

---

**1. Introduction** 1

---

*Frans J. de Bruijn*

Part 1 Background Chapters

---

**2. DNA Reassociation Yields Broad-Scale Information on Metagenome Complexity and Microbial Diversity** 5

---

*Vigdis L. Torsvik and Lise Øvreås*

---

**3. Diversity of 23S rRNA Genes Within Individual Prokaryotic Genomes** 17

---

*Anna Pei, William E. Oberdorf, Carlos W. Nossa, Pooja Chokshi, Martin J. Blaser, Liying Yang, David M. Rosmarin, and Zhiheng Pei*

---

**4. Use of the rRNA Operon and Genomic Repetitive Sequences for the Identification of Bacteria** 29

---

*Andréa Maria Amaral Nascimento*

---

**5. Use of Different PCR Primer-Based Strategies for Characterization of Natural Microbial Communities** 41

---

*James I. Prosser, Shahid Mahmood, and Thomas E. Freitag*

---

**6. Horizontal Gene Transfer and Recombination Shape Mesorhizobial Populations in the Gene Center of the Host Plants *Astragalus Luteolus* and *Astragalus Ernestii* in Sichuan, China** 49

---

*Qiongfang Li, Xiaoping Zhang, Ling Zou, Qiang Chen, David P. Fewer, and Kristina Lindström*

---

**7. Amplified rDNA Restriction Analysis (ARDRA) for Identification and Phylogenetic Placement of 16S-rDNA Clones** 59

---

*Menachem Y. Sklarz, Roey Angel, Osnat Gillor, and Ines M. Soares*

*Satoshi Ishii, Koji Kadota, and Keishi Senoo*

Part 2 The Species Concept

|                                                                                          |           |
|------------------------------------------------------------------------------------------|-----------|
| <b>9. Population Genomics Informs Our Understanding of the Bacterial Species Concept</b> | <b>77</b> |
|------------------------------------------------------------------------------------------|-----------|

*Margaret A. Riley*

|                                                                          |           |
|--------------------------------------------------------------------------|-----------|
| <b>10. The Microbial Pangenome: Implications for Vaccine Development</b> | <b>83</b> |
|--------------------------------------------------------------------------|-----------|

*Annalisa Nuccitelli, Claudio Donati, Michèle A. Barocchi, and Rino Rappuoli*

|                                                        |           |
|--------------------------------------------------------|-----------|
| <b>11. Metagenomic Insights into Bacterial Species</b> | <b>89</b> |
|--------------------------------------------------------|-----------|

*Konstantinos T. Konstantinidis*

|                                                                                                        |           |
|--------------------------------------------------------------------------------------------------------|-----------|
| <b>12. Reports of Ad Hoc Committees for the Reevaluation of the Species Definition in Bacteriology</b> | <b>99</b> |
|--------------------------------------------------------------------------------------------------------|-----------|

*Erko Stackebrandt*

|                                                                               |            |
|-------------------------------------------------------------------------------|------------|
| <b>13. Metagenomic Approaches for the Identification of Microbial Species</b> | <b>105</b> |
|-------------------------------------------------------------------------------|------------|

*David M. Ward, Melanie C. Melendrez, Eric D. Becraft, Christian G. Klatt, Jason M. Wood, and Frederick M. Cohan*

Part 3 Metagenomics

|                                                         |            |
|---------------------------------------------------------|------------|
| <b>14. Microbial Ecology in the Age of Metagenomics</b> | <b>113</b> |
|---------------------------------------------------------|------------|

*Jianping Xu*

|                                                                      |            |
|----------------------------------------------------------------------|------------|
| <b>15. The Enduring Legacy of Small Subunit rRNA in Microbiology</b> | <b>123</b> |
|----------------------------------------------------------------------|------------|

