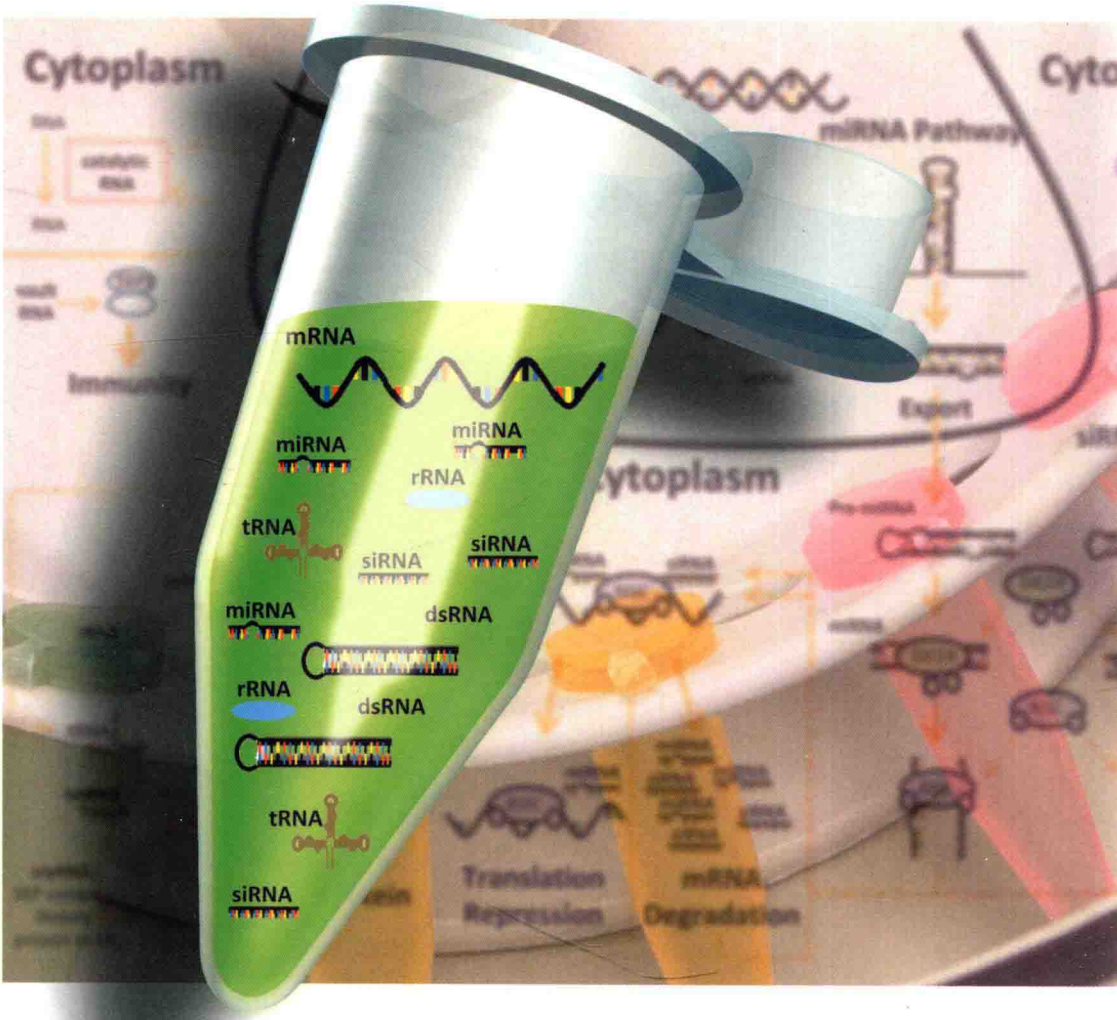
Douglas T. Gjerde, Lee Hoang,
David Hornby

