WHITE PAPER No. 32 I April 2016

Eppendorf Mastercycler®: Meeting all your PCR needs with reliability and flexibility

Executive Summary

Since the polymerase chain reaction (PCR) technique was first developed in 1983, it has become one of the most important basic methods in biological science. The technique, as well as its reagents, accessories and equipment, have gone through tremendous improvements. As a result, commercial products related to PCR are offered in an overwhelming amount of variety. Ultimately however, each experiment, each PCR, is personal, with many factors (sample properties, reagents, consumables, thermal cyclers, area of amplification) influencing PCR outcome. The right combination of materials, protocol and device settings is necessary to achieve results. Precisely because there are so many influencing factors, it is critical that a thermal cycler offers you a maximum of flexibility to adapt to the requirements of all of your experiments. This article outlines the major factors that affect PCR and how an Mastercycler corresponds.

Common factors that affect PCR

Reagents

Each PCR reagent or kit comes with its own recommendation of protocol. This serves as an initial guideline to be modified as needed, with consideration for the properties of the reagent. For example, some Taq polymerase might be more temperature labile than others. In such cases, it is important to ensure that the thermal cycler used does not have a high temperature overshoot (Figure 1; Gerke et. al., 2011) that might affect enzyme stability or cause disruption of template DNA (Lindahl, 1993; Barnes, 1994).

Furthermore, reagent kits validated for specific PCR assays might require the user to follow a certain specified heating/cooling ramp rate. Hence, the modifiable range of the ramp rates of a thermal cycler (Figure 2) will be an important consideration when working with such reagents.

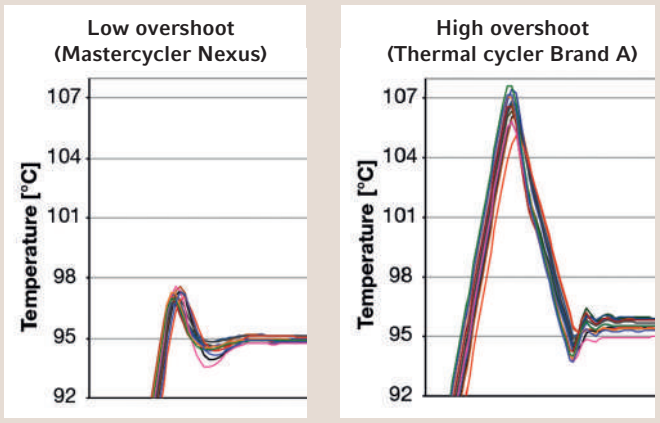

Figure 1: High temperature overshoot (set temperature = 95°C in both cases) in the thermal cycler might affect the activity of enzyme in PCR reagents.

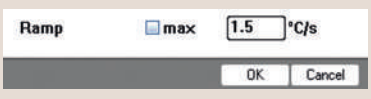

Figure 2: High ramp rate, modifiable in a wide range when required is an important flexibility requirement in a thermal cycler.

Area of amplification (DNA template)

The denaturation temperature in a PCR protocol is dependent mainly on the composition of the PCR buffer used as well as the DNA template. For example, a GC rich template would require a higher denaturation temperature. Unsuitable denaturation temperature will lead to lower PCR yield or total PCR failure (Figure 3). It is therefore crucial that a cycler is able to reliably and accurately deliver the programmed temperature. A temperature gradient (Figure 4) performed at the denaturation stage (Gerke & Hellberg, 2013) can also help to determine the optimal denaturation temperature.

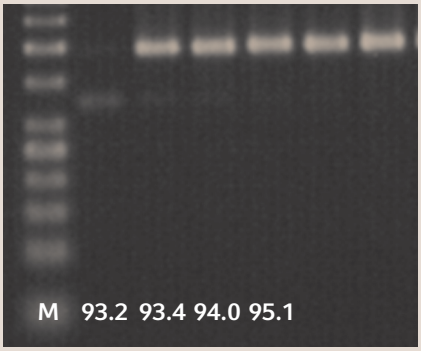

Figure 3: Denaturation temperature differences of just 0.2°C (e.g. 93.2 vs. 93.4°C in this figure) can affect PCR success. Optimization with gradient function (Figure 4) should be done for best results.

