Real-Time PCR: Advanced Technologies and Applications


This book brings together contributions from 20 experts in the field of polymerase chain reaction (PCR), providing a broad perspective of the applications of quantitative real-time PCR (qPCR). The editors state in the preface that the aim is to provide detailed insight into underlying principles and methods of qPCR to provide the novice and the experienced user with an invaluable point of reference for this technology. They have succeeded in reaching this goal and in so doing, the book has, perhaps inadvertently, provided a powerful guide to use in designing qPCR experiments.

The first chapter begins the book with a thorough discussion of fluorescent chemistries for amplicon detection and their advantages and disadvantages. Beginning with simple DNA-binding dyes and progressing through nuclease activity-based probes, to sequence-specific probes, to self-probing amplicons, the authors provide invaluable details of how these options function and what results may be expected. In addition, this chapter discusses specific machines available and their capabilities, and dedicates a section to intellectual-property concerns and how that may need to be considered as one prepares to conduct qPCR experiments.

As the design of an experiment continues, Chapters 2, 7, 8, and 11 can be consulted for valuable information on methods and options. Chapter 2 provides a thorough discussion of internal and external controls. Creative design and use of “molecular mimic” internal controls are described with easily understood diagrams and examples of results. Chapter 7 provides a succinct but thorough review of the methods that have been used to isolate nucleic acids and prepare them for PCR, and Chapter 8 discusses a number of ways to “fine-tune” the specificity of primers using chemical modifications. Chapter 11 provides a discussion of the “minimum standard for the provision of information for qPCR experiments (MIQE).” Chapter 11, although only nine pages long, provides a wealth of guidance on what steps to take to ensure that qPCR results are sound, reproducible, and obtained in such a way that the underlying hypotheses can be tested reliably.

Chapter 3 takes the reader through the “nuts and bolts” of performing a qPCR experiment, including sample preparation and quality assessment, PCR setup, data generation, and data analysis. Potential pitfalls and ways to avoid them are also discussed, and several pages and examples are dedicated to optimization of the PCR.

Chapters 9 and 10 are dedicated to qPCR arrays and their capabilities, constraints, and validation. The background information provided here is sure to be of interest to anyone who is contemplating assaying hundreds of genes, and the suggestions presented in Chapter 10 for validating the results should be useful to anyone hoping to use PCR arrays to relate phenotypes to qPCR results.

Three chapters provide a series of “real-world” examples of using qPCR to detect and quantify microorganisms and begin with Chapter 4 providing an excellent overview of how qPCR, or the less precise qualitative PCR, can be used in very rapid detection of biological warfare threats, be they to public health, agricultural production, or water supply infrastructure. Chapter 5 discusses how qPCR may be applied in the rapid detection and diagnosis of veterinary infectious diseases, especially important in the case of rapidly transmissible...