Amplification of a 100 bp fragment in 1 minute and 59 seconds

Amplification of DNA, using PCR, is a common and, in many cases, inevitable step in molecular biology procedures. Using standard micro tubes or plates, the time needed for the temperature changes accounts for most of the total time of the reaction. Here we report the use of the NEXTGENPCR 1 system to perform a 30 cycle, 3 temperature PCR in less than 2 minutes.

Microplate

Sample plates are SBS-compliant frames holding a thin polypropylene foil with 96 (5 or 20 µl) or 384 (5 µl) wells. All wells are sealed by a second foil, using a heat sealer.

Nextgen PCR

MBS has developed a novel thermocycler, NEXTGENPCR 1, which can perform PCR in less than 2 minutes. The NEXTGENPCR 1 cycler employs 3 temperature zones with two heated blocks each. Zones are set to denaturing, extension and annealing temperatures. Microplates with samples embedded in polypropylene foil are moved between zones, where they are slightly compressed by the temperature blocks. This ensures sample mixing and optimal heat transfer. Temperature transition is practically instantaneous.

Thermocycling

Samples where heated to 98°C for 10 second followed by 5 cycles of denaturation (98°C, 2 seconds), annealling (60°C, 2 seconds), and extension (75°C, 2 seconds), followed by 25 cycles of denaturation (98°C, 0.5 seconds), annealing (60°C, 0.5 seconds), and extension (75°C, 0.5 seconds). PCR product where visualised on 2% agarose (Figure 1.).

Amplification of a 100 bp DNA fragment

A 100 bp fragment of the NAD(P)H dehydrogenase (quinone) 1 (NQO1) gene is amplified in 5 µl reaction volume containing 1XPCR buffer (Kapa Biosystems), 0.3 mM of each dNTP (Kapa Biosystems), 4 mM $MgCl_2$, 2.5 ng template DNA, 1 µM of each primer, 0.5 Units of Kapa 2G Fast HotStart polymerase.

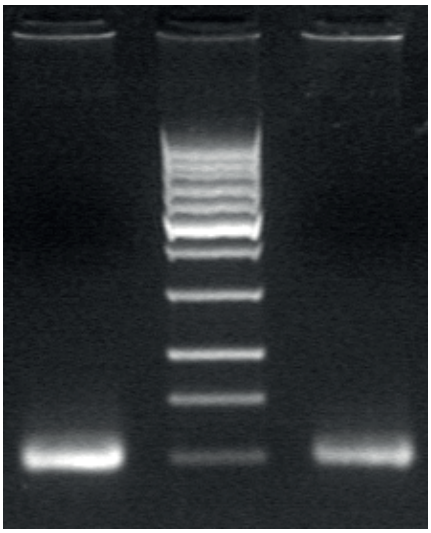

Figure 1. PCR product of a 100 bp fragment compared to a 100 bp ladder, visualised on 2% agarose