

Contents

Contributors	xiii
Foreword	xvii
Preface	xix
Acknowledgments	xxi
Chapter 1: Metagenomics: A Paradigm Shift in Microbiology.....	1
Andrey V. Mardanov, Vitaly V. Kadnikov, Nikolai V. Ravin	
1.1 The Birth of Metagenomics	1
1.2 Function-Based and Sequencing-Based Metagenomics	3
1.3 An Overview of Methods Used in Sequencing-Based Metagenomics	5
1.4 Interplay of Single-Cell Genomics and Metagenomics	8
1.5 Concluding Remarks	9
References.....	10
Chapter 2: Metagenomic Protocols and Strategies	15
Celia Méndez-García, Rafael Bargiela, Mónica Martínez-Martínez, Manuel Ferrer	
2.1 Introduction.....	15
2.2 Wet-Lab Techniques for Sample Collection, Preservation, and Transport	15
2.3 Wet-Lab Techniques for Sample Pretreatment and Nucleic Acid Extraction (DNA)	16
2.3.1 Sample Handling for Superficial Sea- and Freshwater Sample Treatment	17
2.3.2 Sample Handling for Solid and Sediment Sample Treatment	17
2.3.3 Isolation of High-Quality DNA by Phenol:Chloroform Method Followed by DNA Cleaning	18
2.3.4 Isolation of High-Quality DNA by the Hexadecyltrimethylammonium Bromide Method	19
2.3.5 Isolation of High-Quality DNA by Commercial Kits	20
2.3.6 Handling Instructions for the Isolation of High-Quality Genomic DNA From Acidic Samples	21
2.3.7 Isolation of High-Quality DNA From Fecal Samples	21
2.3.8 Rapid Amplification of DNA Using Phi29 DNA Polymerase	22
2.4 Wet-Lab Techniques for RNA Isolation	22
2.5 Meta-Taxonomy Through 16S rRNA Gene: From Wet-Lab Techniques to Bioinformatics	23

2.5.1 Wet-Lab Techniques for Amplification and Sequencing of 16S rRNA	24
2.5.2 Bioinformatic and Statistical Analysis of 16S rRNA Amplicons.....	28
2.5.3 Expression of Results	31
2.6 Metagenomics: Sequencing Methods and Bioinformatics	32
2.6.1 Sequencing Methods for Community DNA and cDNA Analysis	33
2.6.2 Bioinformatic Analysis of Shotgun Sequencing Data From Metagenomic Samples	37
2.6.3 Metagenomes Databases	48
2.7 Metagenomic: Wet-Lab Techniques for Clone Library Construction	50
Acknowledgments	53
References.....	53
Further Reading	54
Chapter 3: Strategies for Taxonomic and Functional Annotation of Metagenomes	55
Johan Bengtsson-Palme	
3.1 Introduction.....	55
3.2 Assessing Taxonomic Composition Using Metagenomic Data.....	55
3.3 Metagenomic Assembly.....	59
3.4 Detection and Quantification of Ecosystem Processes Using Metagenomics.....	61
3.5 Comparing Communities and Estimating Diversity.....	66
3.6 Computational "Pipelines" for Metagenomic Analyses.....	70
3.7 Conclusions and Outlook.....	73
References.....	74
Chapter 4: Computational and Statistical Considerations in the Analysis of Metagenomic Data.....	81
Fredrik Boulund, Mariana Buongiorno Pereira, Viktor Jonsson, Erik Kristiansson	
4.1 Introduction.....	81
4.2 Computational Challenges in the Analysis of Shotgun Metagenomics	82
4.2.1 Choosing a Suitable Read Mapper	84
4.2.2 Analysis Workflows and Computational Resources	84
4.2.3 Existing Big Data Frameworks for Gene Quantification	86
4.2.4 A Remark on Reproducibility in Shotgun Metagenomics	88
4.3 Statistical Aspects of the Analysis of Shotgun Metagenomic Data.....	89
4.3.1 Normalization of Gene Abundance Data.....	90
4.3.2 Identification of Differentially Abundant Genes	94
4.4 Conclusions.....	98
References.....	99
Chapter 5: 16S rRNA-Based Taxonomy Profiling in the Metagenomics Era	103
Matteo Ramazzotti, Giovanni Bacci	
5.1 The 16S rRNA Gene as the Golden Phylogenetic Standard for Prokaryotes	103
5.2 Targeted Amplification of 16S rRNA Gene for Next-Generation Sequencing.....	104
5.3 Biases in Targeted Approaches	105

