Contents

Ac	know	ledgments	xiii
1	Intr	roduction to environmental DNA (eDNA)	1
	1.1	Definitions	1
	1.2	A brief history of eDNA analysis	2
		Constraints when working with eDNA	3
		Workflow in eDNA studies and main methods used	4
	1.5	Environmental DNA as a monitoring tool	5
2	DN/	A metabarcode choice and design	7
	2.1	Which DNA metabarcode?	7
	2.2	Properties of the ideal DNA metabarcode	8
	2.3	In silico primer design and testing	9
		2.3.1 Prerequisites	10
		2.3.2 Reference sequences: description, filtering, and formatting	
		for ecoPrimers	10
		2.3.3 In silico primer design with ecoPrimers	11
		2.3.3.1 The ecoPrimers output	11
		2.3.4 In silico primer testing with ecoPCR	11
		2.3.4.1 The ecoPCR output	14
		2.3.4.2 Filtering of the ecoPCR output	16
		2.3.4.3 Evaluation of primer conservation	16
		2.3.4.4 Taxonomic resolution and Bs index	17
	2.4	Examples of primer pairs available for DNA metabarcoding	19
3	Ref	erence databases	21
	3.1	Extracting reference databases from EMBL/GenBank/DDBJ	21
		3.1.1 Downloading a local copy of EMBL	21
		3.1.2 Identifying sequences corresponding to the relevant metabarcode	23
	3.2	Marker-specific reference databases	23
		3.2.1 Nuclear rRNA gene reference databases	23
		3.2.2 Eukaryote-specific databases	24
	3.3	Building a local reference database	25
		3.3.1 PCR-based local reference database	26
		3.3.2 Shotgun-based local reference database	27
	3.4	Current challenges and future directions	. 27

4	Sam	bling	28		
	4.1	The cycle of eDNA in the environment	28		
		4.1.1 State and origin	28		
		4.1.2 Fate	29		
		4.1.3 Transport	29		
	4.2	Sampling design	30		
		4.2.1 Focusing on the appropriate DNA population	31		
		4.2.2 Defining the sampling strategy	32		
	4.3	Sample preservation	33		
5	DNA extraction				
	5.1	From soil samples	35		
		From sediment	39		
	5.3	From litter	39		
	5.4	From fecal samples	39		
	5.5	From water samples	40		
6	DNA	amplification and multiplexing	41		
	6.1	Principle of the PCR	41		
	6.2	Which polymerase to choose?	43		
	6.3	The standard PCR reaction	44		
	6.4	The importance of including appropriate controls	45		
		6.4.1 Extraction negative controls	45		
		6.4.2 PCR negative controls	45		
		6.4.3 PCR positive controls	46		
		6.4.4 Tagging system controls	46 46		
	< F	6.4.5 Internal controls	40 46		
	6.5 6.6	PCR optimization How to limit the risk of contamination?	40		
	6.7	Blocking oligonucleotides for reducing the amplification of undesirable	40		
		sequences	50		
	6. 8	-	51		
	6.9	Multiplexing several metabarcodes within the same PCR	52		
	6.10	Multiplexing many samples on the same sequencing lane	52		
		6.10.1 Overview of the problem	52		
		6.10.2 Strategy 1: single-step PCR with Illumina adapters	54		
		6.10.3 Strategy 2: two-step PCR with Illumina adapters	55		
		6.10.4 Strategy 3: single-step PCR with tagged primers	55		
7	7 DN	A sequencing	58		
	7.1	Overview of the first, second, and third generations of sequencing			
		technologies	58		
	7.2	The Illumina technology	59		
		7.2.1 Library preparation	59		
		7.2.2 Flow cell, bridge PCR, and clusters	60		

		7.2.3	Sequencing by synthesis	62
		7.2.4	Quality scores of the sequence reads	63
8	DNA	metab	parcoding data analysis	65
	8.1	Basic s	sequence handling and curation	65
		8.1.1	Sequencing quality	65
			8.1.1.1 The pros and cons of read quality-based filtering	65
			8.1.1.2 Quality trimming software	68
		8.1.2	Paired-end read pairing	68
		8.1.3	1 1 0	69
		8.1.4	1 1	69
		8.1.5	0 1	70
	8.2	-	nce classification	70
		8.2.1	Taxonomic classification	71
		8.2.2	+	73
	~ ~	8.2.3		75
	8.3	-	g advantages of experimental controls	76
		8.3.1	Filtering out potential contaminants	76
		8.3.2	Removing dysfunctional PCRs	78
	8.4		al considerations on ecological analyses	80
		8.4.1	Sampling effort and representativeness	81 01
			8.4.1.1 Evaluating representativeness of the sequencing per PCR	81
			8.4.1.2 Evaluating representativeness at the sampling unit or site level	81
		8.4.2	Handling samples with varying sequencing depth	83
		8.4.2 8.4.3	Going further and adapting the ecological models to metabarcoding	83 84
		0.4.0	Conte futurer and adapting the ecological models to metabarcounte	01
9	Sing	le-spec	ies detection	85
	9.1	Princi	ple of the quantitative PCR (qPCR)	85
		9.1.1	Recording amplicon accumulation in real time via fluorescence	
			measurement	85
		9.1 .2		86
		9.1.3	~ 0 1	86
	9.2	•	n and testing of qPCR barcodes targeting a single species	87
		9.2.1	1 1 5	87
		9.2.2	1 1 1	88
			Candidate qPCR barcodes	88
	9.3		ional experimental considerations	88
		9.3.1	General issues associated with sampling, extraction, and PCR	~~
			amplification	88
		9.3.2	The particular concerns of contamination and inhibition	88
10	Envi	ronmer	ntal DNA for functional diversity	90
	10.1	Functi	onal diversity from DNA metabarcoding	90
			Functional inferences	90
		10.1.2	Targeting active populations	92

