METHODS IN MOLECULAR BIOLOGY™ 402

PCR Primer Design

Edited by

Anton Yuryev

HUMANA PRESS

METHODS IN MOLECULAR BIOLOGY™

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Edited by

Anton Yuryev

Application Science Department, Ariadne Genomics Inc.,
Rockville, MD

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999 Riverview Drive, Suite 208
Totowa, New Jersey 07512

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Cover illustration: Fig. 2 from Chapter 1 (Reprinted with permission from the Annual Review of Biophysics and Biomolecular Structure, Volume 33 (c) 2004 by Annual Reviews- www.annualreviews.org), Fig. 2 from Chapter 5, Fig. 2 from Chapter 2, and Fig. 6 from Chapter 18.

Production Editor: Rhukea J. Hussain
Cover design by: Nancy K. Fallatt

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Printed in the United States of America. 10 9 8 7 6 5 4 3 2 1

ISBN 13: 978-1-58829-725-9

eISBN 978-1-59745-528-2

Library of Congress Control Number: 2007925517

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Preface

In the past decade, Molecular Biology has been transformed from the art of cloning a single gene to a statistical science measuring and calculating properties of entire genomes. New high-throughput methods have been developed for genome sequencing and studying the cell at different systematic levels such as transcriptome, proteome, metabolome and other "...omes". At the heart of most high-throughput methods is the technique of polymerase chain reaction (PCR). PCR allows amplification of specific DNA sequences from sub-picomole concentrations to amounts sufficient for gene detection and quantification. The gene expression microarray experiments, the construction of cDNA libraries for two-hybrid experiments for studying protein-protein interaction, and the genome-wide genotyping of single nucleotide polymorphism (SNP) are all impossible without PCR. The performance and accuracy of these methods directly depend on the efficiency of the PCR reaction. Therefore, the improvement of the PCR has been a focus of much attention among molecular biologists.

The principal ingredients of the PCR reaction are DNA template, reaction buffer, DNA polymerase, and two primers that determine the specificity of the amplification. All of these ingredients have been thoroughly studied and optimized in the last few years. This book focuses on primer design, which is critical to both the efficiency and the accuracy of the PCR. The necessity of simultaneously amplifying a large variety of DNA sequences for high-throughput experiments yielded novel PCR approaches that are described in this book. Ultimately, primer design strategy is determined by the goal of the PCR method. However, there are basic oligonucleotide properties for which optimal combination is important for the success of any method. These properties are now well-understood and predictable with great accuracy. The availability of the whole-genome sequences allowed the development of highly sophisticated mathematical methods to calculate thousands of primers in order to maximize the efficiency of the amplification. This book contains the description of basic approaches for PCR primer design in addition to specialized methods. They can be used for both genome-scale experiments and for small-scale individual PCR amplifications. This book will be useful for organizations performing whole

genome studies, for companies designing instruments that utilize PCR, as well as for individual scientists who routinely use PCR in their research.

Dr. Anton Yuryev
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I

Basic Principles and Software for PCR Primer Design

1

Physical Principles and Visual-OMP Software for Optimal PCR Design

John SantaLucia, Jr.

Summary

The physical principles of DNA hybridization and folding are described within the context of how they are important for designing optimal PCRs. The multi-state equilibrium model for computing the concentrations of competing unimolecular and bimolecular species is described. Seven PCR design "myths" are stated explicitly, and alternative proper physical models for PCR design are described. This chapter provides both a theoretical framework for understanding PCR design and practical guidelines for users. The Visual-OMP (oligonucleotide modeling platform) package from DNA Software, Inc. is also described.

Key Words: Thermodynamics; nearest-neighbor model; multi-state model; Visual-OMP; secondary structure; oligonucleotide design; software.

1. Introduction

Single-target PCR is generally regarded as a robust and reliable technique for amplifying nucleic acids. This reputation is well deserved and is a result of the inherent nature of PCR technology, the creativity of a wide variety of scientists and engineers, and the huge financial investment of private industry as well as government funding. An incomplete list of some of the important innovations includes a variety of engineered thermostable polymerases, well-engineered thermocycling instruments, hot-start PCR, exonuclease-deficient polymerases, addition of dimethylsulfoxide (DMSO), buffer optimization, aerosol-blocking pipette tips, and use of uracil DNA glycosylase to minimize contamination artifacts. Despite these innovations and the large investment, there are many

From: *Methods in Molecular Biology, vol. 402: PCR Primer Design*
Edited by: A. Yuryev © Humana Press, Totowa, NJ

aspects of PCR that are still not well understood (such as the detailed kinetic time course of reactions that occur during thermocycling). These gaps in our knowledge result in less-than-perfect design software; the human experts are not perfect either. Nonetheless, there is a series of widely believed myths about PCR that result in poor designs. This chapter is devoted to stating explicitly some of these myths and providing explanations and guidelines for improved PCR design. These principles are fully implemented in the commercial package from DNA Software, Inc. (Ann Arbor, MI, USA) called Visual-OMP (oligonucleotide modeling platform) *(1,2)*. I co-founded DNA Software in year 2000 to implement the advanced thermodynamic prediction methods that were discovered in my academic laboratory as well as the best of what was available in the literature from other laboratories *(2)*. This chapter is organized into a series of sections that provide the background for understanding DNA thermodynamics and sections that specifically address each of seven myths about PCR design.

2. Background: DNA Thermodynamics

The detailed methods for predicting the thermodynamics of DNA folding and hybridization were recently reviewed *(2)*. A full description of solution thermodynamics is beyond the scope of this chapter, but a brief description is given. Review articles on the details of solution thermodynamics of nucleic acids have also been published *(3–5)*. This topic can be difficult and confusing for non-experts and can be the source of many misconceptions about PCR design. However, the serious molecular biologists should be familiar with these topics and should make the effort to educate themselves. This chapter will serve to demystify the topic of DNA thermodynamics and make it clear why thermodynamics is important for PCR design. Such knowledge is crucial for effective use of available software packages.

2.1. Solution Equilibrium and Calculation of the Amount Bound

The process of duplex hybridization for a forward bimolecular reaction is given by

$$A + B \rightarrow AB \quad (1)$$

where A and B imply strands A and B in the random coil state and AB implies the ordered AB duplex state. This is called the two-state approximation

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