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 heating and cooling 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.

While the Mastercycler® X50s as expected from its fast ramp rate, achieved the shortest total PCR run time in these evaluations, some thermal cyclers made by other manufacturers showed noticeably slower run times despite similar cited ramp rates.

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

48 positions of a 96 well plate (Eppendorf twin.tec® PCR Plate 96) were filled with 25 µL water, respectively (Fig. 1). In general, low profile plates were used for all thermal cyclers except the Proflex, SimpliAmp® and T100 cyclers as these thermal cyclers are only compatible with high profile PCR consumables. In these instances, high profile plates were used.

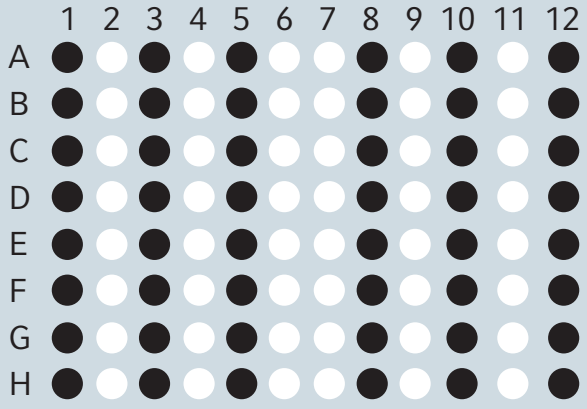

Figure 1: Positions indicated in black of the 96-well PCR plate were filled with 25 µL water each.

The plate was subsequently sealed with the Heat Sealing PCR Film (Eppendorf), centrifuged for 1 min at 500–1000 ×g, placed into the thermal cycler and subjected to a standard 3-step PCR program (Fig. 2).
The run times were determined for the Mastercycler® X50s, Mastercycler® X50l, Mastercycler® nexus gradient, and nine 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 the lowest volume setting were chosen.

Measurement of total run time was initiated immediately following commencement of the first temperature step, and it ended immediately after the temperature of the final step had been reached. These measurements were performed on only one device per model. However, the measurements were verified through multiple repeats on the same device performed on different days and time. The data reported in this paper was randomly chosen among the set of measurements performed for each thermal cycler model respectively. For thermal cyclers that record and save a detailed run protocol, it was possible to determine the total run time following completion of the run by consulting the records.
Apart from the Mastercycler® X50 and Mastercycler® nexus models, whose run protocols may be exported as pdf files for documentation purposes subsequent to the run (Fig. 3), a detailed, exportable record could be obtained from only three of the competing thermal cyclers.

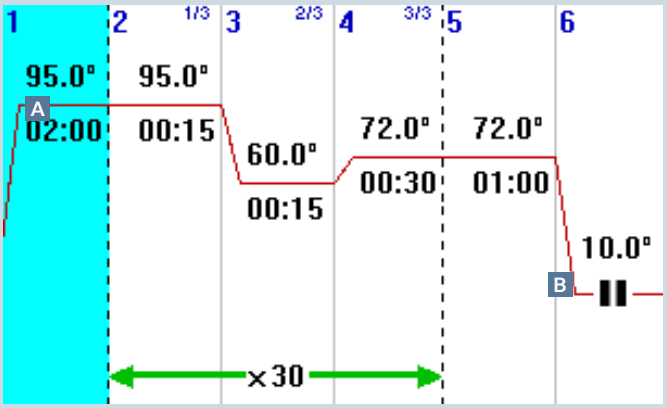

Figure 2: 3-step PCR program for run time determination.
A - Start of run time measurement
B - End of run time measurement

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Program run protocol

Program properties

Program	Total run time
Last edited by	(anonymous), 2017-03-27 12:36:39
Run identification	Totalruntime_2
User	(unknown user)

Header

Lid temperature	105°C
Energy-saving mode	On
Temperature mode	Fast
Block settings	Any
Heating rate limit	Off
Cooling rate limit	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		--:--		

Protocol properties

Cycler name:	Mastercycler X50s
Serial number:	6311FQ000069
Software version:	0.32.0
Last verification:	2017-03-23
Auto Restart:	Off
Program start:	2017-03-27, 12:36:44
Pogram end:	2017-03-27, 13:17:42

Figure 3: 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, is not displayed in this section).

Results and Discussion

The evaluation of the 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 (silverblock) showed, as expected, the shortest total PCR run time, in accordance with the fast ramp rate cited. On the other hand, the run times of certain competing thermal cyclers were considerably longer than would be expected from the ramp rates stated in the manufacturers' 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 repeat runs have confirmed these differences to only be within a few seconds range (full data not shown in this paper). Thus, while the results reported in Table 1 were randomly chosen from a set of measurements for each thermal cycler, each data is representative of the performance of each respective cycler.

It is evident that the thermal cyclers Veriti® Fast, SimpliAmp® and T100 were considerably slower in their actual PCR run times than the Mastercycler nexus gradient, despite the fact that the respective manufacturers had cited faster ramp rates for these thermal cyclers in their technical specifications. Similarly, thermal cyclers TAdvanced S, TAdvanced and SureCycler® all which were cited to have higher ramp rate than Mastercycler X50l also take longer to complete a similar PCR protocol. Furthermore, when comparing thermal cyclers with the same ramp rate (e.g. Mastercycler X50l, PeqSTAR, Proflex® and C1000), there was obvious difference in total PCR run time.

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

Thermal cycler	**Run time** [hh:mm:ss]	**Ramp rate** accord. to techn. data [°C/s]
Mastercycler X50s	00:39:29	10
Mastercycler X50l	00:45:02	5
TAdvanced 96S	00:47:05	8
PeqSTAR 96X	00:47:10	5
TAdvanced 96	00:47:37	6
C1000	00:49:18	5
SureCycler 8800	00:50:33	6
Proflex (96-well)*	00:50:54	6
Mastercycler nexus gradient	00:51:15	3
Veriti Dx Fast	00:56:06	5
SimpliAmp*	00:56:44	4
T100*	01:03:52	4

* Performed in high profile twin.tec plate because the cyclers cannot accommodate low profile plates.

It can be assumed that the following parameters contribute strongly to the observed discrepancies:
> For the different thermal cyclers the maximum 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].

Conclusion

These comparative investigations have shown that the isolated consideration of 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.
For accurate evaluation of the ramping performance of a thermal cycler it is imperative that the manufacturer makes the information of all relevant parameters, e.g. detailed description of selectable temperature control modes, available to the user. 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	Order no. North America
Mastercycler® X50a	6313 000.018	6313000018
Mastercycler® X50p	6315 000.015	6315000015
Mastercycler® X50h	6316 000.019	6316000019
Mastercycler® X50l*	6303 000.010	6303000010
Mastercycler® X50r*	6305 000.017	6305000017
Mastercycler® X50t*	6306 000.010	6306000010
Mastercycler® X50i*	6301 000.012	6301000012
Accessories		
Ethernet cable, 5 m	6313 070.008	6313070008

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

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