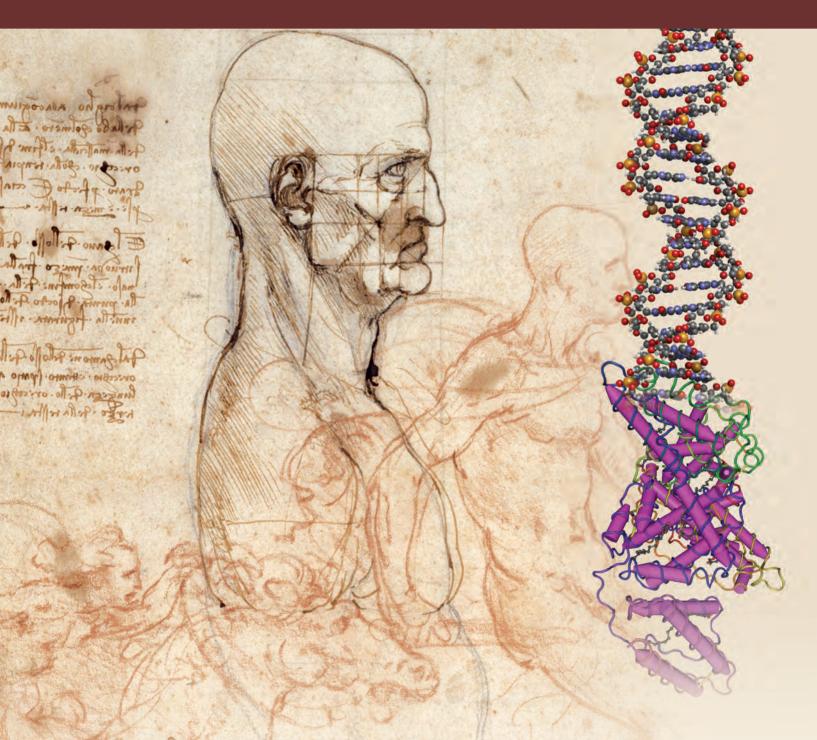
BIOINFORMATICS AND FUNCTIONAL GENOMICS third edition



Jonathan Pevsner

WILEY Blackwell

BIOINFORMATICS AND FUNCTIONAL GENOMICS

Bioinformatics and Functional Genomics

Third Edition

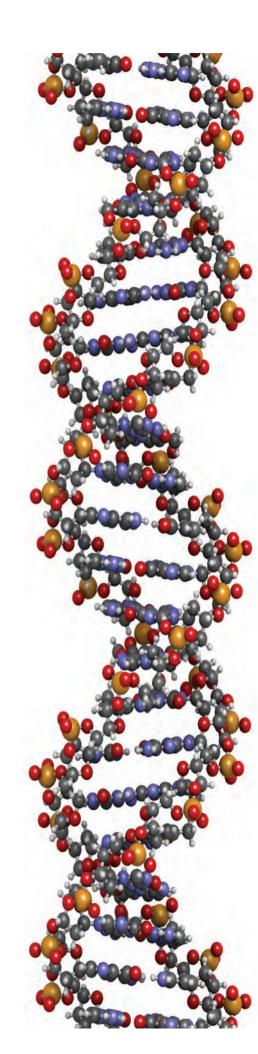
Jonathan Pevsner

Department of Neurology, Kennedy Krieger Institute, Baltimore, Maryland, USA

and

Department of Psychiatry and Behavioral Sciences, The Johns Hopkins School of Medicine, Baltimore, Maryland, USA





This edition first published 2015 © 2015 by John Wiley & Sons Inc

Registered office: John Wiley & Sons, Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK

Editorial offices: 9600 Garsington Road, Oxford, OX4 2DQ, UK The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK 111 River Street, Hoboken, NJ 07030-5774, USA

For details of our global editorial offices, for customer services and for information about how to apply for permission to reuse the copyright material in this book please see our website at www.wiley.com/wiley-blackwell.

The right of the author to be identified as the author of this work has been asserted in accordance with the UK Copyright, Designs and Patents Act 1988.

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, except as permitted by the UK Copyright, Designs and Patents Act 1988, without the prior permission of the publisher.

Designations used by companies to distinguish their products are often claimed as trademarks. All brand names and product names used in this book are trade names, service marks, trademarks or registered trademarks of their respective owners. The publisher is not associated with any product or vendor mentioned in this book.

Limit of Liability/Disclaimer of Warranty: While the publisher and author(s) have used their best efforts in preparing this book, they make no representations or warranties with respect to the accuracy or completeness of the contents of this book and specifically disclaim any implied warranties of merchantability or fitness for a particular purpose. It is sold on the understanding that the publisher is not engaged in rendering professional services and neither the publisher nor the author shall be liable for damages arising herefrom. If professional advice or other expert assistance is required, the services of a competent professional should be sought.

Library of Congress Cataloging-in-Publication Data

Pevsner, Jonathan, 1961-, author.
Bioinformatics and functional genomics / Jonathan Pevsner.—Third edition.
p.; cm.
Includes bibliographical references and indexes.
ISBN 978-1-118-58178-0 (cloth)
I. Title.
[DNLM: 1. Computational Biology—methods. 2. Genomics. 3. Genetic
Techniques. 4. Proteomics. QU 26.5]
QH441.2
572.8'6-dc23

2015014465

A catalogue record for this book is available from the British Library.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic books.

