

Comparative run time evaluations of PCR thermal cyclers

Nils Gerke, Eppendorf AG, Hamburg, Germany

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. Whereas the

Mastercycler® pro S and the Mastercycler® nexus GSX1, as expected from their fast ramp rates, achieved the shortest total PCR run times in these evaluations, some thermal cyclers made by other manufacturers showed noticeably slower run times despite similar cited ramp rates.

Introduction

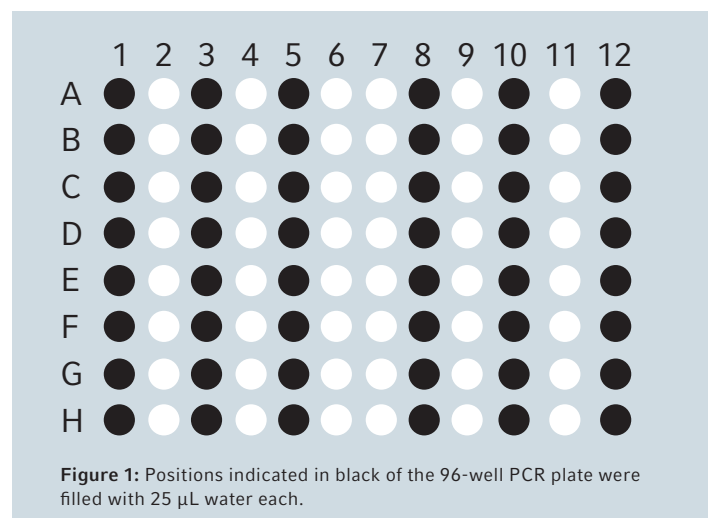
Besides control accuracy and temperature homogeneity, the customary technical details of a thermal cycler also include the ramp rate of the thermoblock. The ramp rate, in particular, is not subject to a uniform standard; instead, manufacturers state a variety of parameters, such as:

- > 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, low profile) were filled with 25 µL water, respectively (Fig. 1).

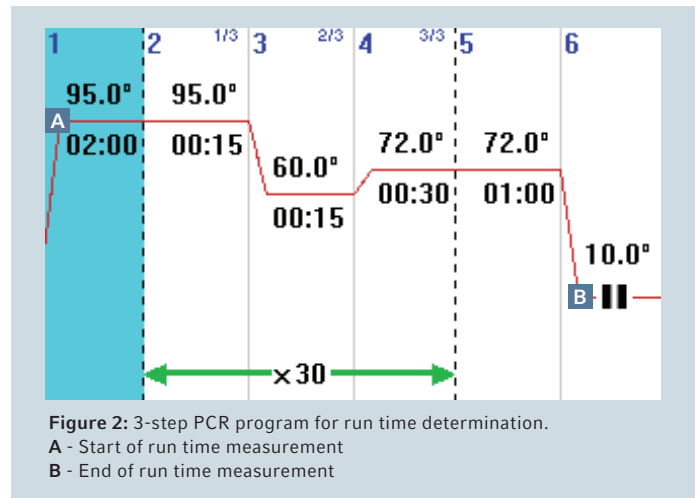


The plate was subsequently sealed with the Heat Sealing PCR Film (Eppendorf), centrifuged for 1 min at 500-1000xg, placed into the thermal cycler and subjected to a standard 3-step PCR program (Fig. 2).

The run times were determined for the Mastercycler® pro S, Mastercycler® nexus GSX1, Mastercycler® nexus gradient and Mastercycler® nexus, and eight 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. For thermal cyclers which 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 pro 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.



