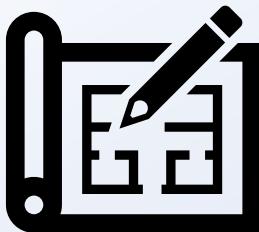




Workflow considerations

Sten Anslan <sten.anslan@ut.ee>





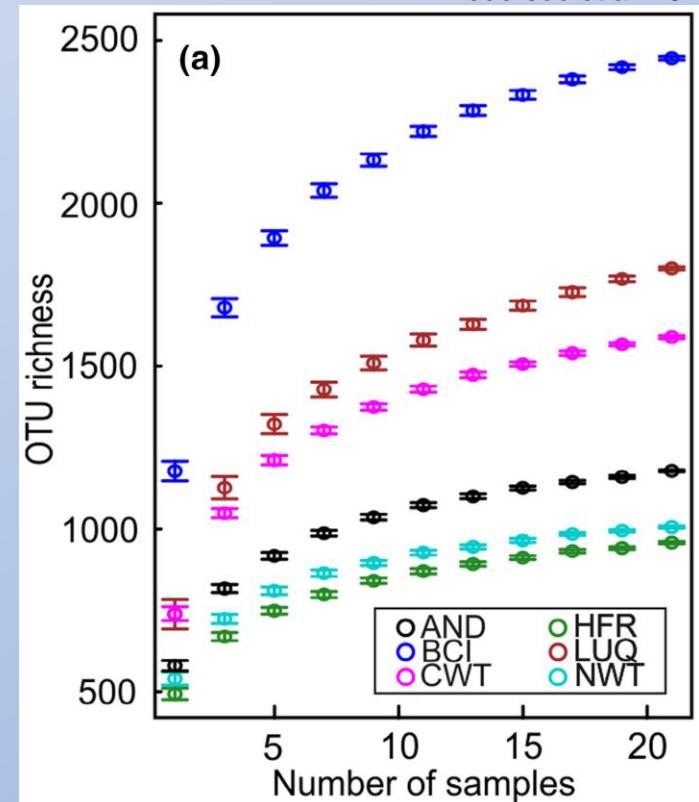
Planning a metabarcoding study

- Experimental design
- Replicates – representativeness, spatiotemporal independence of replicates
(*spatial autocorrelation range for soil fungi ~5–10 meters*)
- Pooling
 - when covering large geographic scale;
 - temporal sampling (single samples can't be resampled)

Post-field working space:

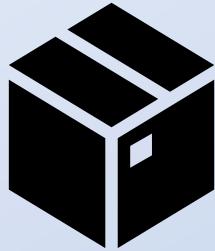
- Sample processing
- DNA extraction
- PCR
- Post-PCR

Tedersoo et al. 2022





Sampling and storing



- Avoid contamination – wear gloves; clean sampling tools (e.g with bleach, DNase, flame; **NOT ONLY EtOH**).
- Homogenize pooled subsamples

Storage

- Freezing: **-80 °C (<-20 °C)**; „The Golden Standard“ ~~freeze-thaw~~
- Drying:
 - freeze drying
 - silica gel: **only for few grams**
 - active air-drying at room temperature, oven, lamps [$<37\text{ °C}$].
Store in dark, constant temp.
- Liquid preservatives – e.g. >95% EtOH for bulk samples

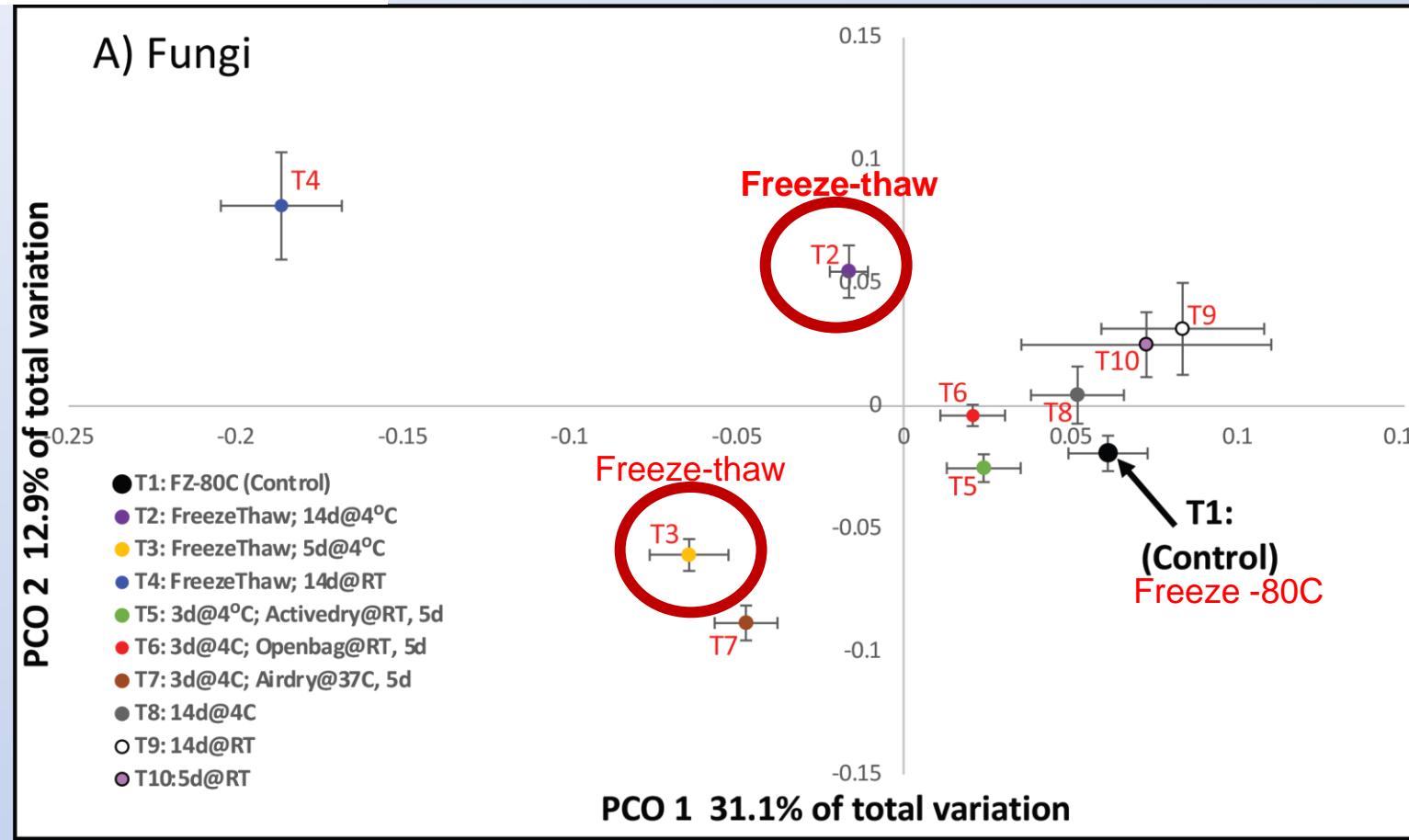


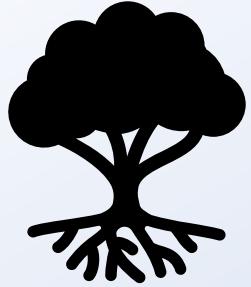
**Soil stabilisation for DNA metabarcoding of plants and fungi.
Implications for sampling at remote locations or via third-parties**

Lina A. Clasen¹, Andrew P. Detheridge¹, John Scullion¹, Gareth W. Griffith¹

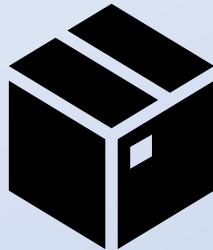
¹ Institute of Biological, Environmental and Rural Sciences, Aberystwyth University; Adeilad Cledwyn, Penglais, Aberystwyth, Ceredigion SY23 3DD, Wales, UK

Clasen et al. 2020





Sampling and storing



Short term storage:
storing soil samples at 4°C (~14 days)

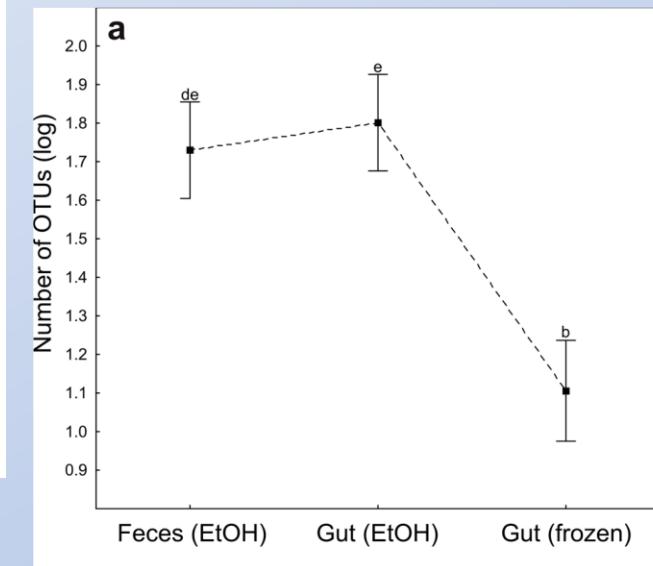
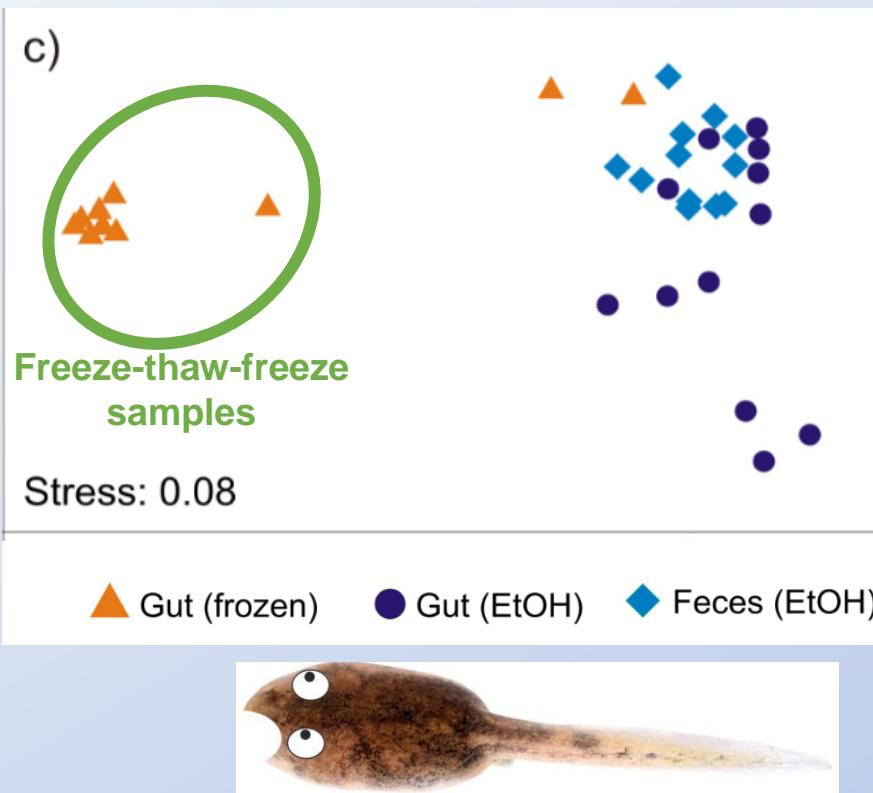
ORIGINAL RESEARCH

Ecology and Evolution Open Access WILEY

Keeping it cool: Soil sample cold pack storage and DNA shipment up to 1 month does not impact metabarcoding results

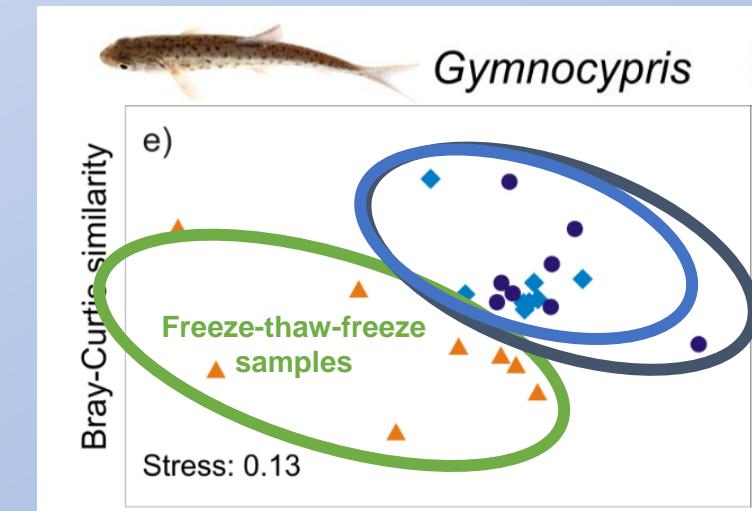
Camille S. Delavaux^{1,2}  | James D. Bever^{1,2} | Erin M. Karppinen³  | Luke D. Bainard³

Sample storage



Microbiomes from feces vs. gut in tadpoles:
distinct community compositions between
substrates and preservation methods

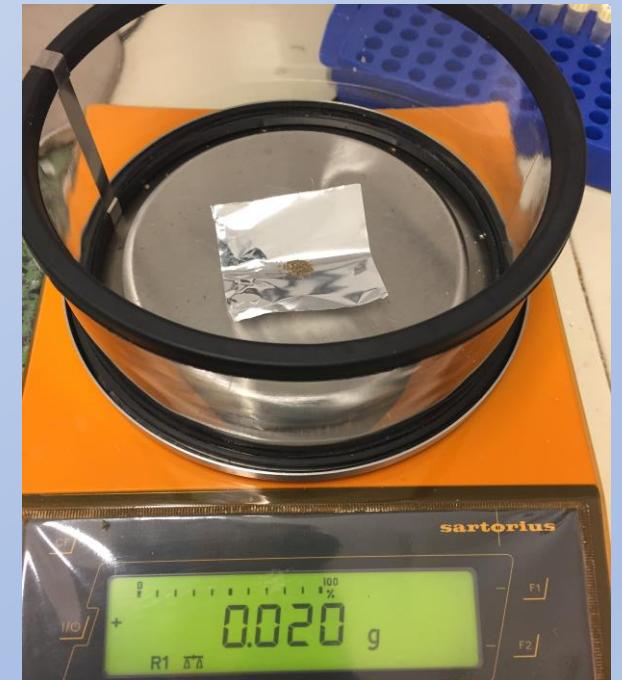
STEN ANSLAN¹, HUAN LI², SVEN KÜNZEL³ & MIGUEL VENCES¹





Pre-molecular processing

- Dedicated working space
- Homogenize the sample prior DNA extraction
- Include blank controls



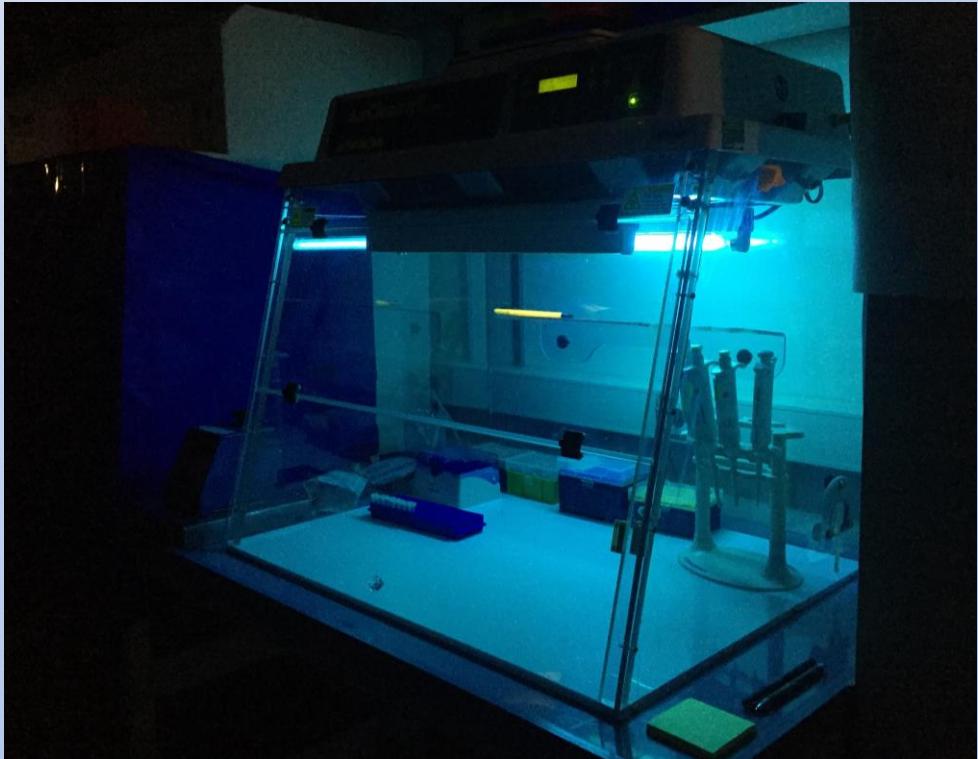


Molecular analysis

DNA extraction

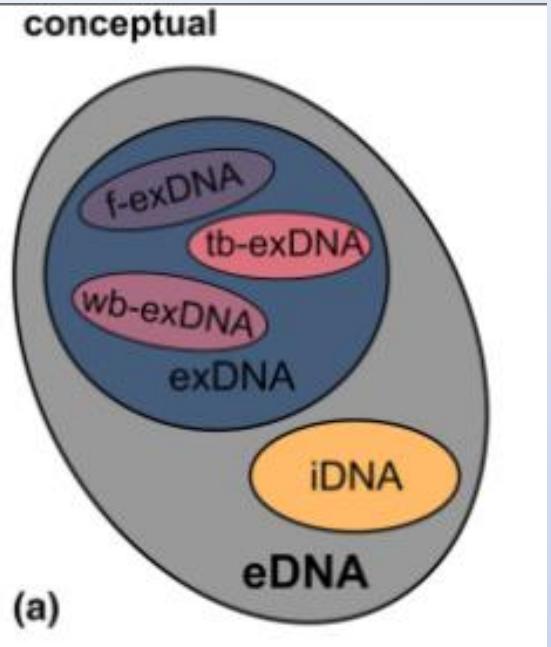
- Dedicated working space for DNA extraction
- Blank DNA extractions (controls)

Consider removal extracellular DNA for e.g. time-series analyses!