WILEY-VCH

RNA Purification and Analysis

Sample Preparation, Extraction, Chromatography



Douglas T. Gjerde, Lee Hoang, and David Hornby

RNA Purification and Analysis

Sample Preparation, Extraction, Chromatography

WILEY-VCH Verlag GmbH & Co. KGaA

The Authors

Dr. Douglas T. Gjerde
PhyNexus, Inc.
3670, Charter Park Drive
San José, CA 95136
USA

Dr. Lee Hoang
PhyNexus, Inc.
3670, Charter Park Drive
San José, CA 95136
USA

Dr. David Peter Joseph Hornby
University of Sheffield
Department of Molecular Biology
Firth Court, Western Bank
Sheffield S10 2TN
United Kingdom

Library of Congress Card No.: applied for

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library.

Bibliographic information published by the Deutsche Nationalbibliothek
The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available on the Internet at http://dnb.d-nb.de

Printed in the Federal Republic of Germany
Printed on acid-free paper

Cover design Schulz Grafik-Design, Fußgönheim
Typesetting SNP Best-set Typesetter Ltd., Hong Kong
Printing betz-druck GmbH, Darmstadt
Bookbinding Litges & Dopf Buchbinderei GmbH, Heppenheim

ISBN: 978-3-527-32116-2

Douglas T. Gjerde, Lee Hoang,
and David Hornby

RNA Purification and Analysis

Further Reading

J.S. Fritz, D.T. Gjerde

Ion Chromatography

2009
ISBN: 978-3-527-32052-3

G. Carta, A. Jungbauer

Protein Chromatography

Process Development and Scale-Up

2009
ISBN: 978-3-527-31819-3

P. Herdewijn (Ed.)

Modified Nucleosides

in Biochemistry, Biotechnology and Medicine

2008
ISBN: 978-3-527-31820-9

L.W. Miller (Ed.)

Probes and Tags to Study Biomolecular Function

for Proteins, RNA, and Membranes

2008
ISBN: 978-3-527-31566-6

J. von Hagen (Ed.)

Proteomics Sample Preparation

2008
ISBN: 978-3-527-31796-7

R.K. Hartmann, A. Bindereif, A. Schön, E. Westhof (Eds.)

Handbook of RNA Biochemistry

2005
ISBN: 978-3-527-30826-2

Preface

Unlocking the role of RNA in biological cellular processes has proved to be more challenging than perhaps many investigators had first believed would be the case. However, it is also becoming apparent that, as the mysteries of RNA function are revealed, greater rewards will be gained than had been imagined. While life is indeed complicated, this book takes an approach to understanding life by examining it as a set of discrete, simple chemical reactions – the control of which generates the complexity. In continuing with this theme of reductionism, RNA is particularly amenable to *in vitro* analysis and, by isolating the RNA and asking questions in a very controlled manner, the details of specifics relating to the catalysis, affinities and mechanisms of RNA can be identified. As a result, major advances should be achieved in our fundamental understanding of biology, and perhaps even greater medical rewards can be gained.

RNA is fragile and complex in both its structure and function, and advanced tools are required for the reliable capture, purification and analysis of its various types. It is not the purpose of this book to provide a series of "cookbook" procedures for RNA's extraction, separation, and analysis; rather, it is intended as a "tool book", in which we describe the chemical principles that can be used as the foundations for the various methods and tools used to investigate RNA. For that reason, some examples are provided to illustrate the various concepts. By understanding these basic chemical concepts, and how to apply them in terms of the way in which RNA can be manipulated, the biologist should be able to better use the routine products and methods that are currently available, or perhaps develop new techniques for coping with any new-found problems associated with RNA.

In this book, we have brought together the concepts of both biology and chemistry to describe what these tools are, and how they work. Some of the tools described, such as spin columns and precipitation procedures, will already be familiar to the biologist, as will the various procedures described for the different types of RNA. Some other tools may be unfamiliar to the reader, however, and consequently the potential of high-performance liquid chromatography is described in detail, as are the basic instrumentation, the separation columns, and the means by which these separations are controlled.

