3).

(b) **DNA methylation studies**: Methylation of dC to 5-methyl-dC, when it occurs in CpG sites near promoters is associated with gene silencing, and is an important epigenetic mechanism in living organisms. Oligonucleotides incorporating 5-Me-dC have been used by a number of research groups as research tools to study the epigenetic effects of DNA methylation in such areas as tumorigenesis and the effects of cocaine on fetal heart development (4-6). References

1. Lebedev, Y.; Akopyants, N.; Azhikina, T.; Shevchenko, Y.; Potapov, V.; Stechenko, D.; Berg, D.; Sverdlov, E.. Oligonucleotides containing 2-aminoadenine and 5-methylcytosine are more effective as primers for PCR amplification than their nonmodified counterparts.


