A-Z of Quantitative PCR

edited by
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Preface

This is not just a cook book for real-time quantitative PCR (qPCR). Admittedly, there are lots of recipes from distinguished contributors and I have attempted to collect, sift through and rationalize the vast amount of information that is available on this subject. And yes, this book was conceived as a comprehensive hands-on manual to allow both the novice researcher and the expert to set up and carry out qPCR assays from scratch. However, this book also sets out to explain as many features of qPCR as possible, provide alternative viewpoints and methods and, perhaps most importantly, aims to stimulate the researcher into generating, interpreting and publishing data that are reproducible, reliable, and biologically meaningful.

The first of the reviews in part I describes the background to quantification using PCR-based assays (S. A. Bustin), the second one provides a fascinating insight into the numerous factors that influence a successful PCR experiment (J. M. Phillips), and the third review discusses in detail the principles underlying real-time quantification (M. Pfaffl). Part II forms the core of this book and presents a detailed dissection of every one of the steps involved in conducting a qPCR experiment. Its emphasis is on providing explanations at each critical step in the PCR assay, starting from sample collection and ending with the interpretation of the quantitative result. Tried and tested sample protocols are included for the main chemistries, together with a “getting started” section for the complete novice and an extensive troubleshooting section which details and explains problems encountered during everyday qPCR assays.

The third part of the book provides an alternative viewpoint and protocol for mRNA quantification (J. C. Willey et al.), specific guidelines for the standardization of qPCR assays (R. Mueller et al.) and protocols designed to optimize the extraction of RNA from formalin-fixed tissue (F. Lewis and N. J. Maughan), perform RT-PCR assays without the need to isolate the RNA in the first place (Q. Hoang and B. Pasloske) and detailed instructions on how to optimize multiplex PCR assays (H. K. Srere et al.). The remaining chapters are concerned with specific applications of real-time PCR assays in breast (P. Pinzani et al.) and colorectal (S. A. Bustin and S. Dorudi) cancer, quantification in single cells (C. Hartshorn et al.; G. Brady and T. Nolan), and SNP analyses (I. A. Afonina et al. and J. Theaker). Each chapter contains an abundance of practical hints and reveals technical information that the authors have acquired as part of their extensive exposure to this technique.

The very nature of the technology means that new chemistries, protocols, and instruments come and go. Any book would struggle to keep up-to-date with such developments. However, by emphasizing and describing the very basic steps that must be right and providing step-by-step guidance on how to achieve reproducible results and interpret them correctly, this book will remain topical. My hope is that this book will contribute to taking quantitative PCR forward to a new stage of use as a standard, reliable, and useful molecular technique. The very nature of the technology means that new chemistries, protocols, and instruments come and go. Any book would struggle to keep up-to-date with such developments. However, by emphasizing and describing the very basic steps that must be right and providing step-by-step guidance on how to achieve reproducible results and interpret them correctly, this book will remain topical. My hope is that this book will contribute to taking quantitative PCR forward to a new stage of use as a standard, reliable, and useful molecular technique.

I am grateful to my numerous friends and contacts at ABI, Ambion, Biorad, Corbett Research,

DXS Genotyping, Oswell, Roche, Stratagene, and Quanta Biotech that keep me supplied with a constant stream of useful information, a lot of which has found a home in this book. I would like to acknowledge financial support from Bowel and Cancer Research.

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London, June 2004