RNA Purification and Analysis: Sample Preparation, Extraction, Chromatography
Douglas T. Gjerde, Lee Hoang, and David Hornby

ISBN: 978-3-527-32116-2

The special instrumentation and columns required for RNA separations have led to this technology being referred to as "RNA Chromatography", with the breadth of the subject being illustrated clearly by the many applications described.

So, we hope that this book will be used by biologists not only as a means of expanding their arsenal of tools for RNA-based investigations, but also to help them use these tools to great effect. With such techniques available it should, in time, be possible to solve – in creative manner – the vast array of problems encountered in RNA-related research.

May 2009

Douglas T. Gjerde
Lee Hoang
David Hornby

Acknowledgments

The authors thank Dr Chris Suh, Applications Scientist at PhyNexus, who helped with this book by editing and providing information for the text. Chris also drew many of the figures and provided the artwork for the book's cover. The authors also held many philosophical discussions with Chris Suh on RNA in general, and how a book of this type should be written.

Dr Mark Dickman, Professor of Chemistry at the Department of Chemical & Process Engineering at the University of Sheffield, provided valuable information for the book. Mark is an expert in chromatography and mass spectrometry detection, and has very successfully combined these tools with the biology of RNA, as evidenced by the quality of his publications that have been cited in this book, and which he continues to produce. We thank Mark for his valuable information and editorial help.

Mrs Jane Broom, mother-in-law to Gjerde and life-long reader, provided her proof-reading expertise for many of the chapters, and her help is greatly appreciated by all of the authors.

In particular, Lee Hoang wishes to thank Dr Karen Artiles for discussions and thoughtful suggestions on the manuscript, as well as all colleagues and mentors at the Center for the Molecular Biology of RNA at the University of California, Santa Cruz.

Finally, the authors wish to thank their respective families. Each of us has been blessed with families that read and love books. Our families are the most important part of our lives; they provide us with our inspiration and the means to enjoy life. We can only hope that we return the favor.

RNA Purification and Analysis: Sample Preparation, Extraction, Chromatography
Douglas T. Gjerde, Lee Hoang, and David Hornby

ISBN: 978-3-527-32116-2

Contents

RNA Purification and Analysis: Sample Preparation, Extraction, Chromatography
Douglas T. Gjerde, Lee Hoang, and David Hornby

ISBN: 978-3-527-32116-2

1
RNA Extraction, Separation, and Analysis

1.1
The Need to Be Able to Extract, Manipulate, and Analyze RNA

RNA is a powerful biological material. It is an active molecule that is directly involved in performing or controlling many biological functions. Unlocking the role of the various types of RNA present in biological cellular processes is critical to developing new methods of diagnosis and understanding and treating disease. New research is being reported daily that someday will lead to new drugs or methods of treating disease. Indeed, some of the work is so promising that it is hoped that a range of diseases will someday be effectively treated and cured. In any case, understanding RNA and its function is one of the keys to understanding life.

There are many different types of RNA, each of which carries out diverse and important functions in the cell, as shown on the book cover and in Figure 1.1. The need to extract and understand the roles of these RNAs is important not only for an understanding of biology but also for developing therapeutics. This particular figure was chosen as the book cover because it illustrates the complexity and importance of RNA in functional mechanisms within the cell. To name a few, RNAs have been shown to function in transcription (pri-RNA, mRNA), splicing (snRNA), translation (tRNA, tmRNA), and post-transcription regulation (siRNA, miRNA). Each type and function of RNA is described in Chapter 2 of this book, while each pathway is discussed in more detail in Chapter 7.

Unlike DNA–which is quite rugged–RNA is transient. RNA is also fragile, elusive, and difficult to recover. Proteins are expressed from DNA using RNA, with the cell carefully controlling which proteins are active, largely through overseeing the synthesis and degradation of RNA. The expression of DNA through RNA to produce new proteins could not be accomplished if the "old" RNA were still present from previous expressions of proteins; consequently, the cells routinely remove the "old" RNA by degrading it with RNase enzymes. Unfortunately, the RNase that is present for biological cellular function can also make RNA collection difficult, and any procedure employed for such collection and use must take this into account.

