

# Contents

<b>Preface</b>	xv
<b>Contributors</b>	xvii
<b>1. Introduction</b>	<b>1</b>
<i>Frans J. de Bruijn</i>	
Part 1 Background Chapters	
<b>2. DNA Reassociation Yields Broad-Scale Information on Metagenome Complexity and Microbial Diversity</b>	<b>5</b>
<i>Vigdis L. Torsvik and Lise Øvreås</i>	
<b>3. Diversity of 23S rRNA Genes Within Individual Prokaryotic Genomes</b>	<b>17</b>
<i>Anna Pei, William E. Oberdorf, Carlos W. Nossa, Pooja Chokshi, Martin J. Blaser, Liying Yang, David M. Rosmarin, and Zhiheng Pei</i>	
<b>4. Use of the rRNA Operon and Genomic Repetitive Sequences for the Identification of Bacteria</b>	<b>29</b>
<i>Andréa Maria Amaral Nascimento</i>	
<b>5. Use of Different PCR Primer-Based Strategies for Characterization of Natural Microbial Communities</b>	<b>41</b>
<i>James I. Prosser, Shahid Mahmood, and Thomas E. Freitag</i>	
<b>6. Horizontal Gene Transfer and Recombination Shape Mesorhizobial Populations in the Gene Center of the Host Plants <i>Astragalus Luteolus</i> and <i>Astragalus Ernestii</i> in Sichuan, China</b>	<b>49</b>
<i>Qiongfang Li, Xiaoping Zhang, Ling Zou, Qiang Chen, David P. Fewer, and Kristina Lindström</i>	
<b>7. Amplified rDNA Restriction Analysis (ARDRA) for Identification and Phylogenetic Placement of 16S-rDNA Clones</b>	<b>59</b>
<i>Menachem Y. Sklarz, Roey Angel, Osnat Gillor, and Ines M. Soares</i>	

<b>8. Clustering-Based Peak Alignment Algorithm for Objective and Quantitative Analysis of DNA Fingerprinting Data</b>	<b>67</b>
<i>Satoshi Ishii, Koji Kadota, and Keishi Senoo</i>	
<b>Part 2 The Species Concept</b>	
<b>9. Population Genomics Informs Our Understanding of the Bacterial Species Concept</b>	<b>77</b>
<i>Margaret A. Riley</i>	
<b>10. The Microbial Pangenome: Implications for Vaccine Development</b>	<b>83</b>
<i>Annalisa Nuccitelli, Claudio Donati, Michèle A. Barocchi, and Rino Rappuoli</i>	
<b>11. Metagenomic Insights into Bacterial Species</b>	<b>89</b>
<i>Konstantinos T. Konstantinidis</i>	
<b>12. Reports of Ad Hoc Committees for the Reevaluation of the Species Definition in Bacteriology</b>	<b>99</b>
<i>Erko Stackebrandt</i>	
<b>13. Metagenomic Approaches for the Identification of Microbial Species</b>	<b>105</b>
<i>David M. Ward, Melanie C. Melendrez, Eric D. Becraft, Christian G. Klatt, Jason M. Wood, and Frederick M. Cohan</i>	
<b>Part 3 Metagenomics</b>	
<b>14. Microbial Ecology in the Age of Metagenomics</b>	<b>113</b>
<i>Jianping Xu</i>	
<b>15. The Enduring Legacy of Small Subunit rRNA in Microbiology</b>	<b>123</b>
<i>Susannah G. Tringe and Philip Hugenholtz</i>	
<b>16. Pitfalls of PCR-Based rRNA Gene Sequence Analysis: An Update on Some Parameters</b>	<b>129</b>
<i>Erko Stackebrandt</i>	
<b>17. Empirical Testing of 16S PCR Primer Pairs Reveals Variance in Target Specificity and Efficacy not Suggested by <i>In Silico</i> Analysis</b>	<b>135</b>
<i>Sergio E. Morales and William E. Holben</i>	
<b>18. The Impact of Next-Generation Sequencing Technologies on Metagenomics</b>	<b>143</b>
<i>George M. Weinstock</i>	
<b>19. Accuracy and Quality of Massively Parallel DNA Pyrosequencing</b>	<b>149</b>
<i>Susan M. Huse and David B. Mark Welch</i>	