	10.2	Metagenomics and metatranscriptomics: sequencing more than a barcode	93
		10.2.1 General sampling constraints	94
		10.2.1.1 Optimization of the number of samples	94
		10.2.1.2 Enrichment in target organisms	94
		10.2.1.3 Enrichment in functional information	95
		10.2.2 General molecular constraints	9 6
		10.2.3 From sequences to functions	96
		10.2.3.1 Assembling (or not) a metagenome	97
		10.2.3.2 Sorting contigs or reads in broad categories	97
		10.2.3.3 Extracting functional information via taxonomic inferences	98
		10.2.3.4 Functional annotation of metagenomes	98
11	Som	e early landmark studies	99
	11.1	Emergence of the concept of eDNA and first results on microorganisms	99
		Examining metagenomes to explore the functional information	
		carried by eDNA	100
	11.3	Extension to macroorganisms	10 2
12	Fres	hwater ecosystems	104
	12.1	Production, persistence, transport, and detectability of eDNA	
		in freshwater ecosystems	104
		12.1.1 Production	104
		12.1.2 Persistence	104
		12.1.3 Transport/diffusion distance	105
		12.1.4 Detectability	106
	12.2	Macroinvertebrates	106
	1 2.3	Diatoms and microeukaryotes	106
		Aquatic plants	107
	12.5	Fish, amphibians, and other vertebrates	107
		12.5.1 Species detection	107
		12.5.2 Biomass estimates	108
	12.6	Are rivers conveyer belts of biodiversity information?	108
13	Mar	ine environments	110
	1 3.1	Environmental DNA cycle and transport in marine ecosystems	110
	13.2	Marine microbial diversity	111
	13.3	Environmental DNA for marine macroorganisms	112
14	Terr	restrial ecosystems	114
	14.1	Detectability, persistence, and mobility of eDNA in soil	114
	14.2	Plant community characterization	116
	14.3	Earthworm community characterization	117
	14.4	Bacterial community or metagenome characterization	117
	14.5	Multitaxa diversity surveys	119

15 Pale	eoenvire	onments	121
15.1	Lake s	sediments	121
	15.1.1	Pollen, macrofossils, and DNA metabarcoding	121
	15.1.2	Plants and mammals from Lake Anterne	121
	15.1.3	Viability in the ice-free corridor in North America	122
15.2	Perma	afrost	125
	15.2.1	Overview of the emergence of permafrost as a source of eDNA	125
	15.2.2	Large-scale analysis of permafrost samples for reconstructing	
		past plant communities	125
15.3		eological midden material	126
	15.3.1	Bulk archaeological fish bones from Madagascar	126
	15.3.2	Midden from Greenland to assess past human diet	126
6 Hos	t-associ	iated microbiota	127
16.1	DNA	dynamics	1 27
		molecular-based works	127
16.3	Post-h	olobiont works	128
7 Diet	t analys	is	131
17.1	Some	seminal diet studies	131
	17.1.1	Proof of concept—analyzing herbivore diet using next-generation	
		sequencing	131
	17.1.2	Assessing the efficiency of conservation actions in Białowieża	
		forest	132
	17.1.3	Characterizing carnivore diet, or how to disentangle predator	
		and prey eDNA	133
	17.1.4	Analyzing an omnivorous diet, or integrating several diets	
		in a single one	133
17.2	Metho	odological and experimental specificities of eDNA diet analyses	135
	17.2.1	eDNA sources	135
		17.2.1.1 Feces	135
		17.2.1.2 Gut content	135
		17.2.1.3 Whole body	135
	17.2.2	Quantitative aspects	136
		17.2.2.1 Relationship between the amount of ingested food and	
		DNA quantity in the sample	136
		17.2.2.2 Quantifying DNA with PCR and next-generation	
		sequencing	137
		17.2.2.3 Empirical correction of abundances	138
		Diet as a sample of the existing biodiversity	138
	17.2.4	Problematic diets	139
8 Ana	lysis of	bulk samples	140
18.1	What	is a bulk sample?	140
18.2	Case s	studies	140

		18.2.1	Bulk insect samples for biodiversity monitoring	140		
		18.2.2	Nematode diversity in tropical rainforest	141		
		18.2.3	Marine metazoan diversity in benthic ecosystems	141		
	18.3	3 Metabarcoding markers for bulk samples				
			ative strategies	143		
19	The	e future of eDNA metabarcoding				
	19.1	19.1 PCR-based approaches				
		19.1.1	Single-marker approach	144		
		19.1.2	Multiplex approach	144		
	19.2	Shotgu	un-based metabarcoding	145		
		19.2.1	Without enrichment by capture	145		
		19.2.2	With enrichment by capture	146		
	19.3	Towar	d more standardization	146		
		19.3.1	For sound comparisons across studies	146		
			For environmental monitoring	147		
	19.4	Next-§	generation reference databases	148		
	19.5	Open questions				
		19.5.1	What will be the impact of new sequencing technologies			
			on eDNA analysis?	14 8		
		19.5.2	Will some specific repositories be developed for DNA			
			metabarcoding?	148		
			Will metabarcoding provide quantitative results?	149		
		19.5.4	Will metabarcoding be fully integrated into ecological models and theories?	150		
		1955	How do we train students and managers to effectively integrate	100		
		17.0.0	this tool into academic and operational ecological research and			
			monitoring?	150		
Ар	pendi	x 1		151		
-	pendi:			217		
•	pendi			220		
Re	ferenc	es		223		
inc	lex			247		