Molecular analysis



Nagler et al. 2022

exDNA may account for up to **60%** of the total **soil** eDNA (Nagler, Insam, et al., [2018](#)) and up to **90%** of the total **marine** eDNA pool (Torti et al., [2015](#)).

Temporal and spatial analyses!!!

Potential false positives

MOLECULAR ECOLOGY RESOURCES

NEWS AND VIEWS | Open Access | ⓘ

Why eDNA fractions need consideration in biomonitoring

Magdalena Nagler, Sabine Marie Podmirseg, Judith Ascher-Jenull, Daniela Sint, Michael Traugott

First published: 02 June 2022 | <https://doi.org/10.1111/1755-0998.13658>

Magdalena Nagler and Sabine Marie Podmirseg contributed equally to this study.

Handling Editor: Andrew P. Kinziger

fast	environmental cell lysis
slow	exDNA degradation
	current organisms
	past / foreign organisms
	total eDNA
	iDNA
	exDNA

Nagler et al. 2022



Molecular analysis

DNA extraction



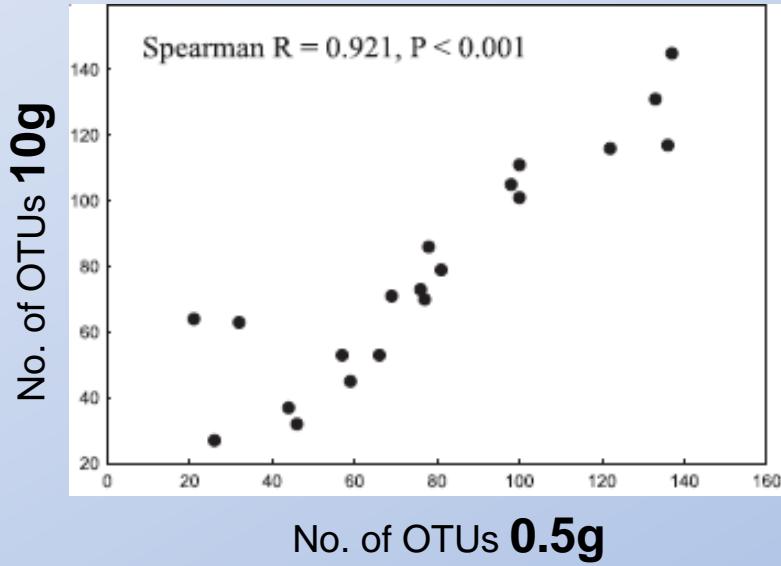
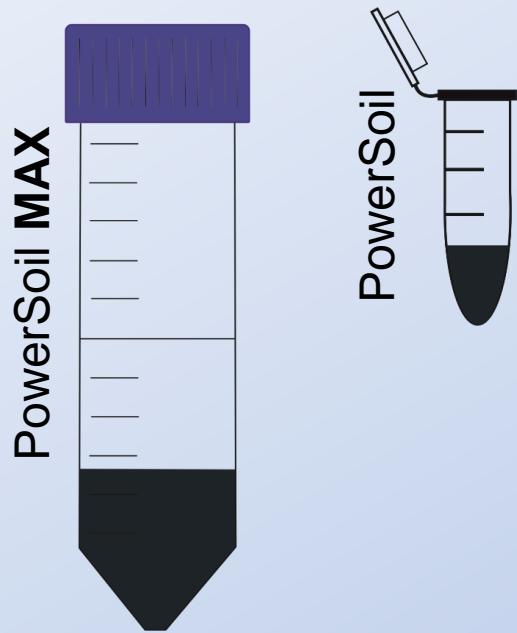
X grams ???





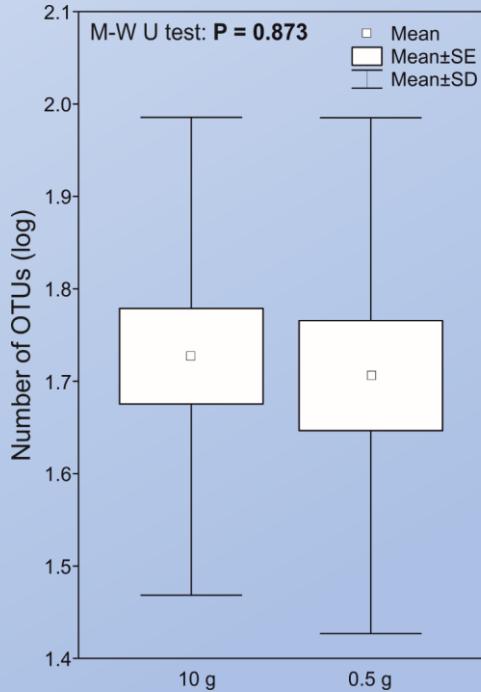
10 grams vs 0.5 grams of substrate

- differences in detecting diatom **richness?**



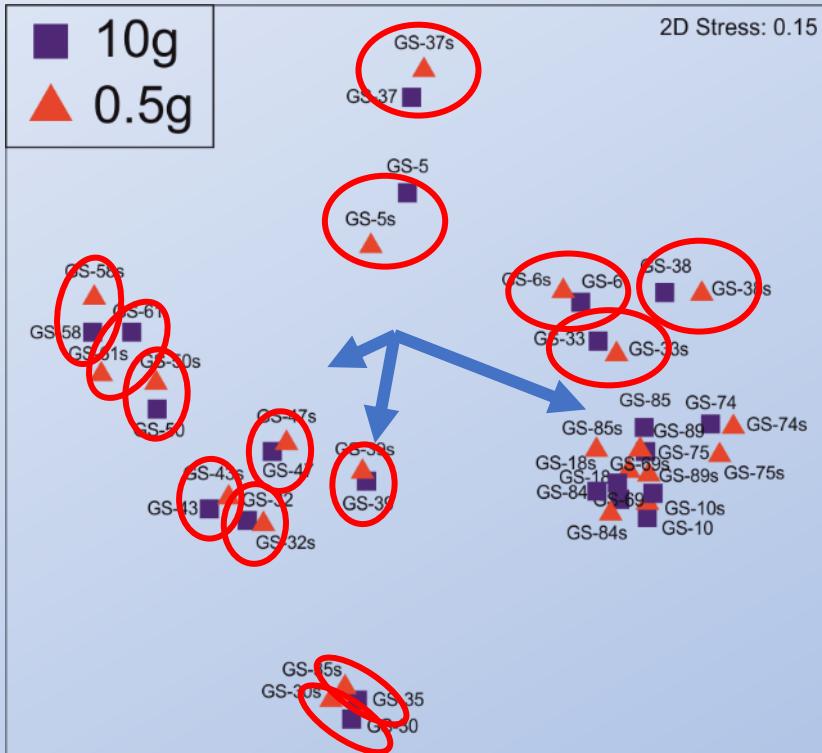
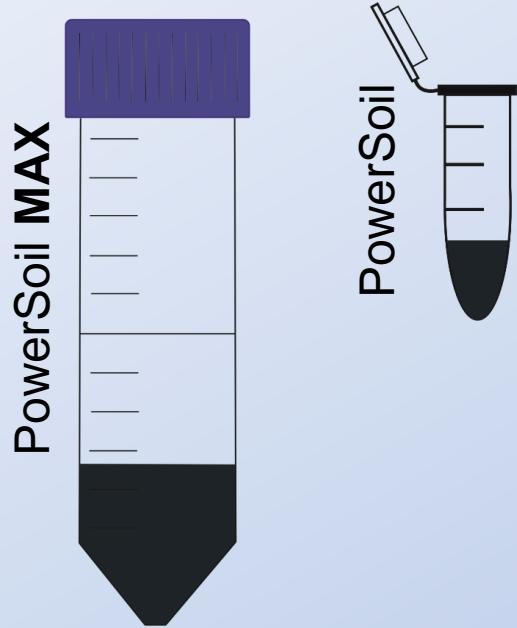
Diatom metabarcoding and microscopic analyses from sediment samples at Lake Nam Co, Tibet: The effect of sample-size and bioinformatics on the identified communities

Wengang Kang^a, Sten Anslan^{b,*}, Nicole Börner^a, Anja Schwarz^a, Robin Schmidt^b, Sven Künzel^c, Patrick Rioual^{d,f}, Paula Echeverría-Galindo^a, Miguel Vences^b, Junbo Wang^c, Antje Schwalb^a



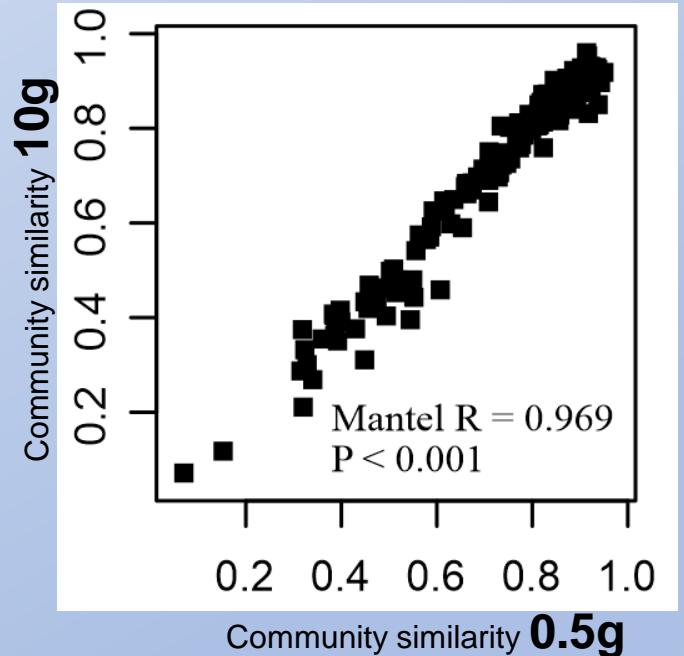
10 grams vs 0.5 grams of substrate

- differences in diatom **community structure?**



Diatom metabarcoding and microscopic analyses from sediment samples at Lake Nam Co, Tibet: The effect of sample-size and bioinformatics on the identified communities

Wengang Kang^a, Sten Anslan^{b,*}, Nicole Börner^a, Anja Schwarz^a, Robin Schmidt^b, Sven Künzel^c, Patrick Rioual^{d,f}, Paula Echeverría-Galindo^a, Miguel Vences^b, Junbo Wang^e, Antje Schwall^a





frontiers
in Microbiology

10 g



ORIGINAL RESE
published: 02 Jui
doi:10.3389/fmicb.2016

Available online at www.sciencedirect.com

SCIENCE @ DIRECT®

Journal of Microbiological Methods 66 (2006) 242–250

Journal
of Microbiological
Methods

www.elsevier.com/locate/jmicmeth

Size Matters: Assessing Optimum Soil Sample Size for Fungal and Bacterial Community Structure Analyses Using High Throughput Sequencing of rRNA Gene Amplicons

C. Ryan Penton^{1,2*}, Vadakattu V. S. R. Gupta³, Julian Yu¹ and James M. Ti...

The effect of sample size in studies of soil microbial community structure

Sanghoon Kang¹, Aaron L. Mills *

Laboratory of Microbiology, Department of Environmental Sciences, 291 McCormick Road, P.O. Box 400123, University of Virginia, Charlottesville, VA 22904-4123, USA

Received 10 October 2005; revised form 30 November 2005; accepted 30 November 2005
Available online 19 January 2006

PLOS ONE

0.25 g

Effort versus Reward: Preparing Samples for Fungal Community Characterization in High-Throughput Sequencing Surveys of Soils

Zewei Song , Dan Schlatter, Peter Kennedy, Linda L. Kinkel, H. Corby Kistler, Nhu Nguyen, Scott T. Bates

Published: May 14, 2015 • <https://doi.org/10.1371/journal.pone.0127234>

OPEN OPEN ACCESS PEER-REVIEWED

RESEARCH ARTICLE

0.25 g



ELSEVIER

Contents lists available at ScienceDirect

Ecological Indicators

0.5 g

journal homepage: www.elsevier.com/locate/ecolind



Diatom metabarcoding and microscopic analyses from sediment samples at Lake Nam Co, Tibet: The effect of sample-size and bioinformatics on the identified communities

Jiengang Kang^a, Sten Anslan^{b,*}, Nicole Börner^a, Anja Schwarz^a, Robin Schmidt^b, Jochen Künzel^c, Patrick Rioual^{d,f}, Paula Echeverría-Galindo^a, Miguel Vences^b, Junbo Wang^e, Antje Schwalb^a



SCIENTIFIC REPORTS



OPEN

Sample size effects on the assessment of eukaryotic diversity and community structure in aquatic sediments using high-throughput sequencing

Francisco J. A. Nascimento^{1,4}, Delphine Lallias², Holly M. Bik³ & Simon Creer^{1,4}

>1 g

Methods in Ecology and Evolution

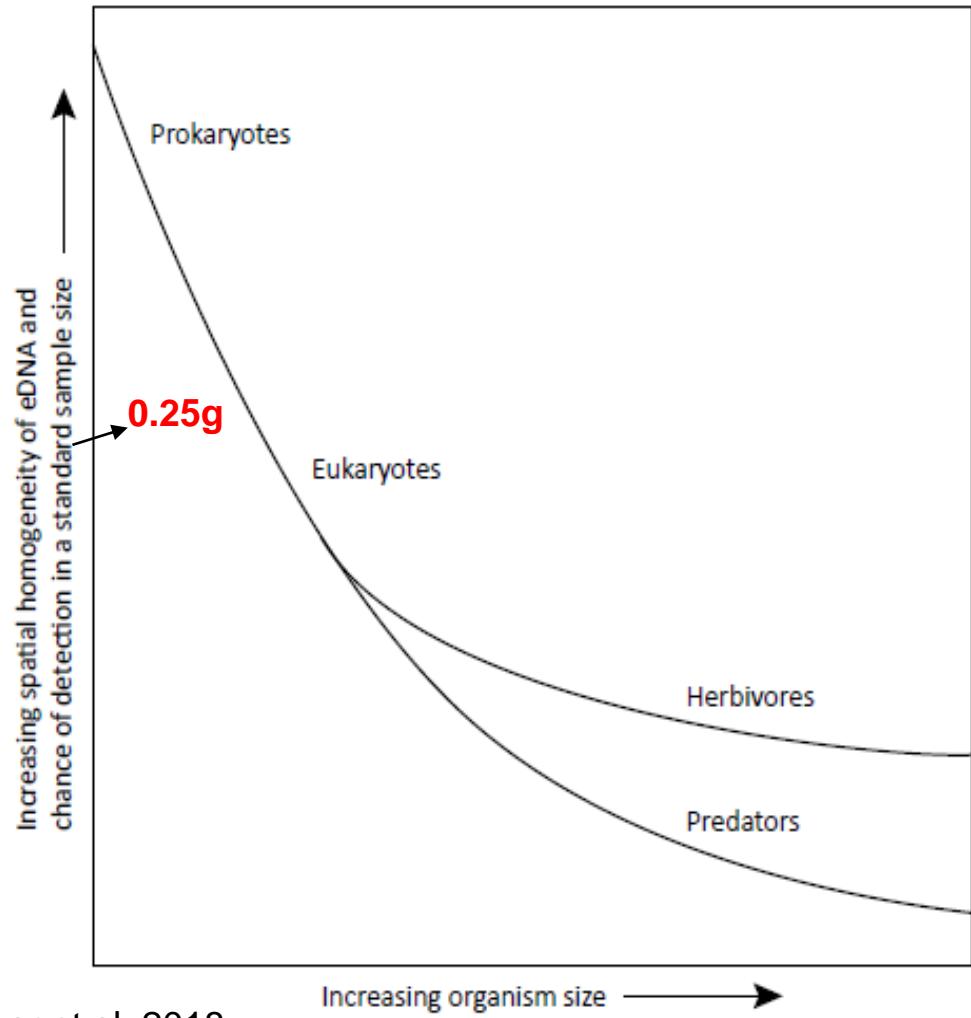


RESEARCH ARTICLE

Impacts of DNA extraction and PCR on DNA metabarcoding estimates of soil biodiversity

Andrew Dopheide^{1,2,3} , Dong Xie⁴ , Thomas R. Buckley^{1,3} , Alexei J. Drummond⁴ , Richard D. Newcomb^{1,2}

Larger soil volumes = higher biodiv estimates for Arthropods but not necessarily for prokaryotes or microeukaryotes.



The spatial distribution of
larger organisms is **less homogenous**
than that of smaller organisms.

Detection of
larger organisms
may require
larger sample sizes.



But which kit?

