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Achieving Fast Amplification with Maverick qPCR Systems

Introduction

In many molecular test applications, time-to-result is of paramount importance. There is a strong demand for fast cycling speed in a qPCR device. The application note describes the methods to complete nucleic acid amplification with Maverick qPCR in less than 30 minutes.

Reagent

The qPCR amplification kit was purchased from Tiansi (Shanghai) Technology Co., Ltd. (TOTOIVD 5G® qPCR Premix, Cat No: QPT-200U); primer probe purchased from homegrown bioengineering (Shanghai) Co., Ltd. (forward primer sequence: AGATTTGGACCTGCGCG; reverse primer sequence: GAGCGGCTGTCTCCACAAGT; probe sequence: TTCTGACCTGAGGCTCTGCGCG; amplified fragment length 70bp).

Equipment

Two Anitoa Maverick MQ 4164 instruments, with identification numbers #1834 #1835 are used to perform this test.

Using the reagent listed above, we tried 3 different fast amplification programs to detect nucleic acids and evaluate repeatability, sensitivity, and linearity.

Procedure 1：37℃ 60s；93℃ 60s；93℃ 1s，64℃ 1s（45 cycles)

Procedure 2：37℃ 60s；95℃ 60s；95℃ 1s，56℃ 1s（45 cycles)

Procedure 3：37℃ 60s；93℃ 60s；93℃ 1s，56℃ 1s（45 cycles)

Note that for the 45 temperature cycles, the denature time and annealing time is only 1 second, this is the shortest time allowed by the software. The actual cycle time is therefore dominated by temperature ramp up and ramp down time, as well as the time the instrument allow for some overshot.

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Experimental results

Amplification time results

Instrument number	Amplification procedure 1	Amplification procedure 2	Amplification procedure 3
1834	24m49s	28m18s	26m52s
1835	25m49s	28m5s	27m9s

Program 2 takes the longest time due to the larger temperature difference between denature and annealing, leading to longer ramping time.

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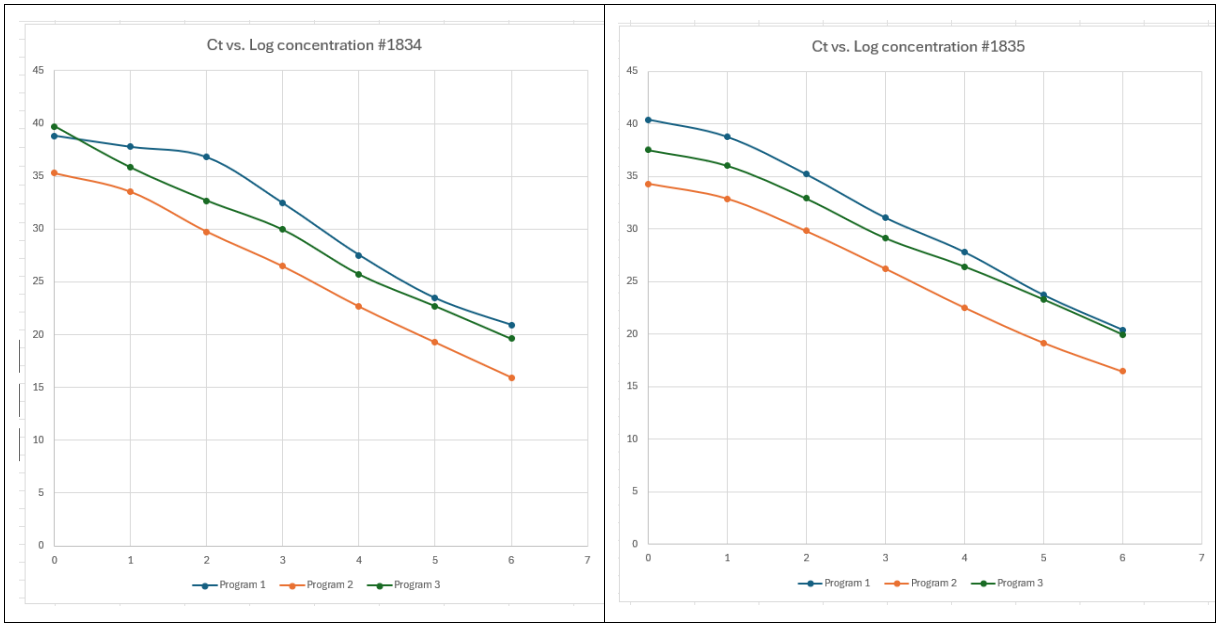
Linearity test results

The table below shows the ct values as a function of the template concentration. Program 2 and Program 3 both show good linearity. Program 2 results in lower Ct values, indicating better amplification efficiency.

Program	1.00E+06	1.00E+05	1.00E+04	1.00E+03	1.00E+02	1.00E+01	1.00E+00	R value
1834-1	20.9	23.44	27.53	32.44	36.8	37.78	38.84	0.9783
1834-2	15.95	19.27	22.66	26.47	29.75	33.53	35.29	0.9995
1834-3	19.62	22.71	25.72	29.94	32.7	35.83	39.73	0.9974
1835-1	20.42	23.74	27.82	31.1	35.23	38.79	40.43	0.9992
1835-2	16.45	19.18	22.53	26.24	29.81	32.89	34.31	0.9982
1835-3	19.94	23.29	26.43	29.14	32.91	36.04	37.53	0.9987



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Repeatability test

In this test, identical reaction mixes are repeated in 4 tubes and their ct variation is evaluated. In all cases, the covariance is below 3%. There is no statistically significant difference between the 3 amplification programs.