The cover image is by Leonardo da Vinci, a study of a man in profile with studies of horse and riders (reproduced with kind permission of the Gallerie d'Accademia, Venice, Ms. 7r [236r], pen, black and red chalk). To the upper right a DNA molecule is shown (image courtesy of Wikimedia Commons) and a protein (human serum albumin, the most abundant protein in blood plasma, accession 1E7I, visualized with Cn3D software described in Chapter 13). Leonardo's text reads: "From the eyebrow to the junction of the lip with the chin, and the angle of the jaw and the upper angle where the ear joins the temple will be a perfect square. And each side by itself is half the head. The hollow of the cheek bone occurs half way between the tip of the nose and the top of the jaw bone, which is the lower angle of the setting on of the ear, in the frame here represented. From the angle of the eye-socket to the ear is as far as the length of the ear, or the third of the face." (Translation by Jean-Paul Richter, *The Notebooks of Leonardo da Vinci*, London, 1883.)

Set in Times LT Std 10.5/13 by Aptara, India

Printed in Singapore

1 2015

For three generations of family: to my parents Aihud and Lucille; to my wife Barbara; to my daughters Kim, Ava, and Lillian; and to my niece Madeline

Contents in Brief

PART I Analyzing DNA, RNA, and Protein Sequences

- 1 Introduction, 3
- 2 Access to Sequence Data and Related Information, 19
- 3 Pairwise Sequence Alignment, 69
- 4 Basic Local Alignment Search Tool (BLAST), 121
- 5 Advanced Database Searching, 167
- 6 Multiple Sequence Alignment, 205
- 7 Molecular Phylogeny and Evolution, 245

PART II Genomewide Analysis of DNA, RNA, and Protein

- 8 DNA: The Eukaryotic Chromosome, 307
- 9 Analysis of Next-Generation Sequence Data, 377
- 10 Bioinformatic Approaches to Ribonucleic Acid (RNA), 433
- 11 Gene Expression: Microarray and RNA-seq Data Analysis, 479
- 12 Protein Analysis and Proteomics, 539
- 13 Protein Structure, 589
- 14 Functional Genomics, 635

PART III Genome Analysis

- 15 Genomes Across the Tree of Life, 699
- 16 Completed Genomes: Viruses, 755
- 17 Completed Genomes: Bacteria and Archaea, 797
- 18 Eukaryotic Genomes: Fungi, 847
- 19 Eukaryotic Genomes: From Parasites to Primates, 887
- 20 Human Genome, 957
- 21 Human Disease, 1011

GLOSSARY, 1075

SELF-TEST QUIZ: SOLUTIONS, 1103

AUTHOR INDEX, 1105

SUBJECT INDEX, 1109

Contents

Preface to the Third Edition, xxxi About the Companion Website, xxxiii

PART ANALYZING DNA, RNA, AND PROTEIN SEQUENCES

1 Introduction, 3

Organization of the Book, 4 Bioinformatics: The Big Picture, 5 A Consistent Example: Globins, 6 Organization of the Chapters, 8 Suggestions For Students and Teachers: Web Exercises, Find-a-Gene, and Characterize-a-Genome, 9 **Bioinformatics Software: Two Cultures, 10** Web-Based Software, 11 Command-Line Software, 11 Bridging the Two Cultures, 12 New Paradigms for Learning Programming for Bioinformatics, 13 Reproducible Research in Bioinformatics, 14 Bioinformatics and Other Informatics Disciplines, 15 Advice for Students, 15 Suggested Reading, 15 References, 16

2 Access to Sequence Data and Related Information, 19

Introduction to Biological Databases, 19 Centralized Databases Store DNA Sequences, 20 Contents of DNA, RNA, and Protein Databases, 24 Organisms in GenBank/EMBL-Bank/DDBJ, 24 Types of Data in GenBank/EMBL-Bank/DDBJ, 26 Genomic DNA Databases, 27 DNA-Level Data: Sequence-Tagged Sites (STSs), 27 DNA-Level Data: Genome Survey Sequences (GSSs), 27 DNA-Level Data: High-Throughput Genomic Sequence (HTGS), 27 RNA data, 27 RNA-Level Data: cDNA Databases Corresponding to Expressed Genes, 27 RNA-Level Data: Expressed Sequence Tags (ESTs), 28 RNA-Level Data: UniGene, 28

Access to Information: Protein Databases, 29 UniProt, 31 Central Bioinformatics Resources: NCBI and EBI, 31 Introduction to NCBI, 31 The European Bioinformatics Institute (EBI), 32 Ensembl, 34 Access to Information: Accession Numbers to Label and Identify Sequences, 34 The Reference Sequence (RefSeg) Project, 36 RefSegGene and the Locus Reference Genomic Project, 37 The Consensus Coding Sequence CCDS Project, 37 The Vertebrate Genome Annotation (VEGA) Project, 37 Access to Information via Gene Resource at NCBI, 38 Relationship Between NCBI Gene, Nucleotide, and Protein Resources, 41 Comparison of NCBI's Gene and UniGene, 41 NCBI's Gene and HomoloGene, 42 Command-Line Access to Data at NCBI, 42 Using Command-Line Software, 42 Accessing NCBI Databases with EDirect, 45 EDirect Example 1, 46 EDirect Example 2, 46 EDirect Example 3, 46 EDirect Example 4, 47 EDirect Example 5, 48 EDirect Example 6, 48 EDirect Example 7, 48 Access to Information: Genome Browsers, 49 Genome Builds, 49 The University of California, Santa Cruz (UCSC) Genome Browser, 50 The Ensembl Genome Browser, 50 The Map Viewer at NCBI, 52 Examples of How to Access Sequence Data: Individual Genes/Proteins, 52 Histones, 52 HIV-1 pol, 53 How to Access Sets of Data: Large-Scale Queries of Regions and Features, 54 Thinking About One Gene (or Element) Versus Many Genes (Elements), 54 The BioMart Project, 54 Using the UCSC Table Browser, 54 Custom Tracks: Versatility of the BED File, 56 Galaxy: Reproducible, Web-Based, High-Throughput Research, 57 Access to Biomedical Literature, 58 Example of PubMed Search, 59 Perspective, 59 Pitfalls, 60 Advice for Students, 60

