

Reduced PCR runtimes and increased yields using Eppendorf Fast PCR Consumables

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Abstract

Since the development of the polymerase chain reaction (PCR) in 1983, it has become one of the key techniques in molecular biology and diagnostics. Although the technique has undergone continuous refinement and frequent efforts were taken to increase the speed of the PCR, a typical protocol to amplify a specific DNA fragment still takes about an hour and is often regarded as time-relevant bottleneck in the workflow.

Here we show that the Eppendorf Fast PCR consumables allow an easy transfer from a standard to a fast PCR protocol without compromising reaction efficiency. The Fast PCR Tube Strips are made from polyethylene, offering better heat transfer properties than polypropylene material that is typically used for PCR consumables. The improved thermal conductivity results in higher amplicon yields under fast PCR conditions in comparison to standard and other fast PCR consumables. This helps to streamline workflows and to increase working efficiency in the lab.

Introduction

PCR is one of the most popular methods in laboratories across the world and is routinely used in research, reference and clinical fields for various applications. Over the years, PCR technology has evolved with the needs of scientists; including developments like real-time PCR and digital PCR. As time is one of the most precious factors in a laboratory, a decrease in amplification time and thus higher throughput are advantageous for every lab. A lot of efforts have been put into speeding up PCR reactions. One approach is the establishment of two-step thermal cycling protocols. Other developments include thermal cyclers with higher ramp rates and special kits containing polymerases with faster extension rates. However, a significant reduction in cycle times often requires specialized equipment and adapted procedures that may not be easily implemented in a standard lab. Therefore, typical standard end-point PCR protocols still take about an hour or more. One reason fast or rapid PCR protocols are not widely-used yet is that the transfer of an established PCR protocol to a fast protocol often compromises PCR yield and specificity.

Furthermore, rapid protocols are often linked to very low reaction volumes (< 5 µL) and these are rarely applicable for standard applications. The use of special fast PCR master mixes does reduce runtimes but their full potential can only be realized in combination with fast thermal cyclers. The limiting factor here is the speed of heat transfer from the block to the sample. In the past, this limitation was mainly addressed by making the vessel walls thinner. Eppendorf's approach is to use an alternative material with better heat transfer properties.

The results of this Application Note show that Eppendorf Fast PCR Tube Strips allow for an easy transfer of a standard PCR protocol to a fast PCR protocol without the need to switch to specialized equipment. Under fast PCR conditions, where standard PCR consumables limit the speed of heat transfer to the sample, the Eppendorf Fast PCR consumables offer a reliable alternative to significantly reduce PCR runtimes without compromising yield or efficiency.

With their thermal conductive properties, they address a relevant end-point PCR bottleneck that still exists today.

Materials and Methods

All experiments were done on Mastercycler® X50 silver block models (X50s and X50i, Eppendorf) with the following settings:

Lid temperature: 105°C, energy-saving mode on
Temperature mode: Fast
Block settings: Silver 96

Human Genomic DNA (Roche®) was used as template for the fast PCR protocols with SpeedSTAR™ HS DNA Polymerase (TaKaRa) or the 2x GeneAmp® Fast PCR Master Mix (Applied Biosystems).

PCR reaction master mix for the SpeedSTAR™ HS DNA Polymerase was prepared using 1x Fast Buffer I, 0.2 mM dNTP-Mix, 0.5 µM of each primer, 30 ng of DNA template and 0.25 U of DNA polymerase in a total volume of 10 µL. PCR reaction master mix for the GeneAmp® Fast PCR Master Mix (2x) was prepared using 1x Fast PCR Master Mix, 0.2 µM of each primer and 20 ng of DNA template in 10 µL total volume.

The following primers were used for the amplification of a 536 bp sequence from the human β-globin fragment:

Forward Primer 5'-GCT CAC TCA GTG TGG CAA AG-3'
 Reverse Primer 5'-GGT TGG CCA ATC TAC TCC CAG G-3'

Cycling conditions:

	SpeedSTAR™ protocol	GeneAmp® Fast protocol
Initial Denaturation	94 °C/60 s	96 °C/15 s
Cycles 35x	96 °C/2 s	96 °C/1 s
	65 °C/6 s	62 °C/16 s
Post Cycle Elongation	72 °C/60 s	72 °C/10 s
Storage	10 °C	10 °C

PCR reactions were carried out in the following consumables:

Eppendorf	Fast PCR Tube Strips
Eppendorf	PCR Tube Strips
ThermoFisher Scientific	ABI MicroAmp™ Fast 8-Tube Strip, 0,1 mL
Bio-Rad	0.2 ml 8-Tube PCR Strips without Caps, low profile
Analytic Jena	8 Well Strip (0.2 ml, Low Profile), transparent with lid

The PCR products were detected via agarose gel electrophoresis using GelRed™ (Biotium) and visualized using the Gel Doc XR+ (Bio-Rad®). The Thermo Scientific GeneRuler 50 bp DNA Ladder was used as marker.