Primers

The optimal annealing temperature changes with primer sequence, concentration and manufacturing process (type of salt used, overall pH & impurities), as well as PCR reagent and the thermal cycler used (due to different heating characteristics). This means that optimization is likely needed for an untried primer sequence. Re-optimization might also be required whenever a) the same primers are ordered from a different supplier; b) there is a change in PCR reagent or c) when using a cycler from a different brand or model. Hence, a thermal cycler with temperature gradient function can cut the amount of time and effort for such an optimization considerably. The higher the number of temperature steps a thermal cycler can perform in a gradient, the more complete the information on primer behavior can be obtained and the higher are the savings.

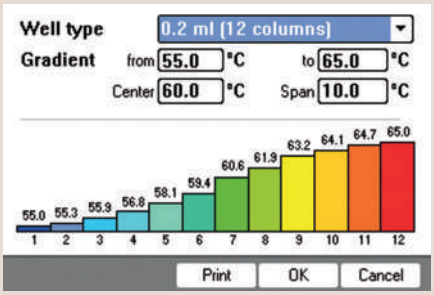

Figure 4: Gradient function is a cost-effective optimization strategy. A 12-temperature gradient provides a comprehensive profile of primer behavior.

Ensuring identical heating and cooling ramp rates across the temperature gradient is important. Such identical ramp rates are also crucial when transferring between a gradient (optimization) and a non-gradient (optimized routine) protocol. These can be easily achieved by using technology specifically designed to have this behavior such as the SteadySlope® technology implemented in all Mastercycler models with gradient function. This allows the user to easily and conveniently optimize new protocols and/or adapt any optimized PCR protocols from any other thermal cyclers. Thermal cyclers that do not maintain identical ramp rates in both gradient and non-gradient mode would mean that the optimized protocol is in fact, "not optimized unless in gradient mode" with respect to the influence of ramp rates (Ong, 2010).

Volume

Higher reaction volumes take longer to heat and cool. However, it is not practical to have different protocols for the same PCR at different volumes. Hence, thermal cyclers that offer the option of adapting their temperature performance according to different reaction volumes in the tubes help to ensure sufficient heating without a change in actual PCR run protocol. Eppendorf Mastercyclers enable this in a quick and safe way with their "fast", "standard" and "safe" temperature control modes (Figure 5). These control settings can also be used to facilitate amplification of "difficult" template (for e.g. "safe" mode can help when amplifying GC-rich target).

Figure 5: Temperature control modes offer easy adaptation to varying PCR reaction volumes and DNA template characteristic.

Reducing the reaction volume saves reagent cost and allows faster PCR completion. However, the smaller the volume, the more likely evaporation can cause concentration changes in the reaction. This may consequently lead to irreproducible results or even PCR failure. Therefore, a good thermal cycler design that provides proper contact and optimal pressure (without damaging the reaction tubes) between the heated lid of the cycler and lid of the reaction vessel is crucial for low volume and/or sensitive PCR applications like sequencing (Gerke & Roth, 2008; Aubertin, 2009). An example of such feature is the Eppendorf patented vapo.protect® lid of the Mastercycler pro that provides maximum protection against evaporation (Figure 6).