Web Resources, 60 Discussion Questions, 61 Problems/Computer Lab, 61 Self-Test Quiz, 63 Suggested Reading, 64 References, 64

3 Pairwise Sequence Alignment, 69

```
Introduction, 69
   Protein Alignment: Often More Informative than DNA Alignment, 70
   Definitions: Homology, Similarity, Identity, 70
   Gaps, 78
   Pairwise Alignment, Homology, and Evolution of Life, 78
Scoring Matrices, 79
   Dayhoff Model Step 1 (of 7): Accepted Point Mutations, 79
   Dayhoff Model Step 2 (of 7): Frequency of Amino Acids, 79
   Dayhoff Model Step 3 (of 7): Relative Mutability of Amino Acids, 80
   Dayhoff Model Step 4 (of 7): Mutation Probability Matrix for the
     Evolutionary Distance of 1 PAM, 82
   Dayhoff Model Step 5 (of 7): PAM250 and Other PAM Matrices, 84
   Dayhoff Model Step 6 (of 7): From a Mutation Probability Matrix to a
     Relatedness Odds Matrix, 88
   Dayhoff Model Step 7 (of 7): Log-Odds Scoring Matrix, 89
   Practical Usefulness of PAM Matrices in Pairwise Alignment, 91
   Important Alternative to PAM: BLOSUM Scoring Matrices, 91
   Pairwise Alignment and Limits of Detection: The "Twilight Zone", 94
Alignment Algorithms: Global and Local, 96
   Global Sequence Alignment: Algorithm of Needleman and Wunsch, 96
     Step 1: Setting Up a Matrix, 96
     Step 2: Scoring the Matrix, 97
     Step 3: Identifying the Optimal Alignment, 99
   Local Sequence Alignment: Smith and Waterman Algorithm, 101
   Rapid, Heuristic Versions of Smith–Waterman: FASTA and BLAST, 103
   Basic Local Alignment Search Tool (BLAST), 104
   Pairwise Alignment with Dotplots, 104
The Statistical Significance of Pairwise Alignments, 106
   Statistical Significance of Global Alignments, 106
   Statistical Significance of Local Alignments, 108
   Percent Identity and Relative Entropy, 108
Perspective, 110
Pitfalls, 112
Advice for Students, 112
Web Resources, 112
   Discussion Questions, 113
   Problems/Computer Lab, 113
```

Self-Test Quiz, 114 Suggested Reading, 115 References, 116

4 Basic Local Alignment Search Tool (BLAST), 121

Introduction, 121 BLAST Search Steps, 124 Step 1: Specifying Sequence of Interest, 124 Step 2: Selecting BLAST Program, 124 Step 3: Selecting a Database, 126 Step 4a: Selecting Optional Search Parameters, 127 Step 4b: Selecting Formatting Parameters, 132 Stand-Alone BLAST, 135 BLAST Algorithm Uses Local Alignment Search Strategy, 138 BLAST Algorithm Parts: List, Scan, Extend, 138 BLAST Algorithm: Local Alignment Search Statistics and E Value, 141 Making Sense of Raw Scores with Bit Scores, 143 BLAST Algorithm: Relation Between E and p Values, 143 **BLAST Search Strategies**, 145 General Concepts, 145 Principles of BLAST Searching, 146 How to Evaluate the Significance of Results, 146 How to Handle Too Many Results, 150 How to Handle Too Few Results, 150 BLAST Searching with Multidomain Protein: HIV-1 Pol, 151 Using Blast For Gene Discovery: Find-a-Gene, 155 Perspective, 159 Pitfalls, 160 Advice for Students, 160 Web Resources, 160 **Discussion Questions**, 160 Problems/Computer Lab, 160 Self-Test Quiz, 161 Suggested Reading, 162 References, 163

5 Advanced Database Searching , 167

Introduction, 167 Specialized BLAST Sites, 168 Organism-Specific BLAST Sites, 168 Ensembl BLAST, 168 Wellcome Trust Sanger Institute, 170 Specialized BLAST-Related Algorithms, 170 WU BLAST 2.0, 170 European Bioinformatics Institute (EBI), 170

Specialized NCBI BLAST Sites, 170 BLAST of Next-Generation Sequence Data, 170 Finding Distantly Related Proteins: Position-Specific Iterated BLAST (PSI-BLAST) and DELTA-BLAST, 171 PSI-BLAST Errors: Problem of Corruption, 177 Reverse Position-Specific BLAST, 177 Domain Enhanced Lookup Time Accelerated BLAST (DELTA-BLAST), 177 Assessing Performance of PSI-BLAST and DELTA-BLAST, 179 Pattern-Hit Initiated BLAST (PHI-BLAST), 179 Profile Searches: Hidden Markov Models, 181 HMMER Software: Command-Line and Web-Based, 184 BLAST-Like Alignment Tools to Search Genomic DNA Rapidly, 186 Benchmarking to Assess Genomic Alignment Performance, 187 PatternHunter: Nonconsecutive Seeds Boost Sensitivity, 188 BLASTZ, 188 Enredo and Pecan, 191 MegaBLAST and Discontinuous MegaBLAST, 191 BLAST-Like Tool (BLAT), 192 LAGAN, 192 SSAHA2, 194 Aligning Next-Generation Sequence (NGS) Reads to a Reference Genome, 194 Alignment Based on Hash Tables, 194 Alignment Based on the Burrows-Wheeler Transform, 196 Perspective, 197 Pitfalls, 197 Advice For Students, 198 Web Resources, 198 **Discussion Questions**, 198 Problems/Computer Lab, 198 Self-Test Ouiz, 199 Suggested Reading, 200 References, 201

