

# PCR Troubleshooting and Optimization

## The Essential Guide



**Edited by: Suzanne Kennedy and Nick Oswald**

*MO BIO Laboratories, Inc., Carlsbad, CA 92010, USA and BitesizeBio, Edinburgh, UK (respectively)*

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The polymerase chain reaction (PCR) is a fundamental tool in scientific research and clinical testing. Real-time PCR, combining both amplification and detection in one instrument, is a rapid and accurate method for nucleic acid detection and quantification. Although PCR is a very powerful technique, the results achieved are valid only if the appropriate controls have been employed. In addition, proper optimization of PCR conditions is required for the generation of specific, repeatable, reproducible and sensitive data.

This book discusses the strategies for preparing effective controls and standards for PCR, when they should be employed and how to interpret the information they provide. It highlights the significance of optimization for efficiency, precision and sensitivity of PCR methodology and provides essential guidance on how to troubleshoot inefficient reactions. Experts in PCR describe design and optimization techniques, discuss the use of appropriate controls, explain the significance of standard curves and explore the principles and strategies required for effective troubleshooting. Authors highlight the importance of sample preparation and quality, primer design, controlling inhibitors, avoiding amplicon and environmental contamination, optimizing reagent quality and concentration, and modifying the thermal cycling protocol for optimal sensitivity and specificity. In addition, specific chapters discuss the history of PCR, the choice of instrumentation, the applications of PCR in metagenomics, high resolution melting analysis, the MIQE guidelines, and PCR at the microliter scale.

The strategies, tips and advice contained in this concise volume enable the scientist to optimize and effectively troubleshoot a wide range of techniques including PCR, reverse transcriptase PCR, real-time PCR and quantitative PCR. An essential book for anyone using PCR technology.

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## **Pcr Troubleshooting And Optimization The Essential Guide:**

*PCR Troubleshooting and Optimization* Suzanne Kennedy, Nick Oswald, 2011 The polymerase chain reaction PCR is a fundamental tool in scientific research and clinical testing Real time PCR combining both amplification and detection in one instrument is a rapid and accurate method for nucleic acid detection and quantification Although PCR is a very powerful technique the results achieved are valid only if the appropriate controls have been employed In addition proper optimization of PCR conditions is required for the generation of specific repeatable reproducible and sensitive data This book discusses the strategies for preparing effective controls and standards for PCR when they should be employed and how to interpret the information they provide It highlights the significance of optimization for efficiency precision and sensitivity of PCR methodology and provides essential guidance on how to troubleshoot inefficient reactions Experts in PCR describe design and optimization techniques discuss the use of appropriate controls explain the significance of standard curves and explore the principles and strategies required for effective troubleshooting The book highlights the importance of sample preparation and quality primer design controlling inhibitors avoiding amplicon and environmental contamination optimizing reagent quality and concentration and modifying the thermal cycling protocol for optimal sensitivity and specificity In addition specific chapters discuss the history of PCR the choice of instrumentation the applications of PCR in metagenomics high resolution melting analysis the MIQE guidelines and PCR at the microliter scale The strategies tips and advice contained in this concise volume will enable the scientist to optimize and effectively troubleshoot a wide range of techniques including PCR reverse transcriptase PCR real time PCR and quantitative PCR It will be an essential book for anyone using PCR technology

**PCR Troubleshooting** Michael L. Altshuler, 2006 This unique polymerase chain reaction PCR troubleshooting guide is an essential companion for readers with some experience in PCR The book discusses the many and varied problems encountered with PCR together with tips advice and procedures to obviate rather than overcome the PCR problems The advice in *PCR Troubleshooting* is invaluable

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*Advanced Technologies for Meat Processing* Fidel Toldrá, Leo M. L. Nollet, 2017-10-10 As with the first edition the main goal of *Advanced Technologies for Meat Processing* is to provide the reader with recent developments in new advanced technologies for the full meat processing chain This book is written by distinguished international contributors with recognized expertise and excellent reputations and brings together all the advances in a wide and varied number of technologies that are applied in different stages of meat processing This second edition contains 21 chapters combining updated and revised versions of several chapters with entirely new chapters that deal with new online monitoring techniques like hyperspectral imaging and Raman spectroscopy the use of

nanotechnology for sensor devices or new packaging materials and the application of omics technologies like nutrigenomics and proteomics for meat quality and nutrition The book starts with the control and traceability of genetically modified farm animals followed by four chapters reporting the use of online non destructive monitoring techniques like hyperspectral imaging and Raman spectroscopy real time PCR for pathogens detection and nanotechnology based sensors Then five chapters describe different advanced technologies for meat decontamination such as irradiation hydrostatic and hydrodynamic pressure processing other non thermal technologies and the reduction in contaminants generation Nutrigenomics in animal nutrition and production is the object of a chapter that is followed by five chapters dealing with nutritional related issues like bioactive peptides functional meats fat and salt reduction processing of nitrite free products and the use of proteomics for the improved processing of dry cured meats The last four chapters are reporting the latest developments in bacteriocins against meat borne pathogens the functionality of bacterial starters modified atmosphere packaging and the use of new nanotechnology based materials for intelligent and edible packaging

*Insect Molecular Genetics* Marjorie A. Hoy, 2018-09-12 *Insect Molecular Genetics*

*Microbial Food Safety* Omar A. Oyarzabal, Steffen Backert, 2011-12-03 In this book some of the most qualified scientists review different food safety topics ranging from emerging and reemerging foodborne pathogens food regulations in the USA food risk analysis and the most important foodborne pathogens based on food commodities This book provides the reader with the necessary knowledge to understand some of the complexities of food safety However anybody with basic knowledge in microbiology will find in this book additional information related to a variety of food safety topics

