

Contents

Contributors	xi
Abbreviations	xii
Preface	xiii
1 Nucleic acid extraction from environmental samples	1
<i>Annett Milling, Newton C. M. Gomes, Miruna Oros-Sichler, Monika Götz and Kornelia Smalla</i>	
1.1 Introduction	1
1.2 Recovery of cells from environmental matrices	2
1.3 Cell lysis and DNA extraction protocols	3
1.4 Immunochemical isolation of DNA from metabolically active cells	5
1.5 RNA extraction from environmental matrices	6
Protocol 1.1	11
Protocol 1.2	17
Protocol 1.3	21
2 Prokaryotic systematics: PCR and sequence analysis of amplified 16S rRNA genes	25
<i>Wilfred F. M. Röling and Ian M. Head</i>	
2.1 Introduction	25
2.2 Application of PCR in microbial ecology	26
2.2.1 Good working practices in PCR: avoiding PCR inhibition and contamination	28
2.2.2 Pitfalls of PCR: artifacts and differential amplification	30
2.2.3 rRNA versus rDNA: indicative of active versus total microbial communities?	32
2.3 Generation and analysis of clone libraries	34
2.3.1 Generation of clone libraries	34
2.3.2 Screening of clone libraries	35
2.4 Quantitative analysis of clone libraries: coverage and diversity indices	38
2.4.1 Coverage and sampling	38
2.4.2 Diversity indices	40
2.5 Phylogenetic inference	41
2.5.1 Sequencing	41
2.5.2 Phylogenetic analysis	42
2.5.3 Parsimony	44
2.5.4 Distance methods	46

2.5.5	Maximum likelihood	47
2.5.6	Bootstrap analysis	50
2.6	Phylogenetic analysis compared to other molecular techniques	50
2.6.1	Comparing large numbers of samples	51
2.6.2	Quantification	51
2.6.3	Relationship between phylogenetic information and function	52
2.7	Concluding remarks	52
	Protocol 2.1	57
3	DNA fingerprinting of microbial communities	65
	<i>Andreas Felske and A. Mark Osborn</i>	
3.1	Introduction	65
3.1.1	DGGE	66
3.1.2	TGGE	68
3.1.3	SSCP analysis	68
3.1.4	T-RFLP analysis	69
3.1.5	LH-PCR (length heterogeneity PCR)	70
3.1.6	Comparison of the different methods	71
3.2	Applications of microbial community fingerprinting	74
3.2.1	Combination of cloning with fingerprinting	76
3.2.2	Screening isolated strains next to fingerprints	76
3.2.3	Southern blot hybridization and fingerprints	77
3.2.4	Multiple competitor RT-PCR/TGGE for rRNA	78
3.2.5	Environmental fingerprints with protein-coding genes	79
3.3	Analyzing microbial community fingerprints	79
3.3.1	Cutting out fingerprint bands for sequencing	80
3.3.2	The rRNA sequence heterogeneity	81
3.3.3	Quantitative analysis of fingerprint signals	82
3.3.4	Cluster analysis of DNA fingerprint signals	84
3.3.5	Diversity and species richness	84
3.3.6	The 'operational taxonomic unit'	85
3.4	Miscellaneous advice	85
	Protocol 3.1	91
4	Molecular typing of environmental isolates	97
	<i>Jan L. W. Rademaker, Henk J. M. Aarts and Pablo Vinuesa</i>	
4.1	Introduction	97
4.1.1	Role of PCR amplification-based typing for microbial ecology	98
4.2	Microbial typing methods	98
4.2.1	AFLP analysis	99
4.2.2	rep-PCR fingerprinting	103
4.2.3	Mini- and microsatellite fingerprinting	106
4.2.4	Fingerprinting methods using random primers (RAPD, AP-PCR, DAF)	106
4.2.5	Ribosomal RNA gene fingerprinting methods	108
4.3	Computer-assisted analysis of PCR-generated fingerprint profiles	113
4.4	Concluding remarks	114
	Protocol 4.1	127
	Protocol 4.2	131

5	RT-PCR and mRNA expression analysis of functional genes	135
	<i>Balbina Nogales</i>	
5.1	Introduction	135
5.2	Advantages and limitations of the RT-PCR analysis of bacterial functional genes	136
5.3	The RT-PCR reaction	137
5.3.1	The RNA template	137
5.3.2	Primers for reverse transcription	138
5.3.3	Reverse transcriptases	138
5.3.4	One-step and two-step RT-PCR protocols	139
5.4	Quantitative RT-PCR	140
5.5	Analysis of global gene expression	140
5.6	Conclusions	141
	Protocol 5.1	145
	Protocol 5.2	146
6	Quantitative real-time PCR	151
	<i>Cindy J. Smith</i>	
6.1	Introduction	151
6.2	Quantification	152
6.3	qPCR chemistries	154
6.3.1	<i>Taq</i> nuclease assay	154
6.3.2	SYBR green	156
6.4	Applications	156
6.4.1	qPCR analysis of total microbial communities via analysis of amplified ribosomal RNA genes	156
6.4.2	Functional ecology	157
	Protocol 6.1	161
7	Stable-isotope probing	167
	<i>Stefan Radajewski and J. Colin Murrell</i>	
7.1	Introduction	167
7.2	Stable-isotope labeling of DNA	168
7.3	Application of stable-isotope probing	169
7.3.1	Methylotroph populations	169
7.3.2	Ammonia-oxidizing populations	170
7.4	Future prospects	170
	Protocol 7.1	173
8	Applications of nucleic acid hybridization in microbial ecology	179
	<i>A. Mark Osborn, Vivien Prior and Konstantinos Damianakis</i>	
8.1	Introduction	179
8.2	Fundamentals of DNA hybridization	180
8.2.1	Probe design	180
8.2.2	Choice of nucleic acid template	184
8.3	Hybridization applications in microbial ecology	186
8.3.1	Hybridization analysis of cultured bacteria	186

