

SHORT REPORT

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# Hot start reverse transcriptase: an approach for improved real-time RT-PCR performance



Nils Rutschke<sup>1,2\*</sup>, Jan Zimmermann<sup>1</sup>, Ronny Möller<sup>1,2</sup>, Gerd Klöck<sup>2</sup>, Mathias Winterhalter<sup>2</sup> and Annika Leune<sup>1</sup>

## Abstract

**Background:** Reverse transcriptase is an indispensable enzyme for real-time reverse transcriptase (RT)-PCR, a standard method in molecular diagnostics for detection and quantification of defined RNA molecules. The prevention of non-specific products due to elongation of misprimed oligonucleotides by the enzyme at temperatures beneath the specific annealing temperature is one of the biggest challenges in real-time RT-PCR.

In the present study, an aptamer directed against the reverse transcriptase was analyzed for its potential to attain a temperature-dependent reverse transcriptase ("hot start" RT).

**Findings:** The hot start effect was investigated in a one-step real-time RT-PCR assay for the detection of Middle East respiratory syndrome coronavirus (MERS-CoV). Results with aptamer revealed a reduced RT activity at low temperatures while achieving full activity at the specific annealing temperature of 55 °C. Sensitivity (limit of detection (LoD) 95 %) of the MERS-CoV assay was increased by about two times in the presence of aptamer.

**Conclusions:** The study demonstrates the potential of aptamer-dependent hot start RT for the improvement of diagnostic real-time RT-PCR assays.

**Keywords:** Real-time RT-PCR; Reverse transcriptase; Hot start; MERS-CoV

## Findings

### Introduction

Real-time RT-PCR is the method of choice in molecular diagnostics for detection and quantification of defined RNA molecules (Mackay 2004). This technique utilizes reverse transcriptase (RT) to convert RNA into complementary DNA (cDNA), a thermostable DNA-dependent DNA polymerase for the amplification of specific target sequences and target specific probes (oligonucleotides) labelled with fluorophores for the detection of amplified DNA (Gibson et al. 1996).

Real-time RT-PCR is regarded as a method with high sensitivity and specificity (Martel et al. 2002). However, this is challenged by non-specific products generated by elongation of misprimed primer that competes with the synthesis of specific amplification products in each cycle (Chou et al. 1992; Li et al. 1990). The probability of non-specific product formation increases with the complexity

of the real-time RT-PCR system and the background nucleic acid in the specimen (Brownie et al. 1997; Handschlar et al. 2009). Ultimately, non-specific products can severely decrease sensitivity as well as specificity of real-time RT-PCR assays (Sharkey et al. 1994; Birch et al. 1996; Jayasena 1999).

Assays for the detection of RNA viruses are often highly complex (high quantity of different oligonucleotides) due to low sequence conservation of the RNA genome (Gardiner et al. 2004; Brownie et al. 1997). In general, mispriming occurs at temperatures below the specific annealing temperature of the oligonucleotides (Jayasena 1999). Thus, the formation of non-specific products can be reduced by using hot start variants of the enzymes, which are inactive at low temperatures and activated at higher temperatures, appropriate for specific primer annealing to the target nucleic acid (Birch et al. 1996).

Several biological or chemical hot start concepts exist for *Taq* polymerase, a thermostable DNA-dependent DNA polymerases. The *Taq* polymerase can be inactivated by binding of specific antibodies or aptamers, by incubation with chemicals or by altered molecular kinetics

\* Correspondence: N.Rutschke@jacobs-university.de

<sup>1</sup>phona Diagnostics GmbH, Moerkenstr. 12, 22767 Hamburg, Germany  
<sup>2</sup>School of Engineering and Science, Jacobs University, Campus Ring 1,  
26759 Bremen, Germany  
Full list of author information is available at the end of the article

# Hot Start Reverse Transcriptase An Approach For Improved

**Mike Jess**



## **Hot Start Reverse Transcriptase An Approach For Improved:**

Forensic Biology Mr. Rohit Manglik, 2023-07-23 Examines DNA blood and tissue analysis and their applications in solving crimes and identifying individuals