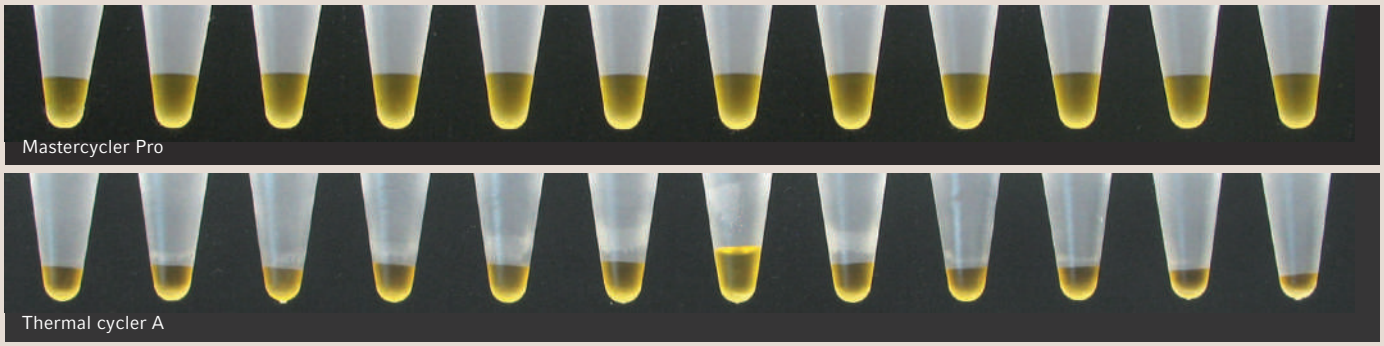

Figure 6: Thermal cyclers with good design protect against evaporation, which is crucial for reproducible results, especially for low volume/ sensitive PCR.

PCR strategy

Apart from optimizing the factors mentioned above, there exist various strategies in order to achieve success in DNA amplification. Alternative primer design (Kunihiro et. al., 2002; Chen et. al. 2008) and modifications of time and/or temperature in PCR protocols are among the most common methods. In conclusion, the variety of temperatures, holding time, and protocols required necessitate a cycler to be flexible enough to handle all types of PCR programming and applications (e.g. nested PCR, long PCR, touch down PCR; Figure 7).

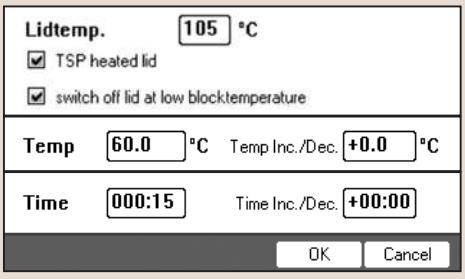

Figure 7: Extra parameter settings are important to cater for any possible PCR application and strategy to achieve results.

Speed

In the matter of speed, many equate high ramp rates to high speed. However, there is no standard on how these ramp rates are determined by the different manufacturers. Thus, thermal cyclers that have a higher ramp rate specification can take longer to complete a PCR compared to another cycler with lower ramp rate (Gerke, 2013; Butts & Vallone, 2014). In order to obtain a relevant measure of the speed of a thermal cycler, compare the total run time of your protocol between the thermal cyclers you are considering, and not only the specified ramp rates.

Flexibility and cost

Other factors that bear consideration in the long run are application flexibility and cost savings. As a researcher you never know what exciting new routes your findings will take you and so you should not be limited in possibility. A student might need a high volume of DNA material in the morning and a co-worker might require efficient low volume PCR in the evening. A thermal cycler should not be the bottleneck in everyday lab work. A thermal block compatible with any vessel format (Figure 8) in combination with a flexible lid design would free the user from being locked to expensive consumables while maintaining flexibility in PCR applications. An example of this is the Eppendorf Mastercycler nexus with its flexlid® that can accommodate low volume plates as well as tall 0.5 mL tubes, all in one cycler. A thermal cycler is a one-time cost, while consumables are a recurring cost that increases over time and number of projects.

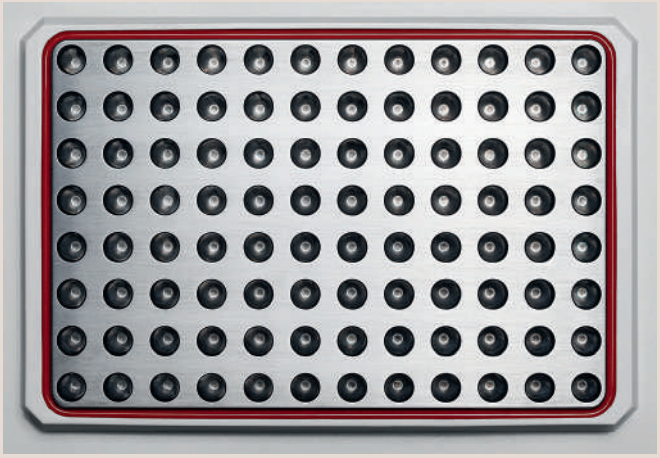

Figure 8: Mastercycler pro/Mastercycler nexus GSX1 silver block.
The design of all block variants of the Mastercyclers enables you to work with all established variants of PCR tubes, tube strips and plates.
Thus giving you the maximum flexibility in choosing your PCR consumables.

