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APPLICATION NOTE No. 289 I December 2013

Straightforward PCR optimization and highly flexible operation on the dual block thermocycler Mastercycler® nexus GX2

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Abstract

The Mastercycler® nexus GX2 provides the user with the utmost flexibility. The combination of a 64-well block and a 32-well block offers optimum utilization of both thermoblocks with respect to the sample number of each PCR preparation. In addition, this thermocycler may be employed for routine applications while simultaneously performing PCR optimization using the temperature gradient function. The PCR applications presented herein show that these flexible possibilities go hand in hand with consistent, high quality results.

Introduction

In many laboratories, flexible application options are the basic condition for optimized use of laboratory equipment. Especially in laboratories in which multiple users rely on one instrument, flexible operation may increase the frequency of use. Similarly, flexible use increases efficiency in routine laboratories with multiple different applications; this may be of special advantage during a step such as PCR, as PCR can take up a significant portion of time allocated to the total workflow. This Application Note will present the extraordinarily flexible application possibilities of the dual block thermocycler Mastercycler® nexus GX2:

> PCR optimization via temperature gradient function
> Reproducibility and comparability of PCR performance on the 64-well block and the 32-well block
> Thermic independence of both thermoblocks.

Materials and Methods

The following PCR runs were carried out on a Mastercycler® nexus GX2. Within the 64-well block a temperature gradient function across 8 positions in horizontal orientation is available to the user. The following primers were used for amplification of a GC-rich fragment (484 bp) from the human β-actin gene (ACTB):

Primer 1: 5'-ATC GCC GCG CTC GTC GTC-3'
Primer 2: 5'-TGG GTC ATC TTC TCG CGG TTG G-3'

The GoTaq® Hot Start Polymerase kit (Promega®) was used in combination with a PCR Nucleotide Mix (Promega) for the setup of the PCRs. Human genomic DNA (Roche®) served as the template. A 20 µL reaction contained the following final concentrations, or amounts, respectively, of the individual components: 300 nM per primer, 200 µM dNTPs, 2.5 mM Mg^{2+}, 2 ng/µL template DNA, 1 x GoTaq Flexi buffer as well as 0.5 U of the GoTaq Hot Start Polymerase. The PCR products were detected using the DNA-binding fluorescent dye ethidium bromide following agarose gel electrophoresis. Further quantification of the PCR products was performed with the Agilent® DNA 1000 kit in accordance with manufacturer's instructions on the Agilent 2100 Bioanalyzer® (Agilent Technologies®).

1) PCR optimization using the temperature gradient function

An initial temperature gradient run was carried out for the purpose of determining a suitable annealing temperature (fig. 1), followed by a second gradient run in order to determine the optimum denaturation temperature. To this end, the annealing temperature of 64 °C, established during the first PCR, and a gradient ranging from 93 to 98 °C for the denaturation step were used – all other conditions were identical.

