5 Summary

Functional genomics tries to understand the information encoded in the genome and its complex network organization. Thereby gene expression analysis and SNP genotyping are central elements of a functional genome analysis. Due to genome complexity, capable analysis methods with high-throughput capability are required in genomics. Limitations of available high-throughput methods such as microarrays are their relatively low sensitivity and reduced flexibility in assay design.

This work aimed at developing a miniaturized high-density micro-well plate platform for liquid PCR-based assays to combine the high density of a miniaturized array format with the features of liquid PCR-based methods. With the polypropylene-based µPCR chip a cost-effective substrate for highly parallel PCR-based analysis was developed. The developed formats of the µPCR chip were verified to permit PCR-based assays in a volume range of 200 to 18 nl.

Real-time PCR-based expression analysis was performed with the 5'-exonuclease assay (TaqMan). For reliable quantification down to 50 nl a workflow with advanced non-contact nanoliter liquid handling, efficient sealing, precise thermocycling and capable signal readout as well as data analysis procedures was developed. The platform demonstrated good reproducibility and high sensitivity down to single molecules in nanoliter volumes. The results of 200 nl assays in a study of tissue-specific gene expression in mouse were consistent with those obtained for 10 µl assays.

Successful TaqMan-based SNP genotyping was performed down to 100 nl. For the analysis a robust allele-calling algorithm was developed. Due to the high sensitivity of the system, reliable genotyping was shown down to 5 initial target molecules of human genomic DNA as well as the equivalent amount of whole genome amplified DNA. In a validation study of 60 patients vs. 16 SNPs per chip, high concordance rates to the results obtained from conventional volumes were achieved.

The newly developed µPCR chip platform proved to be a capable technology for miniaturized PCR-based analyzes in functional genomics. With the reduction of assay volumes down to few nanoliters the µPCR chip platform enables comprehensive large-scale studies in gene expression as well as SNP genotyping fine-mapping requiring only a moderate consumption of biological and financial resources.