<b>20. Environmental Shotgun Sequencing: Its Potential and Challenges for Studying the Hidden World of Microbes</b>	<b>157</b>
<i>Jonathan A. Eisen</i>	
<b>21. A Comparison of Random Sequence Reads Versus 16S rDNA Sequences for Estimating the Biodiversity of a Metagenomic Sample</b>	<b>163</b>
<i>Chaysavanh Manichanh, Charles E. Chapple, Lionel Frangeul, Karine Gloux, Roderic Guigo, and Joel Dore</i>	
<b>22. Metagenomic Libraries for Functional Screening</b>	<b>171</b>
<i>Trine Aakvik, Rahmi Lale, Mark Liles, and Svein Valla</i>	
<b>23. GC Fractionation Allows Comparative Total Microbial Community Analysis, Enhances Diversity Assessment, and Facilitates Detection of Minority Populations of Bacteria</b>	<b>183</b>
<i>William E. Holben</i>	
<b>24. Enriching Plant Microbiota for a Metagenomic Library Construction</b>	<b>197</b>
<i>Ying Zeng, Hao-Xin Wang, Zhao-Liang Geng, and Yue-Mao Shen</i>	
<b>25. Towards Automated Phylogenomic Inference</b>	<b>205</b>
<i>Martin Wu and Jonathan A. Eisen</i>	
<b>26. Integron First Gene Cassettes: A Target to Find Adaptive Genes in Metagenomes</b>	<b>217</b>
<i>Lionel Huang and Christine Cagnon</i>	
<b>27. High-Resolution Metagenomics: Assessing Specific Functional Types in Complex Microbial Communities</b>	<b>225</b>
<i>Ludmila Chistoserdova</i>	
<b>28. Gene-Targeted Metagenomics (GT Metagenomics) to Explore the Extensive Diversity of Genes of Interest in Microbial Communities</b>	<b>235</b>
<i>Shoko Iwai, Benli Chai, Ederson da C. Jesus, C. Ryan Penton, Tae Kwon Lee, James R. Cole, and James M. Tiedje</i>	
<b>29. Phylogenetic Screening of Metagenomic Libraries Using Homing Endonuclease Restriction and Marker Insertion</b>	<b>245</b>
<i>Torsten Thomas, Staffan Kjelleberg, and Pui Yi Yung</i>	
<b>30. ArrayOme- and tRNAcc-Facilitated Mobilome Discovery: Comparative Genomics Approaches for Identifying Rich Veins of Bacterial Novel DNA Sequences</b>	<b>251</b>
<i>Hong-Yu Ou and Kumar Rajakumar</i>	
<b>31. Sequence-Based Characterization of Microbiomes by Serial Analysis of Ribosomal Sequence Tags (SARST)</b>	<b>265</b>
<i>Zhongtang Yu and Mark Morrison</i>	

## Part 4 Consortia and Databases

<b>32. The Metagenomics of Plant Pathogen-Suppressive Soils</b>	<b>277</b>
<i>Jan Dirk van Elsas, Anna Maria Kielak, and Mariana Silvia Cretoiu</i>	
<b>33. Soil Metagenomic Exploration of the Rare Biosphere</b>	<b>287</b>
<i>Tom O. Delmont, Laure Franqueville, Samuel Jacquiod, Pascal Simonet, and Timothy M. Vogel</i>	
<b>34. The BIOSPAS Consortium: Soil Biology and Agricultural Production</b>	<b>299</b>
<i>Luis Gabriel Wall</i>	
<b>35. The Human Microbiome Project</b>	<b>307</b>
<i>George M. Weinstock</i>	
<b>36. The Ribosomal Database Project: Sequences and Software for High-Throughput rRNA Analysis</b>	<b>313</b>
<i>James R. Cole, Qiong Wang, Benli Chai, and James M. Tiedje</i>	
<b>37. The Metagenomics RAST Server: A Public Resource for the Automatic Phylogenetic and Functional Analysis of Metagenomes</b>	<b>325</b>
<i>Elizabeth M. Glass and Folker Meyer</i>	
<b>38. The EBI Metagenomics Archive, Integration and Analysis Resource</b>	<b>333</b>
<i>C. Hunter, G. Cochrane, R. Apweiler, S. Hunter</i>	

