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Fluorescent cyanine dyes for the quantification of low amounts of dsDNA

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ABSTRACT

In this research six cyanine fluorophores for the quantification of dsDNA in the pg-ng range, without amplification, are compared under exactly identical conditions: EvaGreen, SYBR Green, PicoGreen, AccuClear, AccuBlue NextGen and YOYO-1. The fluorescence intensity as a function of the amount of dsDNA is measured at the optimal wavelengths for excitation and emission and for each dye the limit of detection and the response linearity at low levels of dsDNA are determined. No linear range was found for SYBR Green and YOYO-1 for pg-ng quantities of dsDNA. EvaGreen, PicoGreen, AccuClear and AccuBlue NextGen show good linearity in the pg-ng range. AccuClear exhibits the widest linear range of 3 pg −200 ng, whereas AccuBlue NextGen turned out to have the highest sensitivity of the tested dyes with a limit of detection of 50 pg.

1. Introduction

Quantification of low amounts of dsDNA is important, in particular in forensic DNA-analysis. To quantify dsDNA without amplification the absorbance at 260 nm can be measured by UV-spectrophotometry, but such measurements are rather insensitive and influenced by the contribution of nucleotides and single-stranded DNA or contaminants (such as proteins or phenol) [1,2]. Therefore, of the known methods for the detection and quantification of dsDNA, such as nucleic acid stains (e.g. Methyl Violet and Thiazole dyes) and nucleic acid labels (e.g. Texas Red, Cy5 and Carboxy-X-Rhodamine (ROX)), the majority is based on fluorescence detection [3]. Three main classes of nucleic acid stains exist: intercalating dyes (e.g. ethidium bromide (EtBr) and propidium iodide (PI)), minor-groove binders (e.g. DAPI and Hoechst dyes) and other nucleic acid stains (e.g. acridine orange). The possible binding options, i.e. (bis-)intercalation, major and minor groove binding and external binding, are depicted in Fig. 1 [4].

In the past the classic intercalating dyes EtBr and PI were often used, which both bind at a stoichiometry of 1 dye molecule per 4 to 5 base pairs [4]. These dyes have a limited sensitivity and high background fluorescence.

The sensitivity can be increased by adding positively charged side chains to a dye, which increases the electrostatic interaction between dye and DNA. Another possibility is to add a (positively charged) linker to form a dimeric or trimeric dye [5,6]. Cyanine dyes have a high affinity for nucleic acids and show 100- to 1000-fold fluorescence enhancement upon binding to DNA and cyanine dyes are also less mutagenic than the classic dyes [4]. In this research the spectral properties of cyanine fluorescent dyes EvaGreen (EG), SYBR Green (SG), PicoGreen (PG), AccuClear (AC), AccuBlue NextGen (AB) and YOYO-1 (YO) are investigated for direct detection and quantification of low amounts of dsDNA, without any amplification. These dyes are selected, because they are commonly used as nucleic acid stains within real-time PCR, gel analysis, DNA quantification kits (e.g. Qubit PicoGreen Assay) and melting curve analysis. EvaGreen and SYBR Green are dyes from two different manufacturers widely used for real-time PCR and melt curve analysis, but also for agarose gel staining [7,8]. PicoGreen and AccuClear/AccuBlue NextGen are also from two different manufacturers and are developed for the quantification of dsDNA dyes, whereas the last two are specifically designed to quantify low amounts of dsDNA without any amplification step [9]. These newly developed dyes are compared with YOYO-1, a dye that is already available for some decades and used for (counter)staining DNA [10].

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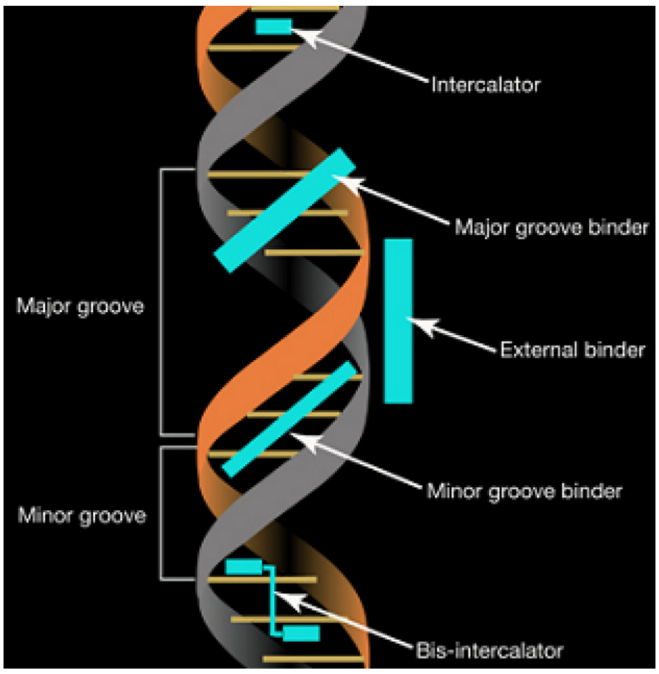

Fig. 1. Schematic diagram showing the possible binding modes of dsDNA dyes, used with permission from Thermo Fisher Scientific, copyrighted 2015 (*www.lifetechnologies.com*) [4].