PROTOCOL

```
Cycler nr:          1
Cycler Serialnr:   6325XJ
Mastercycler model: pro S
Auto restart:      ON
Max powerdown time: 00:01:00
Estimated Runtime: 00:43:03
Program "runtime fast" started at Date: 10.Dec.2012 16:44:54
```

Step-Cyc	Command	Duration	Ramp	Start	End
1	Temp: 95.0 C	Time: 02:00	16:44:55	16:45:05	16:47:05
2 1	Temp: 95.0 C	Time: 00:15		16:47:05	16:47:20
3 1	Temp: 60.0 C	Time: 00:15	16:47:20	16:47:27	16:47:42
4 1	Temp: 72.0 C	Time: 00:30	16:47:42	16:47:44	16:48:14
2 2	Temp: 95.0 C	Time: 00:15	16:48:14	16:48:18	16:48:33

Figure 3: Screenshot from a Mastercycler pro 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 the one hand, the thermal cyclers Mastercycler pro S and the Mastercycler nexus GSX1 (each equipped with silver block) showed, as expected, the shortest total PCR run times, in accordance with the fast ramp rates 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.

It is thus evident that the thermal cyclers V, T and R were considerably slower in their actual PCR run times than the Mastercycler nexus gradient and the Mastercycler nexus, despite the fact that the respective manufacturers had cited faster ramp rates for these thermal cyclers in their technical specifications.

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

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 pro S	00:40:12	6
Mastercycler nexus GSX1	00:42:31	5
C	00:46:50	5
P	00:48:58	5
S	00:50:31	6
Mastercycler nexus gradient	00:51:26	3
Mastercycler nexus	00:51:53	3
V	00:52:22	5
T	00:53:20	4
R	00:56:27	5
G	00:56:38	3.5
A	01:03:13	3

description of selectable temperature control modes, available to the user.

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	International Order no.	North America Order no.
Mastercycler® nexus with silver block		
Mastercycler® nexus GSX1	6345 000.010	6345000028
Mastercycler® nexus SX1	6346 000.013	6346000021
Mastercycler® nexus GSX1e*	6347 000.017	6347000025
Mastercycler® nexus SX1e*	6348 000.010	6348000029
Mastercycler® nexus with universal block		
Mastercycler® nexus gradient	6331 000.017	6331000025
Mastercycler® nexus	6333 000.014	6333000022
Mastercycler® nexus gradient eco*	6334 000.018	6334000026
Mastercycler® nexus eco*	6332 000.010	6332000029
Mastercycler® nexus with flat block		
Mastercycler® nexus flat	6335 000.011	6335000020
Mastercycler® nexus flat eco*	6330 000.013	6330000021
Complete Mastercycler® pro packages		
Mastercycler® pro und Control Panel	6321 000.515	950040015
Mastercycler® pro S und Control Panel	6325 000.510	950040025
Mastercycler® pro 384 und Control Panel	6324 000.516	950040035
Individual Mastercycler® pro modules		
Control Panel, incl. connection cable	6320 000.007	950030050
Mastercycler® pro**	6321 000.019	950030010
Mastercycler® pro S**	6325 000.013	950030020
Mastercycler® pro 384**	6324 000.010	950030030
Accessories		
CAN_BUS connection cable, 50 cm	5341 612.006	950014008
CAN_BUS connection cable, 150 cm	5341 611.000	950014016
Self test dongle	6320 071.001	950030040
Temperature Verification System with 96 well sensor plate	6328 000.006	6328000006
Temperature Verification System with 384 well sensor plate	6328 000.014	6328000014
CycleManager pro, incl. installation manual, online help, and connection cable	5349 810.001	950017007
CycleManager pro, incl. installation manual, online help	5349 820.007	950017202

* To run a Mastercycler® nexus with the suffix »eco« or »e«, a Mastercycler® nexus model without such a suffix is needed. Up to 2 units with the suffix »eco« or »e« can be connected to a Mastercycler® nexus without such a suffix.
 ** A Control Panel or CycleManager pro software (both sold separately) is required for operation. CAN_Bus connection cables are required to link cyclers together as a network.

Your local distributor: www.eppendorf.com/contact
 Eppendorf AG · 22331 Hamburg · Germany
eppendorf@eppendorf.com · www.eppendorf.com

www.eppendorf.com

Eppendorf®, the Eppendorf logo, Mastercycler® and Eppendorf twin.tec® are registered trademarks of Eppendorf AG, Hamburg, Germany.
 All rights reserved, including graphics and images. Copyright © 2012 by Eppendorf AG.