

PCR Troubleshooting and Optimization

The Essential Guide



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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.

Chapter 1. Magic in Solution: An Introduction and Brief History of PCR. *Carl T. Withwer and Jared S. Farrar*

Chapter 2. Difficult Templates and Inhibitors of PCR. *Jack M. Gallup*

Chapter 3. Significance of Controls and Standard Curves in PCR. *Ian Kavanagh, Genwyn Jones and Saima Naveed Nayab*

Chapter 4. Obtaining Maximum PCR Sensitivity and Specificity. *Cameron N. Gundry and Matthew D. Poulson*

Chapter 5. RT-PCR Optimization Strategies. *Martina Rester and Michael W. Ptarm*

Chapter 6. Real-Time PCR Instrumentation: An Instrument Selection Guide. *Sandrine Javorski-Miller and Ivan Delgado Orlic*

Chapter 7. qPCR Data Analysis: Unlocking the Secret to Successful Results. *Jan Hellemans and Jo Vandecastelle*

Chapter 8. The MIQE Guidelines Unleashed. *Gregory L. Shipley*

Chapter 9. PCR Applications for Epigenetics Research. *Gavin Meredith, Miro Dudas, Mark Landers, Vasiliki Anest, Jonathan Wang, Cailu Chen, Peter Jozsi and Christopher Adams*

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Chapter 11. Microfluidic Emulsion PCR. *N. Reginald Beer and John H. Leamon*

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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

Pain Mechanisms and Modulators Editor's Picks 2021 Robert John Vandenberg, 2021-07-21

Current Protocols Essential Laboratory Techniques Sean R. Gallagher, Emily A. Wiley, 2012-03-19 The latest title from the acclaimed Current Protocols series Current Protocols Essential Laboratory Techniques 2e provides the new researcher with the skills and understanding of the fundamental laboratory procedures necessary to run successful experiments solve problems and become a productive member of the modern life science laboratory From covering the basic skills such as measurement preparation of reagents and use of basic instrumentation to the more advanced techniques such as blotting chromatography and real time PCR this book will serve as a practical reference manual for any life science researcher Written by a combination of distinguished investigators and outstanding faculty Current Protocols Essential Laboratory Techniques 2e is the cornerstone on which the beginning scientist can develop the skills for a successful research career

Real-time PCR Kirstin Edwards, Julie M. J. Logan, Nick A.

Saunders,2004 This essential manual presents a comprehensive guide to the most appropriate and up to date technologies and applications as well as providing an overview of the theory of this important technique Written by recognized experts in the field this timely and authoritative volume is an essential requirement for all laboratories using PCR Topics covered include Real time PCR instruments and probe chemistries set up controls and validation quantitative real time PCR analysis of mRNA expression mutation detection NASBA application in clinical microbiology and diagnosis of infection

Understanding PCR Sarah Maddocks,Rowena Jenkins,2016-10-28 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 **Real-time PCR** ,2007 **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 **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 Guru* Ayaz Najafov,Gerta Hoxhaj,2016-11-28 PCR Guru An Ultimate Benchtop Reference for Molecular Biologists is provides researchers in molecular biology with a handy reference for approaching and solving challenging problems associated with PCR setup and optimization As a laboratory guide it emphasizes the technical aspects of employing PCR as a tool in molecular biology laboratories The book covers the history of PCR and the basic science underlying it It then discusses PCR at the bench level starting with detailed description and tips 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 **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 2* M. J. McPherson,B. D. Hames,Graham R. Taylor,1995 PCR is now an indispensable tool for the molecular biologist heralding an era of unparalleled research

productivity The basic PCR technique provides the cornerstone of in vitro DNA amplification upon which further features can be built to expand the range of PCR based applications PCR 2 A Practical Approach is not a revised version of PCR A Practical Approach but sets out to address some of the exciting new applications of PCR and so it is complementary to the original volume The book starts with an essential guide for improving PCRs then deals with the use of modified primers and the direct sequencing of PCR products The focus then switches to mRNA and covers cDNA cloning mRNA quantitation and direct expression of proteins from PCR products Finally genomic DNA analysis is addressed including genome mapping and fingerprinting the analysis of mutations and the direct sequencing and analysis of methylation patterns The book is essential reading for all researchers in molecular biology and related disciplines BOOK JACKET Title Summary field provided by Blackwell North America Inc All Rights Reserved *Quantitative Real-Time PCR* Roberto Biassoni, Alessandro Raso, 2019-10-03 This book expands upon the useful first edition by exploring classic Quantitative Polymerase Chain Reaction qPCR techniques as well as a number of recently developed applications With the changes in instrumentation due to technological advances and the development of new reagents to fulfill ethical and legal issues the qPCR field is now an up to date technology that indeed is widely used in research and clinical diagnostics Written for the highly successful Methods in Molecular Biology series chapters include introductions to their respective topics lists of the necessary reagents step by step readily reproducible laboratory protocols and tips on troubleshooting and avoiding known pitfalls Revised and authoritative *Quantitative Real Time PCR Methods and Protocols Second Edition* is an ideal guide to this expanding and vital field of study *Molecular Diagnostic PCR Handbook* Gerrit J. Viljoen, Louis H. Nel, John R. Crowther, 2005-12-06 PREFACE The Joint FAO IAEA Division of Nuclear Techniques in Food and Agriculture is involved in agricultural research and development and assists Member States of FAO and IAEA in improving strategies to ensure food security through the use of nuclear techniques and related biotechnologies where such techniques have a valuable and often unique role In particular molecular diagnostic methods have rapidly evolved in the past twenty years since the advent of the Polymerase Chain Reaction PCR They are used in a wide range of agricultural areas such as improving soil and water management producing better crop varieties diagnosing plant and animal diseases controlling insect pests and improving food quality and safety The uses of nucleic acid directed methods have increased significantly in the past five years and have made important contributions to disease control country programmes for improving national and international trade These developments include the more routine use of PCR as a diagnostic tool in veterinary diagnostic laboratories However there are many problems associated with the transfer and particularly the application of this technology These include lack of consideration of the establishment of quality assured procedures the required set up of the laboratory and the proper training of staff This can lead to a situation where results are not assured This book gives a comprehensive account of the practical aspects of PCR and strong consideration is given to ensure its optimal use in a laboratory environment This includes the setting up of a PCR laboratory

Good Laboratory Practice and standardised of PCR protocols **PCR Applications in Pathology** David S. Latchman,1995

The polymerase chain reaction has revolutionized molecular biology by allowing the amplification and characterization of minute amounts of nucleic acids As well as being of use to basic scientists this technique is of immense importance to pathologists who routinely need to detect and characterize small amounts of infectious micro organisms or to identify mutations within small amounts of human DNA This essential handbook outlines clearly and concisely the applications of PCR in the area of infectious and inherited diseases so that methods can be readily used by non molecular biologists who need them

Chapter 1: Nucleic Acid Amplification/PCR Applications Promega Corporation,1996-01-01 Chapter 1 of Promega s comprehensive background information and methods resource covering key research topics This chapter focuses on Nucleic Acid Amplification PCR Applications including General Considerations for PCR Optimization General Considerations for RT PCR Thermostable DNA Polymerases Reverse Transcriptases Example of a PCR Protocol Example of an RT PCR Protocol Troubleshooting PCR and RT PCR and References

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