RNA Purification and Analysis: Sample Preparation, Extraction, Chromatography
Douglas T. Gjerde, Lee Hoang, and David Hornby

ISBN: 978-3-527-32116-2

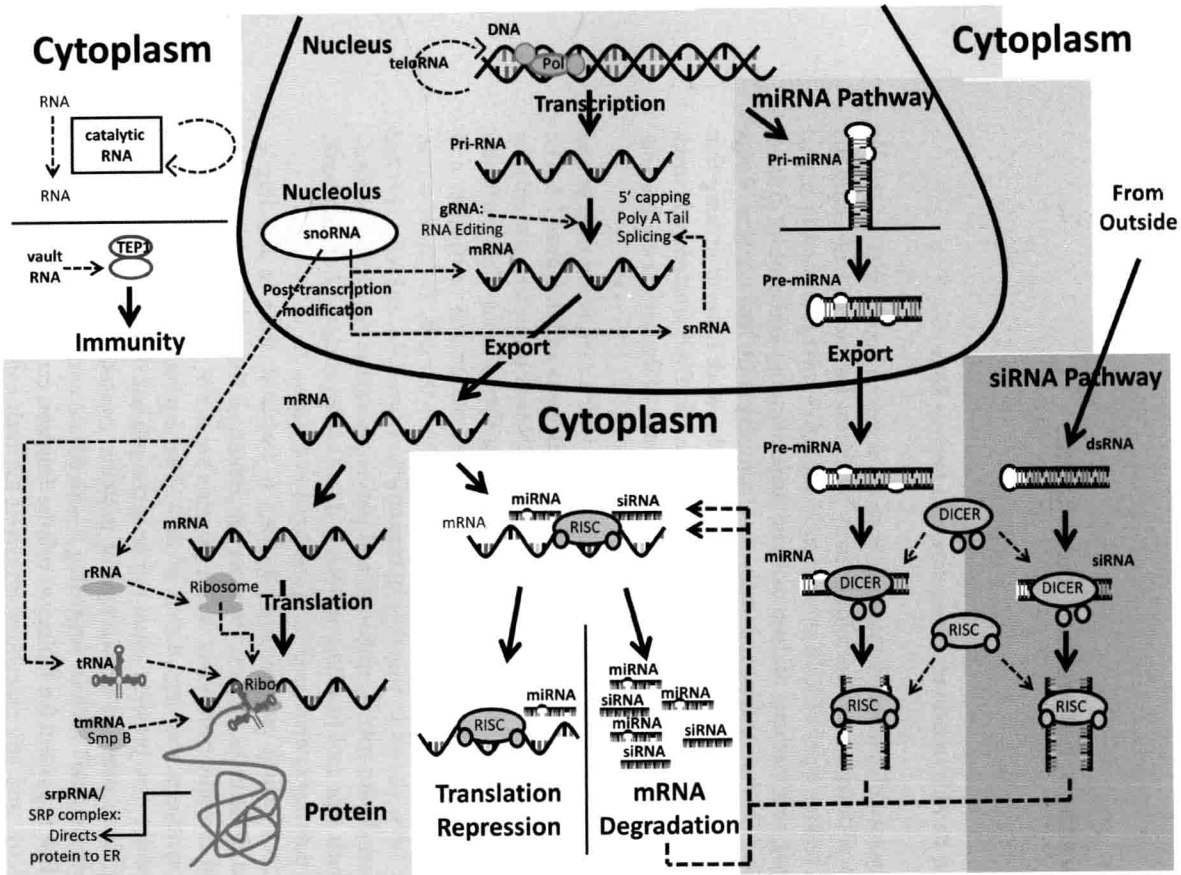


Figure 1.1 The schematic arrangement of different types of RNA, and their functions. These include telomerase RNA (teloRNA), primary RNA (pri-RNA), messenger RNA (mRNA), guide RNA (gRNA), small nucleolar RNA (snoRNA), small nuclear RNA (snRNA), ribosomal RNA (rRNA), transfer RNA (tRNA), transfer messenger RNA (tmRNA), signal recognition particle RNA (srpRNA), micro RNA (miRNA), short interfering RNA (siRNA), vault RNA, and catalytic RNA (ribozyme). The arrows indicated alongside each type of RNA show the cellular functions or processes that they are involved in. Each type and function of RNA is described in Chapter 2, and each pathway is described in more detail in Chapter 7.

