J Biotechnol. 2014. 189:143-149
Doi:10.1016/j.jbiotec.2014.09.007

Hybridisation of N4-methylcytosine-containing-containing amplicons on DNA arrays

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Abstract:
5'-Cy5-labelled PCR amplicons containing the analogue base, N^4-methylcytosine, instead of cytosines were compared in microarray hybridisation experiments with the corresponding amplicons containing the canonical set of bases, with respect to the intensity of the fluorescence signal obtained, and cross hybridisation to non-corresponding probes. In general, higher hybridisation temperatures resulted in reduced signal intensities, particularly in the case of the N^4-methylcytosine containing amplicons. At the lower hybridisation temperatures tested (40 °C, 30 °C), these modified amplicons gave about equal or stronger fluorescence signal than the corresponding regular amplicons. With the two GC-richest amplicons tested, in one instance the N^4-methylated target gave a dramatically higher signal intensity than the unmodified amplicon, interpreted as reflecting the reduced formation of hairpin structures in the target sequence, due to the lower thermodynamic stability of the G:N^4-methylC base pair, making the target more accessible, while in the other case no hybridisation was observed with either version of the amplicon, probably due to interference from a G-tetrad structure. Both for the regular and the N^4-methylated amplicons, no significant cross hybridisation was seen in these experiments.

Full text:

https://doi.org/10.1016/j.jbiotec.2014.09.007