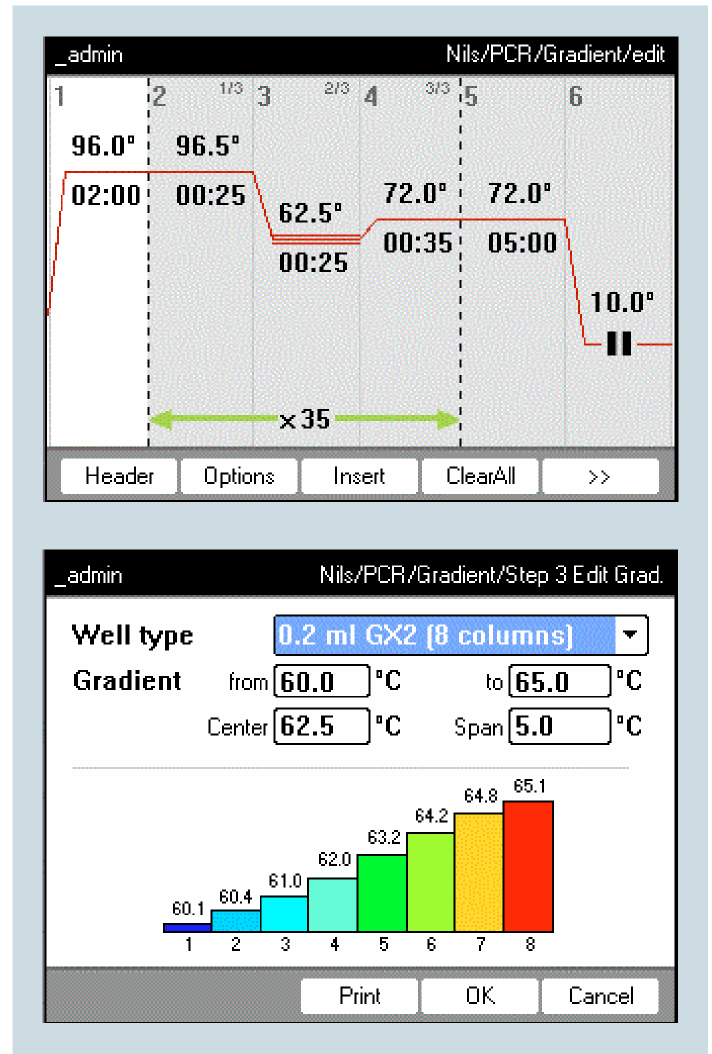

Fig.1: PCR program of the temperature gradient run for determination of a suitable annealing temperature. A screenshot for gradient programming in the Mastercycler nexus GX2 software is shown below.

2) Reproducibility and comparability
Three PCRs were run in parallel on the 64-well block and the 32-well block using the PCR system optimized under 1). For this purpose, the blocks were loaded with 12 samples each, distributed across the entire respective block (fig. 2).

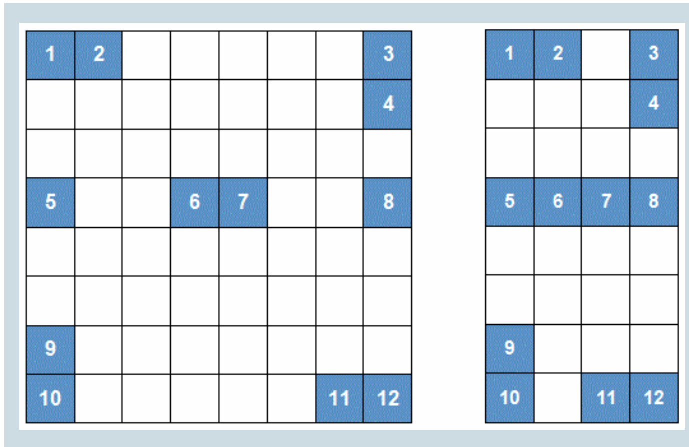

Fig. 2: Positioning of the twelve PCR samples on the 64-well and the 32-well block, respectively.

3) Thermic independence of both thermoblocks
In order to test thermic independence, a PCR run with twelve samples was performed on the 64-well block using the conditions described above, while a continuous holding temperature of 4 °C was programmed on the 32-well block. Subsequently, a second run with twelve samples was carried out on the 32-well block while a temperature of 99 °C was permanently held on the 64-well block.

Results and Discussion

1) PCR optimization via gradient function
The gradient function of the Mastercycler nexus GX2 enables optimization of the annealing temperature as well as the denaturation temperature. During analysis of the PCR products resulting from the annealing temperature gradient run it became apparent that low annealing temperatures led to the formation of non-specific products above 500 bp and at approximately 350 bp (fig. 3). For this reason an annealing temperature of 64 °C was selected for the following runs.

The analysis of the second gradient run revealed the formation of non-specific products at lower denaturation temperature around 93 °C (fig. 3). At higher temperatures, only the specific product associated with a higher yield was observed. Therefore, 97 °C was determined as the suitable future denaturation temperature.

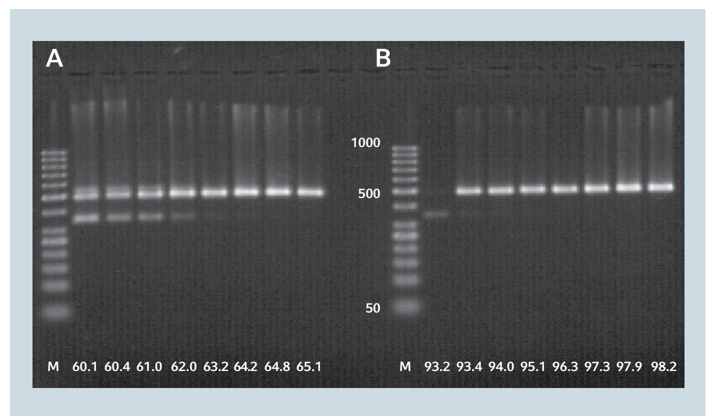

Fig. 3: PCR products resulting from the gradient runs for determination of (A) annealing and (B) denaturation temperatures, respectively. The appropriate gradient temperatures [°C] of both runs are inserted at the bottom of the gel image (M: Length marker, GeneRuler™ 50 bp DNA Ladder, Thermo Fisher Scientific®).