## Part 5 Computer-Assisted Analysis

<b>39. Comparative Metagenome Analysis Using MEGAN</b>	<b>343</b>
<i>Daniel H. Huson and Suparna Mitra</i>	
<b>40. Phylogenetic Binning of Metagenome Sequence Samples</b>	<b>353</b>
<i>Alice Carolyn McHardy and Kaustubh Patil</i>	
<b>41. Gene Prediction in Metagenomic Fragments with Orphelia: A Large-Scale Machine Learning Approach</b>	<b>359</b>
<i>Katharina H. Hoff, Maike Tech, Thomas Lingner, Rolf Daniel, Burkhard Morgenstern, and Peter Meinicke</i>	
<b>42. Binning Metagenomic Sequences Using Seeded GSOM</b>	<b>369</b>
<i>Ching-Hung Tseng, Chon-Kit Kenneth Chan, Arthur L. Hsu, Saman K. Halgamuge, and Sen-Lin Tang</i>	

<b>43. Iterative Read Mapping and Assembly Allows the Use of a More Distant Reference in Metagenome Assembly</b>	<b>379</b>
<i>Bas E. Dutilh, Martijn A. Huynen, Jolein Gloerich, and Marc Strous</i>	
<b>44. Ribosomal RNA Identification in Metagenomic and Metatranscriptomic Datasets</b>	<b>387</b>
<i>Ying Huang, Weizhong Li, Patricia W. Finn, and David L. Perkins</i>	
<b>45. SILVA: Comprehensive Databases for Quality Checked and Aligned Ribosomal RNA Sequence Data Compatible with ARB</b>	<b>393</b>
<i>Elmar Prsse, Christian Quast, Pelin Yilmaz, Wolfgang Ludwig, Jrg Peplies, and Frank Oliver Glckner</i>	
<b>46. ARB: A Software Environment for Sequence Data</b>	<b>399</b>
<i>Ralf Westram, Kai Bader, Elmar Prsse, Yadhu Kumar, Harald Meier, Frank Oliver Glckner, and Wolfgang Ludwig</i>	
<b>47. The Phyloware Project: A Software Framework for Phylogenomic Virtue</b>	<b>407</b>
<i>Daniel N. Frank and Charles E. Robertson</i>	
<b>48. MetaSim: A Sequencing Simulator for Genomics and Metagenomics</b>	<b>417</b>
<i>Daniel C. Richter, Felix Ott, Alexander F. Auch, Ramona Schmid, and Daniel H. Huson</i>	
<b>49. ClustScan: An Integrated Program Package for the Detection and Semiautomatic Annotation of Secondary Metabolite Clusters in Genomic and Metagenomic DNA Datasets</b>	<b>423</b>
<i>John Cullum, Antonio Starcevic, Janko Diminic, Jurica Zucko, Paul F. Long, and Daslav Hranueli</i>	
<b>50. MetaGene: Prediction of Prokaryotic and Phage Genes in Metagenomic Sequences</b>	<b>433</b>
<i>Hideki Noguchi</i>	
<b>51. Primers4clades: A Web Server to Design Lineage-Specific PCR Primers for Gene-Targeted Metagenomics</b>	<b>441</b>
<i>Bernardo Sachman-Ruiz, Bruno Contreras-Moreira, Enrique Zozaya, Cristina Martnez-Garza, and Pablo Vinuesa</i>	
<b>52. A Parsimony Approach to Biological Pathway Reconstruction/Inference for Metagenomes</b>	<b>453</b>
<i>Yuzhen Ye and Thomas G. Doak</i>	
<b>53. ESPRIT: Estimating Species Richness Using Large Collections of 16S rRNA Data</b>	<b>461</b>
<i>Yijun Sun, Yunpeng Cai, Li Liu, Fahong Yu, and William Farmerie</i>	