Results and Discussion

Even though a lot of efforts have been taken to further decrease PCR reaction times, mainstream protocols have not changed in general. Significantly reduced runtimes can be achieved using fast PCR reagents on thermal cyclers with fast ramp rates but standard PCR consumables present a bottleneck. Polypropylene, the standard material for PCR consumables, limits the speed of the heat transfer from the thermal block to the sample even when the tubes are ultra-thin walled. This usually leads to lower amplicon yields and accuracy under fast PCR conditions.

The results presented in this Application Note show a successful conversion of a standard to a fast PCR protocol using the Eppendorf Fast PCR Tube Strips made of polyethylene. PCR protocols using two different fast PCR reagents were successfully established on fast silver block thermal cyclers (Mastercycler X50s and X50i). Whilst the

standard PCR protocol generally takes about an hour, the fast PCR protocols were completed in 12 min (SPEEDStar) to 16 min (GeneAmp). This is a reduction of the runtime by more than 70%.

Furthermore, comparison between Eppendorf Fast PCR Tube Strips, standard PCR consumables and other fast PCR consumables showed the superior PCR performance of the Eppendorf Fast PCR polyethylene consumables. A better heat transfer to the sample results in higher amplicon yields under fast PCR conditions.

Eppendorf Fast PCR Tube Strips allows user to easily transfer PCR protocols, thereby significantly reducing runtimes without sacrificing yield and specificity. This offers the chance to increase relative yield as well as sample throughput in every lab where time is a precious commodity.

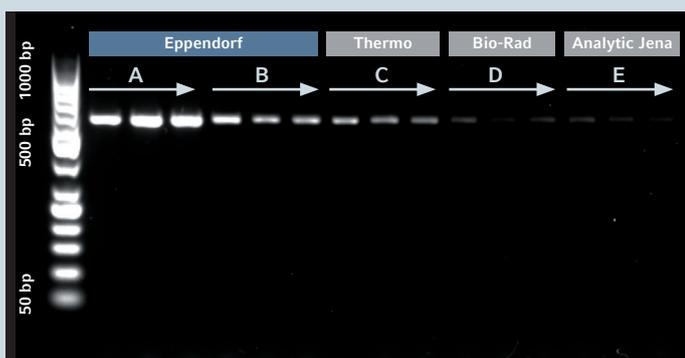


Figure 1: SpeedSTAR™ Protocol

- A: Fast PCR Tube Strips
- B: PCR Tube Stripes
- C: ABI MicroAmp Fast 8-Tube Strips
- D: 0.2 mL 8-Tube Strips
- E: 8 Well Strip (0.2 mL, low profile)

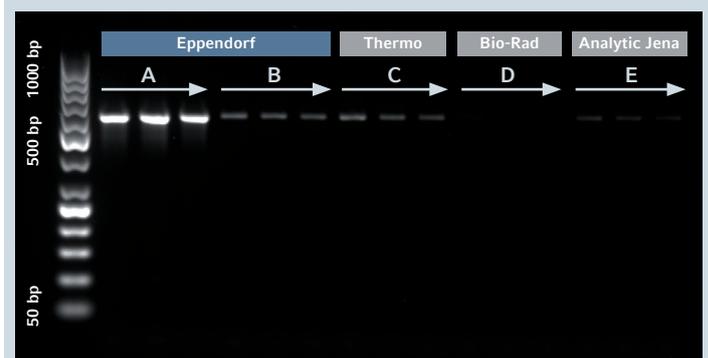


Figure 2: GeneAmp™ Fast PCR Protocol

- A: Fast PCR Tube Strips
- B: PCR Tube Stripes
- C: ABI MicroAmp Fast 8-Tube Strips
- D: 0.2 mL 8-Tube Strips
- E: 8 Well Strip (0.2 mL, low profile)

Conclusion

Due to better heat transfer properties of polyethylene, the new Eppendorf Fast PCR Tube Strips allow for an easy conversion of standard PCR protocols to fast cycling protocols. In combination with fast PCR reagents, it is possible to fully exploit the advantage of high ramp rates on fast thermal cyclers such as Mastercycler X50s/X50i and reduce cycling times by more than 70% without tedious optimization. Compared to standard or other fast PCR consumables being used with fast PCR kits, the Eppendorf Fast PCR Tube Strips made from polyethylene showed a better efficiency and higher yields.

This allows users to reduce the time to get PCR results and to enhance sample throughput using thermal blocks and consumables of standard formats, thus without the need for specialized equipment.

Ordering information

Description	Order no. international	Order no. North America
Eppendorf Fast PCR Tube Strips 0.1 mL	0030 124.901	0030124901
PCR Tube Strips, 0.1mL PCR clean without Lids (10x12 strips)	0030 124.804	0030124804
PCR Tube Strips, 0.1mL PCR clean, with Cap Strips, domed (10x12 strips)	0030 124.812	0030124812
PCR Tube Strips, 0.1mL PCR clean, with Cap Strips, flat (10x12 strips)	0030 124.820	0030124820
Mastercycler® X50s	6311 000.010	6311000010
Mastercycler® X50i*	6301 000.012	6301000012

* Without Touchscreen

Your local distributor: www.eppendorf.com/contact

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