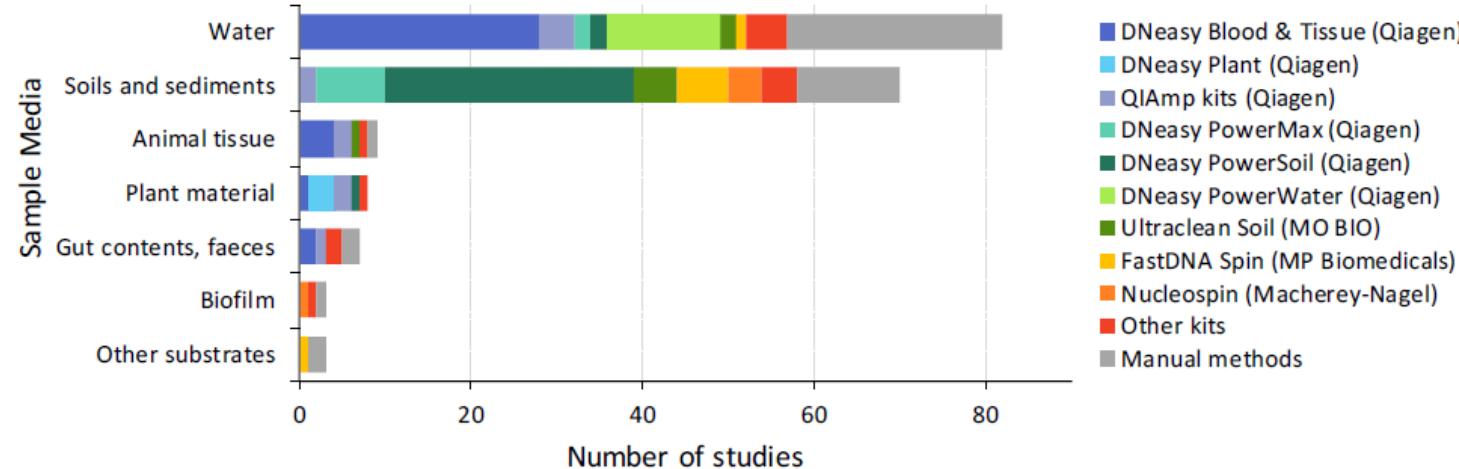
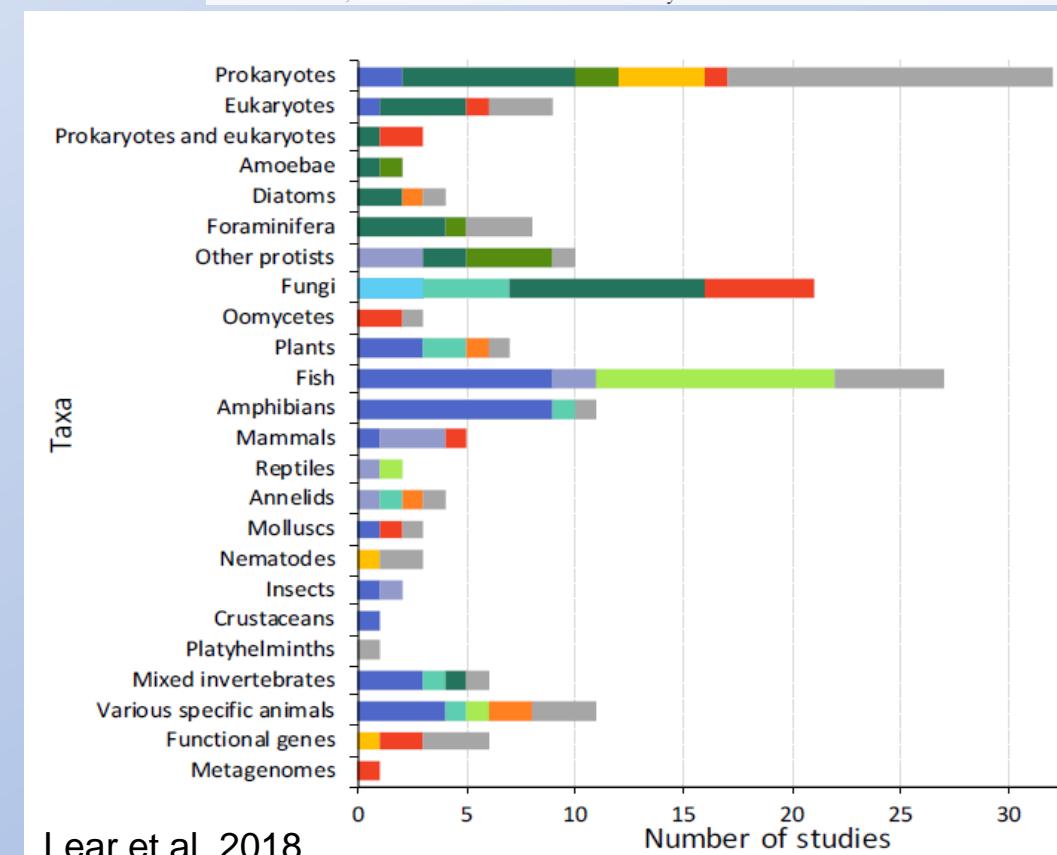


Figure 4. DNA extraction kits and methods used for different sample media. Details of ‘other kits’ used for DNA isolation are provided in Table S4 in Supplementary Material.

REVIEW

Methods for the extraction, storage, amplification and sequencing of DNA from environmental samples

Gavin Lear^{1*}, Ian Dickie², Jonathan Banks³, Stephane Boyer⁴, Hannah L. Buckley⁵, Thomas R. Buckley^{1,6}, Rob Cruickshank⁷, Andrew Dopheide⁶, Kim M. Handley¹, Syrie Hermans¹, Janine Kamke¹, Charles K. Lee⁸, Robin MacDiarmid⁹, Sergio E. Morales¹⁰, David A. Orlovich¹¹, Rob Smissen¹², Jamie Wood¹² and Robert Holdaway¹²





But which kit?



Metabarcoding and Metagenomics 2: 1-12
DOI 10.3897/mbmg.2.26664

Research Article

8

Choice of DNA extraction method affects DNA metabarcoding of unsorted invertebrate bulk samples

Markus Majaneva^{1,2}, Ola H. Diserud³, Shannon H.C. Eagle²,
Mehrdad Hajibabaei², Torbjørn Ekrem¹

Application of high-throughput sequencing (HTS) metabarcoding to diatom biomonitoring:
Do DNA extraction methods matter?

Valentin Vasselon^{1,3}, Isabelle Domaizon^{1,4}, Frédéric Rimet^{1,5}, Maria Kahlert^{2,6}, and Agnès Bouchez^{1,7}

¹CARTEL, INRA, Université de Savoie Mont Blanc, 74200, Thonon-les-Bains, France

²Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, P.O. Box 7050, 75007, Uppsala, Sweden

DNeasy PowerSoil as a universal DNA extraction method
discontinued production
→ PowerSoil Pro Kit

nature
biotechnology

Assessment of variation in microbial community amplicon sequencing by the Microbiome Quality Control (MBQC) project consortium

Rashmi Sinha¹, Galeb Abu-Ali^{2,3}, Emily Vogtmann¹✉, Anthony A Fodor⁴, Boyu Ren², Amnon Amir⁵, Emma Schwager^{2,3}✉, Jonathan Crabtree⁶, Siyuan Ma^{2,3}, The Microbiome Quality Control Project Consortium⁷, Christian C Abnet¹✉, Rob Knight^{5,8}✉, Owen White⁶ & Curtis Huttenhower^{2,3}✉

RESOURCE ARTICLE

WILEY MOLECULAR ECOLOGY RESOURCES

Optimal extraction methods for the simultaneous analysis of DNA from diverse organisms and sample types

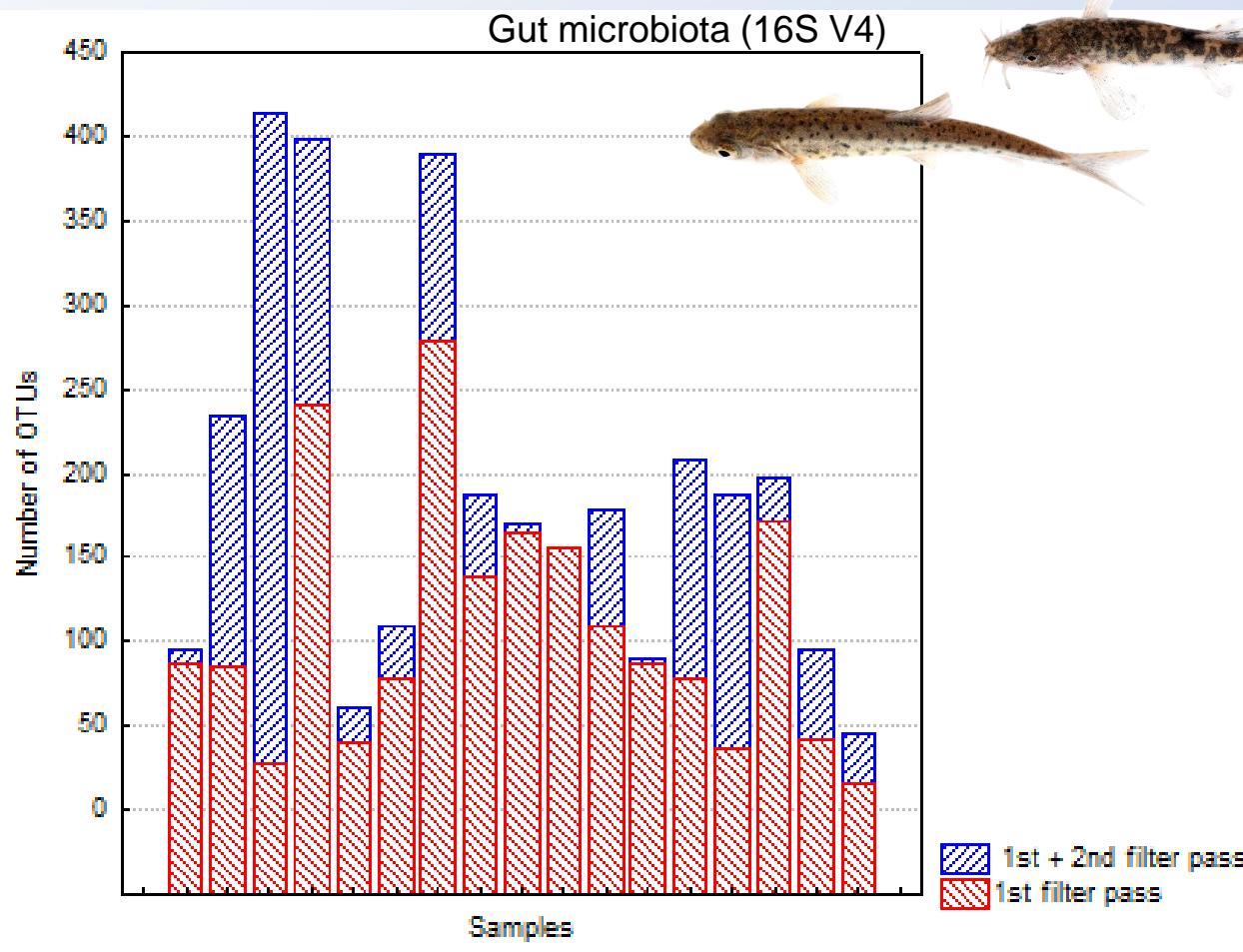
Syrie M. Hermans¹✉ | Hannah L. Buckley²✉ | Gavin Lear¹✉



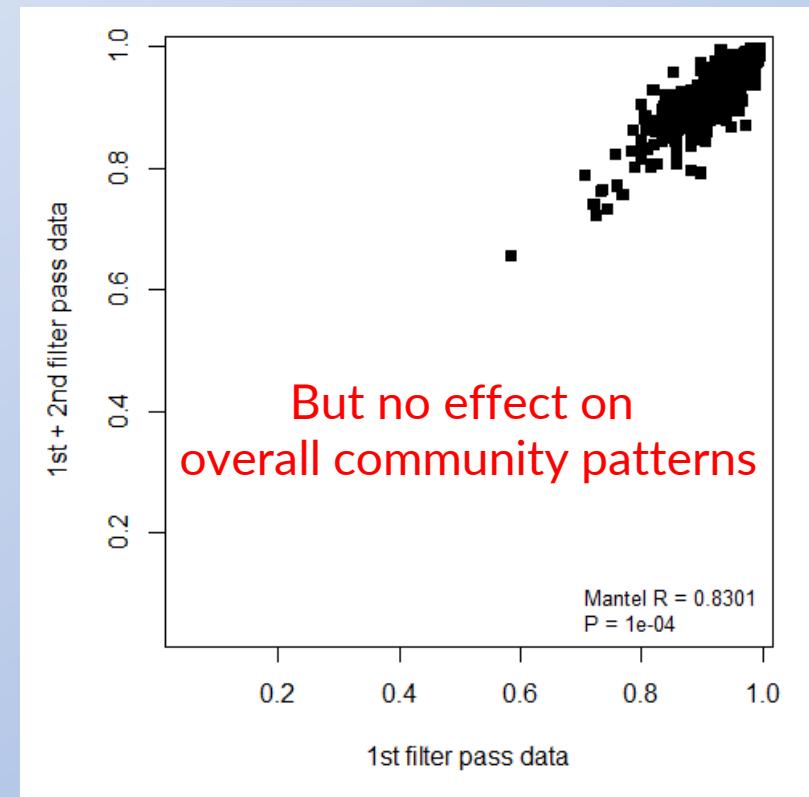
qiagen.com



Anslan, unpublished.



Low biomass samples



longer incubation time = higher DNA yields
(more gene copies)

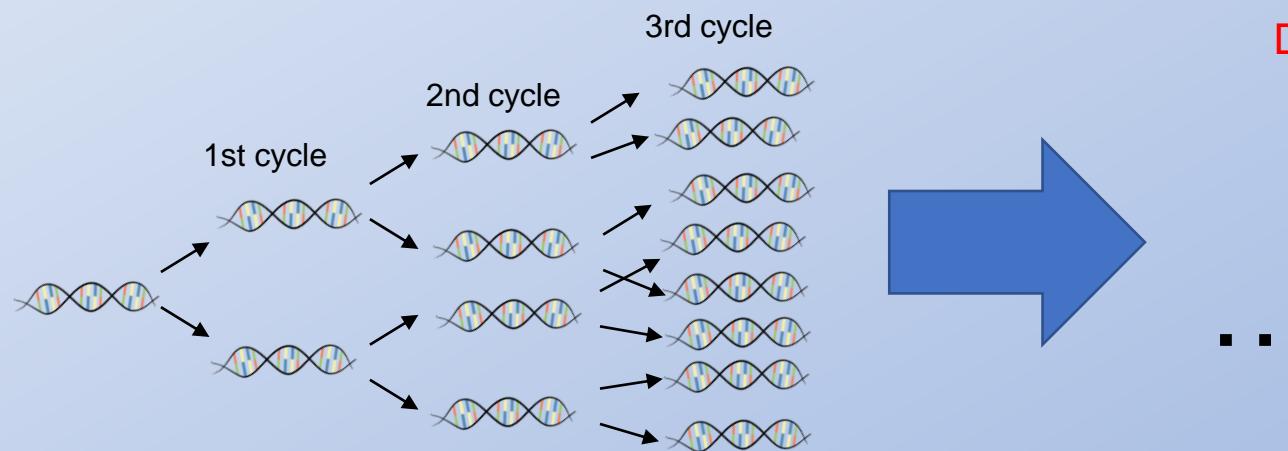
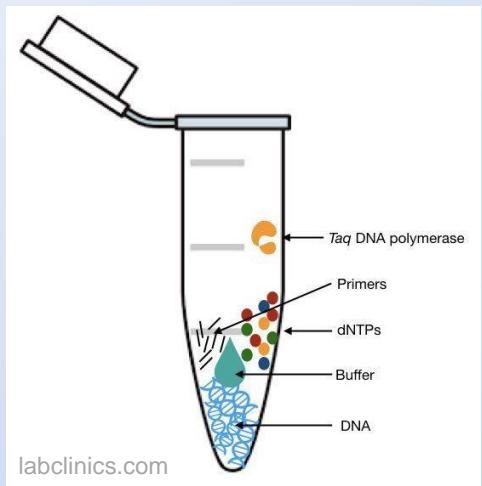


Once DNA is extracted ...

- divide DNA sample into **multiple aliquots** to avoid freeze-thaw cycles



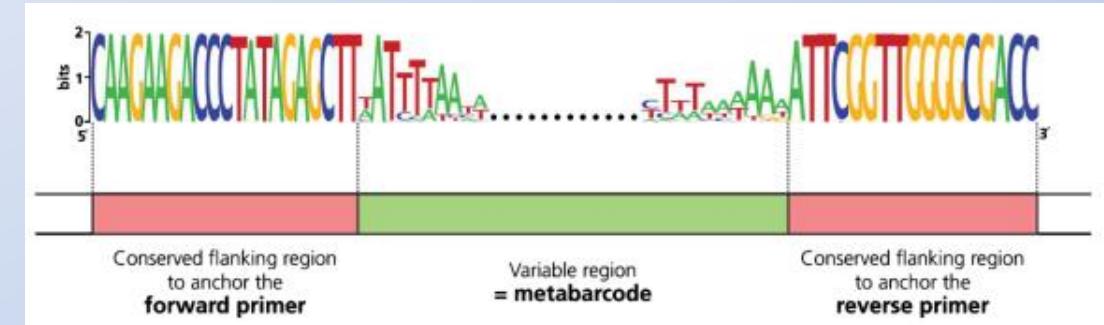
Polymerase Chain Reaction



Dedicated working space for PCR



PCR primer selection



„It is necessary to overcome the idea that a marker **published** for a given clade and application is always the **best marker** in **different context**“ - Taberlet et al 2018.

- eDNA soure
- Coverage
- Resolution
- Amplicon length
- Reference data

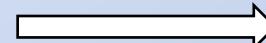


PCR primer selection

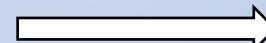
Sampling
DNA extraction
DNA amplification
Sequencing
Sequence analyses
Identification of taxa



DNA quality (*degraded, modern*), source (*wocDNA, soil eDNA*)



Metabarcoding marker/primer choice?



Which strategy/technology?



Which tools/databases?