Although many of these procedures have been described, it is not the purpose of this book to provide "cookbook" methods for RNA extraction, separation, and analysis. Yes, there is a multitude of kits available for RNA, and there is also a place for cookbook procedures, as these can free the researcher to use kits for what is tedious and automatic and allow them to design experiments that are of real interest. The danger lies in when a kit does not exactly fit the research problem at hand, or may not even be available. While it may be possible to modify an existing kit to do the job, that will not be possible if the investigator does not understand the chemistry employed in the kit, or how the chemistry can be controlled, manipulated, and optimized to fit the situation.

In this book, we describe the chemistry that is used in the various methods and tools. By understanding the basic chemical concepts, and how they apply to the way in which RNA is manipulated, the biologist will be able to better use the routine products and methods that are currently available on the market. The purpose of this book is also to provide a working knowledge in the context of the types of samples, types of RNA, what is important, and how to accomplish the various tasks through a basic chemical knowledge of the separation and analysis systems. With this information, it is possible for the biologist to make adjustments to fine-tune a product or procedure to a particular research need, or to develop entirely new procedures and methods to fit the problem at hand.

1.2
Using Chemical Tools to Solve the Problem of Analysis of Biological Processes

The types of RNA chemicals tools described in this book are concerned with: (i) solid-phase extraction; (ii) liquid chromatography; and/or (iii) chemical reagents, including enzymes. Both, RNA solid-phase extraction and RNA separation by liquid chromatography operate on similar principles – that is, an interaction of the RNA molecules with the solid-phase surfaces, or within functional groups in the extraction or chromatography media. The surface chemistry of the media can be quite similar – or even exactly the same – in the two cases. Both technologies operate on similar methods of chemical interaction of RNA with the surface. Moreover, both of the methods used to control this interaction and collect working amounts of RNA material are similar.

Part of the difference between solid-phase extraction and liquid chromatography relates to whether classes of molecules are separated, or whether higher-resolution separations are performed on individual molecules. Whereas, solid-phase extraction can be used to extract a particular type or class of RNA, chromatography can be used to separate classes or individual molecules. Nevertheless, both technologies operate on similar methods of chemical interaction of RNA with the surface. The methods used to control this interaction and to collect working amounts of RNA material are also similar.

1.3 The Principle of Chromatography and Solid-Phase Extraction

1.3.1 Principle of Chromatography

The Russian botanist Mikhail Tswett coined the term "chromatography" in 1903, the name being derived from the Greek words "chroma" and "graphein", meaning "color" and "writing" [1]. Tswett described a method for the separation of pigments found in plant leaves by using an open tubular column filled with a dry solid adsorbent of granular calcium carbonate (chalk). To the top of the chalk column, he added an extract of plant material containing its pigments. He then washed the chalk with an organic solvent, which began to flow down through the column by gravity. As the solvent was added to the top of the column, it carried the mixture of plant pigments down the column. Then, as the washing continued, the various pigments began to separate into a series of discrete colored bands, each of which was found to contain a single pigment from the original mixture. Tswett continued to add solvent, so that gradually the regions between the bands became entirely free of pigments and, after a while, all of the bands had been resolved or separated. Tswett then stopped adding the solvent, waited for the solvent to stop dripping, and finally pushed the moist chalk material out of the tube as an intact cylinder. Each pigment could be recovered by cutting the bands apart with a knife and extracting each individual band with an appropriate solvent.

