APPLICATION NOTE No. 446

# Breaking Barriers: Endpoint PCR in 15 Minutes or Less

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## Abstract

The ever-increasing demand for gene amplification has pushed for faster, more robust tools that improve the speed of endpoint PCR without sacrificing the quality of results. The Mastercycler® X50s PCR cycler has established itself as the market leader in accelerating thermal cycling quickness. With the increased speed of Promega GoTaq® Rapid PCR Master Mix and Rapid 2-Step and 3-step protocols, the combined system provides researchers with a new tool to amplify samples in 15 minutes or less.


## Introduction

The technique of endpoint polymerase chain reaction (PCR) is a mature field that has spurred many developments in related fields. Advancement in instrumentation engineering, biochemical engineering, auxiliary plastic ware as well as hardware and software designs, combined to push this technique to be one of the most robust tools in the biological field. Over time, focus has shifted to the speed of PCR. To be able to complete a PCR in the shortest time possible not only hastens research but also accelerates the introduction of research results into commercial markets. The principle of fast PCR is simple at heart. If the 'correct' temperature is supplied, the process of denaturation, annealing and extension will happen instantaneously in the reaction. However, this 'instantaneous' reaction is limited by a combination of several factors including the technology and speed of the thermocycler, the heat transfer efficiency of PCR vessels, reaction volume, the purity and complexity of the DNA template, as well as the extension rate of the polymerase used.

Manufacturers throughout the years have developed strategies to overcome the various bottlenecks in achieving rapid PCR. Although the nature of the DNA template varies according to the nature of experiment and remains a less controllable variable, the three indispensable technological barriers in PCR – thermal cycler, enzyme and PCR vessel technologies – can be overcome. The Mastercycler X50 developed by Eppendorf is currently the conventional thermal cycler with both the fastest speed and highest ramping rate in the market [1]. The revolutionary Eppendorf Fast PCR Tube Strips made from polyethylene offer better heat transfer properties than polypropylene and allow PCR users to speed up time-to-result using standard thermal cycler and consumables without the need for costly conversion to specialized equipment [2]. And now, using Promega GoTaq Rapid PCR Master Mix and an accelerated 2-step protocol, the time required for PCR can be reduced to under 15 minutes and allow research to keep pace with innovation.

## Materials and Methods

50 ng of Total Human DNA, *E. coli* DNA or *C. albicans* DNA was used as template for the multi-fragments amplification of the human Beta-2-microglobulin (B2M), *E. coli* 16S ribosomal and *C. albicans* KER1 genes, respectively, using GoTaq Rapid Master Mix. Please find the primers used for the amplification in table 1 at the end of the document.

PCR reaction master mix was prepared in polyethylene Fast PCR Tubes. The final 20 µL volume per reaction was run in Mastercycler X50s according to the settings and cycling conditions in Table 1 or 2 below.

**Table 1: 3-step rapid cycling conditions**
Lid temperature: 105 °C
Temperature mode: Fast
Block settings: Silver 96

| Description | Cycles | Time | Temperature |
|---|---|---|---|
| **Activation** | 1 | 1 min | 95 °C |
| **Denaturation** | | 2 sec | 95 °C |
| **Annealing** | 35 | 2 sec | 65 °C |
| **Extension** | | 6 sec | 72 °C |
| **Final extension** | 1 | 1 min | 72 °C |

Approximated run time: 15

**Table 2: 2-step rapid cycling conditions**
Lid temperature: 105 °C
Temperature mode: Fast
Block settings: Silver 96

| Description | Cycles | Time | Temperature |
|---|---|---|---|
| **Activation** | 1 | 1 min | 95 °C |
| **Denaturation** | | 2 sec | 95 °C |
| **Annealing/Extension** | 35 | 6 sec | 65 °C |
| **Final extension** | 1 | 1 min | 72 °C |

Approximated run time: 12 min 30 sec

The PCR products were detected via 1.4-2% of agarose gel electrophoresis using Ethidium Bromide or GelRed® and visualized using the BioRad® Molecular Imager® GelDoc™ XR+.