Biochemistry and Molecular Biology Compendium Roger L. Lundblad, 2019-11-11 This book is an accessible resource offering practical information not found in more database oriented resources The first chapter lists acronyms with definitions and a glossary of terms and subjects used in biochemistry molecular biology biotechnology proteomics genomics and systems biology There follows chapters on chemicals employed in biochemistry and molecular biology complete with properties and structure drawings Researchers will find this book to be a valuable tool that will save them time as well as provide essential links to the roots of their science Key selling features Contains an extensive list of commonly used acronyms with definitions Offers a highly readable glossary for systems and techniques Provides comprehensive information for the validation of biotechnology assays and manufacturing processes Includes a list of Log P values water solubility and molecular weight for selected chemicals Gives a detailed listing of protease inhibitors and cocktails as well as a list of buffers

A Low-Cost Approach to PCR Eva Harris, 1998-12-31 The polymerase chain reaction PCR is a technique used to replicate specific pieces of DNA millions of times which permits the detection and analysis of minute amounts of nucleic acids Since its introduction in the late 1980s this technique has been applied not only in molecular biology research but also in fields as diverse as anthropology phylogeny and forensics However despite the large impact of PCR many of its applications remain within the confines of research and the academic environment Now in A Low Cost Approach to PCR Appropriate Transfer of Biomolecular Techniques Dr Eva Harris makes this elegantly simple technique more accessible to researchers physicians and laboratory workers throughout the world She provides a description of the theoretical basis of the technique the practical details of the method and the philosophy behind the technology transfer program that she developed over the last ten years The book serves as a guide for potential users in developing countries and for scientists in developed countries who may wish to work abroad In addition the low cost approach outlined in this book can be useful for high school undergraduate or continuing education programs in the United States While the specific applications of PCR outlined in the book are immediately useful to the study of infectious diseases the approach presented can be generalized to a number of other technologies and situations The book will help laboratories in many areas of the world generate information on site for use by physicians epidemiologists public health workers and health policy professionals to develop new strategies for disease control

**Molecular Methods in Plant Disease Diagnostics** Neil Boonham, Jenny Tomlinson, Rick Mumford, 2016-04-19 Using molecular methods for plant disease diagnosis provides diagnosticians with a number of advantages over more traditional methods They can allow the identification of morphologically similar species for example or the detection of infection prior to symptom formation Not only can molecular tools help by increasing the efficacy accuracy and speed of diagnosis their common technological basis provides further

benefits especially where resources are limited and traditional skills are hard to sustain This book provides protocols for nucleic acid based methods currently applied to plant pathogen detection and identification It takes the practitioner through the full range of molecular diagnostic and detection methods and as these generic techniques are appropriate for use on any target with minimal modification also provides a useful resource for students of plant pathology and plant pathologists Beginning with the background and future directions of the science it then addresses DNA barcoding microarrays polymerase chain reactions PCR quality assurance and more forming a complete reference on the subject

**Essentials of Nucleic Acid Analysis** Jacquie T Keer, Lyndsey Birch, 2008-02-25 Over the last decade there has been a rapid development of molecular techniques with an increasing range of instrumentation now available The development of accompanying reference literature has not kept pace with technological advances and this poses significant challenges to the analyst Essentials of Nucleic Acid Analysis sets out to guide the analyst through the steps needed to obtain good quality results in DNA analysis The underlying principles for achieving this goal were formulated by LGC formerly the Laboratory of the Government Chemist as the six principles for ensuring valid analytical measurement which are detailed in the introduction The reader is also provided with guidelines for method validation and quality control of established and emerging DNA measurement techniques The authors of each chapter are practitioners of the art of DNA analysis in areas where the quality of the result is critical Technical details and examples of application of key techniques in nucleic acid analysis are provided while highlighting best practice available standards and practical advice on improving measurement quality This book provides an indispensable handbook and premier reference for those working in the widely varying areas and specifically in the fields of food analysis and forensic applications

