SUN Probes from IDT Demonstrate Superior Results to VIC Probes in qPCR

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Objective: The SUN fluorophore is a molecular equivalent to the VIC fluorophore[1]. The SUN fluorphore is thought to offer signal intensity that is either comparable or superior to dyes with same excitation (538 nm) and emission (554nm) spectra[1]. To evaluate how the SUN fluorophore performs in comparison to the VIC fluorophore, we carried out qPCR experiments that targeted the Beta-Actin (ACTB) gene.

Experimental Design: We designed qPCR assays with identical primers and probe sequences to amplify the ACTB gene, employing two different probe configurations. To directly compare two 5' nuclease probes, one was labeled with a 5' SUN fluorophore sourced from Integrated DNA Technologies (IDT), while the other had a 5' VIC fluorophore sourced from Life Technologies Corporation. The double-quenched probe, denoted as 5'SUN/3'ZEN/IABkFQ, exhibited a more robust fluorescence signal compared to the VIC/Tam probe. We used a 542 bp ACTB gBlocks™ Gene Fragment to create quantitative standards which served as templates during amplification. This fragment was 10-fold serially diluted to create 7 standards in 10mM Tris-HCL, pH 8.0 containing 0.1 mg/ml yeast RNA. The most concentrated standard was "S6" (5 $x10^5$ copies/µL) and the lowest concentration standard was labelled "S0" (5 $x10^{-1}$ copies/µL). Each standard was run twelve times (in quadruplicate over three runs) and no template control (NTC) run 6 times (in duplicate over three runs). This was repeated for both SUN and VIC/Tam probe-containing mixes that included primers and Applied Biosystems (ABI) Taqman Fast Polymerase Master Mix. The reactions were run on an ABI Fast Advanced Real-Time PCR System (ThermoFisher Scientific) with the following PCR conditions: 2 minutes at 50°C, 20 seconds at 95°C, and 40 cycles of 3 seconds at 95°C and 30 seconds at 60°C. Using the instrument software and a threshold of 0.1 and auto-baseline setting, the threshold-crossing (C_t) values were determined. C_t was plotted on the y-axis versus the respective log-transformed copies per reaction (x-axis). A linear regression analysis was performed by the instrument software and the slope, y-intercept and linearity (as expressed in the R^2 value from the standard curve) were calculated. From the slope, the PCR amplification efficiency was calculated using the equation (E= (10^(-1/-slope)-1)*100).

Results: The PCR amplification efficiency for the SUN probe is 95.35%, with a slope of -3.438707, an intercept of 37.885590, and an R-squared value of 0.999499 (Table 1). For the VIC probe, the amplification efficiency is approximately 91.12%, with a slope of -3.554989, an intercept of 40.032648, and an R-squared value of 0.999842. The SUN-labeled probe exhibited excellent signal intensity (Figure 1), generating earlier Ct values for the Beta-actin gene target (Table 2A and 2B). All NTC's had the anticipated result of "not detected" indicating a lack of template contamination on the runs.

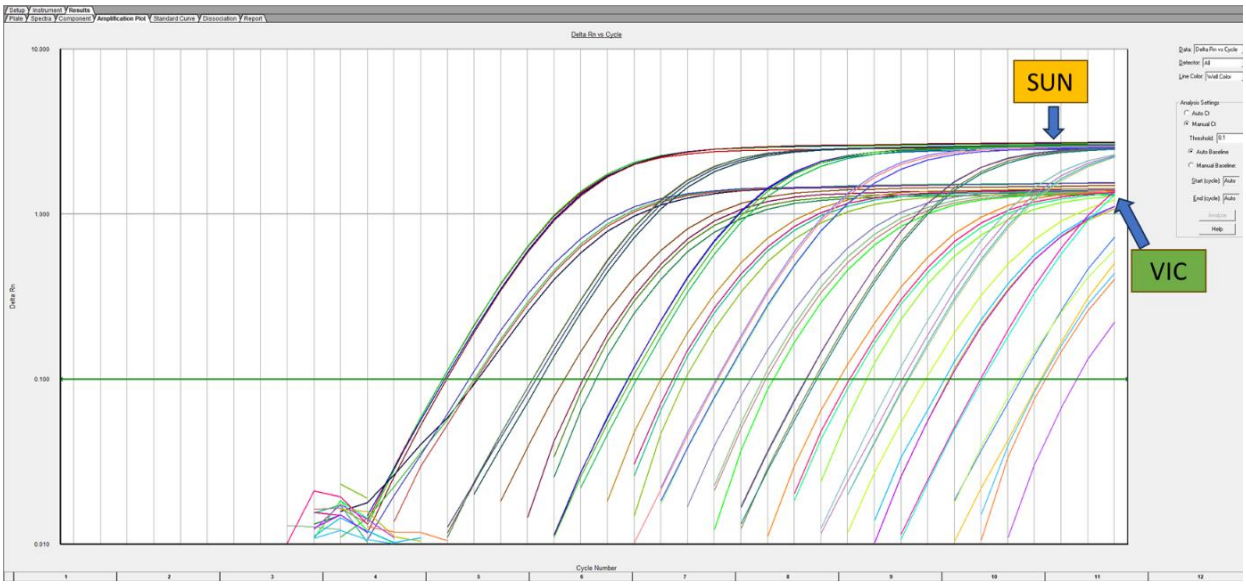

Figure 1: SUN vs VIC PCR amplification plot with 0.1 Threshold and Auto-baseline

Table 1: SUN vs VIC probe performance

Probe	Slope	Intercept	R^2	PCR Amplification Efficiency
SUN/ZEN/IABkFQ	-3.438707	37.885590	0.999499	95.35%
Vic-Tam	-3.554989	40.032648	0.999842	91.12%

Conclusion: In conclusion, the probe possessing the double quenched SUN fluorophore exhibited excellent PCR amplification efficiency, with high R-squared values indicating a strong correlation between cycle threshold and template concentration. These results suggest that both probes are reliable options for PCR applications, with the SUN probe showing a slight advantage in terms of amplification efficiency.

