Droplet Digital PCR

Droplet Digital PCR (ddPCR) is a method for performing digital PCR that is based on water-oil emulsion droplet technology. A sample is fractionated into 20,000 droplets, and PCR amplification of the template molecules occurs in each individual droplet. ddPCR technology uses reagents and workflows similar to those used for most standard TaqMan probe-based assays. The massive sample partitioning is a key aspect of the ddPCR technique.

The technique generates a large amount of nanoliter-sized droplets, each of which carries out a PCR reaction on one template. Therefore, ddPCR provides high precision and absolute quantification of the nucleic acid target, with wide applications for both research and clinical diagnosis.

Wang et al. first conducted droplet digital PCR to quantify the absolute expression levels of the long and short isoforms of the T gene (T-long and T-short, respectively) and revealed that T-long was dominantly expressed in all chordomas and chordoma cell lines, but not in the notochords. The T-long/T-short ratio was significantly different between the chordomas and the notochords. Next, we validated the isoform expression pattern at protein expression level using Western blot in 9 chordomas. Furthermore, the T gene single nucleotide polymorphism site rs2305089, which is the only marker reported to be associated with chordomas, was sequenced in all of the chordoma samples. Association between rs2305089 and T-long/T-short ratio was not significant, indicating it was not involved in T gene alternative splicing. In conclusion, two T gene isoforms were investigated in skull base chordomas and chordoma cell lines, and the longer isoform was dominantly expressed. The distinct expression patterns of these T gene isoforms may contribute to the pathogenesis of skull base chordomas. However, further studies on the function of these isoforms are needed [1)].

Wang et al. collected 62 glioma tissue samples (Grade II to IV) and detected IDH1 mutations by Sanger direct sequencing, ddPCR, and quantitative real-time PCR (qRT-PCR). With the results from Sanger direct sequencing as the standard, the characteristics of ddPCR were compared with qRT-PCR. The data indicated that ddPCR was much more sensitive and much easier to interpret than qRT-PCR. Thus, we demonstrated that ddPCR is a reliable and sensitive method for screening the IDH mutation. Therefore, ddPCR is able to applied clinically in predicting patient prognosis and selecting effective therapeutic strategies [2)].

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Wang K, Hu Q, Wang L, Chen W, Tian K, Cao C, Wu Z, Jia G, Zhang L, Zeng C, Zhang J. T gene isoform expression pattern is significantly different between chordomas and notochords. Biochem Biophys Res Commun. 2015 Nov 13;467(2):261-7. doi: 10.1016/j.bbrc.2015.09.178. Epub 2015 Oct 3. PubMed PMID: 26435504.
2)
Wang J, Zhao YY, Li JF, Guo CC, Chen FR, Su HK, Zhao HF, Long YK, Shao JY, Tony To SS, Chen ZP. IDH1 mutation detection by droplet digital PCR in glioma. Oncotarget. 2015 Oct 14. doi: 10.18632/oncotarget.5630. [Epub ahead of print] PubMed PMID: 26485760.