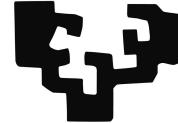




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Genomics of Marine Resources Metagenomics

2018 May 14-17

Antton Alberdi - antton.alberdi@smn.ku.dk
Assistant Professor - University of Copenhagen

0 Introducing myself

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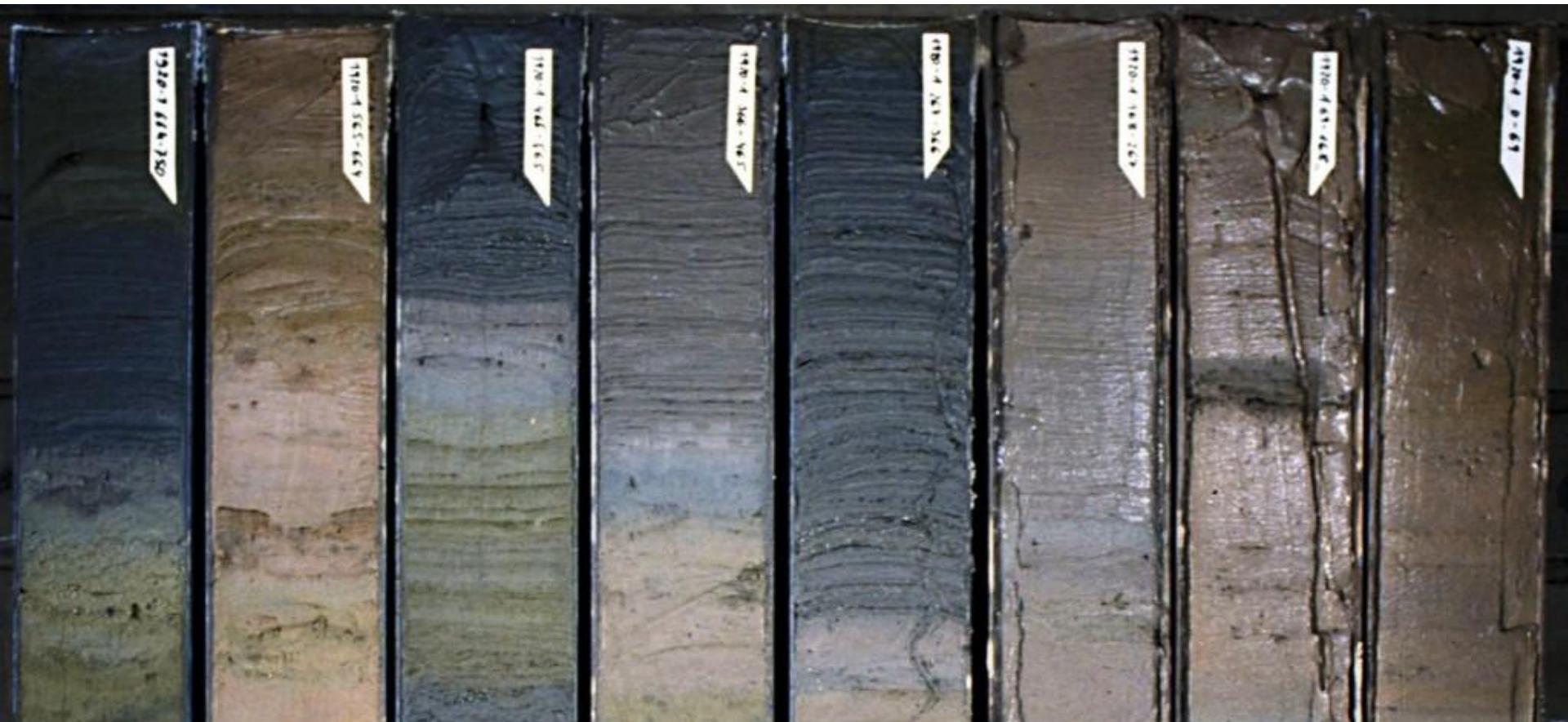
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0 Introducing myself



Trends in Ecology & Evolution

CellPress

Opinion

Do Vertebrate Gut Metagenomes Confer Rapid Ecological Adaptation?

Antton Alberdi,^{1,*} Ostaizka Aizpurua,¹ Kristine Bohmann,^{1,2}
Marie Lisandra Zepeda-Mendoza,¹ and
M. Thomas P. Gilbert^{1,3,4,*}

During times of rapid environmental change, survival of most vertebrate populations depends on their phenomic plasticity. Although differential gene-expression and post-transcriptional processes of the host genome receive focus as the main molecular mechanisms, growing evidence points to the gut microbiota as a key driver defining hosts' phenotypes. We propose that the plasticity of the gut microbiota might be an essential factor determining phenomic plasticity of vertebrates, and that it might play a pivotal role when

Trends

Recent studies highlight the large impact that gut microbiota has on host biology.