In the Data in Brief (DiB) a literature based overview is given of the absorption, excitation and emission wavelengths at which maxima occur for the various dyes, as free dye in solution and as dye/dsDNA complex [22].

The reported linearity of each dye obtained with various detection methods, is shown in Table 1. Since some groups report the linear range in concentration units [2,12,15] whereas others present the range in absolute amounts of dsDNA [11,14,18], comparison is difficult. When reported as concentration range, either the final concentration in the detection volume can be given or the added concentration of dsDNA to the dye. Similar, reports in absolute amounts of dsDNA can give the total amount in the detection volume or the added amount of dsDNA to the dye. Therefore, if it is mentioned in literature, the linear range is converted to absolute amounts of dsDNA (when reported in concentration units) or the end concentration (upon reporting in absolute amounts) and the volume of dsDNA in the total volume is given in Table 1.

From the review in the DiB, and in particular the data in Table 1 and Table 1.1 (DiB) [22], it becomes clear that for each dye the reported spectral data and linear ranges vary significantly. The most striking differences in terms of linearity are found at low dsDNA concentrations, which is likely due to the use of different experimental setups. Therefore the six selected dyes are restudied under exactly similar conditions, in one and the same instrument (a microplate reader), with a focus on the pg-ng range of dsDNA. For AccuClear and AccuBlue NextGen, this will be the first report in an academic journal. Note that in this paper no statements are made about the use and linearity of the dyes in the context of real-time PCR, which is another well-established method to quantify dsDNA. Here only the direct fluorescent quantification is discussed.

2. Materials and methods

Materials. Deoxyribonucleic acid sodium salt from salmon testes (D1626) was obtained from Sigma-Aldrich. SYBR® Green I (10.000X concentrate in DMSO) and Quant-iT™ PicoGreen® (400X concentrate) in DMSO were purchased from Life Technologies, as well as

Table 1
Linearity of dsDNA dyes according to literature for the given dye concentration, detection volume and detection method. If no information on the exact wavelength of excitation and/or emission was reported (e.g. because a LED was used for excitation), mentioned excitation and emission values are maxima of spectra. # = on assumption that the end concentration or final amount of dsDNA has been reported, * = information from the manufacturer of the dye.

	Linear range (DNA)	Concentration (dye)	Volume	Detection method	Incubation	Ref.
EG	5–250 pg/μL 10 μL (1–50 ng)	0.63 μM (0.5X)	200 μL	Microplate reader (485/530 nm)	30 min @ RT	[5]
	10–500 ng 95 μL (0.1–5 ng/μL)#	1.0X (1.33 μM)	100 μL	Spectrofluorometer (503/527 nm)	5 min @ 72 °C	[11]
	0–10 ng/μL n/a (0–2000 ng)#	11.15 μM	200 μL	Microplate reader (490/530 nm)	30 min @ 50 °C	[12]
	2.4–60 pg/μL n/a (n/a)	0.1X (0.133 μM)	~10 nL	CE & LIF (488/520 nm)	Measuring @ RT	[13]
	1–100 ng 23.5 μL (0.04–4 ng/μL)#	1.0X (1.33 μM)	25 μL	Real-time PCR (490/520 nm)	Measuring @ 25 °C	[14]
SG	2.4–30 pg/μL n/a (n/a)	Not known (1/40,000 dilution)	~10 nL	CE & LIF (488/520 nm)	Measuring @ RT	[13]
	0–2 pg/μL n/a (n/a)	1.0X (0.68 μM)	n/a	Spectrofluorometer (480/520 nm)	5 min @ RT	[15]
	0.5–50 ng n/a (n/a)	1.0X (0.68 μM)	n/a	Agarose gel (460/560–700 nm)	not given	[16]
PG	0.025–1000 pg/μL 1 mL (0.05–2000 ng)	1.0X (0.8 μM)	2 mL	Spectrofluorometer (480/520 nm)	2–5 min @ RT	[2], [9],*
	3–450 pg/μL n/a (n/a)	Not given	Not given	Spectrofluorometer (480/520 nm)	not given	[17]
	0–2 pg/μL n/a (n/a)	1.0X (0.8 μM)	n/a	Spectrofluorometer (480/520 nm)	5 min @ RT	[15]
	0.25–150 ng 10 μL (1.67–1000 pg/μL)	Not known (1/200 dilution)	150 μL	Spectrofluorometer (485/520 nm)	not given	[18]
AC	0.03–250 ng 10 μL (0.14–1190 pg/μL)	1.0X	210 μL	Microplate reader (468/507 nm)	5 min @ RT	[19]*
AB	1–3000 pg 10 μL (0.005–14.29 pg/μL)	1.0X	210 μL	Microplate reader (468/507 nm)	5 min @ RT	[20]*
YO	0.5–100 pg/μL n/a (1–200 ng)#	200 nM	2 mL	Fluorometer (470/510 nm)	not given	[21]