Tswett called this process "chromatography", and was the first to recognize that the separation process was based on a general principle. This was the relative degree by which compounds in solution were either adsorbed to a stationary solid phase of the column or dissolved in the liquid mobile phase. Depending on the liquid phase, a particular compound can partly adsorb to the solid stationary phase and partly dissolve in the liquid mobile phase. Since each compound's affinity for the column is likely to be different, the compound will be carried through the column by the solvent, and the rate that a compound moves down the column will depend on how tightly the compound sticks to the column, or how well it is dissolved in the mobile phase. As the mathematical equations developed later in this book will show, the rate of travel of any one compound is proportional to the ratio of the amount of compound in solution divided by the amount of compound adsorbed on the column, for any given solvent.

This elution process of washing the compounds down the column continues until all components of the mixture are separated (Figure 1.2). Thus, the compounds which compose a mixture can be separated based on how each compound differs in its attraction to the stationary phase or the mobile phase. When a mixture of compounds is applied to the top of a column and washed down the column, there are some compounds in the mixture that do not "like" the mobile phase but rather "like" the stationary phase and so stick tightly or adsorb to the column material. These compounds either stay on the column indefinitely, or are eluted very slowly from the column. Other compounds that "like" the mobile phase and

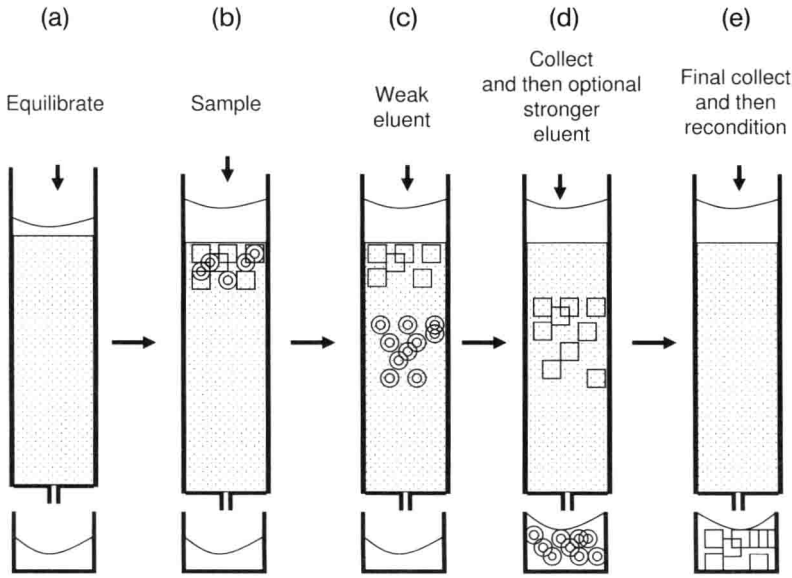


Figure 1.2 The liquid chromatography separation process. (a) The first step is to condition or equilibrate the column to the desired start pH, buffer and/or solvent conditions which ensure that the components are adsorbed into the column. (b) The components of a mixture are added as a concentrated aliquot to the top of the column. (c) Adding eluent to the top of the column starts the separation process. Some components will travel through the column faster because they are solvated by the eluent and interact less with the column packing. Other components interact more strongly with the packing and travel more slowly through the column. Eluent is added from time to time so that the column is not allowed to become dry (the solvent level should not be allowed to fall below the top of the column bed. (d) Component 1 (circles) is eluted and collected. (e) Component 2 (squares) is eluted and collected.

do not adsorb or stick tightly to the stationary phase will travel down the column and be eluted more quickly.

1.3.2 Mobile Phase Gradient Controls Elution

It is quite easy to adjust and control the rate of travel of a compound through a column. The RNA that elutes from a column can be detected by ultraviolet absorbance and recorded either on a chart recorder or electronically. The assessment of the separations involves an analysis of the peaks. Changing the mobile phase to a solvent that does not dissolve the compound as well as the previous solvent will slow the rate of travel of the compounds through the column and improve the separation, albeit at the expense of taking longer to perform the separation. Conversely, a stronger mobile phase will speed up the movement, but (perhaps) at the expense of some of the peaks running down the column together and coeluting.