

# New reporter dyes for real-time PCR binding in the minor groove

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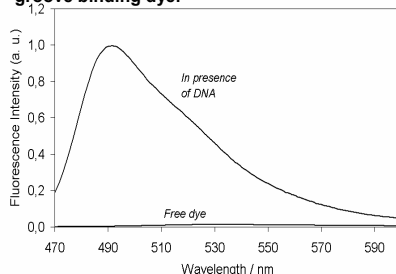
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The fluorescent unsymmetrical cyanine dyes TO and BO show highly increased fluorescence upon binding to double stranded DNA. This property allows them to be used for detection and quantification of nucleic acids in many applications. Neither of them, however, is suitable as reporter dye in real-time PCR because they inhibit of the polymerase chain reaction.

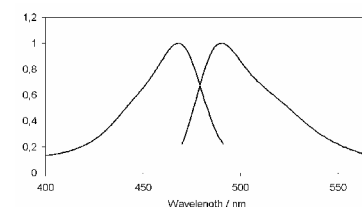
We have modified the intercalating dyes TO and BO by benzothiazole groups giving them a crescent shape that fits the path of the DNA double helix. This design makes them prefer minor groove binding to intercalation. This results in high selectivity for double stranded DNA and minimize interference with the PCR. Similar bent shapes are found in other established minor groove binding dyes such as DAPI and Hoechst 33258. Here we have tested the utility of five minor groove binding dyes as reporters in real-time PCR.

**Fluorescence increase in presence of double stranded DNA is at least 200-fold. This is much higher than of any other known minor groove binding dye.**

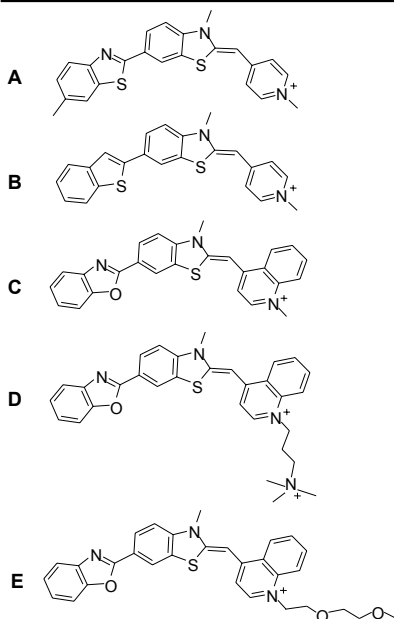


**Figure 1** Emission spectra of BEBO free in buffer and bound to calf thymus DNA at a dye:bp ratio of 1:50 in 25 mM sodium phosphate buffer (pH 7.0 at 25 °C). Excitation wavelength is 467 nm.

Absorption and emission maxima for the BO-based dyes (BEBO and BETIBO) are 468 nm and 492 nm respectively. For the TO-based dye (BOXTO) they are 530 nm and 555 nm. Most real-time PCR instruments allow measurements near these wavelengths, though no filters are currently available to evaluate the dyes at their optimum absorption and emission wavelengths.

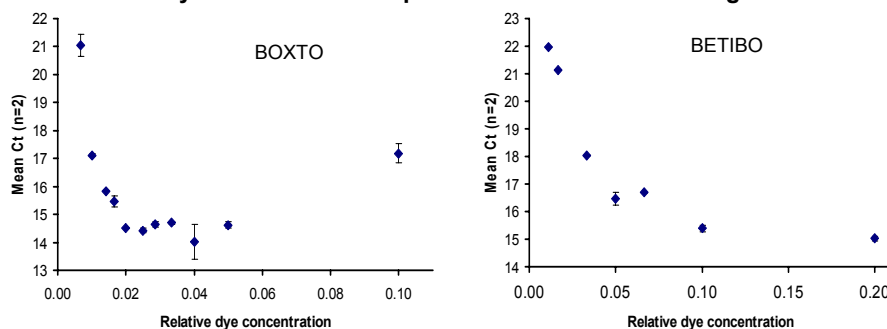


**Figure 2** Normalised absorption and emission spectra of BEBO bound to dsDNA.



**Figure 3** Chemical structures of (A) BEBO, (B) BETIBO, (C) BOXTO, (D) BOXTO-Pro and (E) BOXTO-Gly

## The dyes saturate the amplified DNA without inhibiting the PCR



**Figure 4** Different concentrations of BOXTO and BETIBO were used as reporter dyes for real-time PCR of a 200-bp amplicon. The three other dyes were also tested and showed similar behaviour.

Dye	Ct	Efficiency
BOXTO only	18.0	0.95
SYBR only	17.1	0.95
BOXTO mix	18.7	0.85
SYBR mix	18.3	0.86

BOXTO and SYBR Green I were used as reporter dyes either alone or in combination (i.e. both dyes present simultaneously) in a template dilution series. Ct for the most concentrated sample and PCR-efficiency were determined. Same threshold was used for all samples. A direct comparison with SYBR Green I is however difficult due to different gain settings and properties of the filters and optics used.

## Conclusions

- The new minor groove binding unsymmetrical cyanine dyes are excellent alternatives to SYBR Green as reporter molecules in real-time PCR.
- At working concentration the DNA is saturated with dye molecules and does not inhibit the PCR. For BEBO optimum concentration is 0.1 – 0.5  $\mu$ M.
- The dyes are stable for at least 1 week of storage in water at 5x working concentration.

## Dye stability

All dyes were diluted to 5 times working concentration in water and stored in refrigerator. No sign of dye degradation or precipitation was observed after 1 week of storage.

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