## Results and Discussion

### First step towards Fast PCR protocols: a 3-step fast protocol

When transferring from standard master mix to one formulated for fast PCR, reducing the holding times of each step significantly lowers the total PCR run time. The GoTaq Rapid Master Mix was used to amplify human DNA targets up to 1.4 kb. The amplified product resulted in clean bands with good yield on an agarose gel after a total run time of 15 min 10 seconds. The results showed conversion to a fast PCR master mix provides researchers with good experimental outcome while significantly increasing the productivity in the lab.

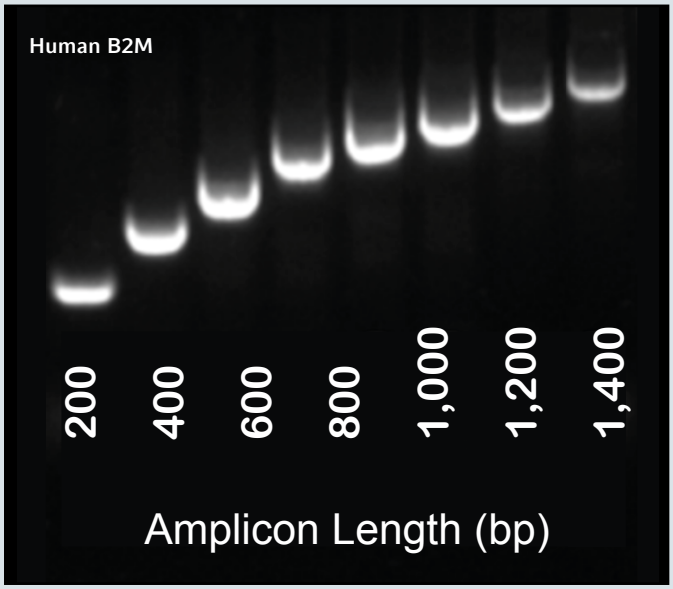


**Figure 1:** Amplification of human DNA targets using Fast PCR system with 3-step protocol

### Stable amplification of >1 kb amplicon in 13 min

The speed potential of the GoTaq Rapid Master Mix was evaluated further by converting to a rapid 2-step protocol. Using the same Human B2M PCR system, amplification with distinct bands and good yields was successfully obtained using the combined annealing/extension time of 6 sec at 65 °C. The parameters used to complete this PCR were thus able to cut down the total run time from the previous 3-step protocol to only 13 min 30 sec from the start of the program to the final holding step at 10 °C. The results showed that the GoTaq Rapid Master Mix allows PCR completion in under 15 min compared to common enzymes in the market that would generally take 90 minutes or more to amplify amplicons of the same length.

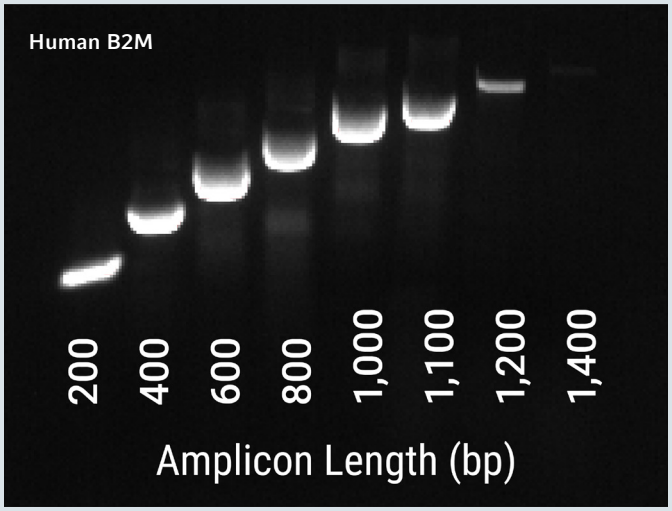


**Figure 2:** Amplification of > 1 kb of human DNA targets with 2-step Fast PCR system protocol

### Robust enzyme with wide application use

The robustness and flexibility of the GoTaq Rapid Master Mix was put to test by using it for amplification of different DNA types. Using the same 2-step protocol, the results showed the new master mix can successfully amplify DNA from bacterial and fungal sources, including longer target of up to 1.6 kb (Figure 3B). The robustness of this fast master mix was evidenced by its ability to rapidly complete various PCR runs in 13 min 30 sec.