5.4	Choosing the Appropriate Target Region	106
5.5	Taxonomic Classification: The Growing of 16S rRNA Databanks	108
5.6	Methods for the Estimation of Taxonomic Abundance	110
5.7	From Taxonomy to Metagenomics: Is It Reasonable?	111
5.8	Taxonomy in Nontargeted Approaches.....	112
5.9	Taxonomy via 16S rRNA Assembling: The Issue of Genomic Complexity	112
5.10	Ribosomal Read Recruitment From Nontargeted Approaches.....	113
5.11	Experiment Repositories and Analysis Pipelines	114
5.12	Concluding Remarks	116
	References.....	116

Chapter 6: Analyzing High-Throughput Microbial Amplicon Sequence Data Using Multiple Markers..... 121

Ilya Y. Zhbannikov, James A. Foster

6.1	Introduction.....	121
6.2	The MetAmp Algorithm and Software	123
6.3	Validation and Efficiency	128
6.4	Discussion.....	133
6.5	Conclusion	136
	Acknowledgments	136
	References.....	136

Chapter 7: Human Body to Water Reservoirs: Estimating Viral Population Characteristics Using High-Throughput Sequencing..... 139

Duleepa Jayasundara, Sen-Lin Tang, Saman K. Halgamuge

7.1	Introduction.....	139
7.2	Viruses as Quasispecies Populations	140
7.2.1	Viruses and Quasispecies Theory	140
7.2.2	Medical Importance.....	140
7.3	Quasispecies Spectrum Reconstruction.....	141
7.3.1	Theoretical Solutions to QSR.....	142
7.3.2	Experimental Noise in NGS Reads	143
7.3.3	Richness Estimation in Viral Quasispecies	144
7.4	Estimating Population Characteristics in Environmental Viral Populations	146
7.4.1	Current State-of-the-Art Methods	147
7.4.2	Limitations of PHACCS.....	149
7.4.3	Alternatives to PHACCS	150
7.4.4	Use of Metaheuristics.....	150
7.5	Future Research Directions.....	151
	References.....	151