2) Reproducibility and comparability

Triplicate repeat PCR runs, each with 12 samples, served to verify the reproducibility of the PCR results obtained from one block, as well as the comparability between the PCR products resulting from the 64-well block and the 32-well block, respectively (fig. 4a). Analysis of the PCR product concentration, as quantified on the Agilent 2100 Bioanalyzer using the Agilent DNA 1000 kit, shows that product yields are reproducible on both blocks (fig. 4b). With an overall average PCR product concentration of 26.82 ng/µL and a standard deviation of 2.5 ng/µL, the 36 samples from the 32-well block show comparable performance across all three runs. The 64-well block shows similar results, i.e. 27.14 ng/µL and 2.95 ng/µL, respectively. These results further highlight not only consistent quality of results within one block, but also a high degree of consistency between the 32-well block and the 64-well block.

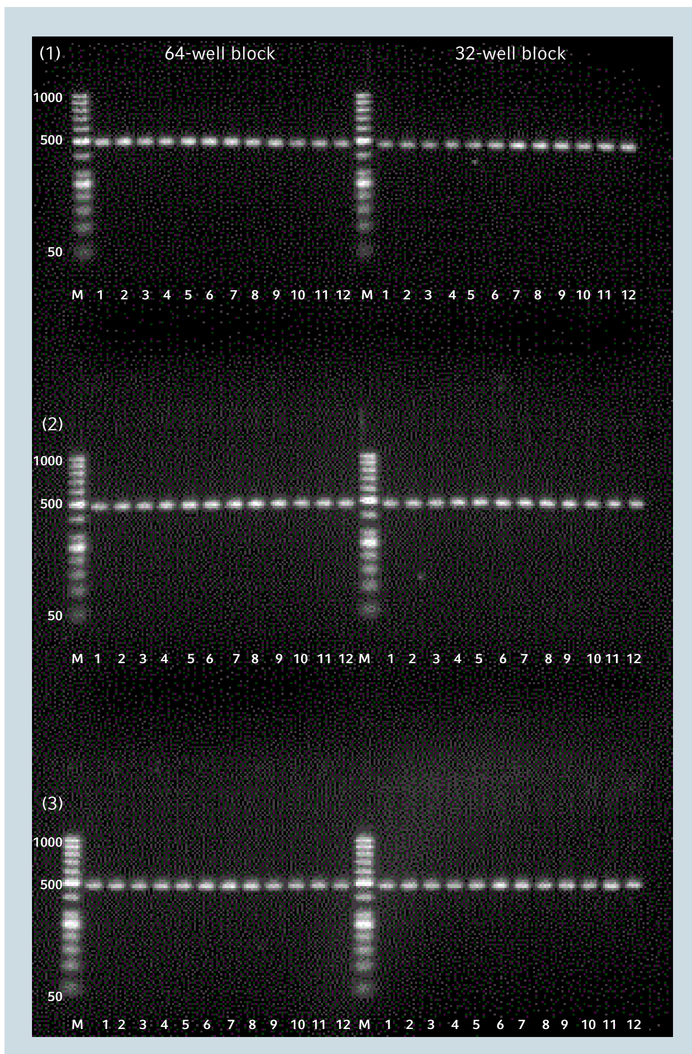

Fig. 4a: Gel image of the PCR products resulting from the triplicate repeat PCR runs on the 64-well block and 32-well block.

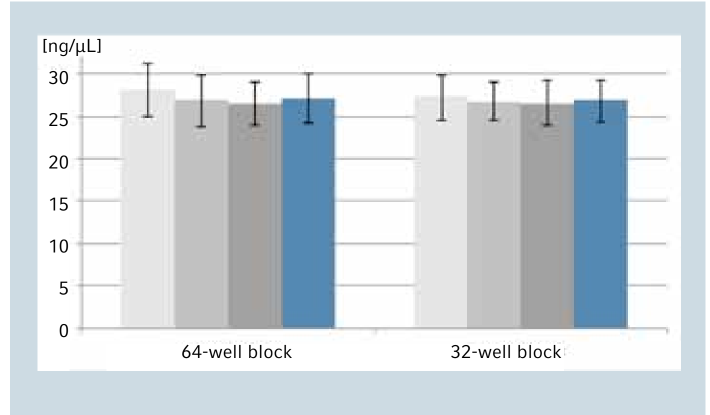

Fig. 4b: Arithmetic means +/- standard deviations of the PCR product concentration of the triplicate repeat PCR runs (light gray bar corresponds to (1) on the gel (fig. 4a), medium gray bar to (2) and dark gray bar to (3)) on the 32-well block and 64-well block. The blue bar displays the overall arithmetic mean +/- standard deviation of the respective block.

3) Thermic independence of both thermoblocks
Both runs established the equally good performance of the PCR across all 12 positions (fig. 5). Evidently no thermic influence was exerted externally, even when the neighboring block was programmed to a permanently extreme high or low holding temperature.