6 Multiple Sequence Alignment, 205

Introduction, 205 Definition of Multiple Sequence Alignment, 206 Typical Uses and Practical Strategies of Multiple Sequence Alignment, 207 Benchmarking: Assessment of Multiple Sequence Alignment Algorithms, 207 Five Main Approaches to Multiple Sequence Alignment, 208 Exact Approaches to Multiple Sequence Alignment, 208 Progressive Sequence Alignment, 208 Iterative Approaches, 214 Consistency-Based approaches, 218 Structure-Based Methods, 220 Benchmarking Studies: Approaches, Findings, Challenges, 221

Databases of Multiple Sequence Alignments, 222 Pfam: Protein Family Database of Profile HMMs, 223 **SMART**, 224 Conserved Domain Database, 226 Integrated Multiple Sequence Alignment Resources: InterPro and iProClass, 226 Multiple Sequence Alignment Database Curation: Manual Versus Automated, 227 Multiple Sequence Alignments of Genomic Regions, 227 Analyzing Genomic DNA Alignments via UCSC, 229 Analyzing Genomic DNA Alignments via Galaxy, 229 Analyzing Genomic DNA Alignments via Ensembl, 231 Alignathon Competition to Assess Whole-Genome Alignment Methods, 231 Perspective, 234 Pitfalls, 234 Advice for Students, 235 **Discussion Ouestions**, 235 Problems/Computer Lab, 235 Self-Test Quiz, 237 Suggested Reading, 238 References, 239

7 Molecular Phylogeny and Evolution, 245

Introduction to Molecular Evolution, 245 Principles of Molecular Phylogeny and Evolution, 246 Goals of Molecular Phylogeny, 246 Historical Background, 247 Molecular Clock Hypothesis, 250 Positive and Negative Selection, 254 Neutral Theory of Molecular Evolution, 258 Molecular Phylogeny: Properties of Trees, 259 Topologies and Branch Lengths of Trees, 259 Tree Roots, 262 Enumerating Trees and Selecting Search Strategies, 263 Type of Trees, 266 Species Trees versus Gene/Protein Trees, 266 DNA, RNA, or Protein-Based Trees, 268 Five Stages of Phylogenetic Analysis, 270 Stage 1: Sequence Acquisition, 270 Stage 2: Multiple Sequence Alignment, 271 Stage 3: Models of DNA and Amino Acid Substitution, 272 Stage 4: Tree-Building Methods, 281 Distance-Based, 282 Phylogenetic Inference: Maximum Parsimony, 287

Model-Based Phylogenetic Inference: Maximum Likelihood, 289 Tree Inference: Bayesian Methods, 290 Stage 5: Evaluating Trees, 293 Perspective, 295 Pitfalls, 295 Advice for Students, 296 Web Resources, 297 Discussion Questions, 297 Problems/Computer Lab, 297 Self-Test Quiz, 298 Suggested Reading, 298 References, 299

PART II GENOMEWIDE ANALYSIS OF DNA, RNA, AND PROTEIN

8 DNA: The Eukaryotic Chromosome, 307

Introduction, 308 Major Differences between Eukaryotes and Bacteria and Archaea, 308 General Features of Eukaryotic Genomes and Chromosomes, 310 C Value Paradox: Why Eukaryotic Genome Sizes Vary So Greatly, 312 Organization of Eukaryotic Genomes into Chromosomes, 310 Analysis of Chromosomes Using Genome Browsers, 314 Analysis of Chromosomes Using BioMart and biomaRt, 314 Example 1, 317 Example 2, 319 Example 3, 319 Example 4, 319 Example 5, 320 Analysis of Chromosomes by the ENCODE Project, 320 Critiques of ENCODE: the C Value Paradox Revisited and the Definition of Function, 322 Repetitive DNA Content of Eukaryotic Chromosomes, 323 Eukaryotic Genomes Include Noncoding and Repetitive DNA Sequences, 323 Interspersed Repeats (Transposon-Derived Repeats), 325 Processed Pseudogenes, 326 Simple Sequence Repeats, 331 Segmental Duplications, 331 Blocks of Tandemly Repeated Sequences, 333 Gene Content of Eukaryotic Chromosomes, 334 Definition of Gene, 334 Finding Genes in Eukaryotic Genomes, 336 Finding Genes in Eukaryotic Genomes: EGASP Competition, 339 Three Resources for Studying Protein-Coding Genes: RefSeq, UCSC Genes, GENCODE, 340 Protein-Coding Genes in Eukaryotes: New Paradox, 342

Regulatory Regions of Eukaryotic Chromosomes, 342 Databases of Genomic Regulatory Factors, 342 Ultraconserved Elements, 345 Nonconserved Elements, 345 Comparison of Eukaryotic DNA, 346 Variation in Chromosomal DNA, 347 Dynamic Nature of Chromosomes: Whole-Genome Duplication, 347 Chromosomal Variation in Individual Genomes, 349 Structural Variation: Six Types, 351 Inversions, 351 Mechanisms of Creating Duplications, Deletions, and Inversions, 351 Models for Creating Gene Families, 353 Chromosomal Variation in Individual Genomes: SNPs, 354 Techniques to Measure Chromosomal Change, 355 Array Comparative Genomic Hybridization, 356 SNP Microarrays, 356 Next-Generation Sequencing, 359 Perspective, 359 Pitfalls, 359 Advice to Students, 360 Web Resources, 360 **Discussion Questions**, 361 Problems/Computer Lab, 361 Self-Test Quiz, 364 Suggested Reading, 365 References, 366

