

PCR

APPLICATIONS

PROTOCOLS FOR FUNCTIONAL GENOMICS

Edited by

Michael A. Innis

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Front cover photograph (paperback edition only): Analysis of the human breast cancer cell line BT474 using comparative genomic hybridization (CGH). During CGH, green fluorescing tumor DNA and red fluorescing normal reference DNA samples were hybridized (along with excess unlabeled cot-1 DNA) to normal metaphase spreads. The chromosomes were then counterstained with DAPI. Chromosomal regions showing green are increased in copy number in BT474, while regions showing red are relatively decreased in copy number.

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PREFACE

When approached about the possibility of editing another PCR volume, I thought the timing was appropriate given the explosion of PCR applications for mRNA quantitation, diagnosis, gene discovery, genomic analysis, and expression profiling. Also, although the newest crop of molecular biologists grew up using PCR routinely, I still find myself devoting significant time teaching first principles of PCR to help young investigators troubleshoot specific applications and to guide them through a confusing maze of choices as to which enzyme, buffer, cycling condition, etc., to use for which purpose.

Our objective in compiling *PCR Applications: Protocols for Functional Genomics* was to combine expert advice with contemporary protocols and perhaps a few personal reflections. *PCR Applications* makes a perfect companion to the earlier sourcebooks, *PCR Protocols* and *PCR Strategies*, because completely new chapters update the reader with the latest practical advice, theoretical information, and protocols without repeating the well-established PCR protocols in the earlier books.

This book, which follows the protocol format of the previous volumes, is divided into four sections. The first section, Key Concepts for PCR, contains chapters that provide practical and theoretical information needed to effectively navigate through the explosion of PCR-based techniques. The second section, Quantitative PCR, provides examples and protocols for real-time quantitative PCR and procedures for measuring gene expression. The last two sections, Gene Discovery, and Genomics and Expression Profiling, contain chapters of unique interest in the postgenomic scientific era.

We hope that, like *PCR Protocols* and *PCR Strategies*, *PCR Applications* will serve as a sourcebook and a practical tool for a wide range of research applications. If it causes you to think differently, then we will have succeeded.

The editors thank all the authors who contributed to this volume. We especially extend our thanks and appreciation to Emelyn

Eldredge, Acquisitions Editor at Academic Press, who inspired us to work together on *PCR Applications*. This book would not have been completed without her encouragement, dedication, and hard work.

Michael A. Innis (for the editors)

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