Table 2A. Standard Screening with SUN probe

Well	Sample Name	Run-1 SUN Ct	Run-2 SUN Ct	Run-3 SUN Ct	Cps/rxn	Log Cps/rxn	Ave Ct
A1	SC-S6	14.3148	15.4980	15.3677	5.00E+06	6.70	15.1089
A2	SC-S6	14.3278	15.4885	15.4216			
A3	SC-S6	14.1966	15.5916	15.5742			
A4	SC-S6	14.2295	15.6051	15.6915			
B1	SC-S5	17.7782	18.3170	18.1917	5.00E+05	5.70	18.1838
B2	SC-S5	17.9307	18.3064	18.2920			
B3	SC-S5	17.6349	18.4527	18.4116			
B4	SC-S5	17.6222	18.6246	18.6441			
C1	SC-S4	21.2759	21.6384	21.5391	5.00E+04	4.70	21.6150
C2	SC-S4	21.2343	21.6836	21.6122			

C3	SC-S4	21.1915	21.8720	21.9383			
C4	SC-S4	21.1701	22.0772	22.1474			
D1	SC-S3	24.6441	25.0814	25.0595	5.00E+03	3.70	25.0566
D2	SC-S3	24.6227	25.0816	25.2248			
D3	SC-S3	24.5853	25.3968	25.3159			
D4	SC-S3	24.6502	25.3674	25.6491			
E1	SC-S2	28.0760	28.4776	28.3887	5.00E+02	2.70	28.5073
E2	SC-S2	28.1815	28.3858	28.5764			
E3	SC-S2	28.0860	28.7636	29.0167			
E4	SC-S2	28.0778	28.8907	29.1663			
F1	SC-S1	31.3044	31.8363	31.9715	5.00E+01	1.70	31.9836
F2	SC-S1	31.4206	32.0747	32.0461			
F3	SC-S1	31.5841	32.3373	32.4112			
F4	SC-S1	31.4935	32.2661	33.0572			
G1	SC-S0	35.1245	35.1858	35.0047	5.00E+00	0.70	35.7063
G2	SC-S0	35.2825	35.0808	35.1762			
G3	SC-S0	35.4638	37.3448	36.6307			
G4	SC-S0	35.0152	36.5648	36.6013			
H5	NTC	Undetermined	Undetermined	Undetermined	N/A		
H6	NTC	Undetermined	Undetermined	Undetermined			

Table 2B. Standard Screening with VIC Probe

Well	Sample Name	Run-1- Vic Ct	Run-2- Vic Ct	Run-3- Vic Ct	Cps/rxn	Log Cps/rxn	Ave Ct
A9	SC-S6	15.2692	17.1169	16.8068	5.00E+06	6.70	16.2975
A10	SC-S6	15.3619	16.8598	16.7140			
A11	SC-S6	15.1999	16.7774	16.9347			
A12	SC-S6	15.2176	16.4347	16.8767			
B9	SC-S5	18.6535	20.4970	20.3425	5.00E+05	5.70	19.7202
B10	SC-S5	19.4145	20.2217	20.1072			
B11	SC-S5	18.7039	20.1096	20.3799			
B12	SC-S5	18.5261	19.4458	20.2407			
C9	SC-S4	22.4044	24.2447	23.7835	5.00E+04	4.70	23.3036
C10	SC-S4	22.8508	23.9129	23.6063			
C11	SC-S4	22.2181	23.7453	23.8692			
C12	SC-S4	22.0628	23.1792	23.7659			
D9	SC-S3	26.0854	27.8985	27.5461	5.00E+03	3.70	26.9390
D10	SC-S3	26.2650	27.5097	27.2963			
D11	SC-S3	25.8176	27.3533	27.7438			
D12	SC-S3	25.7475	26.7037	27.3011			
E9	SC-S2	29.3065	31.2289	31.0205	5.00E+02	2.70	30.3660

E10	SC-S2	29.4735	31.0220	31.0199			
E11	SC-S2	28.9952	30.9045	31.2715			
E12	SC-S2	29.0165	30.2053	30.9278			
F9	SC-S1	33.1045	34.7303	35.0249	5.00E+01	1.70	33.8510
F10	SC-S1	32.9018	34.5583	34.4809			
F11	SC-S1	32.5824	34.5379	34.0436			
F12	SC-S1	32.3706	33.3114	34.5651			
G9	SC-S0	38.2583	38.5332	38.4330	5.00E+00	0.70	37.7027
G10	SC-S0	37.3379	38.1632	36.7498			
G11	SC-S0	35.2101	39.8891	38.4248			
G12	SC-S0	36.0286	36.9590	38.4455			
H7	NTC	Undetermined	Undetermined	Undetermined	N/A		
H8	NTC	Undetermined	Undetermined	Undetermined			

Reference:

1. Nan Pazdernik, PhD. “SunTM Fluorophore: IDT.” Integrated DNA Technologies, October 18, 2022. https://eu.idtdna.com/pages/education/decoded/article/sun-fluorophore-a-molecµLar-equivalent-to-vic.