9 Analysis of Next-Generation Sequence Data, 377

Introduction, 378 DNA Sequencing Technologies, 377 Sanger Sequencing, 379 Next-Generation Sequencing, 379 Cyclic Reversible Termination: Illumina, 382 Pyrosequencing, 384 Sequencing by Ligation: Color Space with ABI SOLiD, 385 Ion Torrent: Genome Sequencing by Measuring pH, 387 Pacific Biosciences: Single-Molecule Sequencing with Long Read Lengths, 387 Complete Genomics: Self-Assembling DNA Nanoarrays, 387 Analysis of Next-Generation Sequencing of Genomic DNA, 387 Overview of Next-Generation Sequencing Data Analysis, 387 Topic 1: Experimental Design and Sample Preparation, 389 Topic 2: From Generating Sequence Data to FASTQ, 390 Finding and Viewing FASTQ files, 392 Quality Assessment of FASTQ data, 393 FASTG: A Richer Format than FASTQ, 394

Topic 3: Genome Assembly, 394 Competitions and Critical Evaluations of the Performance of Genome Assemblers, 396 The End of Assembly: Standards for Completion, 398 Topic 4: Sequence Alignment, 399 Alignment of Repetitive DNA, 400 Genome Analysis Toolkit (GATK) Workflow: Alignment with BWA, 401 Topic 5: The SAM/BAM Format and SAMtools, 402 Calculating Read Depth, 405 Finding and Viewing BAM/SAM files, 405 Compressed Alignments: CRAM File Format, 406 Topic 6: Variant Calling: Single-Nucleotide Variants and Indels, 408 Topic 7: Variant Calling: Structural Variants, 409 Topic 8: Summarizing Variation: The VCF Format and VCFtools, 410 Finding and Viewing VCF files, 413 Topic 9: Visualizing and Tabulating Next-Generation Sequence Data, 413 Topic 10: Interpreting the Biological Significance of Variants, 417 Topic 11: Storing Data in Repositories, 421 Specialized Applications of Next-Generation Sequencing, 421 Perspective, 422 Pitfalls, 423 Advice for Students, 423 Web Resources, 424 **Discussion Questions**, 424 Problems/Computer Lab, 424 Self-Test Ouiz, 425 Suggested Reading, 425 References, 425

10 Bioinformatic Approaches to Ribonucleic Acid (RNA), 433

Introduction to RNA, 433 Noncoding RNA, 436 Noncoding RNAs in the Rfam Database, 436 Transfer RNA, 438 Ribosomal RNA, 441 Small Nuclear RNA, 445 Small Nucleolar RNA, 445 MicroRNA, 445 Short Interfering RNA, 447 Long Noncoding RNA (IncRNA), 447 Other Noncoding RNA, 448 Noncoding RNAs in the UCSC Genome and Table Browser, 448 Introduction to Messenger RNA, 450 mRNA: Subject of Gene Expression Studies, 450 Low- and High-Throughput Technologies to Study mRNAs, 452

Analysis of Gene Expression in cDNA Libraries, 455 Full-Length cDNA Projects, 459 BodyMap2 and GTEx: Measuring Gene Expression Across the Body, 459 Microarrays and RNA-Seg: Genome-Wide Measurement of Gene Expression, 460 Stage 1: Experimental Design for Microarrays and RNA-seg, 461 Stage 2: RNA Preparation and Probe Preparation, 461 Stage 3: Data Acquisition, 464 Hybridization of Labeled Samples to DNA Microarrays, 464 Data acquisition for RNA-seq, 465 Stage 4: Data Analysis, 465 Stage 5: Biological Confirmation, 465 Microarray and RNA-seq Databases, 465 Further Analyses, 465 Interpretation of RNA Analyses, 466 The Relationship between DNA, mRNA, and Protein Levels, 466 The Pervasive Nature of Transcription, 467 eQTLs: Understanding the Genetic Basis of Variation in Gene Expression through Combined RNA-seq and DNA-seq, 468 Perspective, 469 Pitfalls, 470 Advice to Students, 470 Web Resources, 470 **Discussion Questions**, 471 Problems/Computer Lab, 471 Self-Test Ouiz, 471 Suggested Reading, 472 References, 473

11 Gene Expression: Microarray and RNA-seq Data Analysis, 479 Introduction, 479

Microarray Analysis Method 1: GEO2R at NCBI, 482 GEO2R Executes a Series of R Scripts, 482 GEO2R Identifies the Chromosomal Origin of Regulated Transcripts, 485 GEO2R Normalizes Data, 486 GEO2R uses RMA Normalization for Accuracy and Precision, 488 Fold Change (Expression Ratios), 490 GEO2R Performs >22,000 Statistical Tests, 490 GEO2R Offers Corrections for Multiple Comparisons, 494 Microarray Analysis Method 2: Partek, 495 Importing Data, 496 Quality Control, 496 Adding Sample Information, 497 Sample Histogram, 498 Scatter Plots and MA Plots, 498

Working with Log, Transformed Microarray Data, 498 **Exploratory Data Analysis with Principal Components** Analysis (PCA), 498 Performing ANOVA in Partek, 501 From t-test to ANOVA, 503 Microarray Analysis Method 3: Analyzing a GEO Dataset with R, 504 Setting up the Analyses, 504 Reading CEL Files and Normalizing with RMA, 506 Identifying Differentially Expressed Genes (Limma), 508 Microarray Analysis and Reproducibility, 510 Microarray Data Analysis: Descriptive Statistics, 511 Hierarchical Cluster Analysis of Microarray Data, 511 Partitioning Methods for Clustering: k-Means Clustering, 516 Multidimensional Scaling Compared to Principal Components Analysis, 517 Clustering Strategies: Self-Organizing Maps, 517 Classification of Genes or Samples, 517 RNA-Seq, 519 Setting up a TopHat and CuffLinks Sample Protocol, 523 TopHat to Map Reads to a Reference Genome, 524 Cufflinks to Assemble Transcripts, 525 Cuffdiff to Determine Differential Expression, 525 CummeRbund to Visualize RNA-seq Results, 526 **RNA-seq Genome Annotation Assessment Project** (RGASP), 527 Functional Annotation of Microarray Data, 528 Perspective, 529 Pitfalls, 530 Advice for Students, 531 Suggested Reading, 531 Problems/Computer Lab, 532 Self-Test Quiz, 532 Suggested Reading, 533 References, 534

