

Preface

Polymerase chain reaction (PCR) has secured its place in biomedical history as a revolutionary method. Many techniques that have derived from PCR have come and gone. Real-time PCR is based on the conventional principles of PCR and since its beginnings about a decade ago its popularity has kept growing. With the simple shift of emphasis from the end-product to the whole course of the PCR process, real-time PCR has established itself as the most sensitive and specific quantitative PCR method. The real-time PCR concept has also contributed to the development of high-throughput allelic discrimination assays. With a variety of detection chemistries, an increasing number of platforms, multiple choices for analytical methods and the jargon emerging along with these developments, real-time PCR is facing the risk of becoming an intimidating method, especially for beginners. This book aims to provide the basics, explain how they are exploited to run a real-time PCR assay, how the assays are run and where these assays are informative in real life. The book does not intend to cover every aspect of real-time PCR in an encyclopedic fashion but instead to address the most practical aspects of the techniques with the emphasis on 'how to do it in the laboratory'. Keeping with the spirit of the Advanced Methods Series, most chapters provide an experimental protocol as an example of a specific assay. It is left with the reader to adapt the presented protocol to their individual needs.

The book is organized in two parts. The first part begins with a general introduction which is followed by chapters on the basics of data analysis, quantification, normalization and principles of primer and probe design, all contributed by leaders in the field. This part is concluded by chapters on more applied aspects of real-time PCR including an overview of high-resolution melting analysis by the most experienced users of this method. The second part of the book covers specific applications including the less recognized uses of real-time PCR: methylation detection, mitochondrial DNA analysis and immuno-PCR. The following chapters summarize many uses of real-time PCR in the clinic with examples. Clinical microbiology and virology, solid organ and bone marrow transplantation, and prenatal genetic diagnosis are among the topics covered. These chapters have been written with the laboratory and practical uses in mind and the contributors share their most valuable experiences. A comprehensive glossary and index supplement the 17 chapters and aim to make the book more accessible.

Real-time PCR is, like any other modern method in molecular genetics, expanding, with potential applications even in proteomics. This book is expected to provide the basic principles of applied real-time PCR and provide a firm grounding for those who wish to develop further applications. The selection of the chapters reflects the acknowledgment of inevitable future developments. We look forward to covering those in future editions of the book. Real-time PCR is surely here to stay and hopefully this book will help current users of this technique and also help develop further applications for it.

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