
Contents

| | |
|---------------------------|----|
| <i>Preface</i> | v |
| <i>Contributors</i> | xi |

PART I BACKGROUND CONCEPTS

| | |
|--|----|
| 1 Entering the Pantheon of 21 st Century Molecular Biology Tools: A Perspective on Digital PCR | 3 |
| <i>George Karlin-Neumann and Francisco Bizouarn</i> | |
| 2 Basic Concepts and Validation of Digital PCR Measurements | 11 |
| <i>Leonardo Pinheiro and Kerry R. Emslie</i> | |
| 3 Fundamentals of Counting Statistics in Digital PCR: I Just Measured Two Target Copies—What Does It Mean? | 25 |
| <i>Svilen Tzonev</i> | |
| 4 Control Materials and Digital PCR Methods for Evaluation of Circulating Cell-Free DNA Extractions from Plasma | 45 |
| <i>Alexandra S. Whale, Ana Fernandez-Gonzalez, Alice Gutteridge, and Alison S. Devonshire</i> | |

PART II ABSOLUTE QUANTIFICATION

| | |
|--|-----|
| 5 Multiplex Droplet Digital PCR Protocols for Quantification of GM Maize Events | 69 |
| <i>David Dobnik, Bjørn Spilberg, Alexandra Bogožalec Košir, Dejan Štebih, Dany Morisset, Arne Holst-Jensen, and Jana Žel</i> | |
| 6 Using Droplet Digital PCR to Detect Coinfection of Human Herpesviruses 6A and 6B (HHV-6A and HHV-6B) in Clinical Samples | 99 |
| <i>Ashley Vellucci, Emily C. Leibovitch, and Steven Jacobson</i> | |
| 7 Biomarkers in Cerebrospinal Fluid: Analysis of Cell-Free Circulating Mitochondrial DNA by Digital PCR. | 111 |
| <i>Petar Podlesniy and Ramon Trullas</i> | |
| 8 Testing of General and Human-Associated Fecal Contamination in Waters | 127 |
| <i>Yiping Cao, Meredith R. Raith, and John F. Griffith</i> | |

PART III COPY NUMBER VARIATION

| | |
|--|-----|
| 9 Analyzing Copy Number Variation with Droplet Digital PCR | 143 |
| <i>Avery Davis Bell, Christina L. Usher, and Steven A. McCarroll</i> | |
| 10 Assessing <i>HER2</i> Amplification in Plasma cfDNA | 161 |
| <i>Isaac Garcia-Murillas and Nicholas C. Turner</i> | |

| | | |
|--|---|-----|
| 11 | Detection and Quantification of Mosaic Genomic DNA Variation in Primary Somatic Tissues Using ddPCR: Analysis of Mosaic Transposable-Element Insertions, Copy-Number Variants, and Single-Nucleotide Variants | 173 |
| | <i>Bo Zhou, Michael S. Haney, Xiaowei Zhu, Reenal Pattni, Alexej Abyzov, and Alexander E. Urban</i> | |
| PART IV RARE MUTATION AND RARE ALLELE DETECTION | | |
| 12 | Monitoring of Response and Resistance in Plasma of <i>EGFR</i> -Mutant Lung Cancer Using Droplet Digital PCR | 193 |
| | <i>Yanan Kuang, Allison O'Connell, Adrian G. Sacher, Nora Feeney, Ryan S. Alden, Geoffrey R. Oxnard, and Cloud P. Paweletz</i> | |
| 13 | Detection of Cancer DNA in Early Stage and Metastatic Breast Cancer Patients | 209 |
| | <i>Arielle J. Medford, Riaz N. Gillani, and Ben Ho Park</i> | |
| 14 | Droplet Digital PCR for Minimal Residual Disease Detection in Mature Lymphoproliferative Disorders | 229 |
| | <i>Daniela Drandi, Simone Ferrero, and Marco Ladetto</i> | |
| 15 | Quantitation of <i>JAK2</i> V617F Allele Burden by Using the QuantStudio™ 3D Digital PCR System | 257 |
| | <i>Elena Kinz and Axel Muendlein</i> | |
| 16 | Novel Multiplexing Strategies for Quantification of Rare Alleles Using ddPCR | 275 |
| | <i>Miguel Alcaide and Ryan D. Morin</i> | |
| 17 | Identification and Use of Personalized Genomic Markers for Monitoring Circulating Tumor DNA | 303 |
| | <i>Yilun Chen, Anthony M. George, Eleonor Olsson, and Lao H. Saal</i> | |
| 18 | Single Color Multiplexed ddPCR Copy Number Measurements and Single Nucleotide Variant Genotyping | 323 |
| | <i>Christina M. Wood-Bouwens and Hanlee P. Ji</i> | |
| 19 | A Universal Droplet Digital PCR Approach for Monitoring of Graft Health After Transplantation Using a Preselected SNP Set | 335 |
| | <i>Julia Beck, Michael Oellerich, and Ekkehard Schütz</i> | |
| 20 | Detection and Quantification of HDR and NHEJ Induced by Genome Editing at Endogenous Gene Loci Using Droplet Digital PCR | 349 |
| | <i>Tuichiro Miyaoka, Steven J. Mayerl, Amanda H. Chan, and Bruce R. Conklin</i> | |
| 21 | DNA Methylation Analysis Using Droplet Digital PCR | 363 |
| | <i>Ming Yu, Tai J. Heinzerling, and William M. Grady</i> | |
| PART V GENE EXPRESSION AND RNA QUANTIFICATION | | |
| 22 | Simultaneous Quantification of Multiple Alternatively Spliced mRNA Transcripts Using Droplet Digital PCR | 387 |
| | <i>Bing Sun and Yun-Ling Zheng</i> | |

| | | |
|---|--|-----|
| 23 | Using Droplet Digital PCR to Analyze Allele-Specific RNA Expression | 401 |
| | <i>Nolan Kamitaki, Christina L. Usher, and Steven A. McCarroll</i> | |
| 24 | Very Low Abundance Single-Cell Transcript Quantification with 5-Plex ddPCR TM Assays..... | 423 |
| | <i>George Karlin-Neumann, Bin Zhang, and Claudia Litterst</i> | |
| 25 | Quantification of Circulating MicroRNAs by Droplet Digital PCR..... | 445 |
| | <i>Manuela Ferracin and Massimo Negrini</i> | |
| 26 | Droplet Digital PCR for Absolute Quantification of Extracellular MicroRNAs in Plasma and Serum: Quantification of the Cancer Biomarker hsa-miR-141 | 459 |
| | <i>Maria D. Giraldez, John R. Chevillet, and Muneesh Tewari</i> | |
| PART VI OTHER USES OF PARTITIONING | | |
| 27 | Droplet Digital TM PCR Next-Generation Sequencing Library QC Assay | 477 |
| | <i>Nicholas J. Heredia</i> | |
| 28 | Phasing DNA Markers Using Digital PCR..... | 489 |
| | <i>John Regan and George Karlin-Neumann</i> | |
| 29 | ddTRAP: A Method for Sensitive and Precise Quantification of Telomerase Activity..... | 513 |
| | <i>Andrew T. Ludlow, Dawne Shelton, Woodring E. Wright, and Jerry W. Shay</i> | |
| 30 | Highly Efficient and Reliable DNA Aptamer Selection Using the Partitioning Capabilities of ddPCR: The Hi-Fi SELEX Method..... | 531 |
| | <i>Aaron Ang, Eric Ouellet, Karen C. Cheung, and Charles Haynes</i> | |
| | <i>Index</i> | 555 |