Chapter 8: Measuring Microbiome Diversity and Similarity With Hill Numbers 157

Zhanshan (Sam) Ma

8.1	Introduction.....	157
8.2	Rediscovering Hill Numbers and the Partition of Beta Diversity.....	160

8.3 Measuring Phylogenetic Diversity.....	168
8.4 Additional Diversity Metrics Based on Hill Numbers.....	171
8.5 An Example for Measuring Microbiome Diversity With Hill Numbers	174
References.....	175
Chapter 9: Metagenomics: Implications in Oral Health and Disease	179
Muniyandi Nagarajan, Vandana R. Prabhu, Ranganathan Kamalakkannan	
9.1 Human and Microbes: A Perpetual Interplay	179
9.2 An Overview of the Oral Microbiome.....	180
9.3 Oral Microbiome Composition.....	181
9.3.1 Bacteria.....	181
9.3.2 Archaea.....	181
9.3.3 Protozoa.....	181
9.3.4 Fungi.....	182
9.3.5 Virus	182
9.4 The Commensal Residents of Oral Cavity	183
9.5 Oral Microbiome and Its Diseases.....	184
9.5.1 Dental Caries	185
9.5.2 Periodontal Diseases	186
9.5.3 Endodontic Infections	187
9.6 Oral Microbiome-Associated Systemic Infections.....	187
9.6.1 Cardiovascular Disease	188
9.6.2 Alzheimer's Disease.....	188
9.6.3 Diabetes Mellitus.....	189
9.7 Conclusion	189
Acknowledgments	190
References.....	190
Chapter 10: The Human Gut Microbiome in Health and Disease	197
Juan Jovel, Levinus A. Dieleman, Dina Kao, Andrew L. Mason, Eytan Wine	
10.1 Introduction	197
10.2 The Womb and Early Life Microbiome.....	198
10.3 The Human Microbiome and the Immune System.....	201
10.4 The Human Microbiome and Complex Diseases	203
10.5 Fecal Microbiota Transplantation.....	207
10.6 Concluding Remarks	208
References.....	208
Chapter 11: Comprehensive Exploration of the Rumen Microbial Ecosystem With Advancements in Metagenomics.....	215
Ramesh K. Kothari, Neelam M. Nathani, Chandrashekar Mootapally, Jalpa K. Rank, Haren B. Gosai, Bharti P. Dave, Chaitanya G. Joshi	
11.1 Introduction	215
11.2 Rumen Microbial Diversity	216
11.2.1 Rumen Bacterial Diversity	217

11.2.2 Eukaryotic (Protozoa and Fungus) Diversity in Rumen.....	218
11.2.3 Rumen Archaea (Methanogens) Diversity	219
11.3 Rumen Metagenomics	220
11.3.1 Amplicon-Based Rumen Metagenomics.....	220
11.3.2 Shotgun Rumen Metagenomics	221
11.3.3 Gene Mining for Important Agro-Industrial Enzyme Discovery	223
11.4 Applications of Rumen Metagenomics	224
11.4.1 Methanogenesis and Reduction of Methane Gas Emission	225
11.5 Future Perspectives and Conclusions	226
Acknowledgments	227
References.....	227

Chapter 12 The Microbiome of Desert CAM Plants: Lessons From Amplicon Sequencing and Metagenomics.....231

Fonseca-García Citlali, Damaris Desgareñes, Víctor M. Flores-Núñez,
Laila P. Partida-Martínez

12.1 Introduction	231
12.2 Experimental Design	232
12.3 Composition and Diversity of Microbial Communities Associated With Agaves and Cacti.....	235
12.4 Factors Affecting the Assembly of Microbial Communities in CAM Plants.....	239
12.5 The Core Microbiome of CAM Plants	242
12.6 In Silico Functional Analyses of the Rhizosphere and Phyllosphere of CAM Plants and Their Contribution to the Plant Holobiont.....	244
12.7 Conclusions	249
Acknowledgments	249
References.....	250

Chapter 13: Metagenomics of Marine Invertebrate-Microbial Consortium.....255

Muthan Krishnaveni, Santhi Asha, Stephen Sharmin Vini, Stanislaus Mary
Josephine Punitha

13.1 Introduction	255
13.1.1 Ocean Sampling Day.....	255
13.1.2 Applications of Metagenomics.....	256
13.2 Microbial Invertebrate Symbiosis.....	257
13.3 Metagenomic Approach to Explore the Unculturable Microbial World.....	259
13.3.1 Isolation of eDNA	259
13.3.2 Metagenomic Library Construction	260
13.3.3 Screening of Metagenomic Clones	260
13.4 Metaproteomics and Targeted Metagenomics	261
13.4.1 Proteome Analysis.....	263
13.4.2 Single Cell Genomics.....	264
13.5 Conclusion	265
References.....	266

Chapter 14: Novel Biological Resources Screened From Uncultured Bacteria by a Metagenomic Method.....273