*Susannah G. Tringe and Philip Hugenholtz*

|                                                                                            |            |
|--------------------------------------------------------------------------------------------|------------|
| <b>16. Pitfalls of PCR-Based rRNA Gene Sequence Analysis: An Update on Some Parameters</b> | <b>129</b> |
|--------------------------------------------------------------------------------------------|------------|

*Erko Stackebrandt*

|                                                                                                                                                     |            |
|-----------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| <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> |
|-----------------------------------------------------------------------------------------------------------------------------------------------------|------------|

*Sergio E. Morales and William E. Holben*

|                                                                                  |            |
|----------------------------------------------------------------------------------|------------|
| <b>18. The Impact of Next-Generation Sequencing Technologies on Metagenomics</b> | <b>143</b> |
|----------------------------------------------------------------------------------|------------|

*George M. Weinstock*

|                                                                          |            |
|--------------------------------------------------------------------------|------------|
| <b>19. Accuracy and Quality of Massively Parallel DNA Pyrosequencing</b> | <b>149</b> |
|--------------------------------------------------------------------------|------------|

*Susan M. Huse and David B. Mark Welch*

|                                                                                                                                                                                 |            |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| <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>                                                                                                                                           |            |

|                                                                                                                                  |            |
|----------------------------------------------------------------------------------------------------------------------------------|------------|
| <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 Jacquioud, 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, Maïke 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>                                                                                                      |            |

**54. Metagenomic Approaches in Systems Biology** 475

*María-Eugenia Guazzaroni and Manuel Ferrer*

**55. Towards “Focused” Metagenomics: A Case Study Combining DNA Stable-Isotope Probing, Multiple Displacement Amplification, and Metagenomics** 491

*Yin Chen, Marc G. Dumont, Joshua D. Neufeld, and J. Colin Murrell*

**56. Suppressive Subtractive Hybridization Reveals Extensive Horizontal Transfer in the Rumen Metagenome** 497

*Elizabeth A. Galbraith, Dionysios A. Antonopoulos, Karen E. Nelson, and Bryan A. White*

Part 6A Microarrays

**57. GeoChip: A High-Throughput Metagenomics Technology for Dissecting Microbial Community Functional Structure** 509

*Joy D. van Nostrand, Zhili He, and Jizhong Zhou*

**58. Phylogenetic Microarrays (PhyloChips) For Analysis of Complex Microbial Communities** 521

*Eoin L. Brodie*

**59. Phenomics and Phenotype Microarrays: Applications Complementing Metagenomics** 533

*Barry R. Bochner*

**60. Microbial Persistence in Low-Biomass, Extreme Environments: The Great Unknown** 541

*Parag Vaishampayan, James N. Benardini, Myron T. La Duc, and Kasthuri Venkateswaran*

**61. Application of Phylogenetic Oligonucleotide Microarrays in Microbial Analysis** 551

*Pankaj Trivedi and Nian Wang*

Part 6B Metatranscriptomics

**62. Isolation of mRNA From Environmental Microbial Communities for Metatranscriptomic Analyses** 569

*Peer M. Schenk*

**63. Comparative Day/Night Metatranscriptomic Analysis of Microbial Communities in the North Pacific Subtropical Gyre** 575

*Rachel S. Poretsky and Mary Ann Moran*

|                                                                                                                        |            |
|------------------------------------------------------------------------------------------------------------------------|------------|
| <b>64. The “Double-RNA” Approach to Simultaneously Assess the Structure and Function of a Soil Microbial Community</b> | <b>587</b> |
|------------------------------------------------------------------------------------------------------------------------|------------|

*Tim Ulrich and Christa Schleper*

|                                                                     |            |
|---------------------------------------------------------------------|------------|
| <b>65. Soil Eukaryotic Diversity: A Metatranscriptomic Approach</b> | <b>597</b> |
|---------------------------------------------------------------------|------------|

*Roland Marmeisse, Julie Bailly, Coralie Damon, Frédéric Lehembre, Marc Lemaire, Micheline Wésolowski-Louvel, and Laurence Fraissinet-Tachet*

