Application Note

Using Pico Green to Quantify DNA

Introduction

A common task in molecular biology labs is the quantification of small amounts of DNA. Knowing the amount of DNA in a sample is necessary for a number of research applications, such as setting up PCR and dye-terminator sequencing reactions and synthesizing cDNA for library production.

The most widely used method of measuring DNA concentration is using the A260 absorbance reading. While it is a quick and easy process, there are several significant disadvantages to this technique.

- There is a large signal contribution made by single-stranded DNA, RNA, and free nucleic acids.
- Contaminants (such as proteins) often found in sample preparations can interfere with the signal.
- The sensitivity is poor, with an A260 reading of 0.1 corresponding to 5ug/mL.

PicoGreen is a DNA probe that can be used to quantitate small amounts of dsDNA without purification of the sample preparation. It is not affected by contaminants such as protein, and shows little or no selectivity to AT or GC sequences. (1)

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Protocol

Preparation of Standards

In this experiment, standards were prepared from Lambda DNA with an initial concentration of 100ul/mL. The standards were prepared in the first row of a 96 well plate in the following way.

Position	Volume standard + diluent	Concentration (ng per 200ul)
A1	4ul of stock + 196ul TE	200
A2	100ul of A1 + 100ul TE	100
A3	100ul of A2 + 100ul TE	50
A4	100ul of A3 + 100ul TE	25
A5	100ul of A4 + 100ul TE	12.5
A6	100ul of A5 + 100ul TE	6.25
A7	100ul of A6 + 100ul TE	3.125
A8	100ul of A7 + 100ul TE	1.5625
A9	100ul of A8 + 100ul TE	0.78125
A10	100ul of A9 + 100ul TE	0.390625
A11	100ul of A10 + 100ul TE	0.1953125
A12	100ul of A11 + 100ul TE - 100ul	0.09765625

100ul was removed from well A12 to give a total volume of 100ul (equal to the volumes of the other standard wells).

Preparation of Samples

2ul of each sample were added to 98ul of TE, and samples were placed into the 96 well plate.

Preparation of PicoGreen

The PicoGreen is stored in DMSO. A 200-fold dilution of this stock was made in TE. 100ul of diluted PicoGreen was needed per sample or standard.

100ul of diluted PicoGreen was added to each well in the 96 well plate. The plate was incubated in the dark (to avoid photodegradation of the PicoGreen) for 5 minutes to completely bind fluorescent dye to the DNA.

The plate was then read on the Tecan Genios plate reader.

Setting Measurement Parameters for Relative Fluorescent Unit (RFU) readings in Magellan and Xfluor

1. Open the Edit Measurement Parameters window. This is the fourth window of the Magellan wizard for Create/Edit a Method, and is in the Xfluor4 menu of Xfluor.
2. General Tab: Select Fluorescence Intensity.
3. Plate Tab: Select the pdf file for the microtiter plate in use.
4. Meas Params Tab: Under Fluorescence wavelengths, choose Other and set the excitation filter to 485nm and the emission filter to 535nm. Set the Gain to Optimal, and the Read Mode to Top. Integration and Flash values can be left at the default settings.
5. Kinetics Tab: Leave Kinetics box unchecked.
6. Temperature Tab: Leave checkboxes unchecked.
7. Shaking Tab: Leave checkboxes unchecked. When finished, click OK.

Results

Excel was used to graph the results from the read.

Rawdata-Reading #1 Temperature: 25.5 °C

<>	1	2	3	4	5	6	7	8	9	10	11	12
A	51978	28482	12951	6693	3247	1883	1112	700	507	405	347	283
B	51954	49426	39424	38501	15812	38162	35700	33098	52271	39883	295	324
C	1194	22312	23821	40153	26349	29973	47212	48837	39802	14363	293	302
D	777	585	13944	629	790	12274	8474	5097	10776	13395	10992	13062
E	11558	12585	10897	14210	12629	11995	12255	14339	11150	10037	2310	12921
F	12982	1181	11299	10354	14778	6780	4671	6493	362	615	369	299
G												
H												

Rawdata-Reading #2 Temperature: 25.5 °C

<>	1	2	3	4	5	6	7	8	9	10	11	12
A	50360	27679	12893	6600	3225	1909	1112	697	505	399	336	282
B	51685	48493	38485	37684	15547	37001	34721	32536	51060	38896	288	321
C	1170	21924	23501	39350	26102	29421	46281	47862	38952	14077	290	298
D	774	573	13765	615	770	12042	8323	4969	10511	13006	10727	12863
E	11160	12276	10723	13905	12407	11810	11856	14000	10991	9823	2256	12838
F	12672	1146	11053	10178	14501	6522	4585	6352	347	589	350	294
G												
H												