Solution & Benefits

In summary, below is a checklist of essential things to have in a thermal cycler:

> Technical performance (no higher overshoot than necessary)
> Modifiable ramp rate (required for certain optimized reagent kits)
> Gradient function (for fast and easy optimization of denaturation and annealing temperatures)
> Modifiable temperature modes (for different volume and template needs)
> Block and lid design that covers all types and shapes of standard PCR consumables (no matter which ones are provided with some reagent kits)
> Evaporation protection (especially for low volume and sensitive PCR)
> Speed (evaluate total run time instead of ramp rates)
> Flexible programming functions for all possible PCR applications (touch down, nested, etc.)

The Eppendorf Mastercycler comes equipped with all of these as standard features, allowing the highest user flexibility. They have been successfully used in many different applications with different PCR reagents, consumables and DNA sources. Below are some references that may serve as a helpful starting point in your own PCR work:

Thermal cycler models	PCR reagents	References
Mastercycler pro system		
Mastercycler pro	BigDye terminator V.3.1 Cycle Sequencing Kit (Applied Biosystems®)	Fava et. al. (2013) BioMed Research International, Article ID 875048
	2X PCR Master Mix (Fermentas Life Science®)	Ji, et. al. (2013) J Bioremed Biodeg 4: 209
	2X Phusion® High-Fidelity PCR Master Mixes (Thermo Fisher®)	Ji, et. al. (2013) J Bioremed Biodeg 4: 209
	PLATINUM® Taq High Fidelity Supermix (Invitrogen®)	Campana, et al. (2010) PLoS ONE 5(12): e15172
	Pwo DNA-polymerase (Roche Diagnostics®)	Dyachenko et al. (2012) Parasites & Vectors 5: 49
	Taq DNA polymerase (Invitrogen)	Argimon & Caufield (2011) J. Clin. Microbiol. 49(3): 984
	AccuPower® PCR PreMix, (Bioneer®)	Koo, Cho & Jeong (2013) Journal of the Korean Society for Applied Biological Chemistry 56(3): 295
Mastercycler pro S	Taq DNA polymerase (New England Biolabs®)	Vatolin, Khan & Reu (2012) PLoS ONE 7(9): e44690
	KPC Resistance Assay kit (Ibis Biosciences Inc.®)	Endimiani et. al. (2010) J Antimicrobial Chemotherapy 65(8): 1833
	Premix Ex Taq Perfect Real Time 2× mastermix (Takara Bio USA®) plus SpeedSTAR HS DNA Polymerase (Takara Bio)	Butts & Vallone (2014) Electrophoresis, 35: 3053
	Taq DNA polymerase (Qiagen®)	Halary, et al. (2013) PLoS ONE 8(11): e80729
Mastercycler (MC) nexus family		
Mastercycler nexus	Taq DNA polymerase (Qiagen)	Blyton, Shaw & Banks (2014) Conservation Gent Resour 6: 95
	Phusion® DNA Polymerase (Thermo Fisher)	Lerksuthirat, et al. (2015) PLoS ONE 10(3): e0118547
	GoTaq DNA polymerase (Promega®)	Kraus et. al. (2014) PLoS ONE 9(8): e104568
	AccuPrime Taq DNA polymerase (Invitrogen)	Kraus et. al. (2014) PLoS ONE 9(8): e104568
	Q5 High-Fidelity polymerase (New England Biolabs®)	Kostovcik et. al. (2015) The ISME Journal 9: 126
Mastercycler nexus flat	BioTherm™ Taq DNA Polymerase (Ares Bioscience®)	Jörns (2013) Eppendorf Application Note 249
Mastercycler nexus flat / nexus gradient	2× Power Taq Plus PCR MasterMix (Bio-TeKe Corporation®)	Xu et. al. (2015) Lab Chip 15(13): 2826
Mastercycler nexus gradient	PlatinumTaq DNA Polymerase (Life Technologies®)	Amjad et. al. (2014) PLoS ONE 9(8): e105234
	TaKaRa Ex Taq (Takara Bio®)	Zhou et. al. (2014) PLoS ONE 9(7): e103041
	2xTaq PCR kit (LifeFeng Biotechnology Co.®)	Fu et. al. (2014) BioMed Research International, Article ID 527042
	Maxima Hot Start Taq DNA polymerase (Thermo Fisher)	Rajput et. al. (2015) PLoS ONE 10(9): e0138235
	HotMasterMix (5 PRIME®)	Eppendorf Internal testing
	Phire Hot Start II DNA Polymerase (Thermo Fisher)	Eppendorf Internal testing
	TrueStart Hot Start Taq DNA Polymerase (Thermo Fisher)	Eppendorf Internal testing
	TEMPase Hot Start DNA Polymerase (VWR®)	Eppendorf Internal testing
	AmpliTaq® 360 (Applied Biosystems)	Eppendorf Internal testing
	AmpliTaq Gold® 360 (Applied Biosystems)	Eppendorf Internal testing
	Phusion Green High-Fidelity DNA Polymerase (Thermo Fisher)	Eppendorf Internal testing
Mastercycler nexus GSX1	GoTaq DNA polymerase (Promega)	Burt et. al. (2015). PLoS ONE, 10(10): e0139450