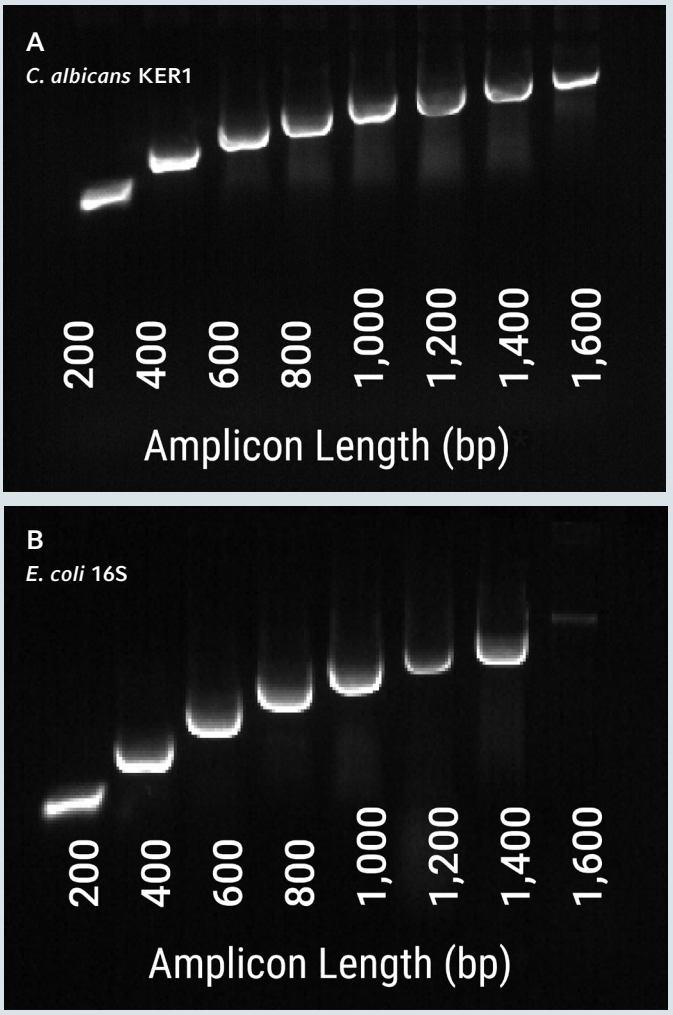


**Figure 3:** Amplification of various targets of (A) fungal and (B) bacterial DNA.

## Conclusion

Traditional endpoint PCR can now be completed exceptionally fast when all limiting factors of thermal cycler, reaction vessels and master mix have been addressed.
In this Application Note, we have shown that the Promega GoTaq Rapid Master Mix is able to amplify DNA amplicons in a very short amount of time when used in conjunction with Eppendorf's fast-ramping thermal cycler, the Mastercycler X50s, and fast thermal transfer PCR consumables.

This complete combination breaks the technical barriers that previously limited the speed of PCR and allows users to continue using the conventional, comfortable format of traditional thermal cyclers. It is now entirely realistic to complete amplification in 15 minutes or less without the need for specialized equipment or closed-format thermal cycler systems.

