Thermal cyclers: key thermal cycling concepts and ramp rates

Key specifications of thermal cyclers are often found in the instruments' literature and on the Web with very little explanation of how the numbers are derived or used. Ramp rates, thermal overshoot, and other thermal cycling characteristics are key to PCR performance, and are explained in depth here using a variety of Applied Biosystems™ thermal cyclers as benchmarks. In addition, ramp rates are tested and compared to published specifications.

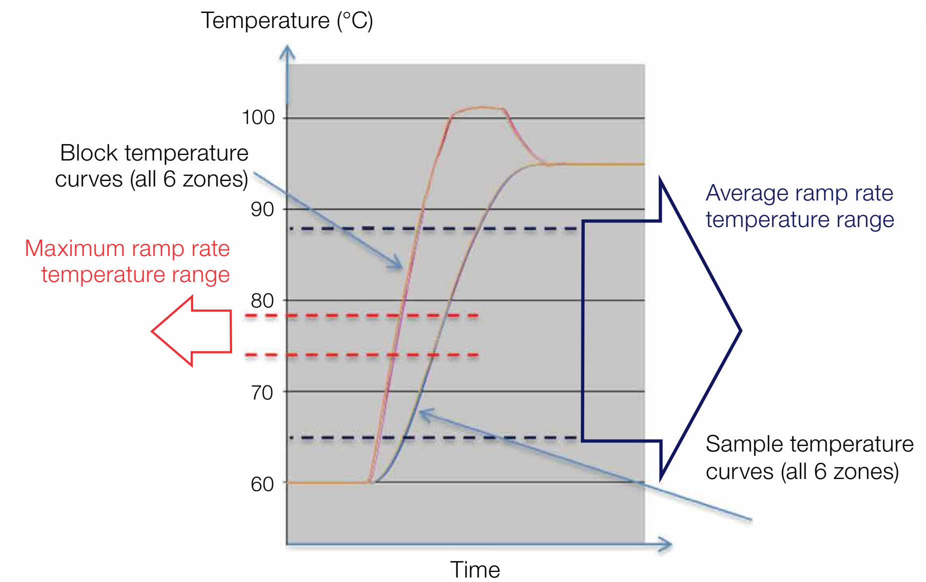

Figure 1. Real block and sample temperature curves from all six zones during up ramp on the Applied Biosystems™ ProFlex™ 96-well PCR system. The dotted lines indicate the temperature ranges sampled for the maximum ramp rate and the average ramp rate.

Ramp rates: maximum vs. average, and block vs. sample

Temperature cycling is fundamental to all PCR reactions, and how fast a thermal cycler can ramp between temperatures will dictate the overall speed and duration of the PCR run. Because of this, thermal cycler manufacturers publish their ramp rates to indicate the change in temperature over time. Ramp rates are typically expressed in °C/second. Looking at this concept graphically on a plot of temperature vs. time, the ramp rate is simply expressed by the slope of the curve. A steeper curve represents a higher ramp rate, and means that a specific temperature range can be covered in a shorter time. The terms "up ramp" and "down ramp" refer to the ramp rates when heating and cooling, respectively. Of course, a faster-ramping block will result in a faster PCR run, so many thermal cycler manufacturers seek to show the highest possible ramp rate. However, the way ramp rates are measured may make them appear to be higher than they really are.

The metal blocks of thermal cyclers must be heated and cooled between the steps in PCR such as denaturation, annealing, and extension. A commonly published specification is the **maximum block ramp rate**, also known as the peak block ramp rate. The maximum block ramp rate corresponds to the highest achievable block performance. This maximum performance may only be achieved during a very brief period during the ramp. Thermal cycler manufacturers may also publish the **average block ramp rate**, which represents the rate of temperature change across a longer portion of the ramp, and is more representative of the thermal cycler's performance and speed (Figure 1).

However, just because the block is ramping quickly, it does not mean that the liquid of your PCR reaction (sample) is ramping just as quickly. It takes time for the heat from the thermal cycler's block to be transferred to the sample. Because of this, ramp rates for Applied Biosystems thermal cyclers are expressed in terms of **average sample ramp rate** and **maximum sample ramp rate**. Ramp rates that are based on the block temperature alone, without accounting for sample volume, do not reflect the ramp rate of the sample.