Ostracodes metabarcoding from sedimentary DNA

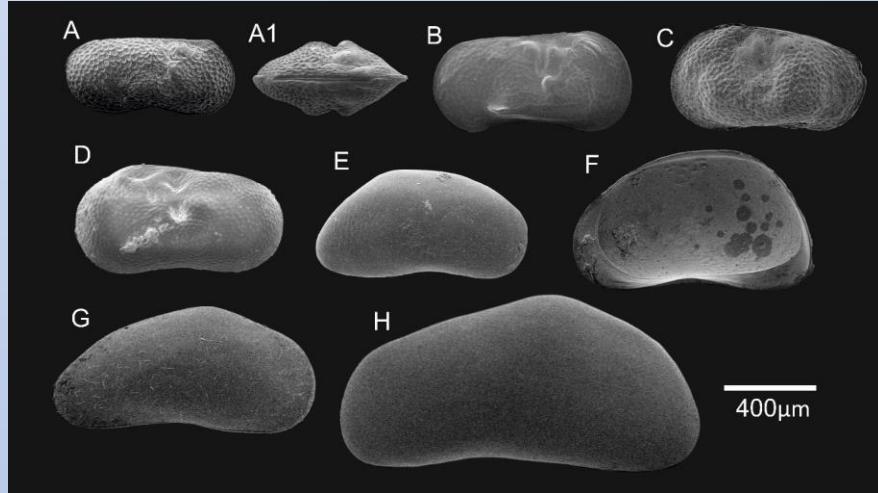
Highest *in-silico* amplification rate

for **COI** gene primers

(matched with 99.3% of ostracodes ref seqs in the database)

High-throughput identification of non-marine Ostracoda from the Tibetan Plateau: Evaluating the success of various primers on sedimentary DNA samples

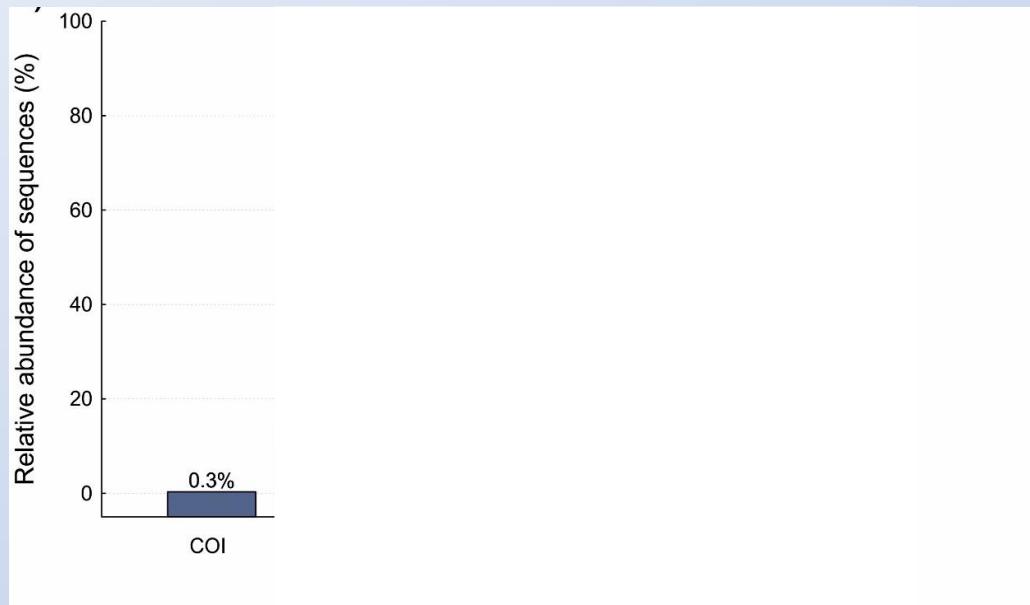
Paula Echeverría-Galindo¹ | Sten Anslan² | Peter Frenzel³ | Sven Künzel⁴ |
Miguel Vences² | Liseth Pérez¹ | Nicole Börner¹ | Wengang Kang¹ |
Anja Schwarz¹ | Junbo Wang⁵ | Ping Peng⁵ | Liping Zhu⁵ | Antje Schwalb¹



Ostracodes metabarcoding from sedimentary DNA

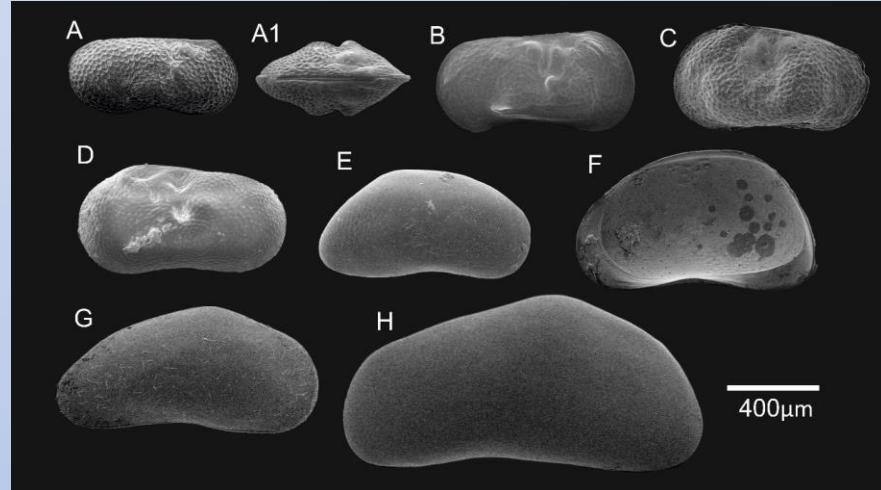
Highest *in-silico* amplification rate
for **COI** gene primers

(matched with 99.3% of ostracodes ref seqs in the database)



High-throughput identification of non-marine Ostracoda from the Tibetan Plateau: Evaluating the success of various primers on sedimentary DNA samples

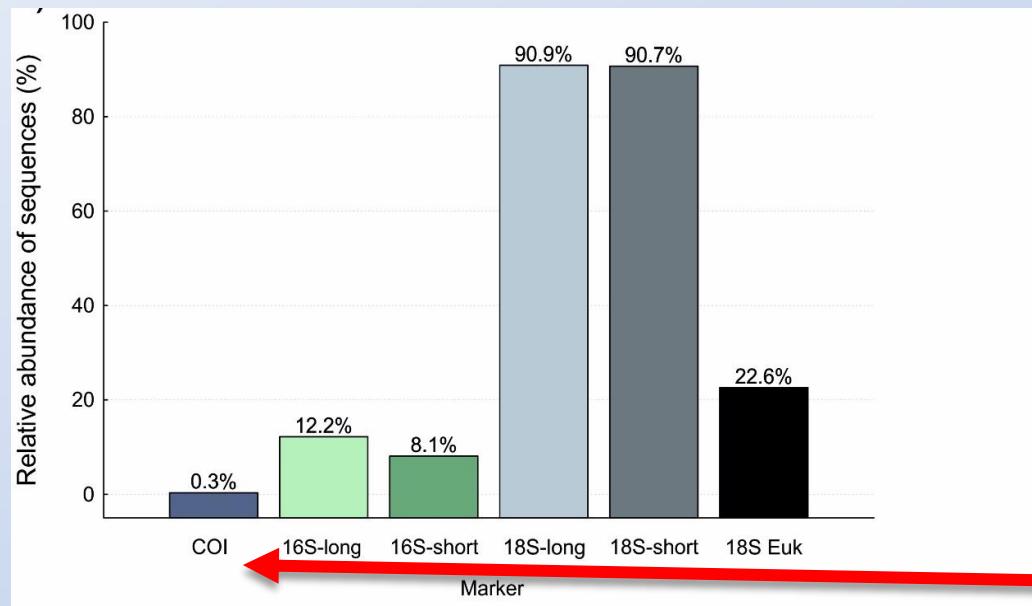
Paula Echeverría-Galindo¹ | Sten Anslan² | Peter Frenzel³ | Sven Künzel⁴ |
Miguel Vences² | Liseth Pérez¹ | Nicole Börner¹ | Wengang Kang¹ |
Anja Schwarz¹ | Junbo Wang⁵ | Ping Peng⁵ | Liping Zhu⁵ | Antje Schwalb¹



Ostracodes metabarcoding from sedimentary DNA

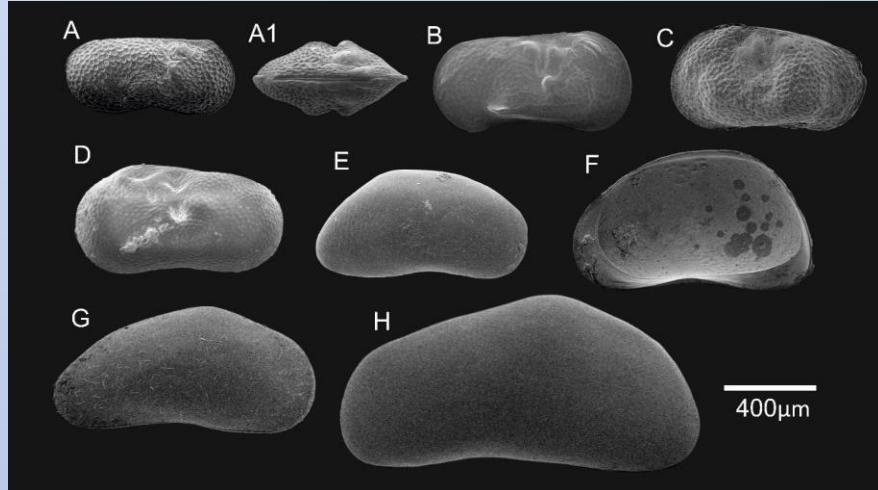
Highest *in-silico* amplification rate
for **COI** gene primers

(matched with 99.3% of ostracodes ref seqs in the database)



High-throughput identification of non-marine Ostracoda from the Tibetan Plateau: Evaluating the success of various primers on sedimentary DNA samples

Paula Echeverría-Galindo¹ | Sten Anslan² | Peter Frenzel³ | Sven Künzel⁴ |
Miguel Vences² | Liseth Pérez¹ | Nicole Börner¹ | Wengang Kang¹ |
Anja Schwarz¹ | Junbo Wang⁵ | Ping Peng⁵ | Liping Zhu⁵ | Antje Schwalb¹



Higher sequencing depth would resolve this issue???

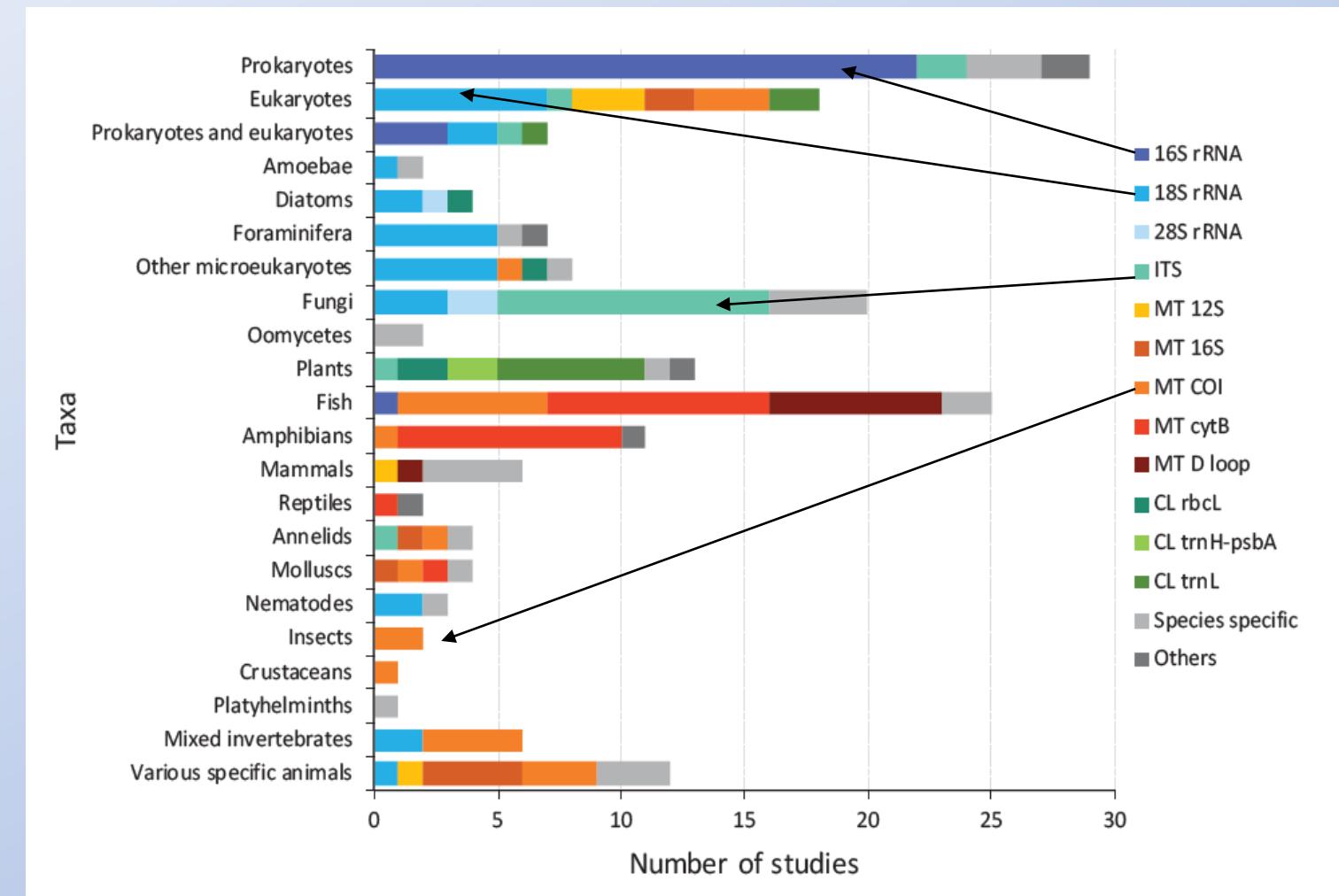


PCR primer selection

Name	
✓ 1. EU587263	Asteropella monambon
✓ 2. EU587260	Asteropella slatteryi
✓ 3. EU587262	Asteropella sp
✓ 4. EU587261	Asteropella slatteryi
✓ 5. EU587257	Cylindroleberidae sp
✓ 6. EU587255	Parasterope gamurru
✓ 7. AY624729	Cylindroleberis sp
✓ 8. AY624728	Parasterope sp
✓ 9. EU587264	Leuroleberis mackenziei
✓ 10. AY624730	Leuroleberis surugaensis
✓ 11. KM503090	Skogsbergia lernerii
✓ 12. KC999396	Skogsbergia sp
✓ 13. KC999397	Skogsbergia sp
✓ 14. AY624736	Skogsbergia sp
✓ 15. AY624735	Paravargula sp
✓ 16. AY624733	Melavargula japonica
✓ 17. AY624734	Paradolabella pellicula
✓ 18. AY624731	Cyprinida noctiluca
✓ 19. AY624737	Vargula hilgendorfi
✓ 20. AY624738	Vargula sp
✓ 21. AY624739	Vargula sp
✓ 22. AY624740	Vargula sp
✓ 23. AY624732	Cypridinidae sp
✓ 24. EU57251	Bathyleberis osculata
✓ 25. EU587252	Synasterope sp
✓ 26. EU587253	Cylindroleberis sp
✓ 27. EU587258	Postasterope barnesi
✓ 28. EU587259	Postasterope corrugata
✓ 29. AY624741	Euphilomedes japonica
✓ 30. AY624742	Euphilomedes sordida
✓ 31. AY624743	Euphilomedes sp
✓ 32. EU587256	Cylindroleberidae sp
✓ 33. EU587254	Parasterope gamurru
✓ 34. GQ914276	Eucypris crassa
✓ 35. GQ914057	Eucypris virens
✓ 36. GQ914058	Eucypris virens
✓ 37. GQ914081	Eucypris virens
✓ 38. GQ914070	Eucypris virens
✓ 39. GQ914075	Eucypris virens
✓ 40. GQ914272	Eucypris virens
✓ 41. GQ914273	Eucypris virens
✓ 42. GQ914095	Eucypris virens
✓ 43. GQ914062	Eucypris virens
✓ 44. GQ914077	Eucypris virens
✓ 45. GQ91422	Eucypris virens
✓ 46. GQ914063	Eucypris virens
✓ 47. GQ914190	Eucypris virens
✓ 48. GQ914112	Eucypris virens
✓ 49. GQ914230	Eucypris virens



PCR primer selection



Lear et al. 2018



Primer tagging/indexing/barcoding

Heterogeneity spacer	tag/index/barcode	primer	
		ACAAACACTCCGACGCCCTSCSCTTANTDATATGC	sample1
		NACAAGTGCTGCTGCCCTSCSCTTANTDATATGC	sample2
NN		NNACACAGTCCTGACGCCCTSCSCTTANTDATATGC	sample3
NNN		NNNACACCAACACCAACGCCCTSCSCTTANTDATATGC	sample4
NNNN		NNNNACACCGCACAATGCCCTSCSCTTANTDATATGC	sample5



Primer tagging/indexing/barcoding

- Single-end

ACAACACTCCGACGCCCTSCSCTTANTDATATGC → *sample1*

GTACCACGGCTCGCCTSCSCTTANTDATATGC → *sample2*

- Paired-end

- Combinational paired-end (dual) indexes

ACAACACFwdPRIMER TACTGARevPRIMER → *sample1*

ACAACACFwdPRIMER ACCAGTRevPRIMER → *sample2*

- Unique paired-end (dual) indexes (no repetitive indexes among samples);

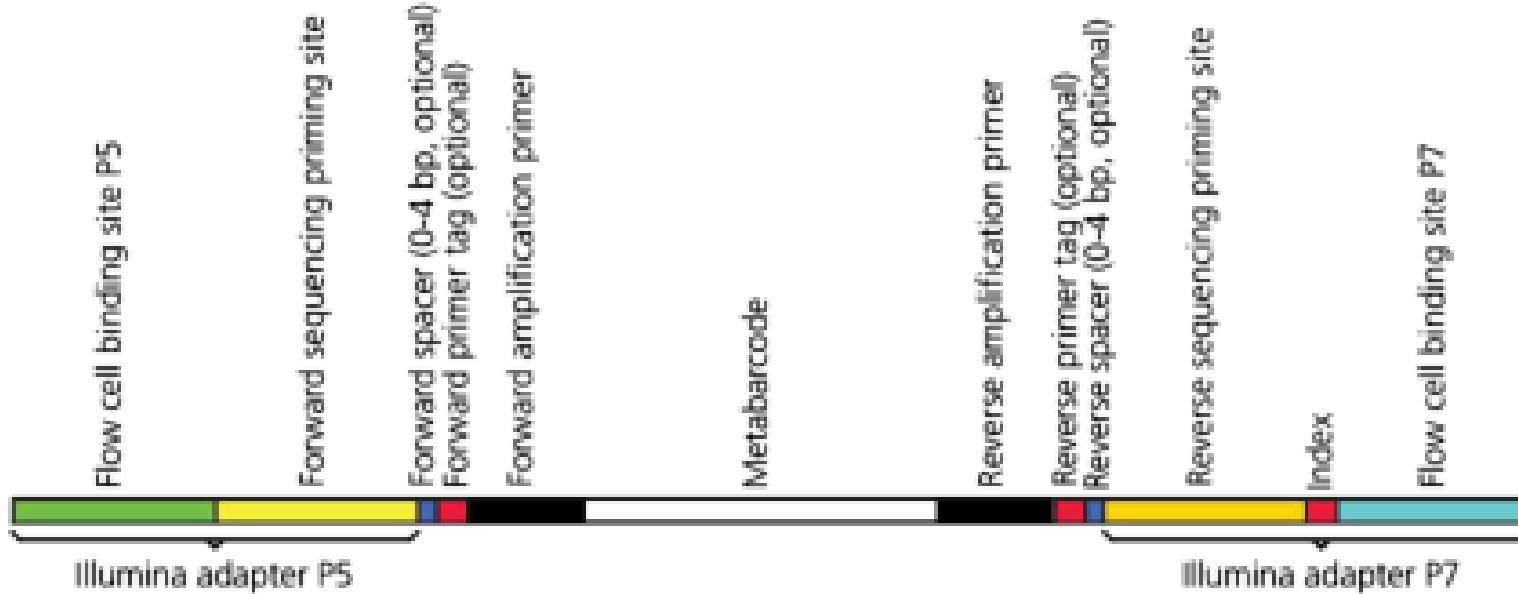
ACAACACFwdPRIMER ACAACACRevPRIMER → *sample1*