12 Protein Analysis and Proteomics, 539

Introduction, 539 Protein Databases, 540 Community Standards for Proteomics Research, 542 Evaluating the State-of-the-Art: ABRF analytic challenges, 542 Techniques for Identifying Proteins, 543 Direct Protein Sequencing, 543 Gel Electrophoresis, 543 Mass Spectrometry, 547 Four Perspectives on Proteins, 551

Perspective 1: Protein Domains and Motifs: Modular Nature of Proteins, 552 Added Complexity of Multidomain Proteins, 557 Protein Patterns: Motifs or Fingerprints Characteristic of Proteins, 557 Perspective 2: Physical Properties of Proteins, 559 Accuracy of Prediction Programs, 561 Proteomic Approaches to Phosphoryation, 563 Proteomic Approaches to Transmembrane Regions, 565 Introduction to Perspectives 3 and 4: Gene Ontology Consortium, 567 Perspective 3: Protein Localization, 568 Perspective 4: Protein Function, 570 Perspective, 575 Pitfalls, 575 Advice for Students, 575 Web Resources, 576 **Discussion Questions**, 578 Problems/Computer Lab, 578 Self-Test Quiz, 579 Suggested Reading, 580 References, 580

13 Protein Structure, 589

Overview of Protein Structure, 589 Protein Sequence and Structure, 590 Biological Questions Addressed by Structural Biology: Globins, 591 Principles of Protein Structure, 591 Primary Structure, 591 Secondary Structure, 594 Tertiary Protein Structure: Protein-Folding Problem, 598 Structural Genomics, the Protein Structure Initiative, and Target Selection, 600 Protein Data Bank, 602 Accessing PDB Entries at NCBI Website, 606 Integrated Views of Universe of Protein Folds, 609 Taxonomic System for Protein Structures: SCOP Database, 610 CATH Database, 613 Dali Domain Dictionary, 615 Comparison of Resources, 617 Protein Structure Prediction, 617 Homology Modeling (Comparative Modeling), 618 Fold Recognition (Threading), 619 Ab Initio Prediction (Template-Free Modeling), 621 A Competition to Assess Progress in Structure Prediction, 621 Intrinsically Disordered Proteins, 622 Protein Structure and Disease, 622 Perspective, 625

Pitfalls, 625 Advice for Students, 625 **Discussion Questions**, 625 Problems/Computer Lab, 626 Self-Test Ouiz, 627 Suggested Reading, 628 References, 628 14 Functional Genomics, 635 Introduction to Functional Genomics, 635 The Relationship Between Genotype and Phenotype, 637 Eight-Model Organisms For Functional Genomics, 638 1. The Bacterium Escherichia coli, 639 2. The Yeast Saccharomyces cerevisiae, 640 3. The Plant Arabidopsis thaliana, 643 4. The Nematode Caenorhabditis elegans, 643 5. The Fruit Fly Drosophila melanogaster, 645 6. The Zebrafish Danio rerio, 645 7. The Mouse Mus musculus, 646 8. Homo sapiens: Variation in Humans, 647 Functional Genomics Using Reverse and Forward Genetics, 648 Reverse Genetics: Mouse Knockouts and the β -Globin Gene, 650 Reverse Genetics: Knocking Out Genes in Yeast Using Molecular Barcodes, 653 Reverse Genetics: Random Insertional Mutagenesis (Gene Trapping), 657 Reverse Genetics: Insertional Mutagenesis in Yeast, 660 Reverse Genetics: Gene Silencing by Disrupting RNA, 662 Forward Genetics: Chemical Mutagenesis, 665 Comparison of Reverse and Forward Genetics, 665 Functional Genomics and the Central Dogma, 666 Approaches to Function and Definitions of Function, 646 Functional Genomics and DNA: Integrating Information, 668 Functional Genomics and RNA, 668 Functional Genomics and Protein, 670 Proteomics Approaches to Functional Genomics, 670 Functional Genomics and Protein: Critical Assessment of Protein Function Annotation, 672 Protein–Protein Interactions, 672 Yeast Two-Hybrid System, 673 Protein Complexes: Affinity Chromatography and Mass Spectrometry, 675 Protein–Protein Interaction Databases, 676 From Pairwise Interactions to Protein Networks, 678 Assessment of Accuracy, 680 Choice of Data, 680

Experimental Organism, 680 Variation in Pathways, 681 Categories of Maps, 681 Pathways, Networks, and Integration: Bioinformatics Resources, 682 Perspective, 685 Pitfalls, 686 Advice for Students, 686 Web Resources, 686 Discussion Questions, 686 Problems/Computer Lab, 686 Self-Test Quiz, 687 Suggested Reading, 688 References, 688

PART III GENOME ANALYSIS

15 Genomes Across the Tree of Life, 699

Introduction, 700 Five Perspectives on Genomics, 701 Brief History of Systematics, 701 History of Life on Earth, 705 Molecular Sequences as the Basis of the Tree of Life, 705 Role of Bioinformatics in Taxonomy, 709 Prominent Web Resources, 710 Ensembl Genomes, 710 NCBI Genome, 710 Genome Portal of DOE JGI and the Integrated Microbial Genomes, 710 Genomes On Line Database (GOLD), 710 UCSC, 710 Genome-Sequencing Projects: Chronology, 711 Brief Chronology, 711 1976–1978: First Bacteriophage and Viral Genomes, 711 1981: First Eukaryotic Organellar Genome, 712 1986: First Chloroplast Genomes, 714 1992: First Eukaryotic Chromosome, 715 1995: Complete Genome of Free-Living Organism, 715 1996: First Eukaryotic Genome, 715 1997: Escherichia coli, 715 1998: First Genome of Multicellular Organism, 716 1999: Human Chromosome, 716 2000: Fly, Plant, and Human Chromosome 21, 716 2001: Draft Sequences of Human Genome, 716 2002: Continuing Rise in Completed Genomes, 717 2003: HapMap, 717 2004: Chicken, Rat, and Finished Human Sequences, 717