Nobutada Kimura

14.1 Introduction	273
14.2 Approaches to Metagenome: Function-Based Screening Method	274
14.2.1 Samples Preparation	274
14.2.2 Library Construction	275
14.2.3 Screening	277
14.3 Approaches to Metagenome: Sequence-Based Screening Method	279
14.4 Screening Targets of Natural Microbial Resources	279
14.4.1 Novel Genes and Enzymes	279
14.4.2 Natural Compounds	280
14.5 Eukaryotic Cells as a Target Resource for a Metagenomic Screening	281
14.6 Concluding Remarks and Future Prospects	282
References	283

Chapter 15: Advances of Functional Metagenomics in Harnessing Thermozyms.....289

Juan-José Escuder-Rodríguez, María-Eugenia DeCastro, Manuel Becerra,
Esther Rodríguez-Belmonte, María-Isabel González-Siso

15.1 Introduction	289
15.2 Methodologies for Functional Metagenomics: Library Construction and Screening	290
15.2.1 Sampling	291
15.2.2 DNA Extraction	291
15.2.3 Vector Choice	294
15.2.4 DNA Cloning	294
15.2.5 Functional Screening	295
15.3 Concluding Remarks and Perspectives	302
Acknowledgments	302
References	303

Chapter 16: Direct Cellulase Gene Amplification From Hot Spring Using the Guidance of 16S rRNA Amplicon Metagenomics.....309

Kok Jun Liew, Chia Chiu Lim, Chia Sing Chan, Kwek Yan Wei, Madiah Md Salleh,
Rajesh Kumar Sani, Kok-Gan Chan, Kian Mau Goh

16.1 Introduction	309
16.1.1 Biofuel Produced From Lignocellulosic Biomass	310
16.1.2 Enzymes Involved in Lignocellulose Biomass Degradation	311
16.1.3 Gene Mining Strategies	312
16.2 Methodology	314
16.2.1 Sample Collection	314
16.2.2 Metagenome Extraction	314
16.2.3 16S rRNA Amplicon Sequencing	315

16.2.4 Screening of Potential Genera With Cellulose Degrading Ability and Direct Gene Amplification.....	315
16.2.5 Expression and Purification of Recombinant Endoglucanase (EglDG) of <i>Dictyoglomus</i> sp. Origin	316
16.2.6 Enzyme and Protein Assay	317
16.2.7 Effect of pH and Temperature on EglDG.....	317
16.2.8 Substrate Specificity of EglDG	317
16.3 Results and Discussion	318
16.3.1 Proof of Concept	318
16.3.2 Characterization of Endoglucanase (EglDG) Originating From <i>Dictyoglomus</i> sp. in US Hot Spring	320
16.4 Conclusions	322
Acknowledgments	323
References.....	323
Chapter 17: Comparative Metaproteomics to Study Environmental Changes	327
Sabine Matallana-Surget, Pratik D. Jagtap, Timothy J. Griffin, Mélanie Beraud, Ruddy Wattiez	
17.1 Introduction	327
17.1.1 Towards a Better Understanding of the Functioning of an Ecosystem.....	327
17.1.2 Environmental Metaproteomics: Past and Future Trends	328
17.1.3 Aquatic Metaproteomics	332
17.2 Metaproteomics on Complex Natural Communities	335
17.2.1 Metaproteomics Workflow	335
17.2.2 Example of a Targeted Comparative Metaproteomics Study	339
17.3 Metaproteomics on Simplified Artificial Communities	339
17.3.1 Design of an Artificial Community	339
17.3.2 Stability of the Artificial Community.....	342
17.3.3 Sample Preparation, Mass Spectrometry Acquisition and Data Analysis.....	342
17.4 Data Analysis.....	345
17.4.1 Database Generation	345
17.4.2 Database Search	347
17.4.3 Taxonomy Analysis	348
17.4.4 Functional Characterization	349
17.4.5 Galaxy Platform: Metaproteomic and Taxonomic Data Analysis.....	351
17.5 Conclusions	356
17.5.1 Pros for Natural Community Approach	357
17.5.2 Pros for Artificial Community Approach	357
Acknowledgments	358
References.....	358
Index.....	365