TGCATCAFwdPRIMER TGCATCARevPRIMER → *sample2*



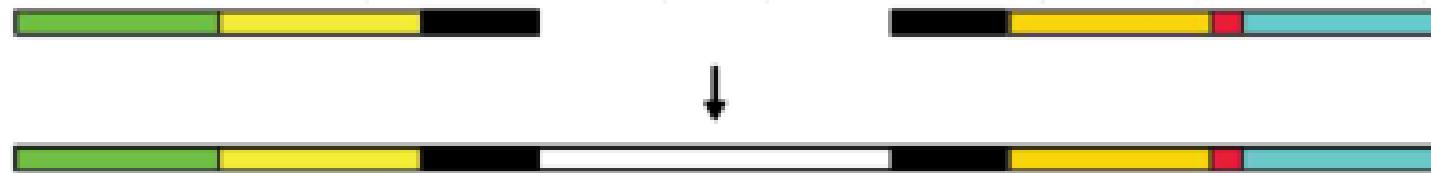
indexing

Structure of a DNA fragment that can be sequenced on Illumina platforms

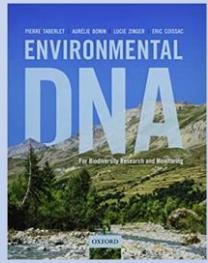


Strategy 1: single-step PCR with Illumina adapters

PCR with P5 and P7 adapters on 5'-ends of specific primers (each library index is specific to a sample)



Simple,
but
long primers
have lower mobility

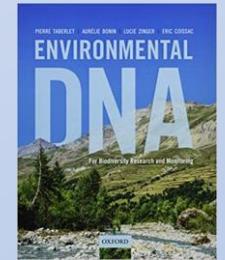
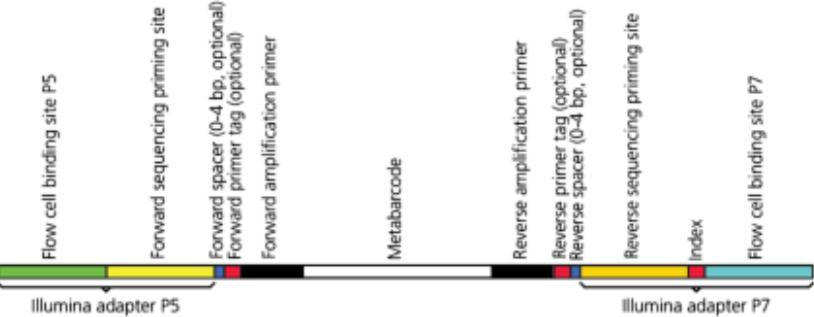


Taberlet et al. 2018



indexing

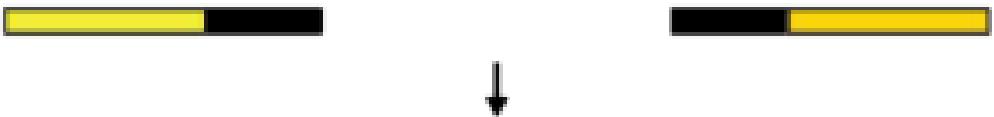
Structure of a DNA fragment that can be sequenced on Illumina platforms



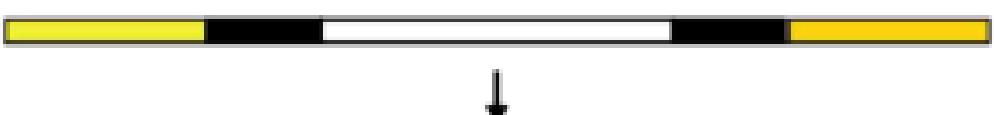
Taberlet et al. 2018

Strategy 2: two-step PCR with Illumina adapters

Step 1: PCR with a linker on the 5'-end of the primers



Step 2: PCR for adding P5 and P7 adapters (each library index is specific to a sample)



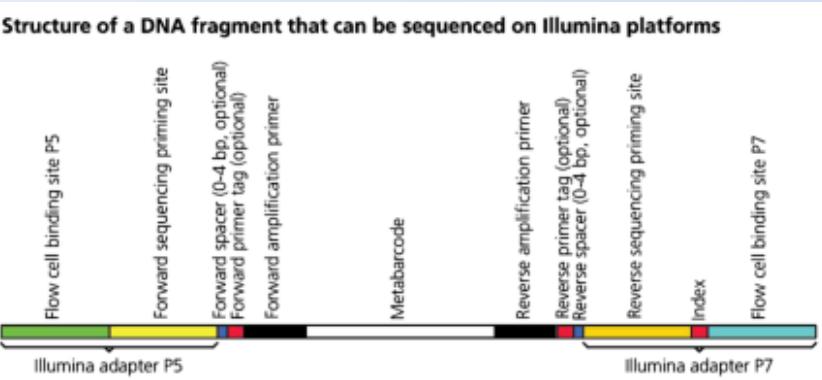
Cost-effective.
But higher
contamination risk.



indexing

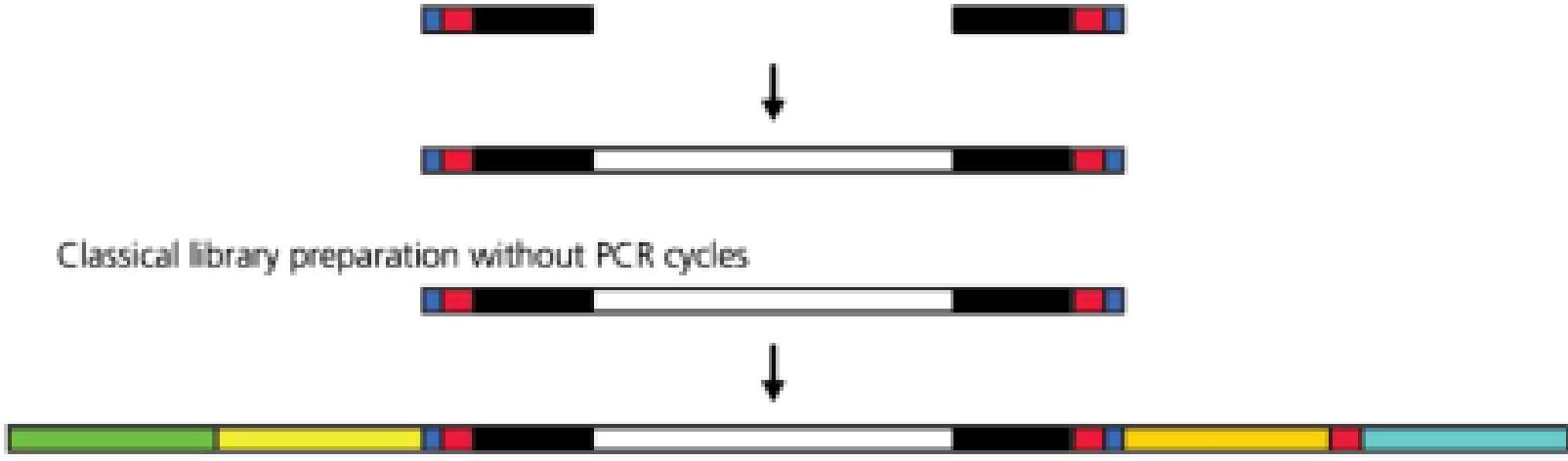
Simple and efficient amplification.

even for
low DNA
concentration samples



Strategy 3: single-step PCR with tagged primers

PCR with tags and spacers on the 5'-end of the primers (each tag combination is specific to a sample)



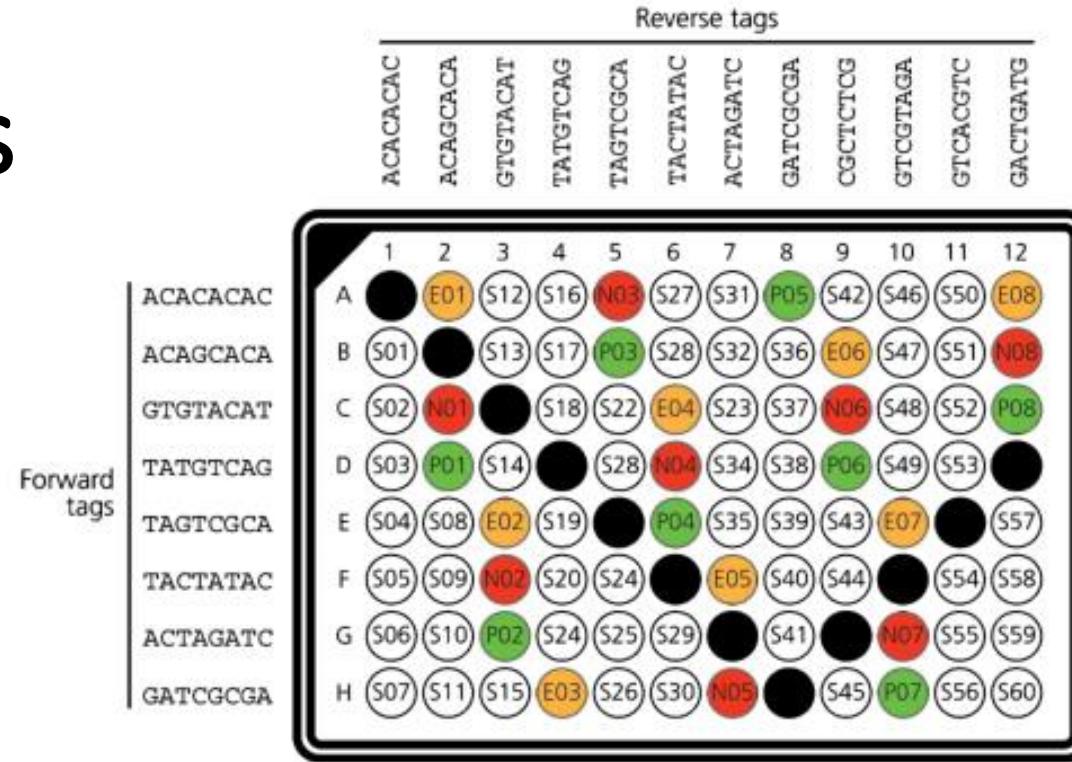


Control samples

- **Negative controls** for DNA extraction and PCR → contamination control
- **Positive controls** for PCR → tag-switching control, PCR conditions control
- **Mock community control** → tag-switching control, PCR conditions control, quantitative capacity
- **(tag-switching controls** for combinational tagging method)



Control samples



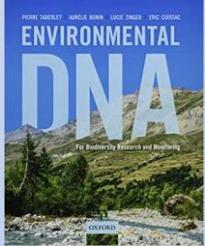
(S01) to (S60) : 60 samples

(●) to (●) : 12 blanks (no primer, no template)

(○) to (○) : 8 extraction controls (template = extraction blanks)

(●) to (●) : 8 PCR negative controls (template = water)

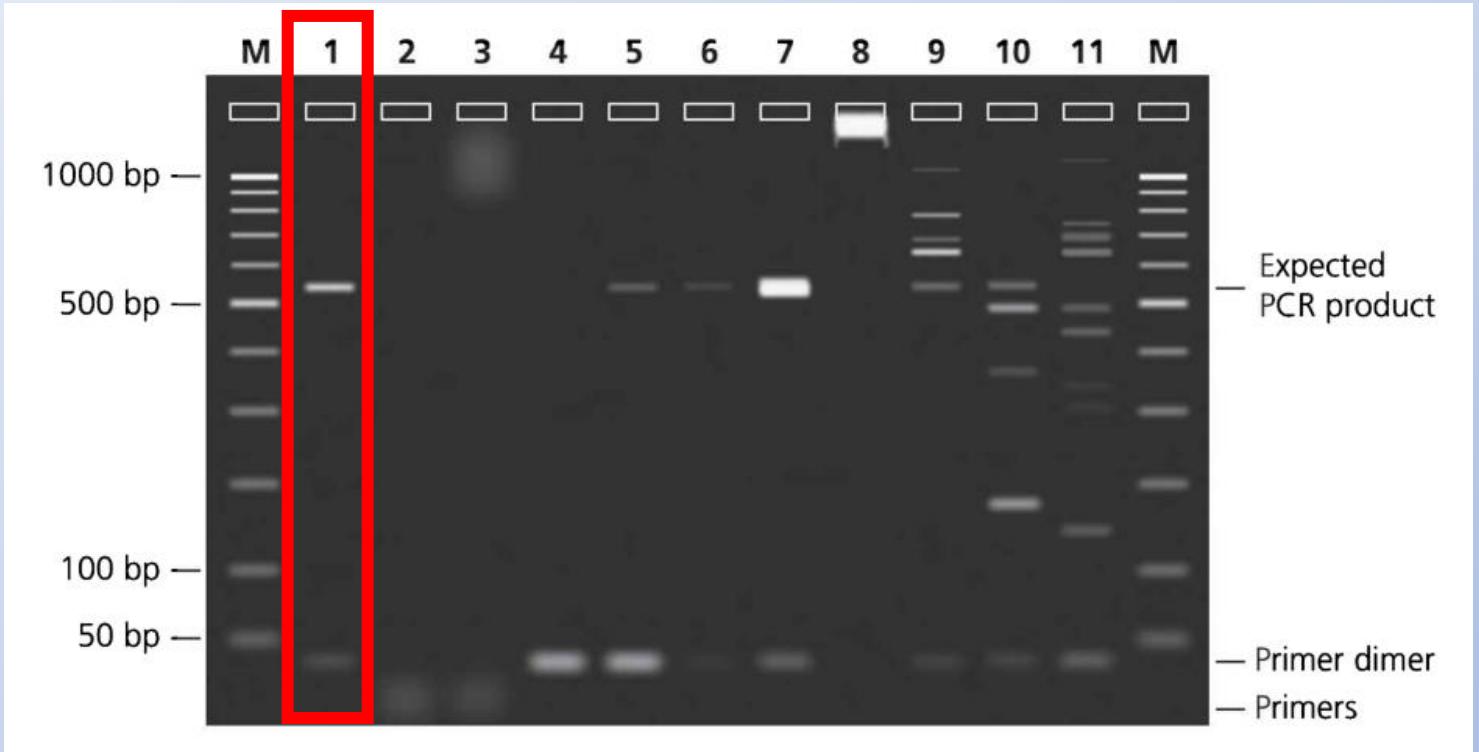
(○) to (○) : 8 positive controls



Taberlet et al. 2018



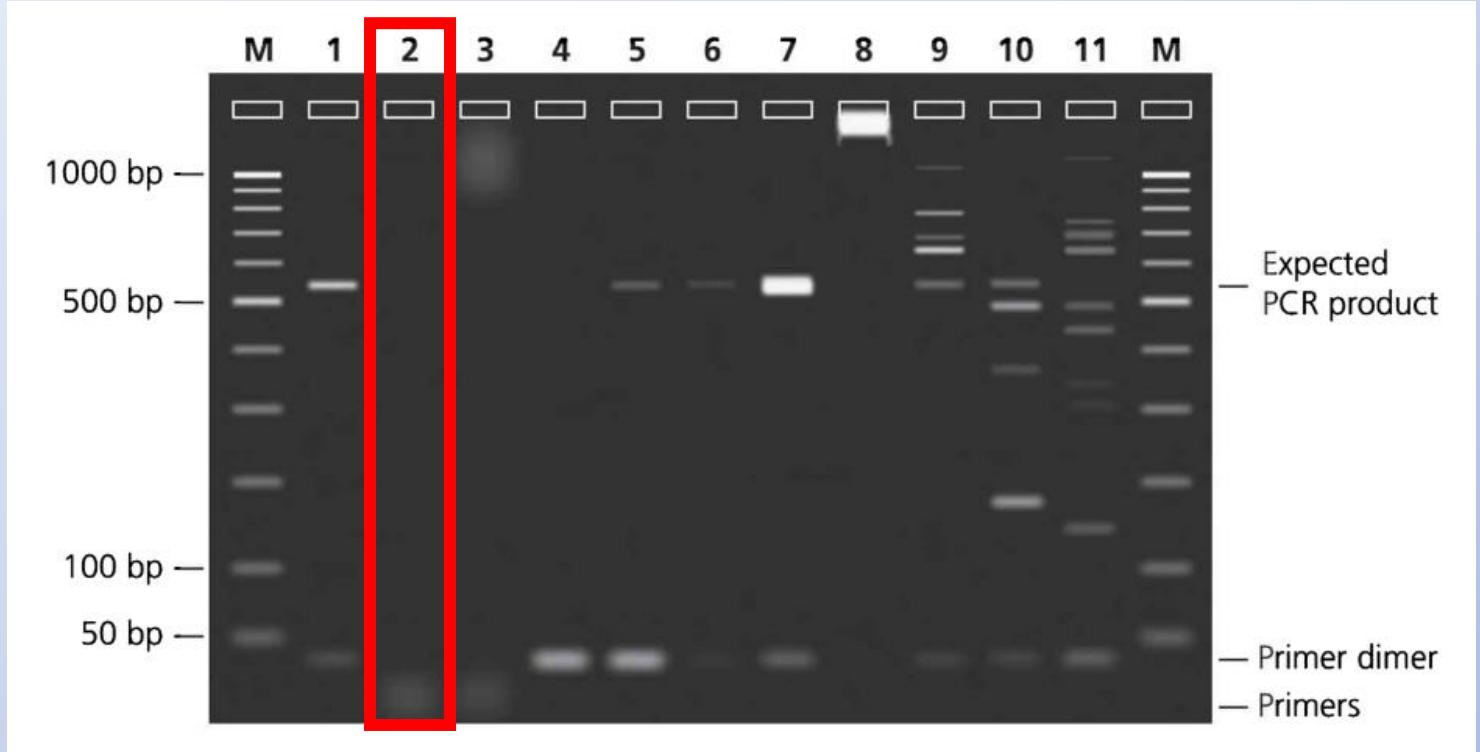
PCR optimization



Looks good.