Part 6 Complementary Approaches	
54. Metagenomic Approaches in Systems Biology	475
<i>María-Eugenia Guazzaroni and Manuel Ferrer</i>	
55. Towards "Focused" Metagenomics: A Case Study Combining DNA Stable-Isotope Probing, Multiple Displacement Amplification, and Metagenomics	491
<i>Yin Chen, Marc G. Dumont, Joshua D. Neufeld, and J. Colin Murrell</i>	
56. Suppressive Subtractive Hybridization Reveals Extensive Horizontal Transfer in the Rumen Metagenome	497
<i>Elizabeth A. Galbraith, Dionysios A. Antonopoulos, Karen E. Nelson, and Bryan A. White</i>	
Part 6A Microarrays	
57. GeoChip: A High-Throughput Metagenomics Technology for Dissecting Microbial Community Functional Structure	509
<i>Joy D. van Nostrand, Zhili He, and Jizhong Zhou</i>	
58. Phylogenetic Microarrays (PhyloChips) For Analysis of Complex Microbial Communities	521
<i>Eoin L. Brodie</i>	
59. Phenomics and Phenotype Microarrays: Applications Complementing Metagenomics	533
<i>Barry R. Bochner</i>	
60. Microbial Persistence in Low-Biomass, Extreme Environments: The Great Unknown	541
<i>Parag Vaishampayan, James N. Benardini, Myron T. La Duc, and Kasthuri Venkateswaran</i>	
61. Application of Phylogenetic Oligonucleotide Microarrays in Microbial Analysis	551
<i>Pankaj Trivedi and Nian Wang</i>	
Part 6B Metatranscriptomics	
62. Isolation of mRNA From Environmental Microbial Communities for Metatranscriptomic Analyses	569
<i>Peer M. Schenk</i>	
63. Comparative Day/Night Metatranscriptomic Analysis of Microbial Communities in the North Pacific Subtropical Gyre	575
<i>Rachel S. Poretsky and Mary Ann Moran</i>	

64. The "Double-RNA" Approach to Simultaneously Assess the Structure and Function of a Soil Microbial Community	587
<i>Tim Urich and Christa Schleper</i>	
65. Soil Eukaryotic Diversity: A Metatranscriptomic Approach	597
<i>Roland Marmeisse, Julie Bailly, Coralie Damon, Frédéric Lehembre, Marc Lemaire, Micheline Wésolowski-Louvel, and Laurence Fraissinet-Tachet</i>	
Part 6C Metaproteomics	
66. Proteomics for the Analysis of Environmental Stress Responses in Prokaryotes	605
<i>Ksenia J. Groh, Victor J. Nesatyy, and Marc J.-F. Suter</i>	
67. Microbial Community Proteomics	627
<i>Paul Wilmes</i>	
68. Synchronicity between Population Structure and Proteome Profiles: A Metaproteomic Analysis of Chesapeake Bay Bacterial Communities	637
<i>Jinjun Kan, Thomas E. Hanson, and Feng Chen</i>	
69. High-Throughput Cyanobacterial Proteomics: Systems-Level Proteome Identification and Quantitation	645
<i>Saw Yen Ow and Phillip C. Wright</i>	
70. Protein Expression Profile of an Environmentally Important Bacterial Strain: The Chromate Response of <i>Arthrobacter</i> Species Strain FB24	663
<i>Kristene L. Henne, Joshua E. Turse, Cindy H. Nakatsu, and Allan E. Konopka</i>	
Part 6D Metabolomics	
71. The Small-Molecule Dimension: Mass-spectrometry-based Metabolomics, Enzyme Assays, and Imaging	677
<i>Trent R. Northen</i>	
72. Metabolomics: High-Resolution Tools Offer to Follow Bacterial Growth on a Molecular Level	683
<i>Lucio Marianna, Agnes Fekete, Moritz Frommberger, and Philippe Schmitt-Kopplin</i>	
73. Metabolic Profiling of Plant Tissues by Electrospray Mass Spectrometry	697
<i>Heather Walker</i>	
74. Metabolite Identification, Pathways, and Omic Integration Using Online Databases and Tools	709
<i>Matthew P. Davey</i>	



