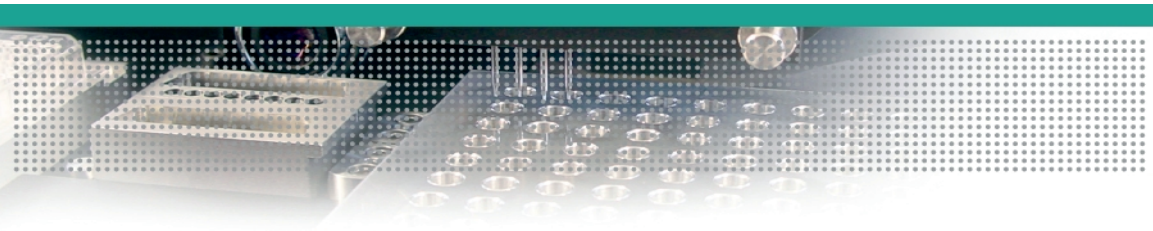

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sciFLEXARRAYER Application Note No. 08004

Increasing speed while decreasing cost: PCR in nanoliter volumes

The combination of precise dispensing in the pico- and nanoliter range and miniaturized microtitreplate formats enables to perform PCR in nanoliter volumes. Small reaction volumes, typically being in the 2-200 nanoliter range, allow for ultrafast heating and cooling operations and to finish a complete PCR in as little as 10 minutes. When compared to standard systems, typically only 10% of reagents are used in nanoliter PCR (nPCR). This leads especially in high throughput environments to massive cost reductions. Combined with an online detection system for real-time or end-point detection the combination described here allows for sensitive, cost-effective and quick analysis of nucleic acids.

Materials and methods

PCR mixes of various vendors were applied to specifically developed polymer microplates in a way that primers, reaction mixes and templates are deposited in nanoliter volumes. Different reaction volumes are composed by varying the number of droplets from a single or multiple dispensers with typical error rates being less than 2.5%. The speed of the deposited droplets (2-3 m/s) results in an effective and rapid mixing of the reagents in the microplate well before the plate is sealed with a transparent lid. The prepared plate is cycled and detected in a customized PCR device.

Results and discussion

In order to successfully perform PCR experiments in the nanoliter range (nano-PCR), several prerequisites were necessary. The used materials are optimised to show no inhibition effects for the assays and polymerases used. Specifically treated polyethylene microplates led to the best results (Fig. 1).
nanoPCR reactions can be finished in only 10 minutes. When compared with standard PCR set-ups, the cycle times of nanoPCRs are much quicker. This is mainly due to shorter diffusion ways and an efficient heat transfer present in the small volumes with large surface contact. In addition the overall energy consumption of the system is drastically decreased, too. Already at present the platform described here enables users to increase throughput tenfold at a cost level which is only 10% of the current running costs.Central applications include real-time expression profiling and SNP genotyping based on end-point detection.

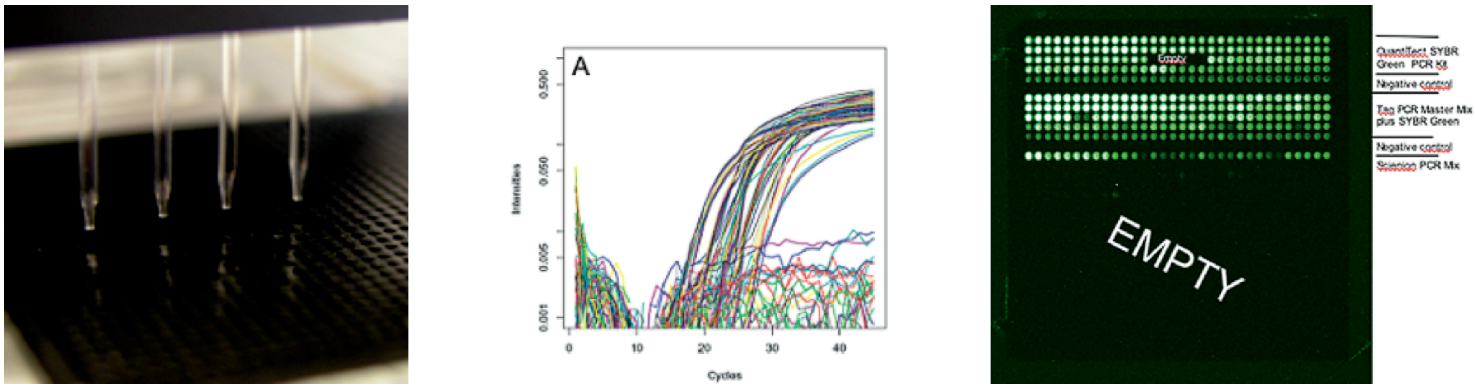

Fig. 1 Loading of a miniaturized microwell plate on a cooled substrate carrier within a sciFLEXARRAYER S5 system (left). Original image of a typical 200 nl reaction real-time readout used in a study for tissue specific expression profiling in mice (middle) and original readout of a reaction done in a miniaturized microplate with 200 nl volume with three different PCR systems (right) .

This work has been done in cooperation with Dr. Andreas Dahl, Max Planck Institute for Molecular Genetics, Berlin, Germany. An article on this topic has been published by Dahl A, Sultan M, Jung A, Schwartz R, Lange M, Steinwand M, Livak KJ, Lehrach H, Nyarsik L.:Quantitative PCR based expression analysis on a nanoliter scale using polymer nano-well chips. Biomed Microdevices. 2007

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