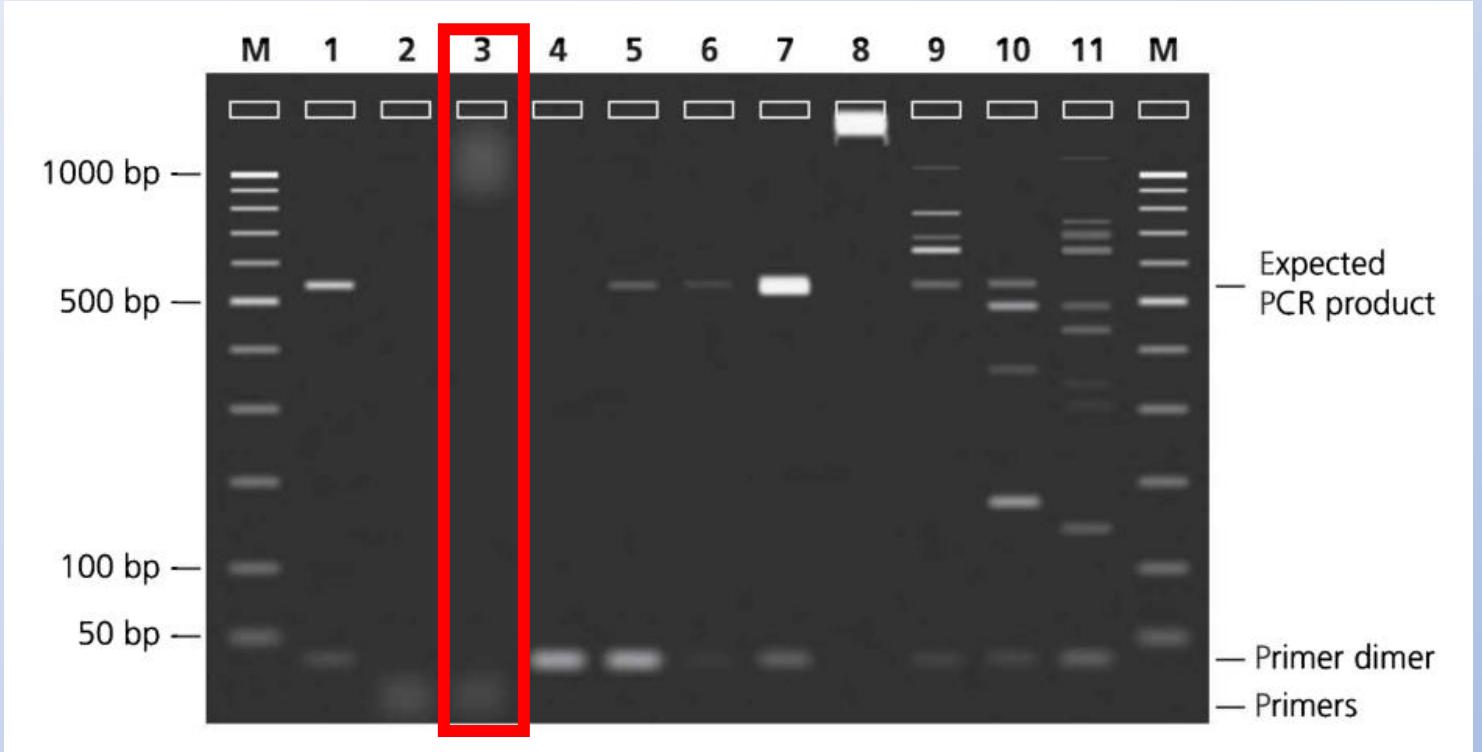
PCR optimization



Check the PCR settings, replace PCR reagents, decrease the annealing temp;
Use positive control to confirm that PCR settings and reagents are working!



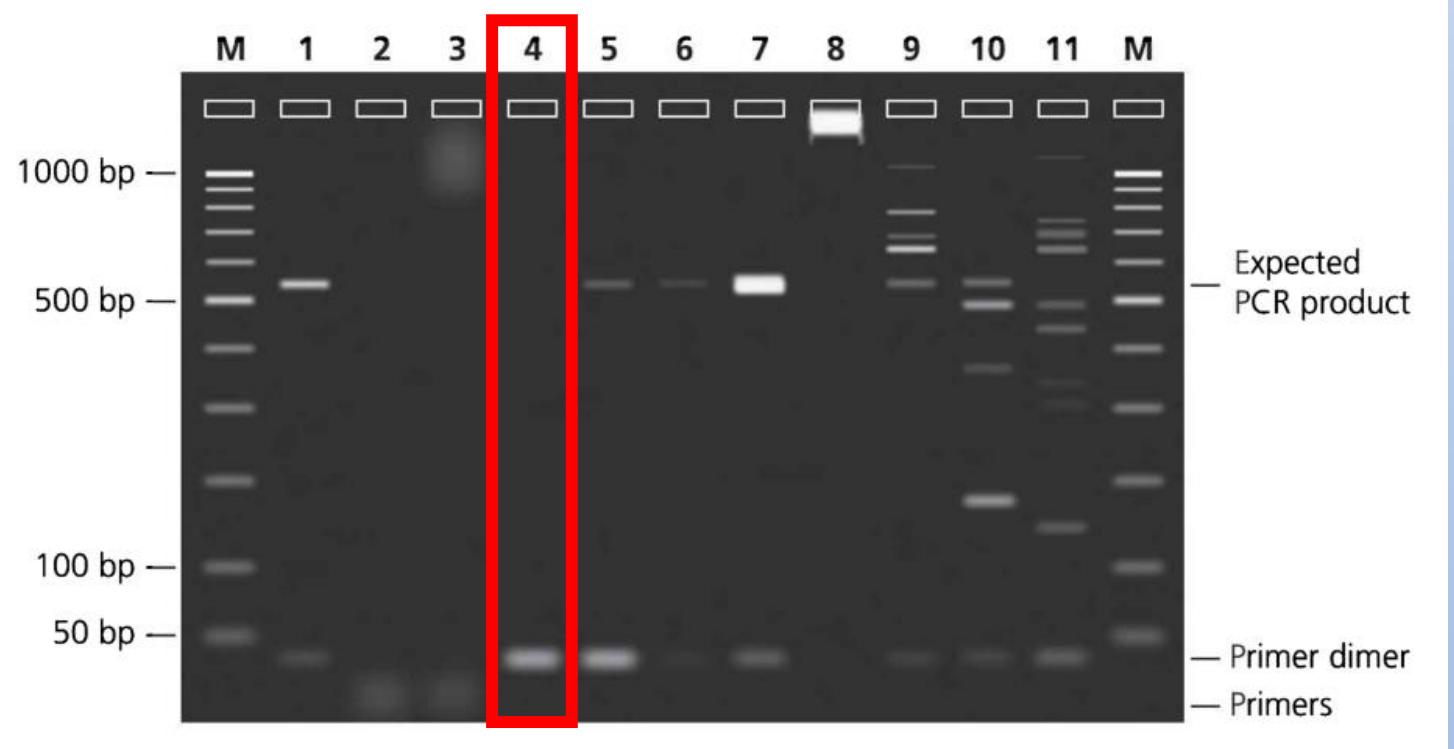
PCR optimization



Dilute the template DNA (by 10, 100 or even 1000). Repeat the analysis.



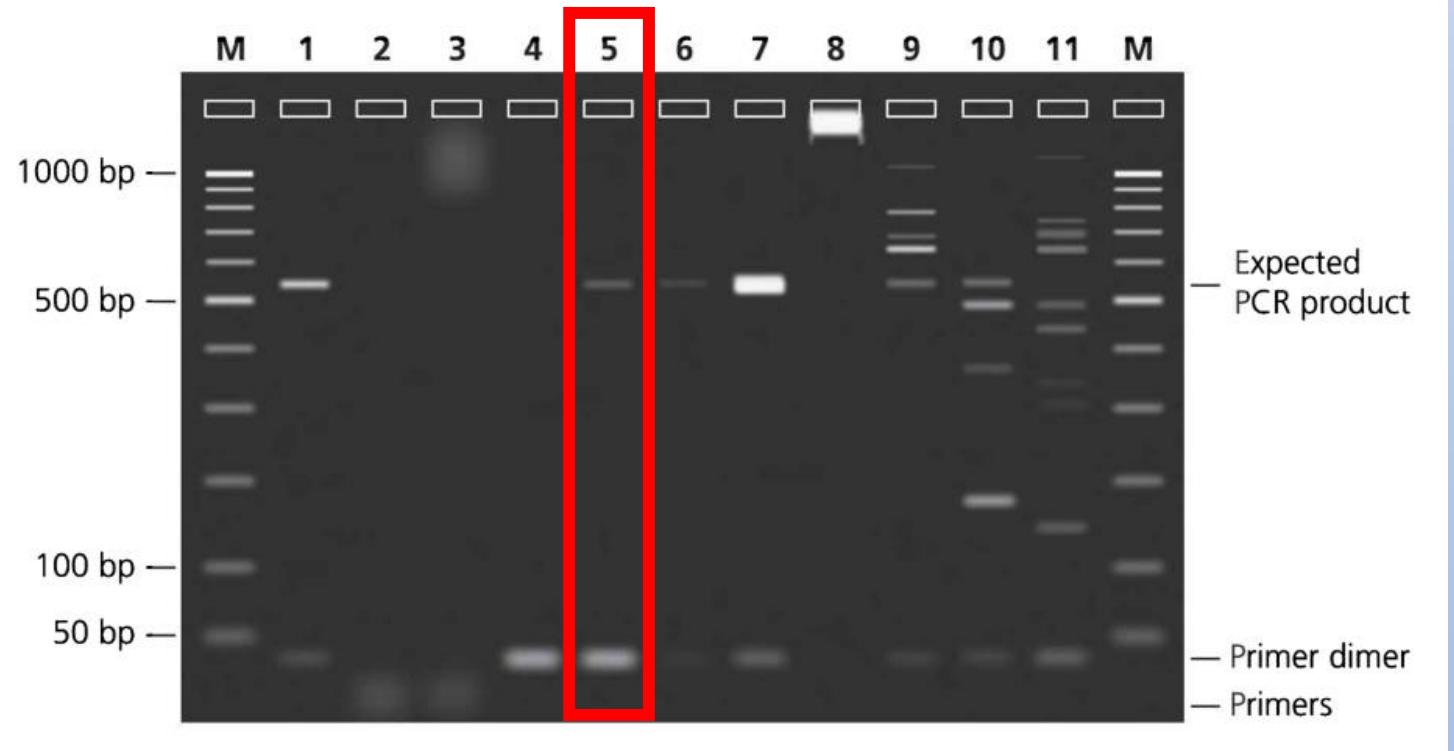
PCR optimization



Decrease the annealing temperature by up to 10 C.



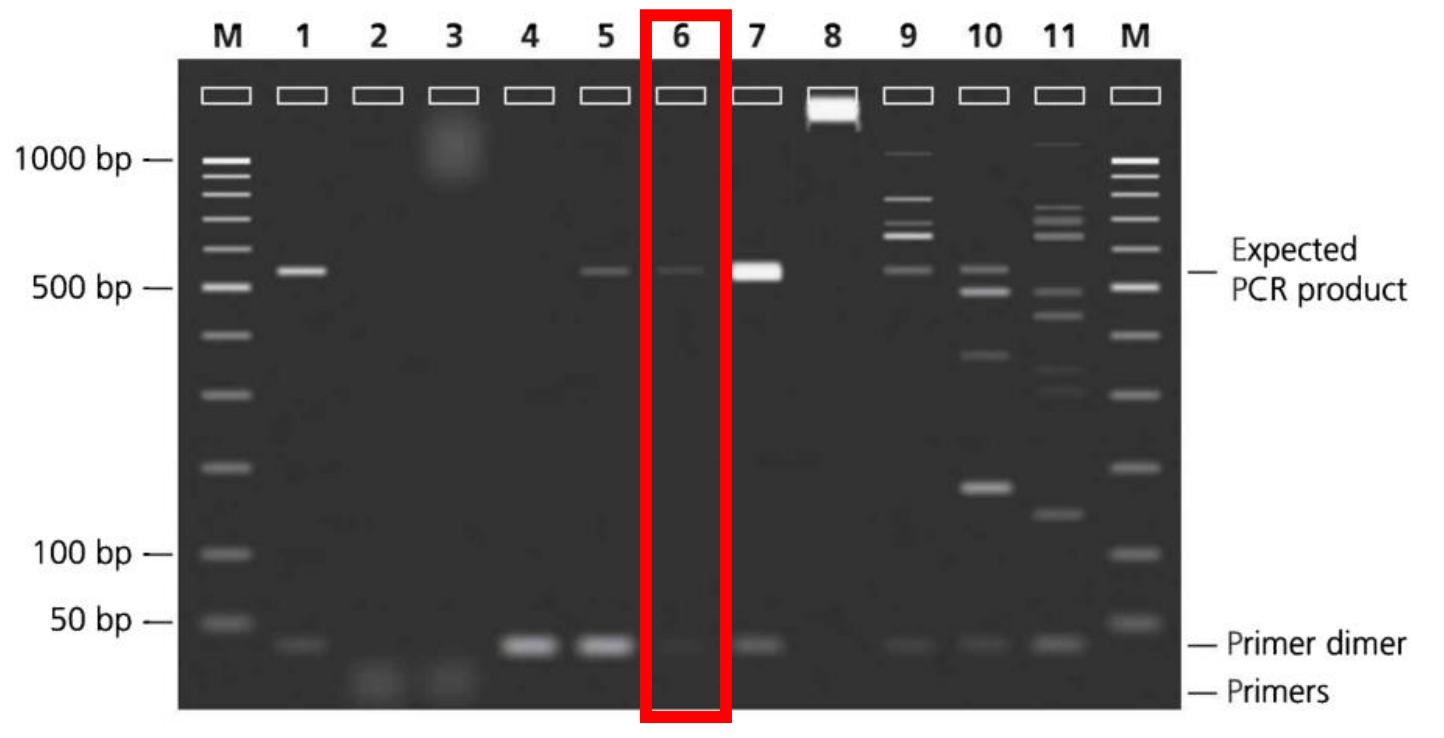
PCR optimization



Decrease the primer concentration.



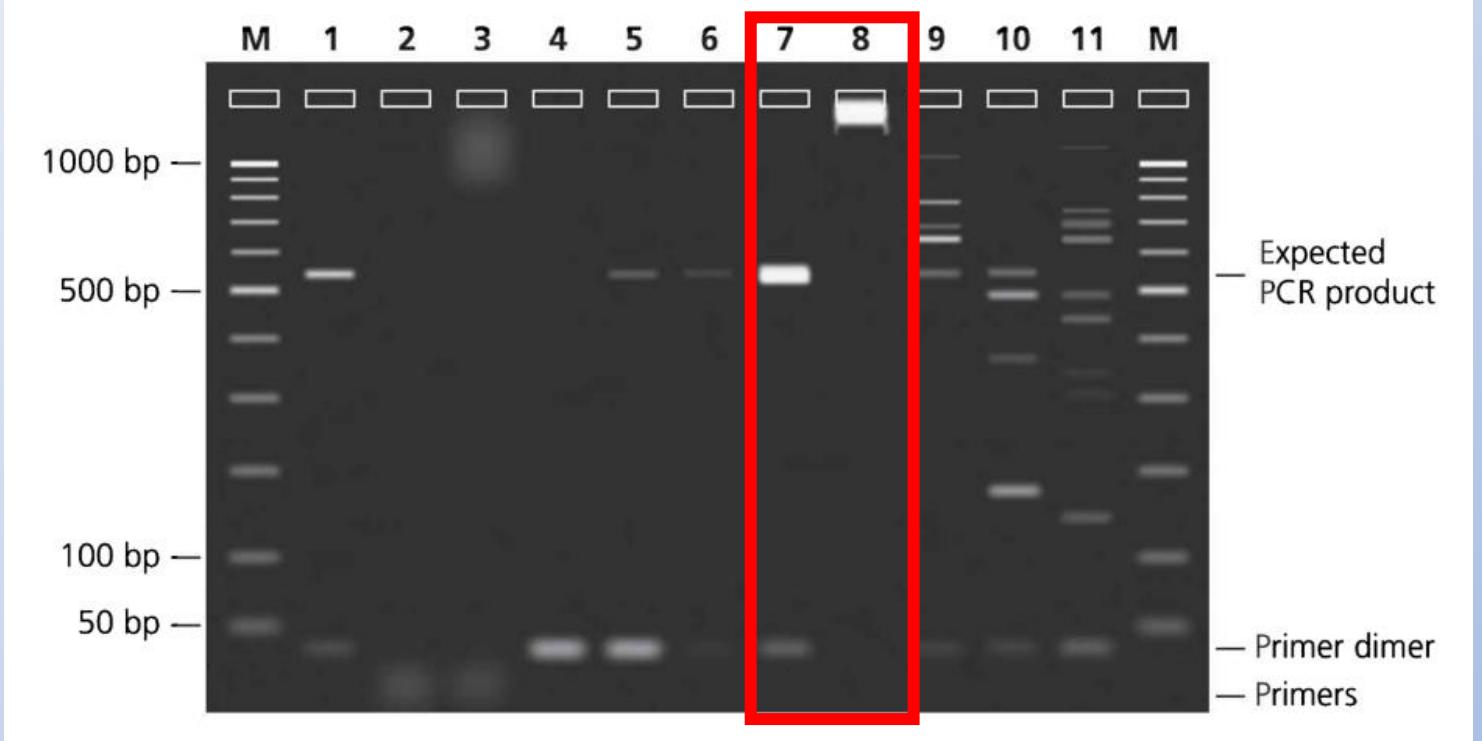
PCR optimization



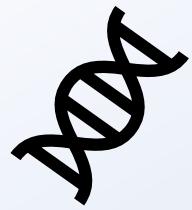
Increase the number of PCR cycles.
Decrease the annealing temp (by 1-5 C).



