

METHODS IN MOLECULAR BIOLOGY

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Next Generation Sequencing

Methods and Protocols

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Dedication

This volume is dedicated to the memory of our friend, colleague, mentor, and surf buddy, Daniel R. Salomon, M.D.

Preface

The revolution in high-throughput sequencing technology—Next Generation Sequencing (NGS)—has transformed the science of genomics in the last decade. The resulting deluge of new methods, protocols, and techniques to answer fundamental questions in biology has enabled highly efficient strategies for addressing problems of DNA, RNA, and their interactions with proteins. Each chapter in this *Methods in Molecular Biology* book describes a current state of the art in NGS application and is intended as a resource for researchers with all levels of NGS experience who wish to expand their knowledge and practical skills in high-throughput sequencing. Examples of the challenges of the role of NGS in basic research protocols data analysis, as well as clinical applications are included.

This book covers a wide range of various fields of research, with the common thread being NGS-related methods and applications, as well as some analysis and interpretation of the data obtained. The first two chapters focus on the highly dynamic processes of translational and transcriptional profiling of a cell. In the first chapter, polysome and ribosome isolation by sucrose gradient is used to investigate translational activity, both approaches are described, and the information obtained from each is discussed. In the second chapter, isolation of cell nuclei and the nascent RNA transcripts therein allow a look at the nascent transcriptome of a cell. The third chapter focuses on a method to detect copy number alterations (CNAs) using whole genome amplification and low pass whole genome sequencing. Chapters 4 and 5 touch on more targeted sequencing applications. The first uses small “bait” oligonucleotides to “fish” out long pieces of DNA (~2 Kb) from the genome combined with long read sequencing using the MinION (Oxford Nanopore, Inc.) allowing for the interrogation of these unknown flanking regions not contained in the baits. Chapter 5 deals with an extremely efficient and cost-effective method called “Hi-Plex” to characterize known polymorphic loci using a highly multiplexed amplicon-based approach to look for genetic variants.

Chapter 15 focuses on DNA structural rearrangements using a method called, “Hi-C,” which maps chromatin interactions in nuclei using NGS. The NGS libraries were generated for resting and activated human CD4 T cells to study activation-induced chromatin structural rearrangement. Another chapter (Chapter 13) dealing with chromosomal changes uses CRISPR-based knockout libraries in genome-wide screens to systematically investigate gene function in biological systems. Specifically, the Genome-Scale CRISPR Knock Out (GeCKO v2) library is used and methods for NGS library generation and sequencing are discussed.

There are several chapters (Chapters 7, 8, 10, and 14) dealing with a single cell of DNA or RNA in NGS. Chapter 7 describes a post-bisulfite treatment adapter tagging strategy to generate single-cell data looking at genome-wide cytidine methylation states. Chapter 10 describes the use of modified adapters in a small RNA protocol termed “CleanTag” (TriLink Biotechnologies Inc.) to prepare NGS libraries. The use of these adapters prevents adapter dimer formation, the principal artifactual product in a standard small RNA library prep. The authors demonstrate that the technique can work with very low inputs down to the single-cell level (~10 pg). Chapter 14 uses a single-cell analysis approach to identify and

characterize rare circulating CD4 T cells using a Biomark HD and profiles 96 different genes by quantitative PCR (qPCR) and generates NGS libraries using the Biomark HD Access Array. Chapter 8 describes a protocol for isolation, extraction, and sequencing of single bacterial and archaeal cells using FACS, MDA, a 16S rRNA screen, and a computational approach for quality assurance.

Two other chapters (Chapters 9 and 12) have more of a computational focus. Chapter 9 uses low pass whole genome sequencing and reference-guided assembly of non-model organisms for SNP discovery allowing for the genotyping of populations. Chapter 12 describes a computational pipeline for RNAseq analysis from tissue samples containing a complex heterogeneous population of cell types (e.g., a blood sample). The pipeline is able to estimate cell type composition and other statistical analysis information generated from bulk RNAseq profiles.

Another important component to almost all NGS-related work, especially those involving microbiome studies, is contamination. To this end, Chapter 11 focuses on the best practices and approaches for sample handling, DNA and/or RNA extraction, and library preparation from microbial and viral samples to generate NGS libraries. Chapter 6 discusses a novel method for generating sequencing libraries from viral RNA termed, “Clickseq,” which uses “Click Chemistry” to attach one of the NGS adapters preventing artifactual generation of chimeras, and consequently, greatly increasing the ability to detect rare recombination events in viral RNA. The last chapter in the book (Chapter 16) describes another RNAseq library prep method for profiling reverse transcription termination sites. It is an efficient protocol and generates good NGS library yields from low RNA inputs generated from protocols such as nascent RNA sequencing, RNA Immunoprecipitation (RIPseq), and 5'-RACE structural probing.

Next Generation Sequencing technology has brought together disparate fields of research from bacterial and viral studies to the study of plants, non-model organisms, and of course human disease. This requires collaboration between the users of NGS data and those needed to design and perform the enzymatic procedures for the preparation of sequencing libraries and ensure the desired target is being captured, targeted, or amplified. Other critical players are the engineers that develop microfluidics and sequencing hardware systems or the bioinformatics experts necessary to ensure data generated is being analyzed correctly and translated into a meaningful analysis. We hope you enjoy this book and find it informative and a useful reference.

La Jolla, CA, USA

*Steven R. Head
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