The vertebrate gut microbiota is a plastic element that can vary considerably as a response to environmental change. This variation can influence host phenomes,

Received: 18 May 2017 | Accepted: 26 June 2017

DOI: 10.1111/2041-210X.12849

RESEARCH ARTICLE



Scrutinizing key steps for reliable metabarcoding of environmental samples

Antton Alberdi¹  | Ostaizka Aizpurua¹ | M. Thomas P. Gilbert^{1,2,3} | Kristine Bohmann^{1,4}

¹Section for Evolutionary Genomics, Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark

²Trace and Environmental DNA Laboratory, Department of Environment and Agriculture, Curtin University, Perth, WA, Australia

³NTNU University Museum, Trondheim, Norway

⁴School of Biological Sciences, University of East Anglia, Norwich, Norfolk, UK

Abstract

1. Metabarcoding of environmental samples has many challenges and limitations that require carefully considered laboratory and analysis workflows to ensure reliable results. We explore how decisions regarding study design, laboratory set-up, and bioinformatic processing affect the final results, and provide guidelines for reliable study of environmental samples.
2. We evaluate the performance of four primer sets targeting COI and 16S regions characterizing arthropod diversity in bat faecal samples, and investigate how metabarcoding results are affected by parameters including: (1) number of PCR replicates;

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Trends in Biotechnology

CellPress
REVIEWS

Opinion

Applied Hologenomics: Feasibility and Potential in Aquaculture

Morten T. Limborg,^{1,*,@} Antton Alberdi,¹ Miyako Kodama,¹ Michael Roggenbuck,² Karsten Kristiansen,^{3,4} and M. Thomas P. Gilbert^{1,5}

Aquaculture will play an essential role in feeding a growing human population, but several biological challenges impede sustainable growth of production. Emerging evidence across all areas of life has revealed the importance of the intimate biological interactions between animals and their associated gut microbiota. Based on challenges in aquaculture, we leverage current knowledge in molecular biology and host microbiota interactions to propose an applied holo-omic framework that integrates molecular data including genomes, transcriptomes, epigenomes, proteomes, and metabolomes for analyzing fish and their gut microbiota as interconnected and coregulated systems. With an eye towards aquaculture, we discuss the feasibility and potential of our holo-omic framework to improve growth, health, and sustainability in any area of food production, including livestock and agriculture.

Highlights

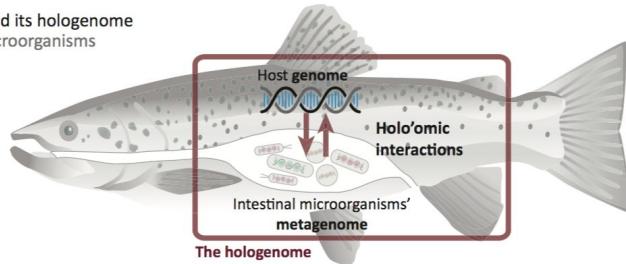
Both the host genotype and gut microbiota of an animal play significant roles in shaping key phenotypes of aquacultural relevance, including growth metabolism and immune functions.

Traditional approaches to improve production have relied on selecting for direct genotype–phenotype correlations or on directly modulating gut microbiome communities.

The hologenome theory argues that the genomes of host organisms and their associated microbial communities are subject to biological interactions, and

