Fluorescence Quenching by Proximal G-bases

Mark A. Behlke[1,2], Lingyan Huang[2], Lisa Bogh[3], Scott Rose[2], and Eric J. Devor[1]
[1]Molecular Genetics and Bioinformatics
[2]Molecular Genetics and Biophysics
[3]Analytical Services
Integrated DNA Technologies

Detection of dye-labeled nucleic acids via fluorescence reporting has become a routine technique in molecular biology laboratories. Given this, it is important to note that the quantum yield of fluorophores attached to nucleic acids is dependent upon a number of factors. One of these is the choice of the base that lies adjacent to the fluorescent molecule. Fluorescence quenching by an adjacent guanosine nucleotide is an under-appreciated phenomenon that can significantly effect quantum yield. Depending upon the fluorophore, this effect can be as much as 40%.

The mechanism of fluorophore quenching has been explained by electron sharing/donor properties of the adjacent base (Nazarenko et al., 2002). Quenching of 2-aminopurine fluorescence in DNA is dominated by distance-dependent electron transfer from 2-aminopurine to guanosine (Kelly and Barton, 1999). Seidel et al. (1996) found that photo-induced electron transfer plays an important role in this type of quenching. The order of quenching efficiency is G<A<C<T if the nucleobase is reduced but it is the reverse, G>A>C>T, if the nucleobase is oxidized (Seidel et al., 1996). Nazarenko et al. (2002) also report that quenching by adjacent nucleobases is dependent upon the location of the fluorophore within the oligonucleotide.

We have investigated some of the practical aspects of fluorescence quenching by an adjacent guanosine nucleotide. A series of fluorescence-labeled oligonucleotides sharing the same core sequence was synthesized such that one of three commonly used fluorophores and each of the four possible adjacent nucleotides was present in each construct (Table 1).

The concentration of each oligonucleotide was normalized by OD_{260} in buffer (10mM Tris HCl (pH 8.3), 50mM KCl, 5mM $MgCl_2$). Fluorescence measurements were made for a 200nM solution of each oligonucleotide on a PTI (Photon Technologies International) scanning fluorometer. Results for each of the three dyes are presented in figure 1. As can be seen both 3' fluorescein and 5' HEX™ (hexachlorofluorescein) displayed significant quenching when the adjacent nucleotide was guanosine. In contrast, the 3' Cy3™ was little affected by the choice of adjacent nucleotide.

Table 1. Fluorescent Test Oligonucleotides Studied		
5'-Dye	DNA Sequence	3'-Dye
	GGAAACAGCTATGACCATA	-Fluorescein
	GGAAACAGCTATGACCATG	-Fluorescein
	GGAAACAGCTATGACCATC	-Fluorescein
	GGAAACAGCTATGACCATT	-Fluorescein
	GGAAACAGCTATGACCATA	-Cy3™
	GGAAACAGCTATGACCATG	-Cy3™
	GGAAACAGCTATGACCATC	-Cy3™
	GGAAACAGCTATGACCATT	-Cy3™
Hex™-	TGGAAACAGCTATGACCAT	
Hex™-	GGGAAACAGCTATGACCAT	
Hex™-	CGGAAACAGCTATGACCAT	
Hex™-	AGGAAACAGCTATGACCAT	

Fluorescence intensities at the emission maximum for each dye were normalized relative to the value obtained when the adjacent base is adenine. These data are shown in figure 2. Here, it is clear that an adjacent guanosine has the greatest affect on all three fluorophores even though it is minimal for Cy3™. These results suggest that adjacent guanosine nucleotides should be avoided when designing oligonucleotides that contain a fluorescent reporter molecule.

References

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Nazarenko, I., Pires, R., Lowe, B., Obaidy, M., and Rashtchian, A. (2002) "Effect of Primary and Secondary Structure of oligodeoxyribonucleotides on the fluorescent properties of Conjugated dyes. *Nuc. Acids. Res.* **30**:2089-2195.

Seidel, C.A.M., Schulz, A., and Sauer, M.H.M.(1996) Nucleobase-Specific Quenching of Fluorescent Dyes. 1. Nucleobase One-Electron Redox Potentials and Their Correlation with Static and Dynamic Quenching Efficiencies. *J. Phys. Chem.* **100**:5541-5553.