2005: Chimpanzee, Dog, Phase I HapMap, 718 2006: Sea Urchin, Honeybee, dbGaP, 718 2007: Rhesus Macaque, First Individual Human Genome, ENCODE Pilot, 718 2008: Platypus, First Cancer Genome, First Personal Genome Using NGS, 718 2009: Bovine, First Human Methlyome Map, 718 2010: 1000 Genomes Pilot, Neandertal, Exome Sequencing to Find Disease Genes, 719 2011: A Vision for the Future of Genomics, 719 2012: Denisovan Genome, Bonobo, and 1000 Genomes Project, 719 2013: The Simplest Animal and a 700,000-Year-Old Horse, 719 2014: Mouse ENCODE, Primates, Plants, and Ancient Hominids, 719 2015: Diversity in Africa, 720 Genome Analysis Projects: Introduction, 720 Large-Scale Genomics Projects, 721 Criteria for Selection of Genomes for Sequencing, 722 Genome Size, 722 Cost, 722 Relevance to Human Disease, 723 Relevance to Basic Biological Questions, 724 Relevance to Agriculture, 724 Sequencing of One Versus Many Individuals from a Species, 724 Role of Comparative Genomics, 724 Resequencing Projects, 725 Ancient DNA Projects, 725 Metagenomics Projects, 725 Genome Analysis Projects: Sequencing, 728 Genome-Sequencing Centers, 728 Trace Archive: Repository for Genome Sequence Data, 728 HTGS Archive: Repository for Unfinished Genome Sequence Data, 730 Genome Analysis Projects: Assembly, 730 Four Approaches to Genome Assembly, 730 Genome Assembly: From FASTQ to Contigs with Velvet, 733 Comparative Genome Assembly: Mapping Contigs to Known Genomes, 734 Finishing: When Has a Genome Been Fully Sequenced?, 735 Genome Assembly: Measures of Success, 735 Genome Assembly: Challenges, 735 Genome Analysis Projects: Annotation, 737 Annotation of Genes in Eukaryotes: Ensembl Pipeline, 738 Annotation of Genes in Eukaryotes: NCBI Pipeline, 739 Core Eukaryotic Genes Mapping Approach (CEGMA), 739 Assemblies from the Genome Reference Consortium, 741 Assembly Hubs and Transfers at UCSC, Ensembl, and NCBI, 741 Annotation of Genes in Bacteria and Archaea, 741 Genome Annotation Standards, 741 Perspective, 742

Pitfalls, 742 Advice for Students, 743 Discussion Questions, 743 Problems/Computer Lab, 743 Self-Test Quiz, 745 Suggested Reading, 743 References, 745

16 Completed Genomes: Viruses, 755

Introduction, 755 International Committee on Taxonomy of Viruses (ICTV) and Virus Species, 756 Classification of Viruses, 758 Classification of Viruses Based on Morphology, 758 Classification of Viruses Based on Nucleic Acid Composition, 758 Classification of Viruses Based on Genome Size, 758 Classification of Viruses Based on Disease Relevance, 760 Diversity and Evolution of Viruses, 762 Metagenomics and Virus Diversity, 764 Bioinformatics Approaches to Problems in Virology, 765 Human Immunodeficiency Virus (HIV), 766 NCBI and LANL resources for HIV-1, 766 Influenza Virus, 771 Measles Virus, 774 Ebola Virus, 775 Herpesvirus: From Phylogeny to Gene Expression, 776 The Pairwise Sequence Comparison (PASC) Tool, 780 Giant Viruses, 782 Comparing genomes with MUMmer, 783 Perspectives, 785 Pitfalls, 786 Advice for Students, 786 Web Resources, 786 **Discussion Questions**, 787 Problems/Computer Lab, 787 Self-Test Quiz, 788 Suggested Reading, 789 References, 789

17 Completed Genomes: Bacteria and Archaea, 797

Introduction, 797 Classification of Bacteria and Archaea, 798 Classification of Bacteria by Morphological Criteria, 800 Classification of Bacteria and Archaea Based on Genome Size and Geometry, 801

Classification of Bacteria and Archaea Based on Lifestyle, 805 Classification of Bacteria Based on Human Disease Relevance, 808 Classification of Bacteria and Archaea Based on Ribosomal RNA Sequences, 809 Classification of Bacteria and Archaea Based on Other Molecular Sequences, 810 The Human Microbiome, 811 Analysis of Bacterial and Archaeal Genomes, 814 Nucleotide Composition, 817 Finding Genes, 819 Interpolated Context Model (ICM), 822 GLIMMER3, 824 Challenges of Bacterial and Archaeal Gene Prediction, 825 Gene Annotation, 825 Lateral Gene Transfer, 827 Comparison of Bacterial Genomes, 830 TaxPlot, 830 MUMmer, 833 Perspective, 834 Pitfalls, 835 Advice for Students, 835 Web Resources, 835 Discussion Ouestions, 836 Problems/Computer Lab, 836 Self-Test Quiz, 836 Suggested Reading, 837 References, 837