*Forensic Biology* Richard Li, 2015-03-11 Focusing on forensic serology and forensic DNA analysis this book introduces students to the methods and techniques utilized by forensic biology laboratories Using schematic illustrations to clarify concepts this second edition explores the latest DNA profiling tools contains three new chapters and provides 200 new images It also includes new tables for many chapters Covering the full scope of forensic biology the book uses an accessible style designed to enhance students education and training so they are prepared both in the laboratory and in the field

Microbial-Mediated Induced Resistance: Interactive Effects for Improving Crop Health Debasis Mitra, Bahman Khoshru, Anju Rani, Edappayil Janeeshma, 2025-06-02 The current population of the Earth which is approximately 7.88 billion is projected to reach 9.8 billion by the year 2050 In order to accommodate this growth it is crucial that we prepare for the increased demand for food However the agricultural industry continues to rely heavily on chemical fertilizers pesticides and herbicides These practices have severe environmental consequences leading to a decline in the diversity of soil microorganisms which can ultimately harm food production This situation is further complicated by climate change deteriorating soil health and other stressors Here microbial mediated induced resistance MIR is an intriguing area of study in agriculture that explores the potential of microbes to sustain plant resistance to pathogens This methodology

utilizes specific microorganisms including bacteria and fungi to trigger a systemic response in plants thus enhancing their defense mechanisms against disease The impact of MIR on crop health can be substantial and provide sustainable alternatives to conventional chemical based techniques for disease management Advancing research into the study of microbes in sustainable agriculture will generate interest in adopting novel methods that increase crop yield soil health and fertility Through this Research Topic we aim to showcase the most recent insights about plant soil microbes which play a significant role in microbial mediated induced resistance Specifically we are interested in the rhizospheric soil dynamics and nutrient acquisition contributing to plant growth and development Soil microbes are crucial for plant nutrient uptake inducing Induced Systemic Resistance and managing stressful climatic conditions through plant signaling compounds and crosstalk mechanisms Beneficial symbiotic microorganisms and other soil microbial interactions with plant roots help to utilize nutrients efficiently and induce plant defense mechanisms for sustainable production Topics welcomed into this Research Topic Mechanisms of plant defense Induced Systemic Resistance by Beneficial Microbes Soil biodiversity and microbial community Phytohormone signaling pathways Soil nutrient dynamics and nutrients transport Arbuscular mycorrhizal fungi

**Tietz Textbook of Clinical Chemistry and Molecular Diagnostics - E-Book** Carl A. Burtis,Edward R. Ashwood,David E. Bruns,2011-12-16 As the definitive reference for clinical chemistry Tietz Textbook of Clinical Chemistry and Molecular Diagnostics 5th Edition offers the most current and authoritative guidance on selecting performing and evaluating results of new and established laboratory tests Up to date encyclopedic coverage details everything you need to know including analytical criteria for the medical usefulness of laboratory procedures new approaches for establishing reference ranges variables that affect tests and results the impact of modern analytical tools on lab management and costs and applications of statistical methods In addition to updated content throughout this two color edition also features a new chapter on hemostasis and the latest advances in molecular diagnostics Section on Molecular Diagnostics and Genetics contains nine expanded chapters that focus on emerging issues and techniques written by experts in field including Y M Dennis Lo Rossa W K Chiu Carl Wittwer Noriko Kusukawa Cindy Vnencak Jones Thomas Williams Victor Weedn Malek Kamoun Howard Baum Angela Caliendo Aaron Bossler Gwendolyn McMillin and Kojo S J Elenitoba Johnson Highly respected author team includes three editors who are well known in the clinical chemistry world Reference values in the appendix give you one location for comparing and evaluating test results NEW Two color design throughout highlights important features illustrations and content for a quick reference NEW Chapter on hemostasis provides you with all the information you need to accurately conduct this type of clinical testing NEW Six associate editors lend even more expertise and insight to the reference NEW Reorganized chapters ensure that only the most current information is included

