Digital PCR - Methods and Protocols
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Sensitivity, reproducibility, precision and accuracy are recurring words in this book. Indeed, digital PCR (dPCR) represents a major step forward in the quantification of nucleic acids. In this field, for many years it has been used the quantitative PCR (qPCR), a technology that allows a real-time monitoring of DNA amplification which has proved useful for obtaining a relative measurement, but that only indirectly, through the use of reference standards, provides an absolute quantification. In dPCR, direct detection of targets is possible, since the DNA fragments are isolated in micro-partitions and subsequently amplified. The endpoint fluorescence measurement of each partition enables an absolute quantification of the target nucleic acid.

"Digital PCR" is an expression used for the first time in an article published twenty years ago, in which it was presented a method for the study of cancer-associated mutations based on the use of limit dilution technique and allele-specific-fluorescent probes (Vogelstein and Kinzler, PNAS 1999;96;9236-41). The evolution of technology has made this method one of the most successful applications of dPCR for the quantification of somatic mutations (Whale *et al*., Anal Chem 2017;89:1724-33).

The book is organized into six parts and contains a total of 30 chapters. The introductory part consists of four chapters. Chapter 1, written by the editors of the publication, is an exhaustive description of the contents of the volume and describes the potentials of the dPCR. Chapter 2 gives a clear introduction to the technology by an explanation of the basic concepts. Warnings about its limitations, a comparison with the qPCR and a description of the different dPCR systems are also provided. Chapter 3 presents an overview of test performance descriptors (sensitivity, specificity, limit of blank, limit of detection and limit of quantification) in the context of a technology that is able to detect up to a single molecule. Chapter 4 concerns the use of control materials for the evaluation of plasma extraction efficiency of circulating cell-free DNA (cfDNA) and for the validation of measurements carried out on specific diagnostic targets. This chapter, like the following, is accompanied by a detailed step-by-step description of the laboratory protocol and a list of reagents and instruments needed to reproduce it.

In the second part, two distinctive features of the dPCR, such as the possibility of obtaining an absolute quantification without the use of reference standards and poor susceptibility to PCR inhibitors, are explored in different contexts: in the detection of GMO in maize (Chapter 5), in the quantification of herpes viruses in different types of clinical samples (Chapter 6), in the analysis of cfDNA of mitochondrial origin present in the cerebrospinal fluid of patients with neurodegenerative diseases (Chapter 7) and to test the presence of fecal contaminants in water (Chapter 8).

The third part covers the topic of the "Copy number variation". Chapter 9 details a protocol that includes the design of the primers up to the interpretation of the results that can be used to determine the number of copies of a genomic segment. Chapter 10 describes a combination of the liquid biopsy technique associated with an assessment of *HER2* amplification to evaluate the disease progression in breast cancer. In Chapter 11 it is explained how to perform an assay and analyze the data deriving from the study of somatic mosaicism due to copy number variation of genomic variants of different sizes in primary or cultured cells.

Part 4 (Rare mutation and rare allele detection) includes numerous examples of quantitative analysis of mutations present in peripheral blood cells genomic DNA and in plasma cfDNA. Chapters 12, 13 and 17 illustrate methods for the analysis of somatic mutation in cfDNA isolated from plasma of patients with non-small cell lung cancer and breast cancer. In Chapter 14, a diagnostic algorithm for the classification of various lymphoproliferative diseases and the relative evaluation of minimal residual disease (MRD), which involves the use of a wide range of dPCR assays, is exhaustively presented. The assessment of the percentage of *JAK2* p.V617F mutated alleles in patients with myeloproliferative diseases is described in Chapter 15. Chapters 16 and 18 show the techniques by which it is possible to analyze multiple mutations in a single multiplex assay. The authors of Chapter 19 present an interesting method that includes a series of assays designed to detect and quantify polymorphic variants (SNPs), which can be used to monitor the progress of transplantation over time. The topic of the CRISP/Cas9 genome editing is discussed in Chapter 20 where a method that takes advantage of the ability to quantify rare alleles, for the simultaneous identification of homologous and non-homologous repair events, is accurately described. The dense fourth part of the book concludes with the description of a protocol for the analysis of the DNA methylation status after bisulphite conversion (Chapter 21).

The applications devoted to the study of gene expression are collected in Part 5. Chapter 22 shows a method for the simultaneous quantification of four different splice variants of the human telomerase gene. Very interesting and well developed is the idea of allele-specific expression measurement, which is presented in Chapter 23. Equally intriguing is the method for the simultaneous (or independent) quantification of 5 transcripts in a single cell, presented in Chapter 24. The two remaining chapters in this part provide guidance for the quantification of miRNAs in serum and plasma using either EvaGreen or probe-based Taq-man chemistry (Chapter 25 and 26).

Part 6 collects methods that employ micro-partitioning for other applications such as, the quantification of NGS libraries (Chapter 27), the determination of physical linkage between two genetic markers located at distances up to 200 Kb (Chapter 28), the quantification of the telomerase enzyme activity (Chapter 29) and a variant of the SELEX method that takes advantage from the possibility of generating thousands of partitions, to simplify and accelerate the selection of aptamers (Chapter 30).

As admitted by the editors in the introductory chapter, a limitation of the collection is that, with the sole exception of the method described in Chapter 15, all the protocols are implemented on a single technological platform, the Bio-rad Droplet Digital PCR System. Also, as a reader interested in exploring the potential of dPCR for the study of gene expression, I wanted to find more applications dedicated to this topic.

Overall, the editors of the volume have succeeded in providing specialists in different sectors with a wide range of technology applications that can be adapted to other platforms or can provide the basis for creating new ones.

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