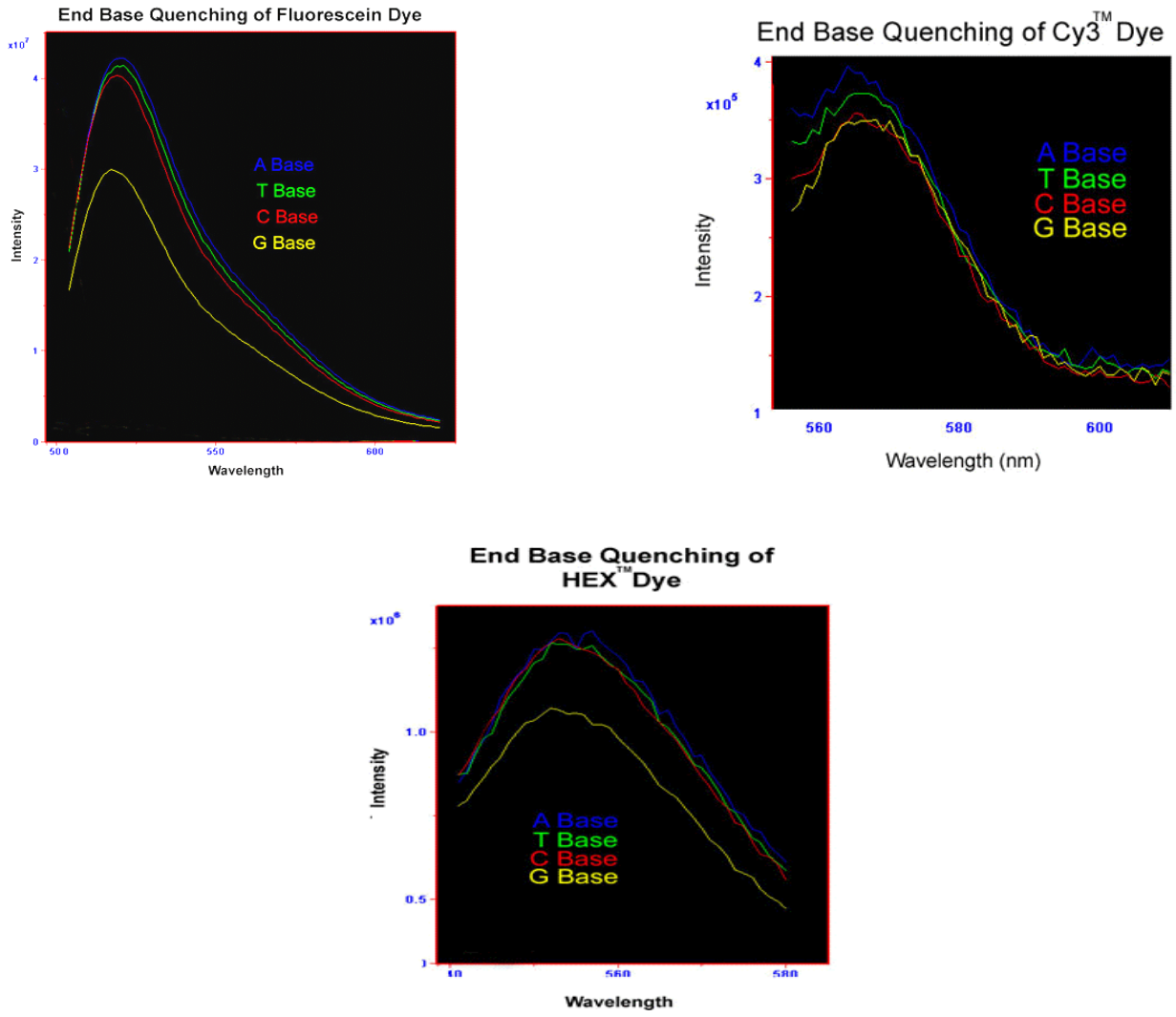

Fig. 1. Scanning fluorometer results obtained with the oligonucleotide constructs shown in Table 3.

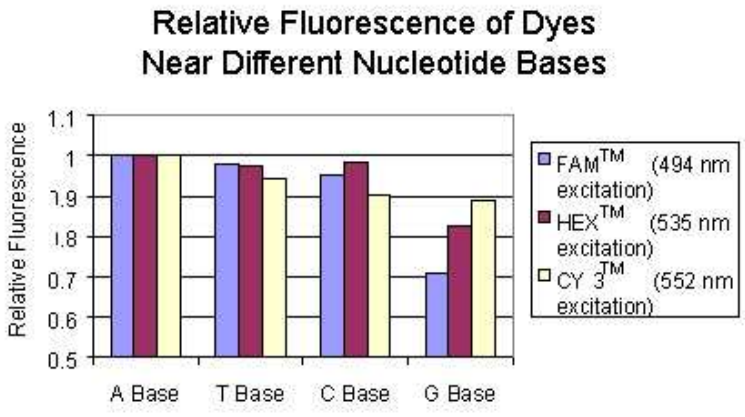

Fig. 2. Relative fluorescence intensities of FAM™, HEX™, and Cy3™ as a function of the nucleotide adjacent to the fluorophore.