SUPPORTING INFORMATION

Detection of Terminal Mismatches on DNA Duplexes with Fluorescent Oligonucleotides

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Fluorescence data

Influence of the presence of mismatches at the penultimate or last two positions of the duplex

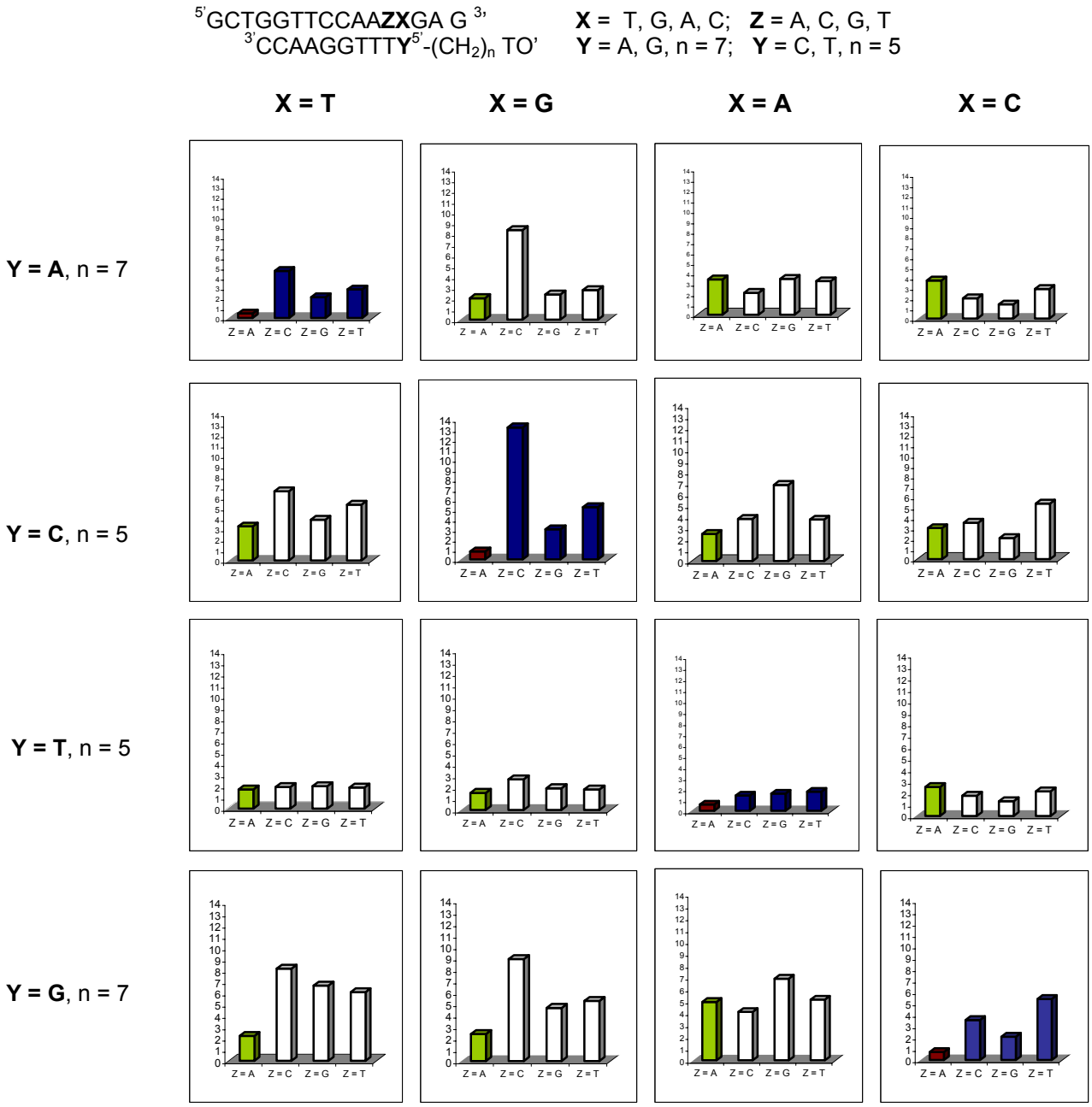

Fig. 9. Fluorescence duplex/fluorescence free probe. Concentrations were [C] = 1μM (each strand) in a 5 mM sodium cacodylate buffer, pH = 6, containing 50 mM NaCl. 45 min hybridization at room temperature.

As previously observed at 6°C (Figure 5), the discrimination factor between the perfectly matched duplexes (red bars) and those involving terminal mismatches (green bars) on the same lines was also superior to 2 at room temperature (25°C). The values observed for the perfectly matched duplexes (red bars) were also inferior to those obtained for the duplexes involving a perfectly matched terminal base-pair and a penultimate mismatched one (blue bars on the same histograms) and also for the duplexes involving two terminal mismatched base-pairs (white bars). Among the combinations studied, the greatest fluorescence increases were observed when a CT mismatch was present at the penultimate position of the duplexes. Another observation was that the hybridization of the four ODN-TO' probes (each column of histograms) with each of the corresponding complementary target sequences gave, in all cases, the lowest fluorescence ratio for the perfect duplexes.

