

Novel thermal cycler amplifies the entire BRCA1 gene within 10 minutes

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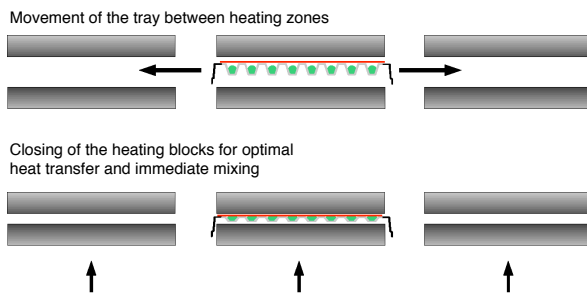
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Stand 524

Introduction

PCR is, in most cases, an inevitable and lengthy step in clinical genetic diagnostics and research. A novel thermocycler enables amplification of DNA in minutes. Here we report amplification of the complete BRCA1 gene (29 fragments) in less than 10 minutes.

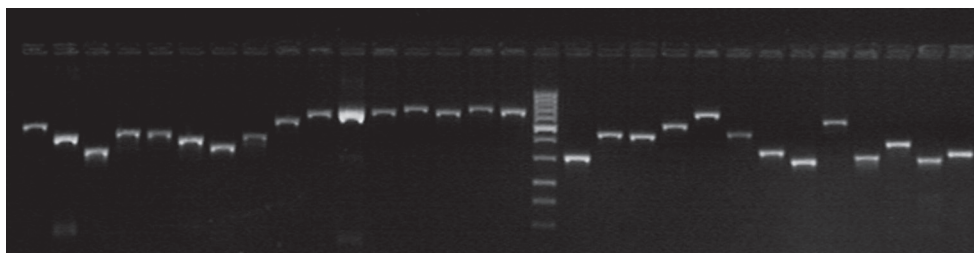
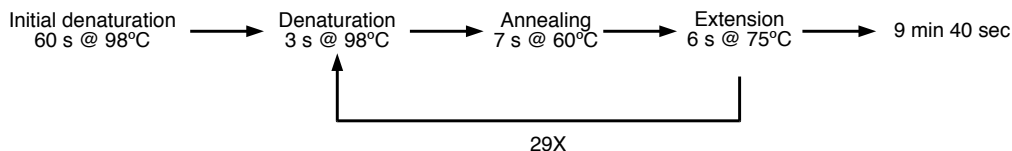


Principle

Amplification is performed in ultra thin wells (<50 micron) in a 96-well SBS microplate format. The cycler consists of three temperature zones for denaturation, annealing and extension, with 2 blocks each. When the tray enters a temperature zone, heated blocks are brought together, thus compressing the wells slightly. This compression results in sample mixing and instantaneous temperature change.

Experimental

For analysis of the BRCA1 gene, the diagnostic setup of the Erasmus MC is used: 29 primer pairs containing M13 tails for subsequent Sanger sequencing. Amplification was performed in 5 µl reaction volume containing 1XPCR buffer (Kapa Biosystems), 0.3 mM dNTPs (Kapa Biosystems), 2 mM MgCl₂, 2.5 ng template DNA, 0.5 µM of each primer, 0.125 Units of Kapa 2G Fast HotStart polymerase.



All 29 fragments were amplified in less than 10 minutes.

Amplified sequences were confirmed with Sanger sequencing.

Discussion

We present the next generation PCR machine, which can reduce PCR time from hours to less than 10 minutes. This novel machine employs three temperature zones for denaturation, annealing and extension, which enables instantaneous temperature changes of the PCR mix. The machine enables us to amplify all 29 amplicons of the BRCA1 gene within 10 minutes. In fact, a 100 basepair fragment could be amplified in less than 2 minutes.

As the time for amplification can be reduced 10-20 times, this novel cycler allows for 10-20 times more reactions per cycler per day. Therefore, this machine results in extremely fast amplification. It also raises the throughput or reduces the number of instruments needed, reducing costs in purchase and maintenance contracts.

A real time version is currently under development.