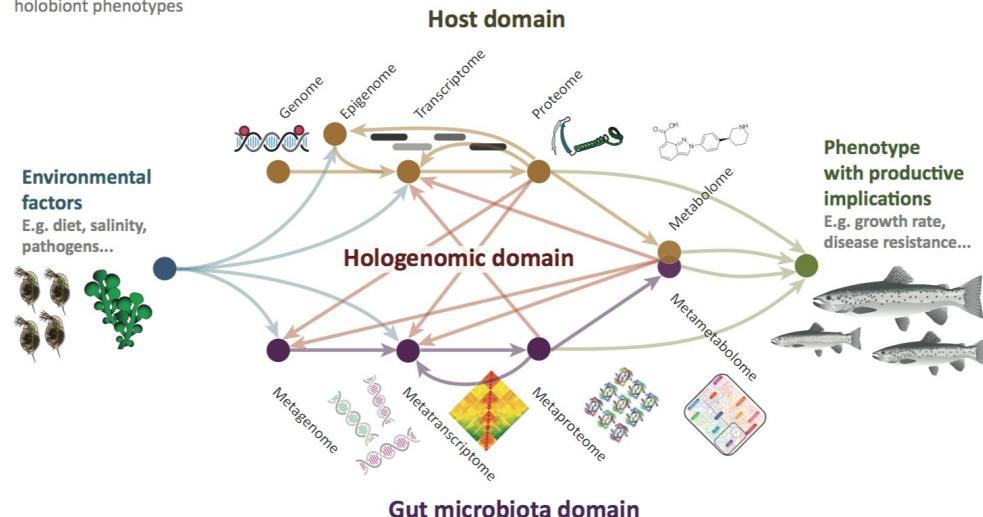
Introducing myself

(A) The holobiont and its hologenome
Host + intestinal microorganisms



(B) Holo'omic interactions

Biomolecular interactions between hosts and symbiotic microorganisms triggered by environmental factors yield different holobiont phenotypes



Trends in Biotechnology

CellPress
REVIEWS

Opinion

Applied Hologenomics: Feasibility and Potential in Aquaculture

0 Introducing the course



0 Introducing the course



Monday afternoon

Tuesday afternoon

Wednesday morning

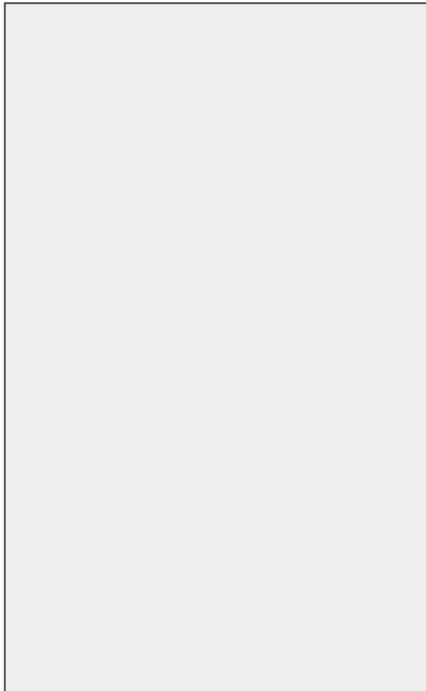
Thursday afternoon

0 Introducing the course

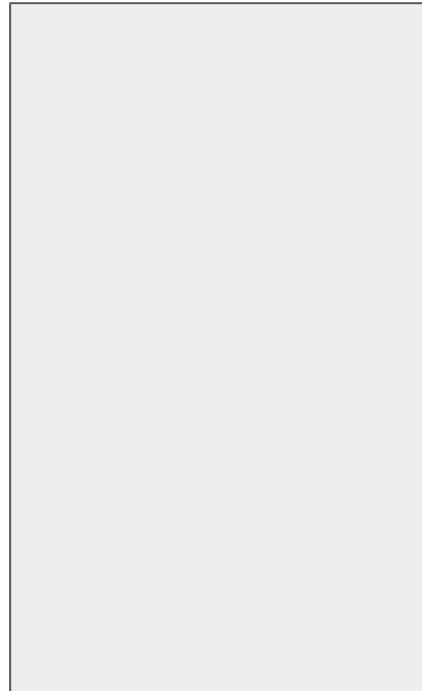
Monday afternoon

- Introduction to eDNA research
- Experimental design
- Introduction to the exercises

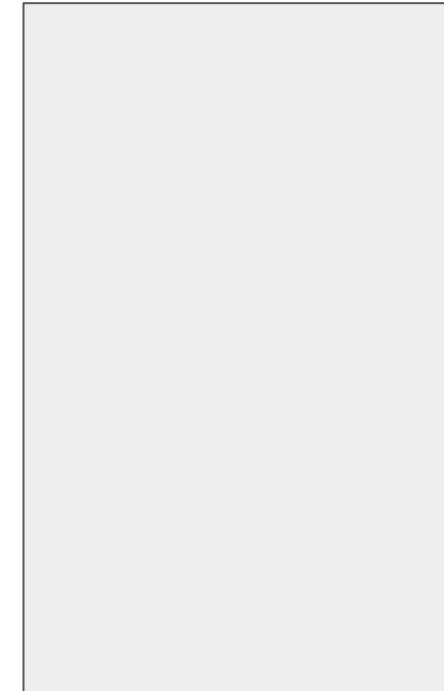
Tuesday afternoon



Wednesday morning



Thursday afternoon



0 Introducing the course

Monday afternoon

- Introduction to eDNA research
- Experimental design
- Introduction to the exercises

Tuesday afternoon

- Introduction to DNA metabarcoding
- Using the terminal and remote servers
- Metabarcoding excercise