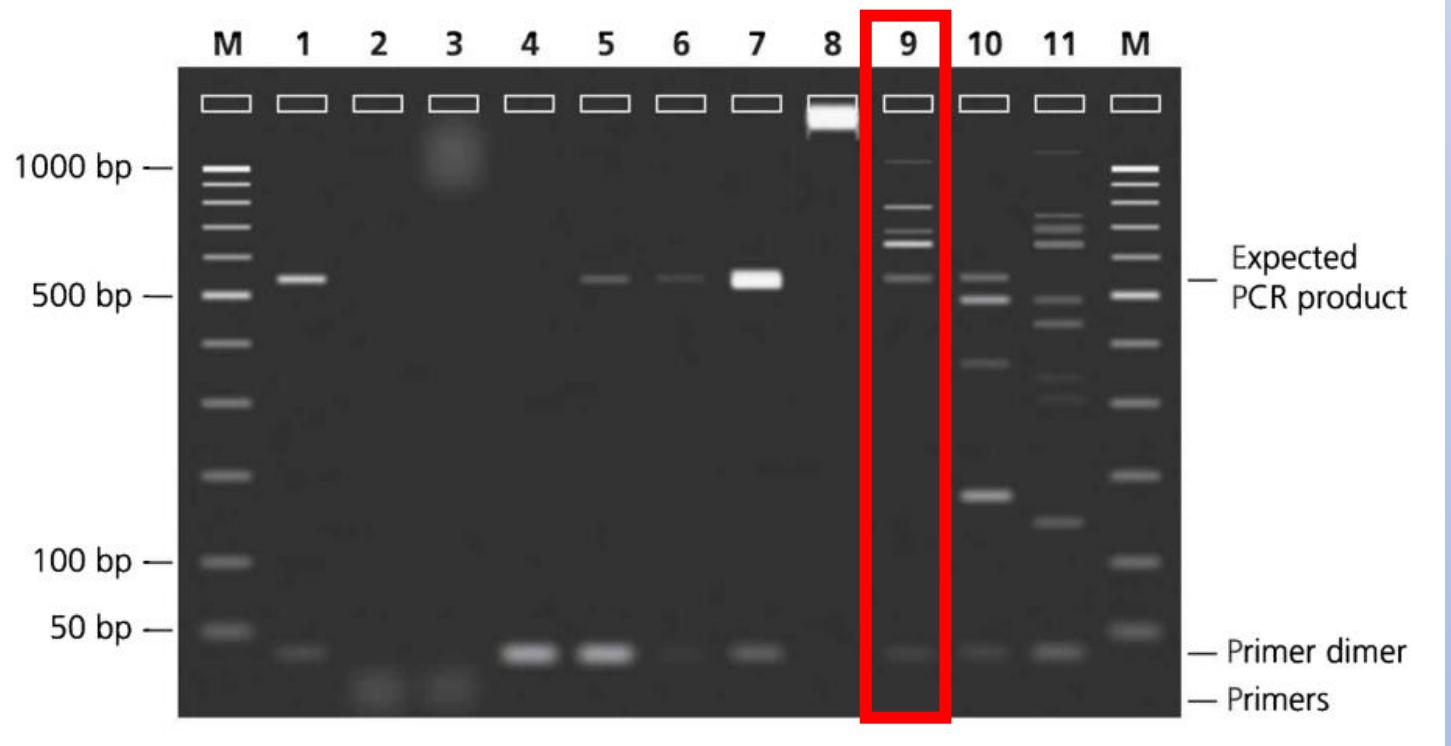
PCR optimization



Decrease the number of PCR cycles.



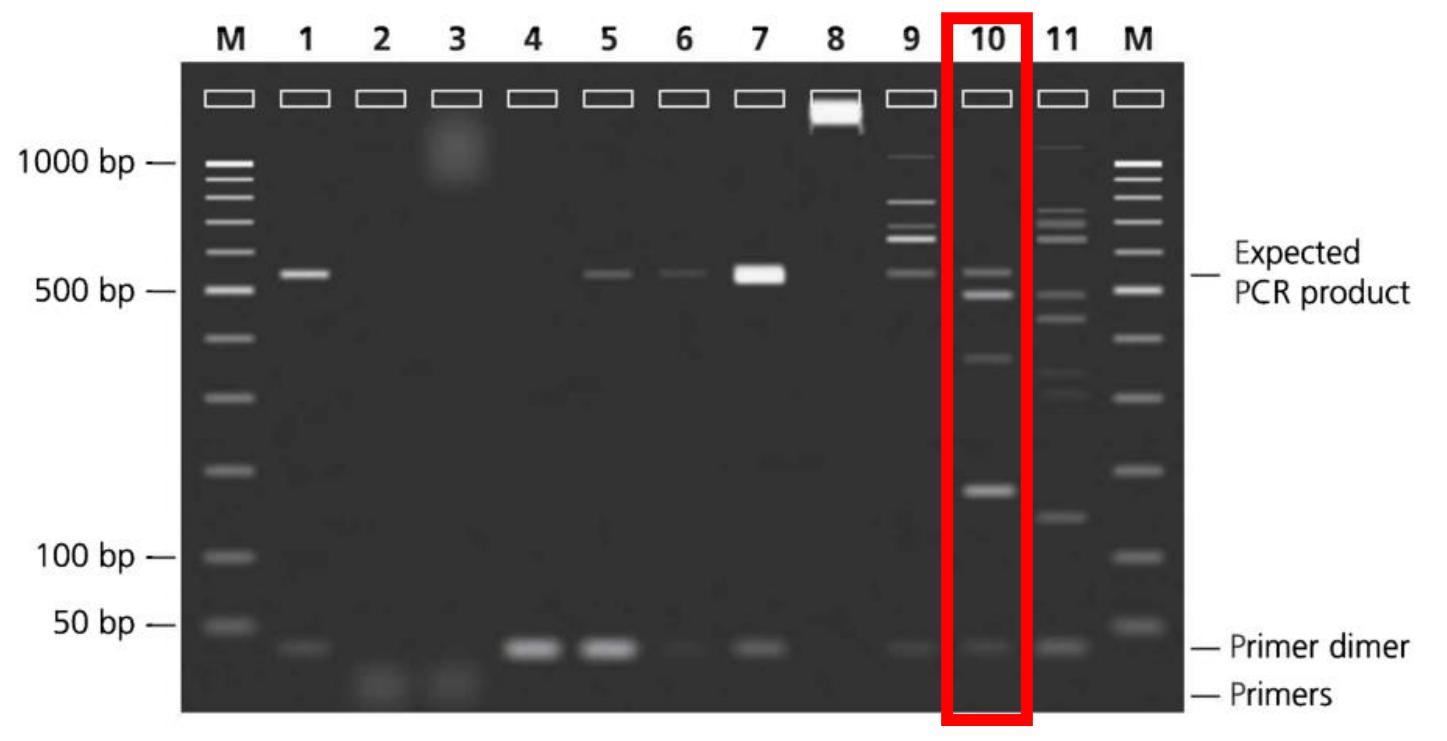
PCR optimization



Increase the annealing temperature.
Decrease the elongation time.



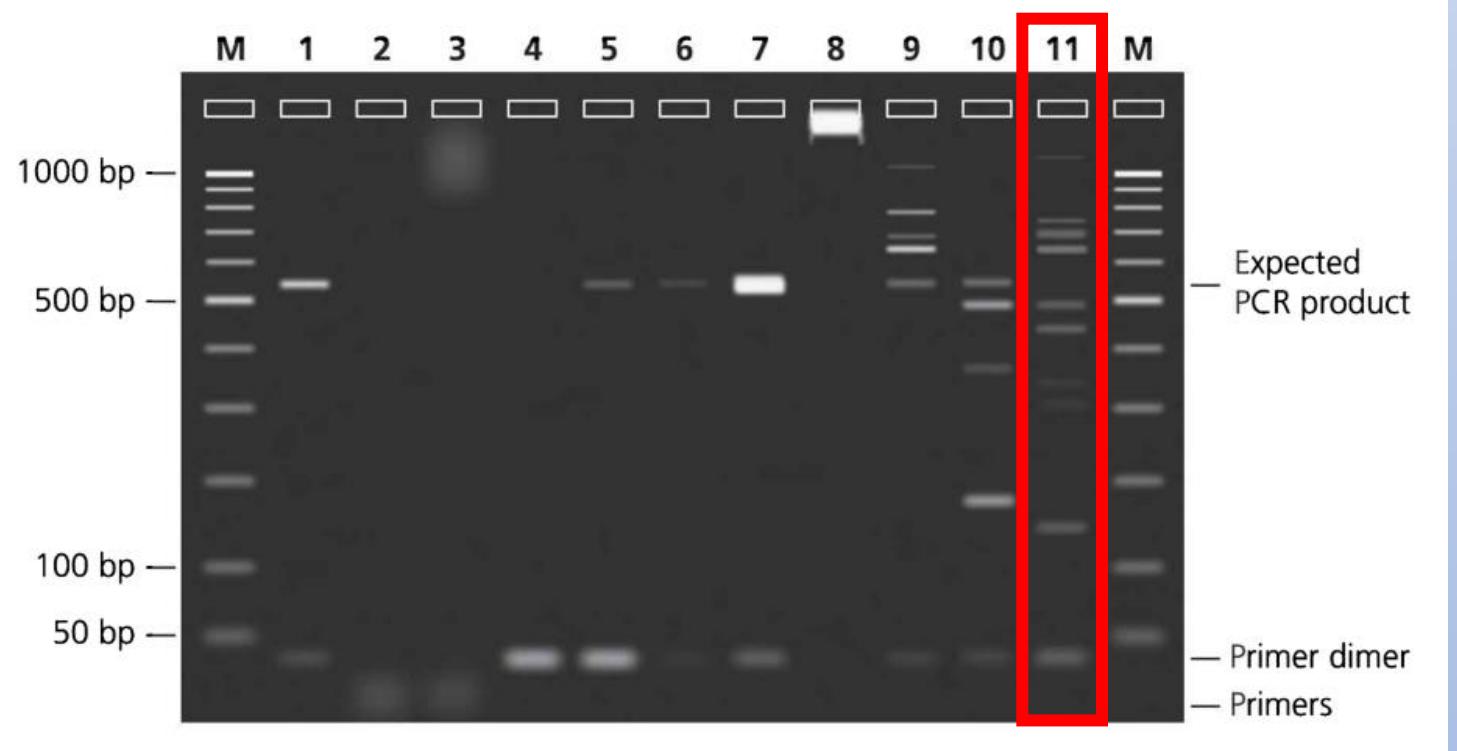
PCR optimization



Increase the annealing temperature.
Increase the elongation time.



PCR optimization

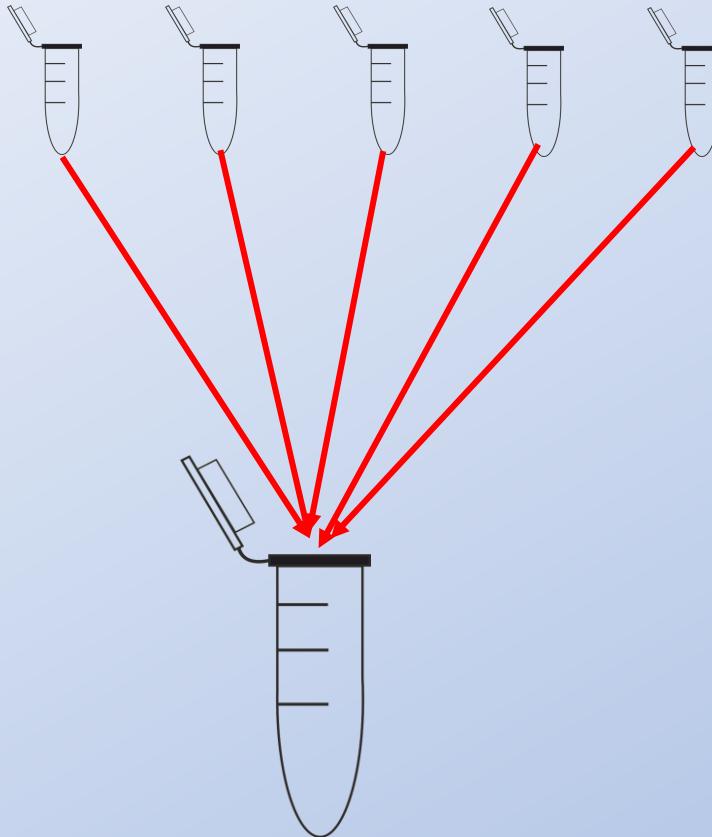


Maybe only a single primer is able to anneal

Decrease the annealing temperature.

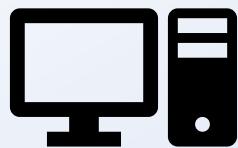


equimolarly pooled samples → sequencing



lib prep.





Sequencing data analyses

nature
biotechnology

Assessment of variation in microbial community amplicon sequencing by the Microbiome Quality Control (MBQC) project consortium

Rashmi Sinha¹, Galeb Abu-Ali^{2,3}, Emily Vogtmann¹ , Anthony A Fodor⁴, Boyu Ren², Amnon Amir⁵, Emma Schwager^{2,3} , Jonathan Crabtree⁶, Siyuan Ma^{2,3}, The Microbiome Quality Control Project Consortium⁷, Christian C Abnet¹ , Rob Knight^{5,8} , Owen White⁶ & Curtis Huttenhower^{2,3}

MycobKeys 39: 29–40 (2018)
doi: 10.3897/mycobkeys.39.28109
<http://mycobkeys.pensoft.net>

RESEARCH ARTICLE



Great differences in performance and outcome of high-throughput sequencing data analysis platforms for fungal metabarcoding

Sten Anslan¹, R. Henrik Nilsson², Christian Wurzbacher³, Petr Baldrian⁴, Leho Tedersoo⁵, Mohammad Bahram^{6,7,8}

Contents lists available at ScienceDirect



Fungal Ecology

journal homepage: www.elsevier.com/locate/funeco



Bioinformatics matters: The accuracy of plant and soil fungal community data is highly dependent on the metabarcoding pipeline

Charlie Pauvert ^a, Marc Buée ^b, Valérie Laval ^c, Véronique Edel-Hermann ^d, Laure Fauchery ^b, Angélique Gautier ^c, Isabelle Lesur ^{a,e}, Jessica Vallance ^f, Corinne Vacher ^{a,*}



RESEARCH ARTICLE

Bioinformatic Amplicon Read Processing Strategies Strongly Affect Eukaryotic Diversity and the Taxonomic Composition of Communities

Markus Majaneva^{1,2*}, Kirsi Hyttäinen^{1,2}, Sirkka Liisa Varvio³, Satoshi Nagai⁴, Jaanika Blomster¹

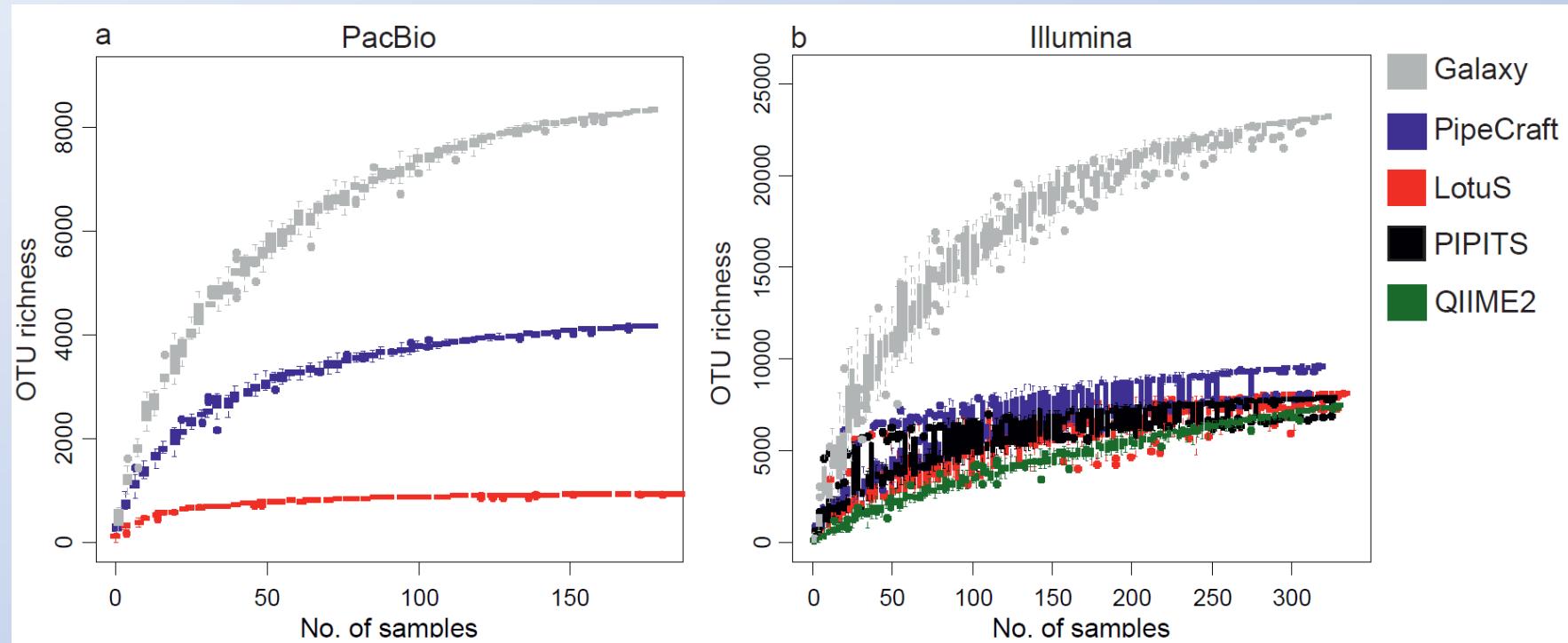


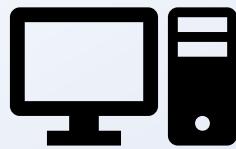
ITS1-5.8S-ITS2

ITS2

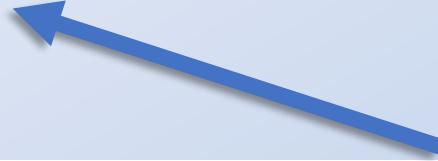
Great differences in performance and outcome of high-throughput sequencing data analysis platforms for fungal metabarcoding

Sten Anslan¹, R. Henrik Nilsson², Christian Wurzbacher³, Petr Baldrian⁴,
Leho Tedersoo⁵, Mohammad Bahram^{6,7,8}