8.3.2	Hybridization analysis of total community DNA	187
8.3.3	Reverse sample genome probing (RSGP) and microarray analysis of microbial communities	189
Protocol 8.1		198
Protocol 8.2		207
Protocol 8.3		211
9	Fluorescence <i>in situ</i> hybridization for the detection of prokaryotes	213
	<i>Holger Daims, Kilian Stoecker and Michael Wagner</i>	
9.1	Introduction	213
9.2	Fundamentals of rRNA FISH	214
9.3	Structure–function analyses: combination of FISH with other techniques	217
Protocol 9.1		223
10	Lessons from the genomes: microbial ecology and genomics	241
	<i>Andrew S. Whiteley, Mike Manefield, Sarah L. Turner and Mark J. Bailey</i>	
10.1	Introduction	241
10.2	Structural genomics	242
10.3	Comparative genomics	242
10.3.1	Small genomes	243
10.3.2	Large genomes	244
10.3.3	The horizontal gene pool	244
10.3.4	Insights into phylogeny	245
10.4	Functional genomics in microbial ecology	246
10.4.1	Characterizing microbial responses to environmental change	246
10.4.2	Relating specific genes to specific functions	247
10.4.3	Expression of genes from components of a genome	248
10.5	The application of genomic tools <i>in situ</i>	249
10.5.1	Organism diversity	249
10.5.2	Functional genes	250
10.5.3	Metagenomics	250
10.5.4	Functional gene arrays	252
10.5.5	Diversity, function and process	253
10.6	Lessons learned and future perspectives	254
11	Metagenomic libraries from uncultured microorganisms	261
	<i>Doreen E. Gillespie, Michelle R. Rondon, Lynn L. Williamson and Jo Handelsman</i>	
11.1	Introduction	261
11.2	Factors affecting experimental design	261
11.2.1	Environmental source	262
11.2.2	DNA isolation	264
11.2.3	Vector selection	264
11.2.4	Host cell selection	264
11.3	Summary	265
Protocol 11.1		269

12 A molecular toolbox for bacterial ecologists: PCR primers for functional gene analysis	281
<i>Michael J. Larkin, A. Mark Osborn and Derek Fairley</i>	
12.1 Introduction	281
12.2 Adopting a strategy to detect sequences from the environment	283
12.3 Primer and probe design	285
12.4 PCR primers to amplify 16S rRNA gene sequences	286
12.5 PCR primers for detecting functional genes	287
13 Molecular detection of fungal communities in soil	303
<i>Eric Smit, Francisco de Souza and Renske Landeweert</i>	
13.1 Introduction	303
13.2 Molecular strategies for fungal community analyses	304
13.3 DNA extraction	305
13.4 Target genes	306
13.5 Primers for fungal populations	307
13.6 <i>In silico</i> analysis of 18S rRNA primers	310
13.7 Specific application of molecular identification techniques for studying mycorrhizal fungi	311
13.7.1 Analyzing arbuscular mycorrhizal fungal diversity	311
13.7.2 Analyzing ectomycorrhizal fungal diversity	312
13.8 Conclusions	313
Protocol 13.1	317
14 Environmental assessment: bioreporter systems	321
<i>Steven A. Ripp and Gary S. Sayler</i>	
14.1 Introduction	321
14.2 Reporter systems	322
14.2.1 β -Galactosidase (<i>lacZ</i>)	322
14.2.2 Chloramphenicol acetyltransferase (CAT)	322
14.2.3 Catechol 2,3-dioxygenase (<i>xylE</i>)	322
14.2.4 β -Lactamase (<i>bla</i>)	323
14.2.5 β -Glucuronidase (<i>gusA</i> , <i>gusA</i> , <i>uidA</i>)	324
14.2.6 Ice nucleation (<i>inaZ</i>)	324
14.2.7 Green fluorescent protein (GFP)	324
14.2.8 Aequorin	326
14.2.9 Uroporphyrinogen (Urogen) III methyltransferase (UMT)	326
14.2.10 Luciferases	326
14.3 Mini-transposons as genetic tools in biosensor construction	330
Case study	343
15 Bioinformatics and web resources for the microbial ecologist	345
<i>Wolfgang Ludwig</i>	
15.1 Introduction	345
15.2 Databases	347
15.2.1 European Bioinformatics Institute (EBI) databases	347
15.2.2 GenBank	358

15.3	Genome projects	359
15.3.1	Munich Information Center for Protein Sequences (MIPS)	359
15.3.2	Sanger Institute	359
15.3.3	The Institute for Genomic Research (TIGR)	359
15.3.4	Mobile genetic elements (MGE) databases	360
15.4	Ribosomal RNA databases	360
15.4.1	Ribosomal Database Project	360
15.4.2	European Ribosomal RNA database	362
15.5	rRNA-targeted probes	363
15.5.1	ProbeBase	363
15.5.2	Roscoff database	363
15.5.3	PRIMROSE	363
15.6	ARB project	363
15.6.1	ARB databases	364
15.6.2	Phylogenetic treeing	366
15.6.3	Future developments	369
15.7	Concluding remarks	369
	Index	373