**Molecular Diagnostics for Dermatology** Gregory A. Hosler,Kathleen M. Murphy,2014-04-30 Molecular diagnostics is an exploding field and recent advances in our understanding of the molecular basis of disease have provided a platform for the development of new

diagnostic tests as well as tests to predict tumor behavior and potential response to targeted therapy This textbook provides a reference and practical guide to molecular diagnostics for dermatologists and dermatopathologists It outlines our current understanding of the molecular underpinnings of dermatologic disease describes the appropriate use of currently available molecular tests and explains the interpretation of these tests in the context of diagnosis and management Tests relating to various disorders are covered including but not confined to melanoma genodermatoses and infectious disease Pitfalls are highlighted and user friendly algorithmic approaches presented

*Cumulated Index Medicus* ,1992 *Molecular Biological and Immunological Techniques and Applications for Food Chemists* Bert Popping,Carmen Diaz-Amigo,Katrin Hoenicke,2010-03-16 A guide to using molecular biology and immunological methods for the analysis of food Many of the analytical problems that food chemists face in the lab cannot be solved by chemistry alone and so analytical chemists are turning to molecular biology and immunology for alternative approaches *Molecular Biological and Immunological Techniques and Applications for Food Chemists* comprehensively explains the most important molecular biology and immunology methods and illustrates their application in food analysis Written by a distinguished group of experts the coverage includes Molecular Biological Methods techniques explained laboratory layout PCR real time PCR RFLP SSCP and sequencing Molecular Biology Applications meat genetically modified organisms GMOs food allergens offal and fish Immunological Methods techniques explained and antibody based detection methods Immunology Applications animal speciation international food allergen regulations except Japanese Japanese regulations and buckwheat allergen detection egg allergen detection soy allergen detection milk allergen detection gluten allergen detection nut allergen detection fish allergen detection lupin allergen detection mustard allergen detection and celery allergen detection Clearly written and consistently edited to provide information to a wide range of readers *Molecular Biological and Immunological Techniques and Applications for Food Chemists* offers an up to date reference for food scientists in government and industry policymakers and graduate level students of food science technology and engineering Note CD ROM DVD and other supplementary materials are not included as part of eBook file

*RNA modifications and epitranscriptomics - volume II* Giovanni Nigita,Xiao Han,Ernesto Picardi,Kunqi Chen,2023-06-30 *Lacrimal Gland, Tear Film, and Dry Eye Syndromes* David A. Sullivan,2012-12-06 During the past decade a significant international research effort has been directed towards understanding the composition and regulation of the precocular tear film This effort has been motivated by the recognition that the tear film plays an essential role in maintaining corneal and conjunctival integrity protecting against microbial challenge and preserving visual acuity In addition research has been stimulated by the knowledge that alteration or deficiency of the tear film which occurs in countless individuals throughout the world may lead to desiccation of the ocular surface ulceration and perforation of the cornea an increased incidence of infectious disease and potentially pronounced visual disability and blindness 7 To promote further progress in this field of vision research the International Conference on

the Lacrimal Gland Tear Film and Dry Eye Syndromes Basic Science and Clinical Relevance was held in the Southampton Princess Resort in Bermuda from November 14 to 17 1992 This meeting was designed to assess critically the current knowledge and state of the art research on the structure and function of lacrimal tissue and tears in both health and disease The goal of this conference was to provide an international exchange of information that would be of value to basic scientists involved in eye research to physicians in the ophthalmological community and to pharmaceutical companies with an interest in the treatment of lacrimal gland tear film or ocular surface disorders e g Sjogren s syndrome

**Dictionary of DNA and Genome Technology** Paul Singleton,2012-10-25 DNA technology is evolving rapidly with new methods and a fast growing vocabulary This unique dictionary offers current detailed and accessible information on DNA technology to lecturers researchers and students throughout the biomedical and related sciences The third edition is a major update with over 3000 references from mainstream journals and data from the very latest research going well beyond the remit of most science dictionaries It provides clear explanations of terms techniques and tests including commercial systems with detailed coverage of many important procedures and methods and includes essay style entries on many major topics to assist newcomers to the field It covers topics relevant to medicine diagnosis genetic disorders gene therapy veterinary science biotechnology biochemistry pharmaceutical science drug development molecular biology microbiology epidemiology genomics environmental science plant science agriculture taxonomy and forensic science