## Part 6C Metaproteomics

|                                                                                         |            |
|-----------------------------------------------------------------------------------------|------------|
| <b>66. Proteomics for the Analysis of Environmental Stress Responses in Prokaryotes</b> | <b>605</b> |
|-----------------------------------------------------------------------------------------|------------|

*Ksenia J. Groh, Victor J. Nesatyy, and Marc J.-F. Suter*

|                                           |            |
|-------------------------------------------|------------|
| <b>67. Microbial Community Proteomics</b> | <b>627</b> |
|-------------------------------------------|------------|

*Paul Wilmes*

|                                                                                                                                               |            |
|-----------------------------------------------------------------------------------------------------------------------------------------------|------------|
| <b>68. Synchronicity between Population Structure and Proteome Profiles: A Metaproteomic Analysis of Chesapeake Bay Bacterial Communities</b> | <b>637</b> |
|-----------------------------------------------------------------------------------------------------------------------------------------------|------------|

*Jinjun Kan, Thomas E. Hanson, and Feng Chen*

|                                                                                                              |            |
|--------------------------------------------------------------------------------------------------------------|------------|
| <b>69. High-Throughput Cyanobacterial Proteomics: Systems-Level Proteome Identification and Quantitation</b> | <b>645</b> |
|--------------------------------------------------------------------------------------------------------------|------------|

*Saw Yen Ow and Phillip C. Wright*

|                                                                                                                                                          |            |
|----------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| <b>70. Protein Expression Profile of an Environmentally Important Bacterial Strain: The Chromate Response of <i>Arthrobacter</i> Species Strain FB24</b> | <b>663</b> |
|----------------------------------------------------------------------------------------------------------------------------------------------------------|------------|

*Kristene L. Henne, Joshua E. Turse, Cindy H. Nakatsu, and Allan E. Konopka*

## Part 6D Metabolomics

|                                                                                                           |            |
|-----------------------------------------------------------------------------------------------------------|------------|
| <b>71. The Small-Molecule Dimension: Mass-spectrometry-based Metabolomics, Enzyme Assays, and Imaging</b> | <b>677</b> |
|-----------------------------------------------------------------------------------------------------------|------------|

*Trent R. Northen*

|                                                                                                      |            |
|------------------------------------------------------------------------------------------------------|------------|
| <b>72. Metabolomics: High-Resolution Tools Offer to Follow Bacterial Growth on a Molecular Level</b> | <b>683</b> |
|------------------------------------------------------------------------------------------------------|------------|

*Lucio Marianna, Agnes Fekete, Moritz Frommberger, and Philippe Schmitt-Kopplin*

|                                                                                   |            |
|-----------------------------------------------------------------------------------|------------|
| <b>73. Metabolic Profiling of Plant Tissues by Electrospray Mass Spectrometry</b> | <b>697</b> |
|-----------------------------------------------------------------------------------|------------|

*Heather Walker*

|                                                                                                       |            |
|-------------------------------------------------------------------------------------------------------|------------|
| <b>74. Metabolite Identification, Pathways, and Omic Integration Using Online Databases and Tools</b> | <b>709</b> |
|-------------------------------------------------------------------------------------------------------|------------|

*Matthew P. Davey*

|                                                                                                                             |            |
|-----------------------------------------------------------------------------------------------------------------------------|------------|
| <b>75. Application of Cytomics to Separate Natural Microbial Communities by their Physiological Properties</b>              | <b>727</b> |
| <hr/>                                                                                                                       |            |
| <i>Susann Müller and David R. Johnson</i>                                                                                   |            |
| <b>76. Capturing Microbial Populations for Environmental Genomics</b>                                                       | <b>735</b> |
| <hr/>                                                                                                                       |            |
| <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> |
| <hr/>                                                                                                                       |            |
| <i>Peter Westermann and Thomas Kvist</i>                                                                                    |            |
| <b>Index</b>                                                                                                                | <b>747</b> |