Applied Biosystems thermal cyclers use a proprietary algorithm that calculates the temperature of the PCR sample itself, based on the volume entered. This allows us to publish sample ramp rates. We also publish block ramp rates to allow more direct comparisons to other manufacturers' thermal cyclers.

Overview: sample temperature control

How thermal cyclers attain and measure temperature differs, depending on the manufacturer. Often, thermal cyclers are programmed to achieve temperatures based on block temperature alone, without accounting for the temperature of the liquid sample.

However, just because the block has achieved its desired temperature, or set point, it does not mean that the sample has achieved the same set point. The proprietary temperature control algorithm used by Applied Biosystems thermal cyclers helps ensure that temperature set points are reached quickly and accurately, by accounting for the sample volume used. This algorithm actually drives the block temperature higher than the set point to permit the sample to reach the set point faster. This is known as "block overshoot thermal energy", depicted in green in Figure 2. It is important to note that although the block overshoots the set point, the sample should reach the set point with no overshoot. In addition, the thermal cycler does not begin to count down the time of that step until the sample reaches the set point. This helps ensure that the sample spends exactly the amount of time at a specific temperature that you programmed into the thermal cycler.

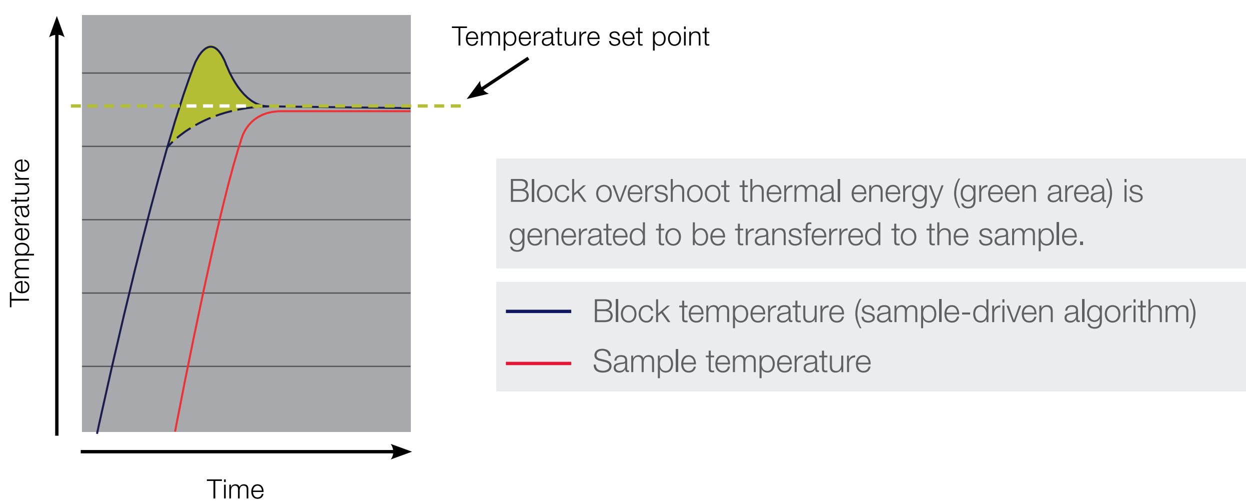

Figure 2. Block overshoot thermal energy. Temperature curves for the reaction block (black) and sample (red), and the associated block overshoot thermal energy (green) during a heating phase that is ideally controlled. The horizontal dotted line indicates the set temperature for this phase.

Our proprietary temperature control algorithm helps ensure that samples reach a stable set point temperature—within a range of ±0.25°C—without overshoot and associated deleterious effects. The algorithm uniquely predicts the temperature and speed of the PCR reaction while accounting for the volume of the PCR reaction and the thickness of the reaction tube. Sample temperature or sample ramp rates are typically not mentioned by other thermal cycler manufacturers.