**Molecular Microbiology** David H. Persing, Fred C. Tenover, Randall T. Hayden, Margareta Ieven, Melissa B. Miller, Frederick S. Nolte, Yi-Wei Tang, Alex van Belkum, 2020-07-24 Presenting the latest molecular diagnostic techniques in one comprehensive volume The molecular diagnostics landscape has changed dramatically since the last edition of *Molecular Microbiology Diagnostic Principles and Practice* in 2011 With the spread of molecular testing and the development of new technologies and their opportunities laboratory professionals and physicians more than ever need a resource to help them navigate this rapidly evolving field Editors David Persing and Fred Tenover have brought together a team of experienced researchers and diagnosticians to update this third edition comprehensively to present the latest developments in molecular diagnostics in the support of clinical care and of basic and clinical research including next generation sequencing and whole genome analysis These updates are provided in an easy to read format and supported by a broad range of practical advice such as determining the appropriate type and quantity of a specimen releasing and concentrating the targets and eliminating inhibitors *Molecular Microbiology Diagnostic Principles and Practice* Presents the latest basic scientific theory underlying molecular diagnostics Offers tested and proven applications of molecular diagnostics for the diagnosis of infectious diseases including point of care testing Illustrates and summarizes key concepts and techniques with detailed figures and tables Discusses emerging technologies including the use

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**Animal Biotechnology** Ashish S. Verma, Anchal Singh, 2013-11-04 Animal Biotechnology introduces applications of animal biotechnology and implications for human health and welfare It begins with an introduction to animal cell cultures and genome sequencing analysis and provides readers with a review of available cell and molecular tools Topics here include the use of transgenic animal models tissue engineering nanobiotechnology and proteomics The book then delivers in depth examples of applications in human health and prospects for the future including cytogenetics and molecular genetics xenografts and treatment of HIV and cancers All this is complemented by a discussion of the ethical and safety considerations in the field Animal biotechnology is a broad field encompassing the polarities of fundamental and applied research including molecular modeling gene manipulation development of diagnostics and vaccines and manipulation of tissue Given the tools that are currently available and the translational potential for these studies animal biotechnology has become one of the most essential subjects for those studying life sciences Highlights the latest biomedical applications of genetically modified and cloned animals with a focus on cancer and infectious diseases Provides firsthand accounts of the use of biotechnology tools including molecular markers stem cells and tissue engineering

*Real-Time PCR* Kirstin J. Edwards, Julie M. J. Logan, Nick A. Saunders, 2004 [Book Review Index Cumulation](#) Dana Ferguson, 2005-09 Book Review Index provides quick access to reviews of books periodicals books on tape and electronic media representing a wide range of popular academic and professional interests The up to date coverage wide scope and inclusion of citations for both newly published and older materials make Book Review Index an exceptionally useful reference tool More than 600 publications are indexed including journals and national general interest publications and newspapers Book Review Index is available in a three issue subscription covering the current year or as an annual cumulation covering the past year

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on primer design and continuing with the standard protocols used to perform PCR Provides troubleshooting tips for various types of modifications of standard protocols Contains unique Good Practices and Tips that are indispensable for the beginner and expert alike Features Special Cases with applications of PCR optimization and troubleshooting Includes detailed appendices with tables figures and key protocols Organized as a systematic concentrated resource to save time when addressing a PCR problem

**Understanding PCR** Sarah Maddocks, Rowena Jenkins, 2016-10-27 Understanding PCR A Practical Bench Top Guide gives you all of the information you need to plan your first PCR from reagents to conditions to analysis and beyond It is a user friendly book that has step by step basic protocols which can be adapted to your needs Includes helpful information such as where to order your reagents and basic troubleshooting hints and tips Includes resources for reagents Explains basic laboratory preparation Provides straightforward experimental protocols Incorporates fundamental analytical techniques Contains a troubleshooting guide

*PCR* Julian Burke, 1996-12-16 This convenient spiral bound laboratory manual saves readers valuable time by providing easily accessible information on key topics and protocols Succinctly describes the most commonly applied techniques and contains useful tips on stopping points troubleshooting and safety

PCR Protocols Michael A. Innis, David H. Gelfand, John J. Sninsky, Thomas J. White, 2012-12-02 The correct procedures you need for frustration free PCR methods and applications are contained in this complete step by step clearly written inexpensive manual Avoid contamination with specific instructions on setting up your lab Avoid cumbersome molecular biological techniques Discover new applications

Principles and Technical Aspects of PCR Amplification Elizabeth van Pelt-Verkuil, Alex van Belkum, John P. Hays, 2008-03-14 Kary Mullis was awarded a Nobel Prize for inventing the PCR technique more than a decade ago in 1993 Since its discovery multiple adaptations and variations of the standard PCR technique have been described This publication aims to provide the reader with a guide to the standard PCR technique and its many available variants with particular emphasis being placed on the role of these PCR techniques in the clinical diagnostic laboratory the central theme of this book

PCR Lucília Domingues, 2023-08-22 This second volume focuses on PCR methods and PCR application specificities to the biotechnology and bioengineering field New and updated chapters detail real time PCR protocols synthetic biology applications pathogen detection microfluidics digital multiplex detection recent advances Written in the highly successful Methods in Molecular Biology series format chapters include introductions to their respective topics lists of the necessary materials and reagents step by step readily reproducible laboratory protocols and key tips on troubleshooting and avoiding known pitfalls Authoritative and cutting edge PCR Methods and Protocols Second Edition aims to be a useful and practical guide to new researchers and experts looking to expand their knowledge

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