**Laboratory Techniques for Fish Disease Diagnosis** Basanta Kumar Das,Vikash Kumar,2025-04-30 This book covers various aspects of fish health disease identification and laboratory procedures Each section of the book is detailed and includes practical information step by step procedures and relevant illustrations and diagrams The recent updates on fish disease diagnosis have been incorporated to address new techniques and technologies in the field Fish disease diagnosis is primarily based on the color and characteristics in the image to target the infected area It is an indispensable part of modern aquaculture and rapid and real time diagnosis is an essential part of the early and precise treatment of the diseases As farmed fishes are affected by viruses bacteria parasites metal pollution and fishing damage accurate disease diagnosis is crucial for effective management interventions It often requires a combination of clinical expertise advanced technology and collaboration among healthcare professionals This book is a comprehensive guide for students researchers and professionals involved in fish disease diagnosis

**Veterinary PCR Diagnostics** Chengming Wang,Bernhard Kaltenboeck,Mark D. Freeman,2012-03-15 PCR Polymerase Chain Reaction technology has become an indispensable component of routine veterinary diagnostics However a number of pitfalls and limiting factors affect its sensitivity and specificity of detection It is imperative that veterinary

**Fluorescence Correlation Spectroscopy** R. Rigler,E.S. Elson,2012-12-06 Fluorescence correlation spectroscopy FCS was developed in order to characterize the dynamics of molecular processes in systems in thermodynamic equilibrium FCS determines transport and chemical reaction rates from measurements of spontaneous microscopic thermally driven

molecular concentration fluctuations Since its inception and particularly in recent years technical and conceptual advances have extended the range of practical applicability and the information obtainable from FCS measurements Improvements in microscopy data acquisition and data processing have greatly shortened the time required for FCS measurements FCS can now be routinely applied to labile systems such as cells and for the acquisition of large volumes of data as required for high throughput screening Cross correlation methods provide a powerful tool for characterizing interactions among different molecular species Analysis of the amplitude of concentration fluctuations can provide a wealth of information about aggregation polymerization process and the compositions of mixtures Furthermore FCS provides a bridge between conventional measurements of dynamic processes on a macroscopic concentration scale and the currently developing field of single molecule measurements Both FCS and single molecule approaches measure directly stochastic fluctuations in molecular properties and so must be analyzed by statistical methods to yield conventional phenomenological parameters As commonly practiced FCS yields these phenomenological parameters e.g diffusion coefficients and chemical rate constants directly in terms of a fluorescence fluctuation autocorrelation function

**Dail and Hammar's Pulmonary Pathology**  
 Joseph F. Tomashefski, 2010-05-17 Dail and Hammar's Pulmonary Pathology has established itself as the definitive reference in the field This third edition is now a two volume full color text led by Dr Tomashefski as Editor in Chief and Drs Cagle Farver and Fraire as Associate Editors The new editorial board has continued to build upon the excellence Dail and Hammar achieved in the previous editions by reorganizing expanding and substantially revising the text This authoritative reference work has been thoroughly updated to cover newly recognized entities and the latest advances in molecular diagnostic techniques Abundantly illustrated with more than 2000 full color illustrations This outstanding contribution to pathology literature is a must have for the library of every surgical and pulmonary pathologist Dail and Hammar's Pulmonary Pathology has set the standard for which all other pathology texts strive to achieve From stellar reviews of the Second Edition When the first edition of Pulmonary Pathology by David H Dail and Samuel P Hammar was published in 1988 it was clear that it was to become the reference book for pulmonary pathology It was difficult to conceive then that a better book on the subject could be assembled The second edition of this encyclopedic work proved convincingly that improvement could occur Indeed the new revised edition is more complete and better illustrated I consider this book to be an outstanding contribution to the pathology literature and a must in the library of surgical and pulmonary pathologists Human Pathology **Investigative Ophthalmology & Visual Science**, 2002 Laboratory Information Bulletin, 2005



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