Published vs. measured ramp rates

Our Applied Biosystems thermal cyclers include information on both the maximum block ramp rate and the more relevant maximum sample ramp rate. The published data [1-4] are based on the averages of measured performance data captured during product testing (Table 1).

Some manufacturers' published thermal cycler ramp rates are known to vary significantly from actual measured ramp rates [5]. In a recent comparison of maximum block ramp rates of multiple thermal cyclers, the smallest differences between the published and measured ramp rates were observed for Applied Biosystems thermal cyclers (Table 2). These differences are useful when directly comparing thermal cycler capabilities.

Table 1. Measured and published data for Applied Biosystems™ ProFlex™, VeritiPro™, SimpliAmp™, MiniAmp™ Plus, and MiniAmp™ thermal cyclers.

	Max. block rate (°C/sec)		Max. sample rate at 1 µL (°C/sec)	
Block type	Up ramp	Down ramp	Up ramp	Down ramp
Measured data				
ProFlex 96-well	6.4	5.9	4.6	4.3
ProFlex 3 x 32-well	6.2	5.6	4.4	4.2
VeritiPro 96-well	6.8	6.0	4.9	3.4
SimpliAmp	4.0	3.7	3.1	3.0
MiniAmp Plus	3.6	3.7	2.7	2.6
MiniAmp	3.5	3.5	2.3	2.0
Published data				
ProFlex 96-well	6.0	4.4		
ProFlex 3 x 32-well	6.0	4.4		
VeritiPro 96-well	6.0	4.4		
SimpliAmp	4.0	3.0		
MiniAmp Plus	3.5	2.7		
MiniAmp	3.5	2.2		

Table 2. Comparison of average maximum block ramp rates (°C/sec) of thermal cyclers from different manufacturers [5].

ProFlex 96-well thermal cycler				ProFlex 3 x 32-well thermal cycler				Eppendorf™ Mastercycler™ X50 thermal cycler				Bio-Rad™ C1000 Touch™ thermal cycler			
Published		Measured		Published		Measured		Published		Measured		Published		Measured	
Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down
6.0	6.0	6.4	5.9	6.0	6.0	6.2	5.6	5.0	2.3	6.7	3.9	5.0	5.0	3.0	2.4

VeritiPro thermal cycler				SimpliAmp thermal cycler				Biometra™ TAdvanced thermal cycler				Bioer™ LifeECO thermal cycler			
Published		Measured		Published		Measured		Published		Measured		Published		Measured	
Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down
6.0	4.4	6.8	6.0	4.0	4.0	4.0	3.7	6.6	4.3	6.6	4.3	4.2	2.7	4.2	2.7

MiniAmp Plus thermal cycler				MiniAmp thermal cycler				Bio-Rad™ T100™ thermal cycler				Biometra™ TOne thermal cycler			
Published		Measured		Published		Measured		Published		Measured		Published		Measured	
Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down
3.5	2.7	3.6	3.7	3.5	2.2	3.5	3.5	4.0	4.0	2.6	1.6	4.0	3.3	3.4	2.5

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Summary

Precise temperature control of thermal cyclers is crucial for the accuracy and efficiency of PCR experiments. Multiple factors specific to thermal cyclers, including the actual thermal cycler ramp rates, sample temperatures, and how the thermal cycler ensures accurate overshoot calculation, contribute to the integrity and performance of PCR assays.

Our proprietary algorithm uniquely enables our thermal cyclers to achieve the correct temperatures of the samples rather than the blocks; the resulting published ramp rates help ensure adherence to the programmed protocol and evaluation of a thermal cycler's true performance.

References

1. ProFlex PCR System User Guide. P/N MAN0007697, Rev B.0.
2. VeritiPro Thermal Cycler User Guide. P/N MAN0019157, Rev. A.0.
3. SimpliAmp Thermal Cycler User Guide. P/N MAN0009889, Rev. C.0
4. MiniAmp and MiniAmp Plus Thermal Cycler User Guide. P/N MAN0017492, Rev. A.0.
5. Kim YH, Yang I, Bae YS, Park SR (2008) Performance evaluation of thermal cyclers for PCR in a rapid cycling condition. *Biotechniques* 44(4):495–505.

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