## Part 6E Single-Cell Analysis

<b>75. Application of Cytomics to Separate Natural Microbial Communities by their Physiological Properties</b>	<b>727</b>
<i>Susann Müller and David R. Johnson</i>	
<b>76. Capturing Microbial Populations for Environmental Genomics</b>	<b>735</b>
<i>Martha Schattenhofer and Annelie Wendeberg</i>	
<b>77. Microscopic Single-Cell Isolation and Multiple Displacement Amplification of Genomes from Uncultured Prokaryotes</b>	<b>741</b>
<i>Peter Westermann and Thomas Kvist</i>	

<b>Index</b>	<b>747</b>
--------------	------------

## Preface

In the last 25 years, microbiology and molecular microbial ecology have undergone drastic transformations that changed the microbiologist's view of how to study microorganisms. Previously, the main problem was the assumption that microorganisms needed to be culturable, in order to classify them and study their metabolic and organismal diversity. The heart of this transformation was the convincing demonstration that the yet-unculturable world was far greater than the culturable one. In fact, the number of microbial genomes has been estimated from 2000 to 18,000 genomes per gram of soil. In 1985, an experimental advance radically changed our perception of the microbial world. After Carl Woese showed that rRNA genes could be used to derive evolutionary relationships, phylogenetic "trees" and evolutionary chronometers, Norman Pace and colleagues created a new chapter in molecular microbial ecology, using the direct analysis of rRNA sequences in the environment to describe the diversity of microorganisms without culturing (Handelsman, 2004). The next major step forward was the development of the PCR reaction, to amplify rRNA genes for subsequent sequence analysis and classification. The subsequent major advance was the notion that one could extract total DNA or RNA from environmental samples, including culturable and yet unculturable organisms, and clone it into a suitable vector for introduction into a culturable organism, followed by analysis by using high throughput shotgun DNA sequencing of cloned DNA, or by direct sequencing. The idea of cloning DNA directly from environmental samples was first proposed by Page; this method was coined "metagenomics" by Handelsman et al. in 1994, and is now used in many laboratories worldwide to study diversity and for the isolation of novel medical and industrial compounds.

These recent studies are reviewed in this book and the companion book, *Handbook of Molecular Microbial Ecology II: Metagenomics in Different Habitats*. Instead of relying only on a limited number of (long) review articles on selected topics, this book provides reviews

as well as a large number of case studies, mostly based on original publications and written by expert "at-the-bench" scientists from more than 20 different countries. Both books highlight the databases and computer programs used in each study, by listing them at the end of the chapter, together with their sites. This special feature of both books, facilitates the computer-assisted analysis of the vast amount of data generated by metagenomic studies. In addition, metagenomic studies in a variety of habitats are described, primarily in Volume II, which present a large number of system dependent different approaches in greatly differing habitats. The latter also results in the presentation of multiple biological systems which are interesting to microbial ecologists and microbiologists in their own right. Both books should be of interest to scientists in the fields of soil, water, medicine and industry who are or are contemplating using metagenomics and complementary approaches to address academic, medical, or industrial questions about bacterial communities from varied habitats, but also to those interested in particular biological systems in general.

## ACKNOWLEDGMENTS

For their support of this project, I gratefully acknowledge:

The Laboratory for Plant Microbe Interactions (LIPM),



the Institut National de Recherche de Agriculture (INRA), and



the Centre National de Recherche Scientifique (CNRS).

I would like to thank Claude Bruand for his help with the computer work.

FRANS J. DE BRUIJN

Castanet, Tolosan, France  
March 2011