Program	Channel	1.00E+03	1.00E+03	1.00E+03	1.00E+03	average value	standard deviation	CV
1834-1	FAM	32.24	32.39	32.25	32.51	32.35	0.128	0.40%
	VIC	33.73	33.31	33.41	32.95	33.35	0.321	0.96%
	ROX	33.4	33.41	33.43	33.35	33.40	0.034	0.10%
	CY5	33.18	33.2	33.15	32.86	33.10	0.159	0.48%
1834-2	FAM	27.27	27.07	27.47	28.15	27.49	0.47	1.71%
	VIC	28.95	28.74	28.99	28.98	28.92	0.12	0.41%
	ROX	29.36	28.95	29.51	29.4	29.31	0.25	0.84%
	CY5	28.23	27.81	27.88	28.17	28.02	0.21	0.74%
1834-3	FAM	31.76	31.44	30.85	30.21	31.07	0.68	2.20%
	VIC	32.31	31.52	30.78	30.6	31.30	0.78	2.49%
	ROX	32.31	31.62	31	30.67	31.40	0.72	2.30%
	CY5	31.03	30.38	29.93	29.55	30.22	0.64	2.11%
1835-1	FAM	32.69	32.65	31.87	31.58	32.20	0.56	1.73%
	VIC	32.57	32.55	32.15	31.76	32.26	0.38	1.19%
	ROX	32.58	32.57	32.47	32.02	32.41	0.26	0.82%
	CY5	32.06	32.18	31.43	31.4	31.77	0.41	1.29%
1835-2	FAM	27.62	28.06	27.92	27.91	27.88	0.18	0.66%
	VIC	27.84	27.61	27.85	27.79	27.77	0.11	0.40%
	ROX	28.59	29.25	29.45	29.36	29.16	0.39	1.34%

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	CY5	27.06	27.31	27.24	27.32	27.23	0.12	0.44%
1835-3	FAM	31.68	31.02	30.55	30.64	30.97	0.51	1.66%
	VIC	31.69	31.24	30.73	30.57	31.06	0.51	1.64%
	ROX	32.12	31.35	31.34	31.22	31.51	0.41	1.31%
	CY5	31.23	30.62	29.76	30.1	30.43	0.64	2.11%

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Sensitivity test

In this test, we tried very low concentration samples and tested the sensitivity. Only program 2 had shown consistent amplification curve for all samples and all channels.

Procedure	passage	1.00E+00	1.00E+00	1.00E+00	1.00E+00	Detection rate
1834-1	FAM	40.04	41.4	40.8	39.77	100%
	VIC	40.59	/	41.41	41.09	75%
	ROX	39.26	40.86	40.02	39.32	100%
	CY5	39.78	/	41.03	39.89	75%
1834-2	FAM	37.89	38.36	36.82	36.59	100%
	VIC	38.26	34.72	37.85	38.46	100%
	ROX	37.98	38.25	37.15	37.64	100%
	CY5	37.6	38	36.84	36.92	100%
1834-3	FAM	38.37	38.3	39.87	40.42	100%
	VIC	38.36	37.02	39.29	/	75%
	ROX	37.99	37.78	38.91	40.92	100%
	CY5	37.41	37.24	38.62	40.6	100%
1835-1	FAM	39.32	40.42	40.04	/	75%
	VIC	38.76	39.56	39.68	/	75%
	ROX	38.43	39.29	39.5	40.71	100%
	CY5	38.92	40.15	40.26	/	75%
1835-2	FAM	37.14	37.03	36.64	37.12	100%
	VIC	36.92	37.22	37.08	37.73	100%
	ROX	37.18	37.45	36.93	37.6	100%

	CY5	36.65	36.71	36.02	36.9	100%
1835-3	FAM	39.62	39.11	38.66	39.46	100%
	VIC	39.32	39.04	37.08	39.32	100%
	ROX	39.03	38.9	38.68	39.01	100%
	CY5	38.94	38.7	38.46	39.03	100%

Discussion

Overall, in terms of efficiency and sensitivity, Program2 > Program 3 > Program 1. The most important factor is the effective annealing temperature. We need to make sure the annealing temperature is reached. In program 2 and 3, the annealing temperature is set to be 56℃, which is what the assay wants. In Procedure 1, the annealing temperature is set higher, resulting in quicker cycling time but at the expense of less amplification efficiency and sensitivity.

Procedure 2 improves over procedure 3 by setting the denature temperature higher too, this ensures successful denature even with very short denature temperature hold time.

Conclusion

Similarly predetermined denature and annealing time, the smaller the temperature difference between denaturation and annealing, the shorter the reaction time. However, reaching desired denature and annealing time will impact amplification efficiency and sensitivity. Reaction procedure 1 is the shortest time, followed by reaction time 2 and reaction time 3 again. The performance of Reaction Procedure 2 and Reaction Procedure 3 is not much different, and the sensitivity of Reaction Procedure 1 will be slightly impaired.