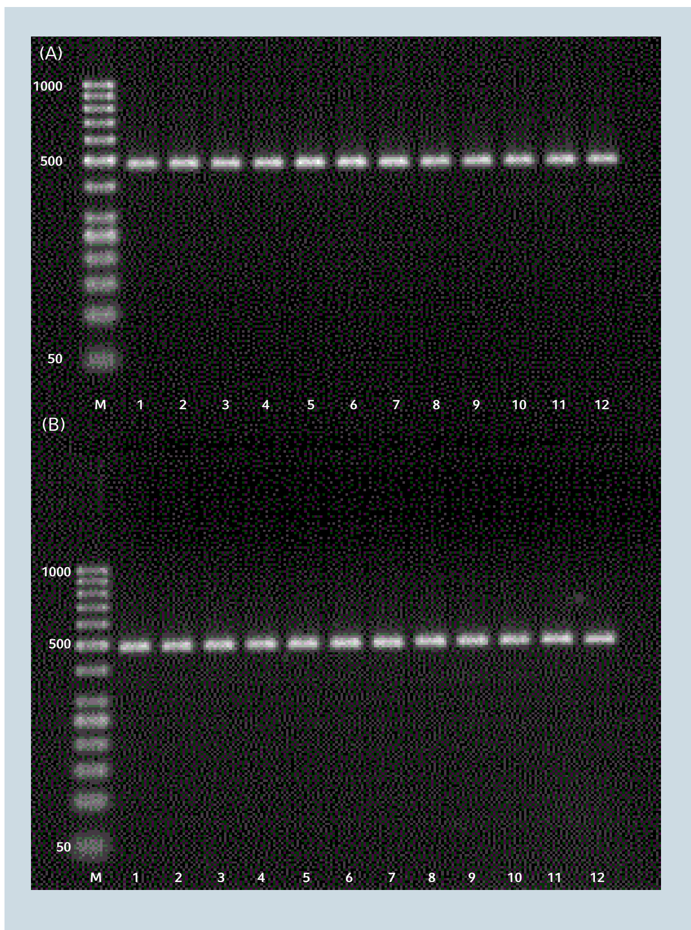

Fig. 5: (A) Twelve PCR products of the 64-well block during a permanent holding temperature of 4 °C on the 32-well block; (B) twelve PCR products of the 32-well block during a permanent holding temperature of 99 °C on the 64-well block (M: Length marker, GeneRuler™ 50bp DNA Ladder, Thermo Fisher Scientific).

Conclusion

In addition to the independent use of two thermoblocks, the user may take maximum advantage of the new Mastercycler nexus X2 and its different 64-well block and 32-well block formats to accommodate different sample numbers. Therefore, the Mastercycler nexus X2 provides the user with the utmost flexibility while simultaneously delivering high quality results. This underscores the instrument’s specific suitability for laboratories in which multiple users rely on the thermocycler, as well as for routine laboratories performing numerous different PCR reactions of low to medium sample number. This flexibility can be further expanded by an instrument variant (Mastercycler nexus GX2) which offers a temperature gradient function to the user.

Ordering Information

Description	Order no. international	Order no. North America
Mastercycler® nexus with universal dual block		
Mastercycler® nexus GX2	6336 000.015	6336000023
Mastercycler® nexus X2	6337 000.019	6337000027
Mastercycler® nexus GX2e*	6338 000.012	6338000020
Mastercycler® nexus X2e*	6339 000.016	6339000024
Mastercycler® nexus with silver block		
Mastercycler® nexus GSX1	6345 000.010	6345000028
Mastercycler® nexus SX1	6346 000.013	6346000021
Mastercycler® nexus GSX1e*	6347 000.017	6347000025
Mastercycler® nexus SX1e*	6348 000.010	6348000029
Mastercycler® nexus with universal block		
Mastercycler® nexus gradient	6331 000.017	6331000025
Mastercycler® nexus	6333 000.014	6333000022
Mastercycler® nexus gradient eco*	6334 000.018	6334000026
Mastercycler® nexus eco*	6332 000.010	6332000029
Mastercycler® nexus with flat block		
Mastercycler® nexus flat	6335 000.011	6335000020
Mastercycler® nexus flat eco*	6330 000.013	6330000021
Accessories		
CAN_BUS connection cable, 50 cm	5341 612.006	950014008
CAN_BUS connection cable, 150 cm	5341 611.000	950014016
Self test dongle	6320 071.001	950030040
Temperature Verification System USB – Single-Channel	0056 000.003	0056000003
Temperature Sensor for Temperature Verification System USB – Single-Channel	0056 001.000	0056001000

* To run a Mastercycler® nexus with the suffix »eco« or »e«, a Mastercycler® nexus model without such a suffix is needed. Up to 2 units with the suffix »eco« or »e« can be connected to a Mastercycler® nexus without such a suffix.

Your local distributor: www.eppendorf.com/contact
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eppendorf@eppendorf.com · www.eppendorf.com

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