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APPLICATION NOTE No. 274

Comparative Run Time Evaluations of PCR Thermal Cyclers

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Abstract

For the purpose of comparing the speed of different thermal cyclers, the isolated consideration of ramp rates cited in the technical specifications often does not reflect the actual run times. An estimate of actual run times based on these technical ramp rates may thus lead to false conclusions.
The Mastercycler® X50s as expected from its high maximum heating ramp rate, achieved the shortest total PCR run time in these evaluations. Some thermal cyclers made by other manufacturers showed noticeably longer run times than the Mastercycler X50a despite similar cited (maximum heating) ramp rates. Although, the Mastercycler X40 has the lowest (maximum heating) ramp rate of all thermal cyclers tested, it achieved a shorter PCR run time than several thermal cyclers from other manufacturers in these evaluations.

Introduction

Besides control accuracy and temperature homogeneity, the customary technical details of a thermal cycler also include the ramp rate of the thermoblock. The ramp rate, in particular, is not subject to a uniform standard; instead, manufacturers state a variety of parameters, such as:
> maximum heating and cooling rate
> maximum ramp rate
> average ramp rate
> maximum sample ramp rate.

Thus, the user is left with the option of estimating the actual ramp rates based on these diverse statements. Therefore, comparative investigations were undertaken in order to evaluate whether the details pertaining to the ramp rates stated in the technical specifications are suitable for estimating the total run times of PCR applications on thermal cyclers.

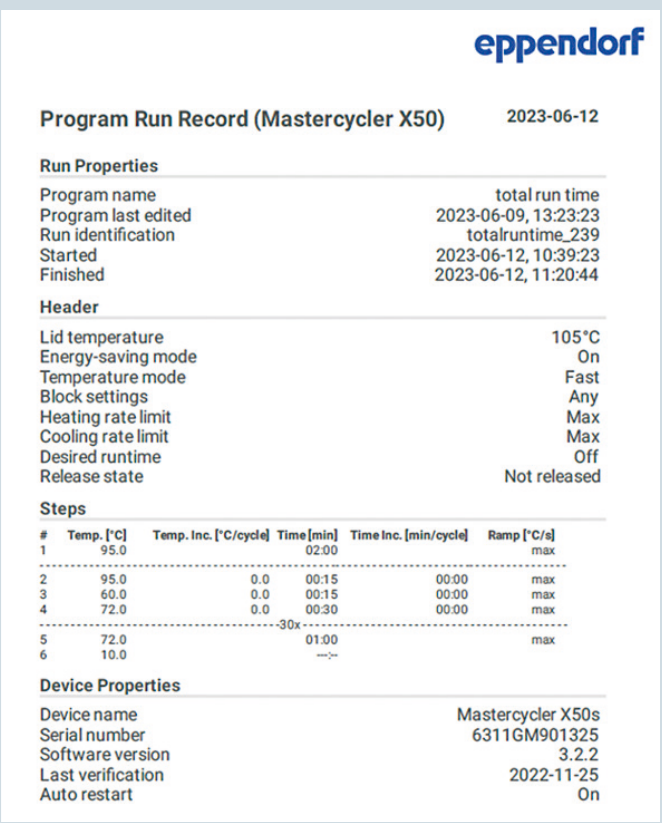
Materials and Methods

All positions of an unskirted 96 well plate (Eppendorf twin.tec® PCR Plate 96) were filled with 10 µL water.
In general, low profile plates were used for all thermal cyclers except the Proflex, AllInOneCycler™, SimpliAmp™ and T100™ as these thermal cyclers are only compatible with high profile PCR consumables. In these instances, high profile plates were used.

The plate was subsequently sealed with the Eppendorf Heat Sealing Foil, centrifuged for 1 min at 500–1000 × g, placed into the thermal cycler and subjected to a standard 3-step PCR program (Fig. 1).
The run times were determined for the Mastercycler® X50s, Mastercycler® X50a, Mastercycler® X40, and ten competing thermal cyclers. In cases where the respective thermal cycler software allowed for different temperature control modes or reaction volume settings, the fastest ramping speed and/or 10 µl volume setting were chosen.

Measurement of total run time was initiated immediately following commencement of the first temperature step (at 95.0 °C see A in Fig. 1), and it ended immediately after 10.0 °C of the final step had been reached (see B in Fig.1). These measurements were performed on only one device per model. However, the measurements were performed 3 times on the same device. Data shown in table 1 represent the mean of these 3 measurements (rounded to the nearest 10 seconds).
The first page of an exemplary program run record of the Mastercycler X50s is shown in Figure 2.

Figure 1: 3-step PCR program for run time determination.
A - Start of run time measurement
B - End of run time measurement



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Program Run Record (Mastercycler X50) 2023-06-12

Run Properties

Program name	total run time
Program last edited	2023-06-09, 13:23:23
Run identification	totalruntime_239
Started	2023-06-12, 10:39:23
Finished	2023-06-12, 11:20:44

Header

Lid temperature	105°C
Energy-saving mode	On
Temperature mode	Fast
Block settings	Any
Heating rate limit	Max
Cooling rate limit	Max
Desired runtime	Off
Release state	Not released

Steps

#	Temp. [°C]	Temp. Inc. [°C/cycle]	Time [min]	Time Inc. [min/cycle]	Ramp [°C/s]
1	95.0		02:00		max
2	95.0	0.0	00:15	00:00	max
3	60.0	0.0	00:15	00:00	max
4	72.0	0.0	00:30	00:00	max
		-30x			
5	72.0		01:00		max
6	10.0		--:--		

Device Properties

Device name	Mastercycler X50s
Serial number	6311GM901325
Software version	3.2.2
Last verification	2022-11-25
Auto restart	On

Figure 2: Screenshot from a Mastercycler X50s run protocol, exported as pdf file, from the instrument software (additional information, e.g. user, program details and additional cycler settings are not displayed in this section).