18 Eukaryotic Genomes: Fungi, 847

Introduction, 847 Description and Classification of Fungi, 848 Introduction to Budding Yeast Saccharomyces Cerevisiae, 849 Sequencing Yeast Genome, 851 Features of Budding Yeast Genome, 851 Exploring Typical Yeast Chromosome, 854 Web Resources for Analyzing a Chromosome, 854 Exploring Variation in a Chromosome with Command-Line Tools, 857 Finding Genes in a Chromosome with Command-Line Tools, 858 Properties of Yeast Chromosome XII, 860 Gene Duplication and Genome Duplication of S. cerevisiae, 860 Comparative Analyses of Hemiascomycetes, 865 Comparative Analyses of Whole-Genome Duplication, 866 Identification of Functional Elements, 868 Analysis of Fungal Genomes, 869 Fungi in the Human Microbiome, 870

Aspergillus, 871 Candida albicans, 871 Cryptococcus neoformans: model fungal pathogen, 872 Atypical Fungus: Microsporidial Parasite Encephalitozoon cuniculi, 873 Neurospora crassa, 873 First Basidiomycete: Phanerochaete chrysosporium, 875 Fission Yeast Schizosaccharomyces pombe, 875 Other Fungal Genomes, 876 Ten Leading Fungal Plant Pathogens, 876 Perspective, 876 Pitfalls, 877 Advice for Students, 877 Web Resources, 877 Discussion Ouestions, 877 Problems/Computer Lab, 878 Self-Test Quiz, 879 Suggested Reading, 880 References, 880

19 Eukaryotic Genomes: From Parasites to Primates, 887

Introduction, 887 Protozoans at Base of Tree Lacking Mitochondria, 890 Trichomonas, 890 Giardia lamblia: A Human Intestinal Parasite, 891 Genomes of Unicellular Pathogens: Trypanosomes and Leishmania, 890 Trypanosomes, 892 Leishmania, 894 The Chromalveolates, 895 Malaria Parasite Plasmodium falciparum, 895 More Apicomplexans, 898 Astonishing Ciliophora: Paramecium and Tetrahymena, 899 Nucleomorphs, 902 Kingdom Stramenopila, 904 Plant Genomes, 906 Overview, 906 Green Algae (Chlorophyta), 908 Arabidopsis thaliana Genome, 910 The Second Plant Genome: Rice, 913 Third Plant: Poplar, 914 Fourth Plant: Grapevine, 915 Giant and Tiny Plant Genomes, 915 Hundreds More Land Plant Genomes, 915 Moss, 916 Slime and Fruiting Bodies at the Feet of Metazoans, 916 Social Slime Mold Dictyostelium discoideum, 916

Metazoans, 917 Introduction to Metazoans, 917 900 MYA: the Simple Animal Caenorhabditis elegans, 918 900 MYA: Drosophila melanogaster (First Insect Genome), 919 900 MYA: Anopheles gambiae (Second Insect Genome), 921 900 MYA: Silkworm and Butterflies, 922 900 MYA: Honeybee, 923 900 MYA: A Swarm of Insect Genomes, 923 840 MYA: A Sea Urchin on the Path to Chordates, 924 800 MYA: Ciona intestinalis and the Path to Vertebrates, 925 450 MYA: Vertebrate Genomes of Fish, 926 350 MYA: Frogs, 929 320 MYA: Reptiles (Birds, Snakes, Turtles, Crocodiles), 929 180 MYA: The Platypus and Opposum Genomes, 931 100 MYA: Mammalian Radiation from Dog to Cow, 933 80 MYA: The Mouse and Rat, 934 5-50 MYA: Primate Genomes, 937 Perspective, 940 Pitfalls, 941 Advice for Students, 941 Web Resources, 942 Discussion Ouestions, 942 Problems/Computer Lab, 942 Self-Test Quiz, 943 Suggested Reading, 944 References, 944

20 Human Genome, 957

Introduction, 957 Main Conclusions of Human Genome Project, 958 Gateways to Access the Human Genome, 959 NCBI, 959 Ensembl, 959 University of California at Santa Cruz Human Genome Browser, 961 NHGRI, 961 Wellcome Trust Sanger Institute, 964 Human Genome Project, 964 Background of Human Genome Project, 964 Strategic Issues: Hierarchical Shotgun Sequencing to Generate Draft Sequence, 966 Human Genome Assemblies, 966 Broad Genomic Landscape, 968 Long-Range Variation in GC Content, 969 CpG Islands, 969 Comparison of Genetic and Physical Distance, 970

Repeat Content of Human Genome, 971 Transposon-Derived Repeats, 972 Simple Sequence Repeats, 973 Segmental Duplications, 973 Gene Content of Human Genome, 974 Noncoding RNAs, 975 Protein-Coding Genes, 975 **Comparative Proteome Analysis**, 975 Complexity of Human Proteome, 978 25 Human Chromosomes, 979 Group A (Chromosomes 1-3), 981 Group B (Chromosomes 4, 5), 982 Group C (Chromosomes 6-12, X), 983 Group D (Chromosomes 13-15), 983 Group E (Chromosomes 16-18), 984 Group F (Chromosomes 19, 20), 984 Group G (Chromosomes 21, 22, Y), 984 Mitochondrial Genome, 985 Human Genome Variation, 986 SNPs, Haplotypes, and HapMap, 986 Viewing and Analyzing SNPs and Haplotypes, 988 HaploView, 988 HapMap Browser, 988 Integrative Genomics Browser (IGV), 988 NCBI dbSNP, 988 PLINK, 992 SNPduo, 990 Major Conclusions of HapMap Project, 994 The 1000 Genomes Project, 995 Variation: Sequencing Individual Genomes, 998 Perspective, 999 Pitfalls, 1000 Advice for Students, 1001 **Discussion Questions**, 1001 Problems/Computer Lab, 1001 Self-Test Quiz, 1003 Suggested Reading, 1004 References, 1004

21 Human Disease, 1011

Human Genetic Disease: A Consequence of DNA Variation, 1011 A Bioinformatics Perspective on Human Disease, 1012 Garrod's View of Disease, 1014 Classification of Disease, 1015 NIH Disease Classification: MeSH Terms, 1017