**Supplementary Table 1: The following primers were used for the amplification of the respective gene fragments.**

| Organism Gene | Amplicon Length (bp) | Forward Sequence | Reverse Sequence |
|---|---|---|---|
| **Human B2M** | 206 | AGA TCA AGG CAG GAG CAG GAA CCA | TGA TGC CCT CTC AGC ACT CAT AGC A |
| **Human B2M** | 395 | TGG GTG TAG ATC AAG GCA GGA GCA | CCA CCT TCC CAA CAA GCC ACC T |
| **Human B2M** | 590 | GCT GAG AGG GCA TCA GAA GTC CTT G | ACA TGG TTC ACA CGG CAG GCA |
| **Human B2M** | 790 | TGG GTG TAG ATC AAG GCA GGA GCA | TCA CAT GGT TCA CAC GGC AGG C |
| **Human B2M** | 990 | AGA TGA GTA TGC CTG CCG TGT GAA C | GGG TAA CCA CCT GCC TTT ATC CTG C |
| **Human B2M** | 1102 | TGC AGC GCA ATC TCC AGT GAC A | GGC TGG CAG AAT AGG CTG CTG T |
| **Human B2M** | 1203 | GCA ATT GCT ATG TCC CAG GCA CTC T | GCT GCC ACA AAA GCT AGA GGA AGC C |
| **Human B2M** | 1392 | TGC AGC GCA ATC TCC AGT GAC A | TGC CAC TCC ACA GGA GAA GGG A |
| **Fungal KER1** | 205 | AAC CGG CCA ACG AAG CCG AG | TCA TCG CCT TTG AGT GCT TCT CCA |
| **Fungal KER1** | 391 | GCC TTG GCA GCC GGT AGT GC | CCG CAG TGG TTT CTC CTG CTG C |
| **Fungal KER1** | 598 | AGC TGA AAA GGA GAA AGC TGC CGA | TCC AGC ACT ACC GGC TGC CA |
| **Fungal KER1** | 793 | GCC TTT CTT CCT GCA CAC CCC C | CGG CTT CGT TGG CCG GTT CT |
| **Fungal KER1** | 997 | GGA GAA GCA CTC AAA GGC GAT GAC A | AAG CAG CAG CAG AGC CAG CC |
| **Fungal KER1** | 1195 | TGG TGT TTT GGC TGG CTC TGC T | TCC AGC ACT ACC GGC TGC CA |
| **Fungal KER1** | 1403 | GGC CCA ACT CGA CAA ACC GGA | ACT GGA GCT GCT GGA ACC GC |
| **Fungal KER1** | 1605 | TCA CCC CTC ACG CCC TCA CTT | TTC GGC CGT TTT GTC GCT GCT |
| **Bacteria 16S** | 203 | GGG GGA CCT TCG GGC CTC TT | CGG CAT GGC TGC ATC AGG CT |
| **Bacteria 16S** | 409 | TAC GGG AGG CAG CAG TGG GG | AGT CTT CGT CCA GGG GGC CG |
| **Bacteria 16S** | 601 | GCT GGC GGC AGG CCT AAC A | TGC AGT TCC CAG GTT GAG CCC |
| **Bacteria 16S** | 792 | GGG GTA GAA TTC CAG GTG TAG CGG T | GCC CTC CCG AAG GTT AAG CTA CCT A |
| **Bacteria 16S** | 1021 | TCA GCG GGG AGG AAG GGA GT | GCC CTC CCG AAG GTT AAG CTA CCT A |
| **Bacteria 16S** | 1196 | AGG CTC ACC TAG GCG ACG ATC C | GCC CTC CCG AAG GTT AAG CTA CCT A |
| **Bacteria 16S** | 1398 | CCG CCT TCG CCA CCG GTA TT | TTG CCC GCA TCA TCC GCA GG |
| **Bacteria 16S** | 1600 | TCC GAT GGC AAG AGG CCC GA | CTG GCG GCG AGC TTT GGT CA |

## References

[1] Gerke, N. & Phang, A. Comparative Run Time Evaluations of PCR Thermal Cyclers. Eppendorf Application Note 274.
[2] Isermann, K. & Phang, A. Reduced PCR runtimes and increased yields using Eppendorf Fast PCR Consumables. Eppendorf Application Note 400.

**Ordering information**

| Description | Order no. Germany | Order no. International |
|---|---|---|
| **Eppendorf FAST PCR Tube Strips 0.1 mL** | | |
| PCR clean without Lids | 0030 124 901 | 3303 124 901 |
| PCR clean, with Cap Strips, domed | 0030 124 928 | 0030 124 928 |
| PCR clean, with Cap Strips, flat | 0030 124 910 | 0030 124 910 |
| Mastercycler® X50s | 6311 000 010 | 6311 000 010 |
| Mastercycler® X50i* | 6301 000 012 | 6301 000 012 |
| CycleManager X50-Software | 6349 000 014 | 6349 000 014 |

*eco model, without touchscreen

| | Order no. International |
|---|---|
| GoTaq® Rapid Master Mix (Promega®) | CS3083A01 |
| Human Genomic DNA (mixed) (Promega®) | G3041 |
| Candida albicans (Robin) Berkhout (ATCC®) | 10231D5™ |
| *Escherichia coli* O111:NM, DNA (10 µg) (Zeptometrix®) | 0801747DNA |

**This application note was done in collaboration with**


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