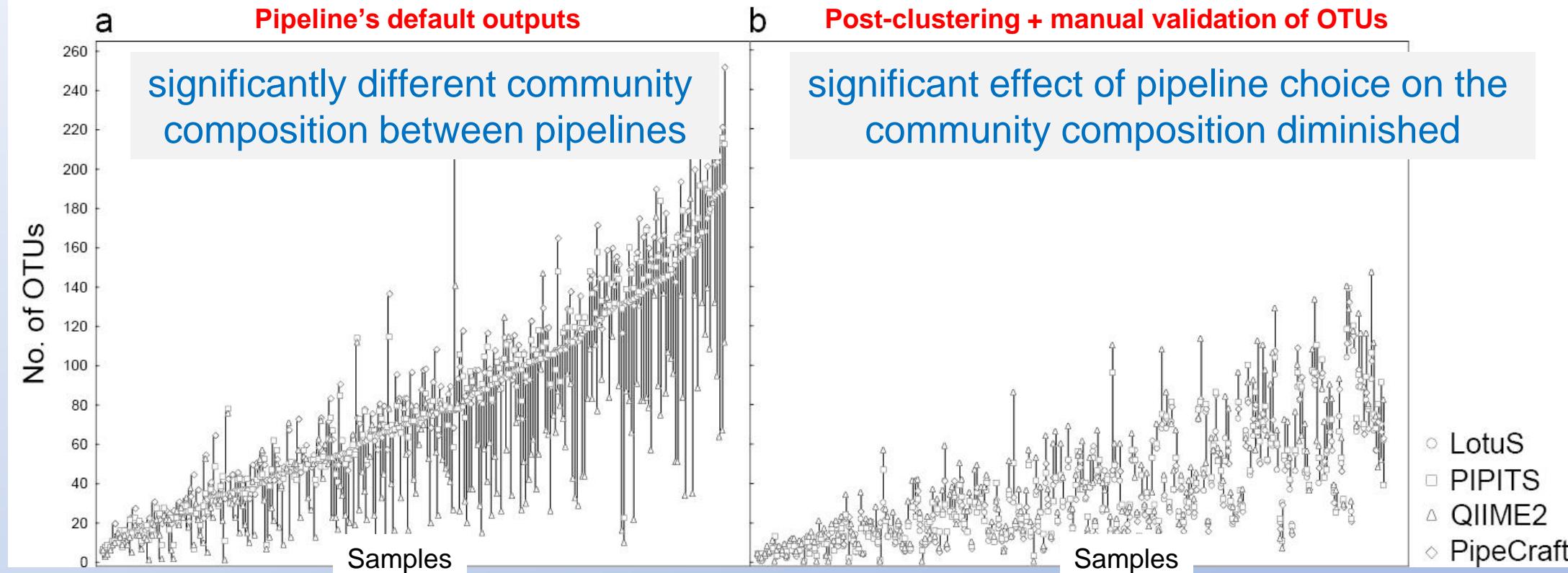


Based on taxonomy assignment values



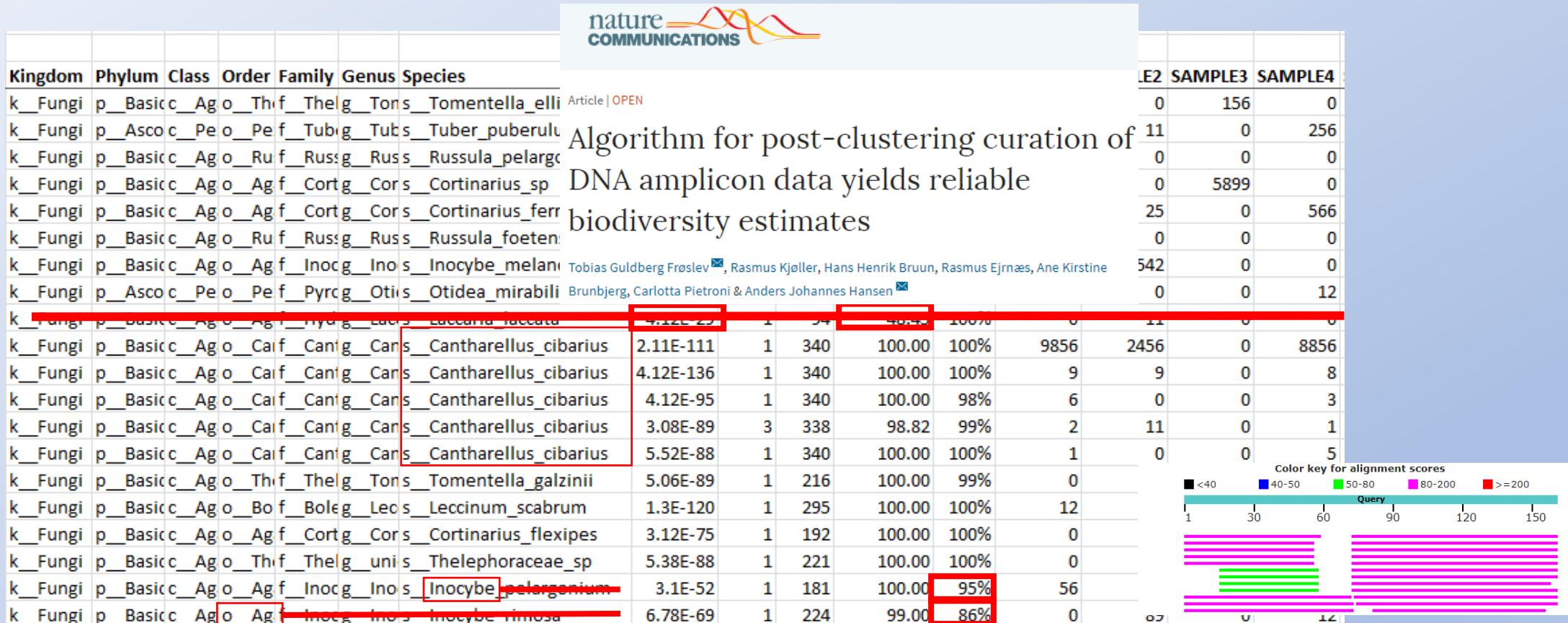
Great differences in performance and outcome of high-throughput sequencing data analysis platforms for fungal metabarcoding

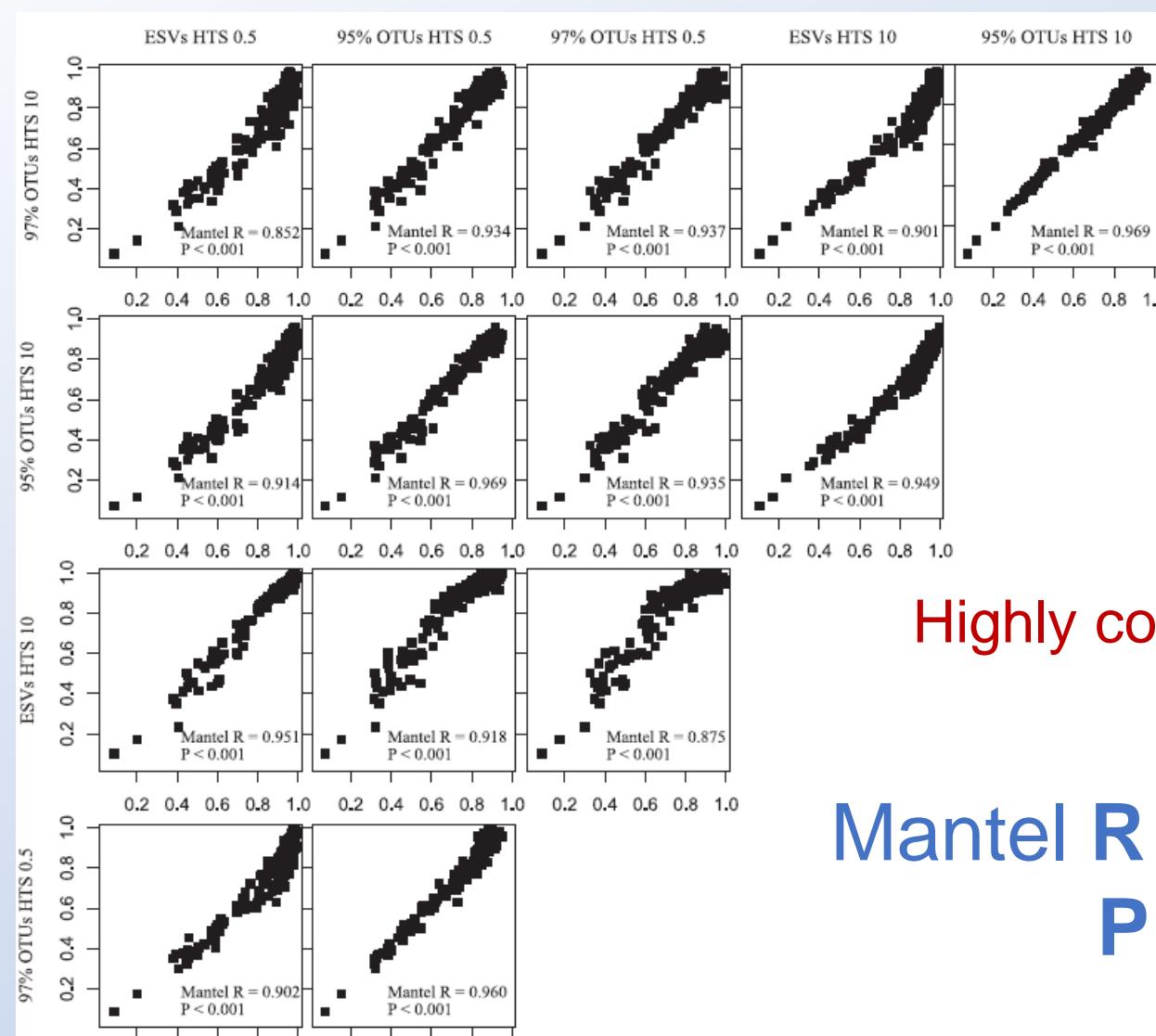
Sten Anslan¹, R. Henrik Nilsson², Christian Wurzbacher³, Petr Baldrian⁴,
Leho Tedersoo⁵, Mohammad Bahram^{6,7,8}





Curation of the OTU matrix





Highly correlating results from different pipelines

Mantel R > 0.85
P < 0.001



Diatom metabarcoding and microscopic analyses from sediment samples at Lake Nam Co, Tibet: The effect of sample-size and bioinformatics on the identified communities

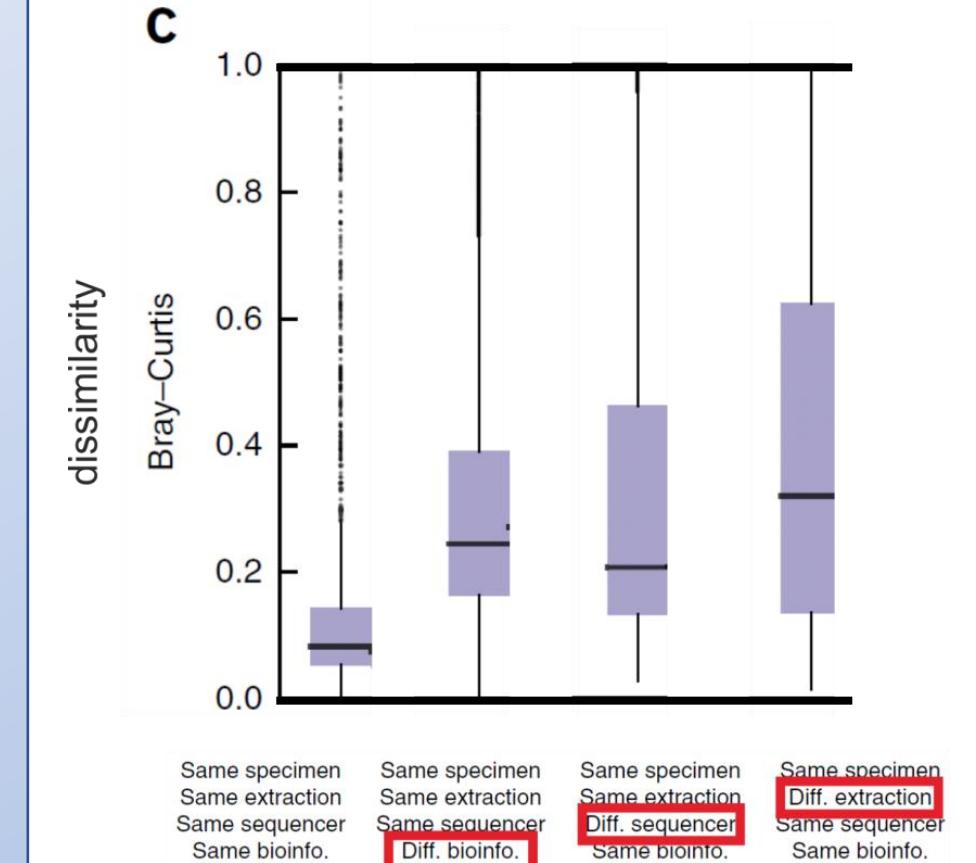
Wengang Kang^a, Sten Anslan^{b,*}, Nicole Börner^a, Anja Schwarz^a, Robin Schmidt^b, Sven Künzel^c, Patrick Rioual^{d,f}, Paula Echeverría-Galindo^a, Miguel Vences^b, Junbo Wang^c, Antje Schwalb^a

Effect size distributions of technical variation

Assessment of variation in microbial community amplicon sequencing by the Microbiome Quality Control (MBQC) project consortium

Rashmi Sinha¹, Galeb Abu-Ali^{2,3}, Emily Vogtmann¹✉, Anthony A Fodor⁴, Boyu Ren², Amnon Amir⁵, Emma Schwager^{2,3}✉, Jonathan Crabtree⁶, Siyuan Ma^{2,3}, The Microbiome Quality Control Project Consortium⁷, Christian C Abnet¹✉, Rob Knight^{5,8}✉, Owen White⁶ & Curtis Huttenhower^{2,3}✉

nature
biotechnology



Sinha et al 2017

Considerable variation in metabarcoding results from similar biodiversity analyses undertaken in different labs

Sources of variation:

- Preservation puffer (*RNAlater vs. LifeGuard*)
- Type of DNA polymerase (*six polymerases used, random variation?*)
- Addition of PCR enhancers (+BSA [bovine serum albumin], preventing inhibitions)

OTU matrix curation alleviates the variation:

- Removing contaminated samples
- Removing samples with low sequence yield
- Removing samples with extremely low number of OTUs

Towards reproducible metabarcoding data: Lessons from an international cross-laboratory experiment

Anastasija Zaiko^{1,2} | Paul Greenfield^{3,4} | Cathryn Abbott⁵ | Ulla von Ammon¹ | Jaret Bilewitz⁶ | Michael Bunce⁷ | Melania E. Cristescu⁸ | Anthony Charlton⁴ | Eddy Dowle⁹ | Jonathan Geller¹⁰ | Alba Ardura Gutierrez¹¹ | Mehrdad Hajibabaei¹² | Emmet Haggard¹⁰ | Graeme J. Inglis¹³ | Shane D. Lavery^{2,14} | Aurelija Samuloviene¹⁵ | Tiffany Simpson¹⁶ | Michael Stat¹⁷ | Sarah Stephenson³ | Judy Sutherland⁶ | Vibha Thakur¹⁴ | Kristen Westfall⁵ | Susanna A. Wood¹ | Michael Wright¹² | Guang Zhang⁸ | Xavier Pochon^{1,2}

It is critical to clearly write the methods in publications



INVITED TECHNICAL REVIEW

WILEY MOLECULAR ECOLOGY RESOURCES

Towards robust and repeatable sampling methods in eDNA-based studies

Ian A. Dickie^{1,2} | Stephane Boyer^{3,4} | Hannah L. Buckley⁵ | Richard P. Duncan⁶ |
Paul P. Gardner² | Ian D. Hogg^{7,8} | Robert J. Holdaway⁹ | Gavin Lear¹⁰ |
Andreas Makiola¹ | Sergio E. Morales¹¹ | Jeff R. Powell¹² | Louise Weaver¹³

Received: 28 September 2021 | Revised: 7 February 2022 | Accepted: 30 March 2022

DOI: 10.1111/mec.16460

INVITED REVIEW

MOLECULAR ECOLOGY WILEY

Best practices in metabarcoding of fungi: From experimental design to results

Leho Tedersoo^{1,2} | Mohammad Bahram^{1,3} | Lucie Zinger^{4,5} | R. Henrik Nilsson⁶ |
Peter G. Kennedy⁷ | Teng Yang⁸ | Sten Anslan⁹ | Vladimir Mikryukov^{1,9}

bioRxiv preprint doi: <https://doi.org/10.1101/2022.05.04.490577>; this version posted May 4, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Best practice recommendations for sample preservation in metabarcoding studies: a case study on diatom environmental samples

Baricevic Ana¹, Chardon Cécile², Kahlert Maria³, Karjalainen Satu Maaria⁴, Maric Pfannkuchen Daniela¹, Pfannkuchen Martin¹, Rimet Frédéric², Smoldlaka Tankovic Mirta¹, Trobajo Rosa⁵, Vasselon Valentin^{2,6}, Zimmermann Jonas⁷, Bouchez Agnès²



(GIGA)ⁿ
SCIENCE

GigaScience, 2022, 11, 1–12

DOI: 10.1093/gigascience/giac065

Review

Toward global integration of biodiversity big data: a harmonized metabarcode data generation module for terrestrial arthropods

Paula Arribas^{1,*}, Carmelo Andújar¹, Kristine Bohmann², Jeremy R. deWaard^{3,4}, Evan P. Economo⁵, Vasco Elbrecht⁶, Stefan Geisen⁷, Marta Goberna⁸, Henrik Krehenwinkel⁹, Vojtech Novotny^{10,11}, Lucie Zinger^{12,13}, Thomas J. Creedy¹⁴, Emmanouil Meramveliotakis¹⁵, Víctor Noguerales¹, Isaac Overcast¹², Hélène Morlon¹², Anna Papadopoulou¹⁵, Alfried P. Vogler^{14,16} and Brent C. Emerson¹

IMBMG
Metabarcoding & Metagenomics

Metabarcoding and Metagenomics 5: 233–247
DOI 10.3897/mbmg.5.71107

Research Article



Towards harmonization of DNA metabarcoding for monitoring marine macrobenthos: the effect of technical replicates and pooled DNA extractions on species detection

Laure Van den Bulcke^{1,2}, Annelies De Backer¹, Bart Ampe¹, Sara Maes¹,
Jan Wittoeck¹, Willem Waegeman², Kris Hostens¹, Sofie Derycke^{1,2}

Received: 3 May 2021 | Revised: 28 July 2021 | Accepted: 23 August 2021

DOI: 10.1111/1755-0998.13502

INVITED TECHNICAL REVIEW

MOLECULAR ECOLOGY RESOURCES WILEY

Coming of age for COI metabarcoding of whole organism community DNA: Towards bioinformatic harmonisation

Thomas J. Creedy¹ | Carmelo Andújar² | Emmanouil Meramveliotakis³ |
Victor Noguerales^{2,3} | Isaac Overcast⁴ | Anna Papadopoulou³ | Hélène Morlon⁴ |
Alfried P. Vogler^{1,5} | Brent C. Emerson² | Paula Arribas²



Teaching new students