Rawdata-Reading #3 Temperature: 25.6 °C

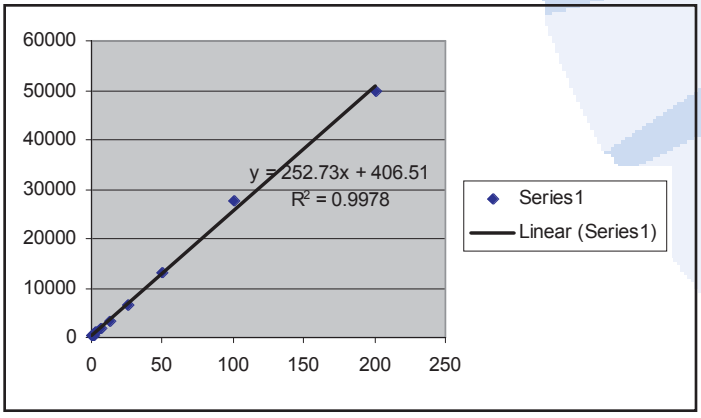
<>	1	2	3	4	5	6	7	8	9	10	11	12
A	49137	27467	12954	6661	3260	1912	1109	708	494	397	333	273
B	51535	48498	37609	36762	15371	35890	34159	31794	50258	38307	288	319
C	1148	21545	23202	38459	25666	28957	45627	46984	37987	13937	291	303
D	764	569	13593	598	759	11838	8175	4889	10326	12759	10546	12681
E	10995	12072	10548	13645	12213	11701	11640	13694	10707	9665	2213	12807
F	12509	1131	10760	10086	14261	6327	4531	6287	343	590	359	288
G												
H												

Rawdata-Reading #4 Temperature: 25.6 °C

<>	1	2	3	4	5	6	7	8	9	10	11	12
A	48462	27326	12944	6645	3274	1917	1102	716	499	391	329	272
B	51151	48529	37080	36214	15153	35294	33307	31631	49824	37696	284	307
C	1145	21254	22905	37806	25376	28563	44997	46466	37346	13797	284	293
D	761	572	13541	582	757	11687	8121	4817	10211	12498	10274	12553
E	10808	11928	10404	13552	12019	11520	11560	13567	10578	9587	2176	12771
F	12360	1114	10680	9894	14120	6255	4507	6178	339	574	349	292
G												
H												

Average

<>	1	2	3	4	5	6	7	8	9	10	11	12
A	49984	27739	12936	6650	3252	1905	1109	705	501	398	336	278
B	51581	48737	38150	37290	15471	36587	34472	32265	50853	38696	289	318
C	1164	21759	23357	38942	25873	29229	46029	47537	38522	14044	290	299
D	769	575	13711	606	769	11960	8273	4943	10456	12915	10635	12790
E	11130	12215	10643	13828	12317	11757	11828	13900	10857	9778	2239	12834
F	12631	1143	10948	10128	14415	6471	4574	6328	348	592	357	293
G												
H												



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ng/200ul	RFU	Actual
200	49984	196.1688
100	27739	108.1470
50	12936	49.5746
25	6650	24.7032
12.5	3252	11.2570
6.25	1905	5.9302
3.125	1109	2.7786
1.5625	705	1.1821
0.78125	501	0.3749
0.390625	398	-0.0337
0.195313	336	-0.2780

Concentration

<>	1	2	3	4	5	6	7	8	9	10	11	12
Stand-ards	98.0844	54.0735	24.7873	12.3516	5.6285	2.9651	1.3893	0.5910	0.1874	-0.0168	-0.1390	-0.2552
A	98.0844	54.0735	24.7873	12.3516	5.6285	2.9651	1.3893	0.5910	0.1874	-0.0168	-0.1390	-0.2552
B	101.2439	95.6159	74.6706	72.9706	29.8030	71.5788	67.3945	63.0282	99.8036	75.7508	-0.2330	-0.1756
C	1.4991	42.2432	45.4057	76.2385	50.3833	57.0213	90.2598	93.2433	75.4070	26.9794	-0.2315	-0.2127
D	0.7171	0.3328	26.3211	0.3947	0.7171	22.8579	15.5635	8.9750	19.8819	24.7458	20.2355	24.4990
E	21.2158	23.3624	20.2518	26.5530	23.5637	22.4548	22.5957	26.6955	20.6742	18.5405	3.6249	24.5870
F	24.1844	1.4571	20.8552	19.2330	27.7143	11.9980	8.2440	11.7141	-0.1163	0.3670	-0.0984	-0.2241
G												
H												

	= KF PRs		V2 = m1(100ul/50ng/ul)-100ul, NOTE: m1=conc. in ng/ul values of each samples, , -100ul original vol.
	= Probes		V2 = m1(45ul/10ng/ul)-45ul, NOTE: m1=conc. in ng/ul values of each samples, -45 ul original vol.
	= Standard DNA		
	= Blank wells		

Acknowledgements

We would like to thank Gerard Magpantay of Ceres, Inc. for supplying the materials and data from the experimental run.

References

1. *Comparison of two different detection techniques for DNA quantification: Absorbance vs. Fluorescence.* Tecan, Inc. July, 2000.

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