Digital PCR in non-invasive prenatal testing

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Background: In the past decade, digital PCR (dPCR) has been widely used in clinical research. dPCR is suitable for the sensitive detection of rare variants and the detection of small quantities of input material and has a higher tolerance to inhibitors. dPCRdivides samples into thousands of individual partitions anduses fluorescent probes to provide highly sensitive absolute quantification of nucleic acids.

Methods: We used various combinations of primers for specific detectionof *SRY* in cfDNA samples and to detect*RhD* and *RhC* status of the fetus. We prepared an allele-specific dPCR assay for the detection of the most common mutation causing achondroplasia. We are also working on a dPCR assay for screening trisomy 21 and 18. We designed multiplex PCR with the primers, which lie to different parts of chromosomes 21 and 18, and universal probes. To validate all of these assays, we used anonymous samples (healthy controls and samples with confirmed pathogenic variants or syndromes) from CMBG in FN Brno. We prepared artificial samples to simulate cfDNA (5% and 10% fetal fraction) and finally, we used these assays in cfDNA samples.

Results: dPCR proved to be a suitable method for fetal sex determination using *SRY* detection, for *RhD* and *RhC* factor determination in *RhD/RhC* negative mothers. This method is also suitable for NIPT of achondroplasia, which is related to advanced paternal age and in most cases arises*de novo*.dPCR was also used for NIPS of the most common aneuploidies (trisomy 21 and 18), where this method appears to be sufficiently sensitive, but further experiments are needed to optimize and validate this method.

Conclusion: In this work, we showed that dPCR has the potential to be used for various NIPT examinations. dPCR has some advantages over NGS, such as simpler workflow, fasterturnaround time, and lower cost.