Results and Discussion

The evaluation of the (maximum heating) ramp rates stated by the manufacturers, in comparison with the empirically determined run times, highlighted the fact that isolated consideration of ramp rates in accordance with technical data is not suitable for the reliable prediction of the actual run time of a PCR program (Tab. 1).
On one hand, the thermal cycler Mastercycler X50s (silver-block) showed, as expected, the shortest total PCR run time, in accordance with the maximum heating ramp rate cited.
On the other hand, the run time of the AllInOneCycler was considerably longer than would be expected from the maximum heating ramp rate stated in the manufacturer's technical specifications.
Slight variances in time taken to complete a PCR may exist due to environmental factors (e.g. room ambient temperature, device placement, etc.). However, multiple repetitions have confirmed these differences to only be within a few seconds range (full data not shown in this application note).
It is evident that the thermal cyclers AllInOneCycler, Veriti™ Fast, SimpliAmp, XT^{96} and T100 were considerably slower in their actual PCR run times than the Mastercycler X40, despite the fact that the respective manufacturers had cited faster (maximum heating) ramp rates for these thermal cyclers in their technical specifications.
Furthermore, when comparing thermal cyclers with the same (maximum heating) ramp rate (e.g. Mastercycler X50a, PeqSTAR, 96X, Prima-96™ C1000 Touch™ and PTC Tempo), there was obvious difference in total PCR run time.
It can be assumed that the following parameters contribute strongly to the observed discrepancies:

> For the different thermal cyclers the (maximum heating) ramp rates stated in the technical manuals are reached for different periods of time during the ramping process from one temperature to the next – possibly for only a short time during each ramping phase for certain thermal cyclers.
> Temperature control modes or reaction volume settings may also exert considerable influence on ramping behavior [1]. This may even lead to the need to re-optimize a reaction following the transfer of a PCR system from one thermal cycler to another [2].

Manufacturer	Thermal cycler	Total run time [hh:mm:ss] (rounded to the nearest 10 seconds)	(Maximum heating) ramp rate according to technical data [°C/s]
Eppendorf	Mastercycler® X50s	00:39:00	10
Eppendorf	Mastercycler® X50a	00:45:10	5
VWR™	PeqSTAR 96X	00:47:20	5
HiMedia®	Prima-96™	00:48:00	5
Bio-Rad®	C1000 Touch™	00:48:20	5
Bio-Rad®	PTC Tempo 96	00:48:20	5
Applied Biosystems	Proflex™ (96-well)*	00:48:40	6.0
Eppendorf	Mastercycler® X40	00:51:20	3.3
Bioneer	AllInOneCycler™#*	00:51:40	9.5
Applied Biosystems	Veriti™ Dx Fast	00:53:40	5.0
Applied Biosystems	SimpliAmp™*	00:54:30	4.0
VWR™	XT^{96}	00:55:30	4
Bio-Rad®	T100*	01:02:50	4

Table 1: Total run time of a standard 3-step PCR protocol using the fastest settings for a sample volume of 10 µl possible in the instrument software. Due to diverse manufacturers' statements of ramp rates, only the (maximum heating) ramp rate which could be found according to the technical data for an instrument is presented here.

#96 well Fast PCR system.
*Performed in high profile twin.tec plate because the cyclers cannot accommodate low profile plates.

Conclusion

These comparative investigations have shown that the isolated consideration of (maximum heating) ramp rates often bears limited meaningfulness and may even lead to false conclusions with regards to the estimation of the actual PCR run time of a given thermal cycler.
When considering thermal cycler speed, especially when the objective is faster PCR completion or the ability to run more number of PCR per day, a comparison of total run times between different cyclers will be vastly more accurate.
Besides consideration of the technical data, for the purpose of an all-encompassing assessment of the performance of a thermal cycler it is strongly recommended to test the instrument in a demo-setting with regards to hardware, software and PCR applications.

References

[1] Application Note 244. www.eppendorf.com/pcr
[2] Hughes S., Moody A. (eds.): PCR. Scion Publishing Limited; 2007.

Ordering information

Description	Order no. International
Mastercycler® X40	6381 000 018
Mastercycler® X50a	6313 000 018
Mastercycler® X50h	6316 000 019
Mastercycler® X50l*	6303 000 010
Mastercycler® X50s	6311 000 010
Mastercycler® X50t*	6306 000 010
Mastercycler® X50i*	6301 000 012
Eppendorf twin.tec® PCR Plate 96, low profile (unskirted, 150 µl)	0030 133 307
Eppendorf twin.tec® PCR Plate 96 (unskirted, 250 µL)	0030 133 366
Eppendorf Heat Sealing Foil	0030 127 854
Eppendorf HeatSealer S200	5392 000 005

* To operate this unit, it needs to be connected to a Mastercycler X50 s,a, or h. Up to 9 units can be connected to a Mastercycler X50 s,a, or h.

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