References

[1] Aubertin, J. (2009) A streamlined gDNA sequencing protocol for low sample consumption and reduced reagent costs using the Mastercycler® pro. Eppendorf Application Note 212.
[2] Barnes, W. M. (1994) PCR amplification of up to 35 kb DNA with high fidelity and high yield from λ bacteriophage templates. Proc Natl Acad Sci USA 91: 2216 – 2220.
[3] Butts, E. L. R. & Vallone, P. M. (2014) Rapid PCR protocols for forensic DNA typing on six thermal cycling platforms. Electrophoresis, 35: 3053–3061.
[4] Gerke, N. (2013) Comparative run time evaluations of PCR thermal cyclers. Eppendorf Application Note 274.
[5] Gerke, N. & Hellberg, A. (2013) Straightforward PCR optimization and highly flexible operation on the dual block thermocycler Mastercycler® nexus GX2. Eppendorf Application Note 289.
[6] Gerke, N. & Roth, R. (2008) Mastercycler® pro with vapo.protect® technology: Improved protection against evaporation of PCR solution. Eppendorf Application Note 199.
[7] Gerke, N., Tasch, H., Schicke, K. & Hellberg, A. (2011) Excellent temperature homogeneity contributes to high PCR performance of the Mastercycler® nexus. Eppendorf Application Note 244.
[8] Lindahl, T. (1993) Instability and decay of the primary structure of DNA. Nature 362: 709 – 715.
[9] Ong, W. K. (2010) Using the gradient technology of the Mastercycler® pro to generate a single universal PCR protocol for multiple primer sets. Eppendorf Application Note 220.
[10] Kunihiro et. al., 2002; Chen et. al. 2008

About Eppendorf

Eppendorf is a leading life science company that develops and sells instruments, consumables, and services for liquid-, sample-, and cell handling in laboratories worldwide. Its product range includes pipettes and automated pipetting systems, dispensers, centrifuges, mixers, spectrometers, and DNA amplification equipment as well as ultra-low temperature freezers, fermentors, bioreactors, CO_2 incubators, shakers, and cell manipulation systems. Associated consumables like pipette tips, test tubes, microtiter plates, and disposable bioreactors complement the instruments for highest quality workflow solutions. Eppendorf was founded in Hamburg, Germany in 1945 and has about 2,900 employees worldwide. The company has subsidiaries in 25 countries and is represented in all other markets by distributors.