Variation of the nucleic base V on the target sequences adjacent to the terminal base-pair of the duplex (on the side of TO' attachment):

$^{5'}$GCTGGTTCCAAA**XV**AG$^{3'}$ **X** = T, G, A, C; **V** = G, C, A, T

$^{3'}$CCAAGGTTT**Y**$^{5'}$-$(CH_2)_n$ TO' **Y** = A, G, n = 7; **Y** = C, T, n = 5

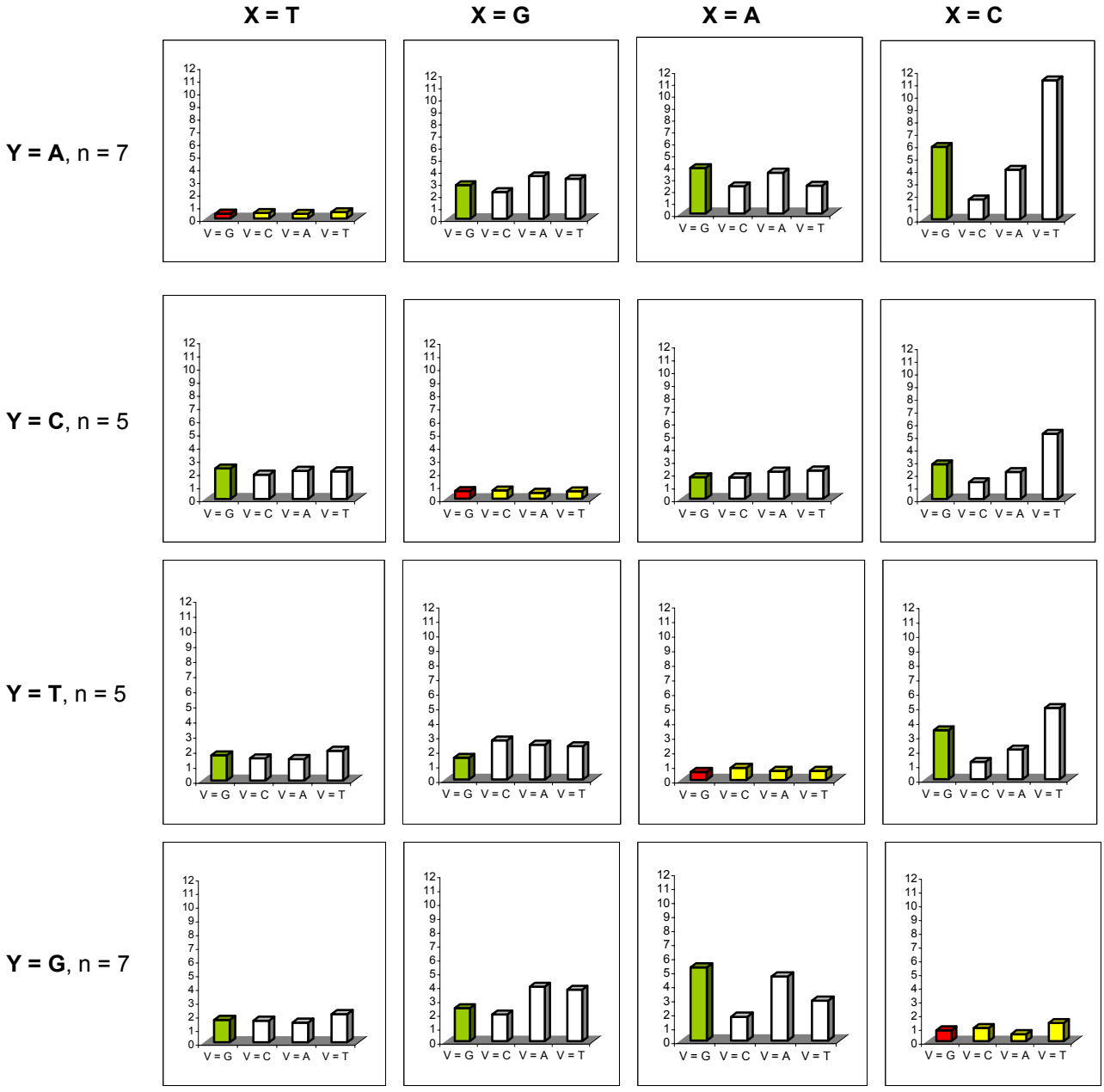

Fig. 10. Fluorescence duplex/fluorescence free probe. Concentrations were [C] = 1μM (each strand) in a 5 mM sodium cacodylate buffer, pH = 7, containing 50 mM NaCl. 30 min hybridization at room temperature.

Sixteen possibilities were studied. A comparison of the results showed that the perfect duplexes formed with the target studied above **V** = G (red bars) and the perfect duplexes formed with the other three targets **V** = C, A or T (yellow bars) could be discriminated from the corresponding mismatched ones [green bars (**V** = G) and white bars (**V** = C, A or T)]. The fluorescence ratio for the duplexes involving terminal mismatches were higher than those corresponding to the perfect duplexes. The discrimination factor was at least equal or superior to 2 except when **X** and **V** were cytosines leading to a reduced value (between 1.7 and 1.4).