Wednesday morning

Thursday afternoon

0 Introducing the course

Monday afternoon

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- Experimental design
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- Metabarcoding excercise

Thursday afternoon

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- Experimental design
- Introduction to the exercises

Tuesday afternoon

- Introduction to DNA metabarcoding
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Wednesday morning

- Metabarcoding excercise
- Introduction to shotgun metagenomics
- Shotgun metagenomics excercise

Thursday afternoon

0 Introducing the course

Monday afternoon

- Introduction to eDNA research
- Experimental design
- Introduction to the exercises

Tuesday afternoon

- Introduction to DNA metabarcoding
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- Metabarcoding excercise

Wednesday morning

- Metabarcoding excercise
- Introduction to shotgun metagenomics
- Shotgun metagenomics excercise

Thursday afternoon

- Shotgun metagenomics excercise
- Summary and final remarks

0 Introducing the course

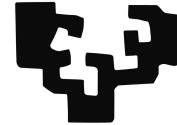


Evaluation:

- Lectures
- 2 exercises > 2 one-page reports
 - Sent by email before May 28th



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1

Introduction to eDNA research

Monday, May 14th

By Antton Alberdi - antton.alberdi@snm.ku.dk

1 Introduction to eDNA research



What is eDNA?

What is eDNA?

Complex mixtures of potentially degraded DNA from many different organisms that can be extracted from environmental samples such as soil, water or even air, without the need for individual extraction of organisms of interest (Taberlet et al. 2012, Molecular Ecology)

What is eDNA?

Complex mixtures of potentially degraded DNA from many different organisms that can be extracted from environmental samples such as soil, water or even air, without the need for individual extraction of organisms of interest (Taberlet et al. 2012, Molecular Ecology)

Faeces? Stomach contents? Saliva?

1 Introduction to eDNA research



Aims:

- Taxonomic profiling
- Functional profiling
- Population genomics

1 Introduction to eDNA research



Taxonomic profiling

- Prokaryotes/Eukaryotes
- Metabarcoding/Shotgun

Functional profiling

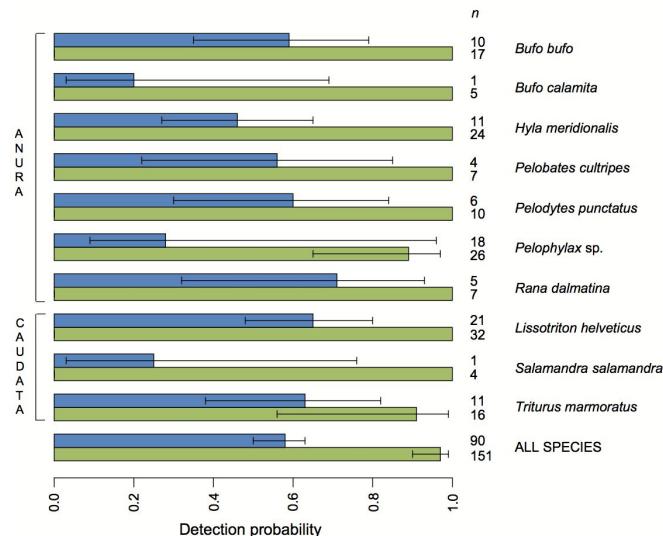
- Prokaryotes
- Shotgun sequencing

Biodiversity
detection

Microbiome
research

1 Introduction to eDNA research

Biodiversity detection



MOLECULAR ECOLOGY

Molecular Ecology (2016) 25, 929–942

doi: 10.1111/mec.13428

Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding

ALICE VALENTINI,* PIERRE TABERLET,†‡ CLAUDE MIAUD,§ RAPHAËL CIVADE,¶ JELGER HERDER,** PHILIP FRANCIS THOMSEN,†† EVA BELLEMAIN,* AURÉLIEN BESNARD,§ ERIC COISSAC,†‡ FRÉDÉRIC BOYER,†‡ COLINE GABORIAUD,* PAULINE JEAN,* NICOLAS POULET,†‡ NICOLAS ROSET,§§ GORDON H. COPP,¶¶**** PHILIPPE GENIEZ,§ DIDIER PONT,¶ CHRISTINE ARGILLIER,††† JEAN-MARC BAUDOIN,††† TIPHAINE PEROUX,††† ALAIN J. CRIVELLI,††† ANTHONY OLIVIER,††† MANON ACQUEBERGE,§§§ MATTHIEU LE BRUN,¶¶¶ PETER R. MØLLER,**** ESKE WILLERSLEV†† and TONY DEJEAN*

1 Introduction to eDNA research

Biodiversity detection

Received: 23 August 2017 | Revised: 6 November 2017 | Accepted: 8 December 2017

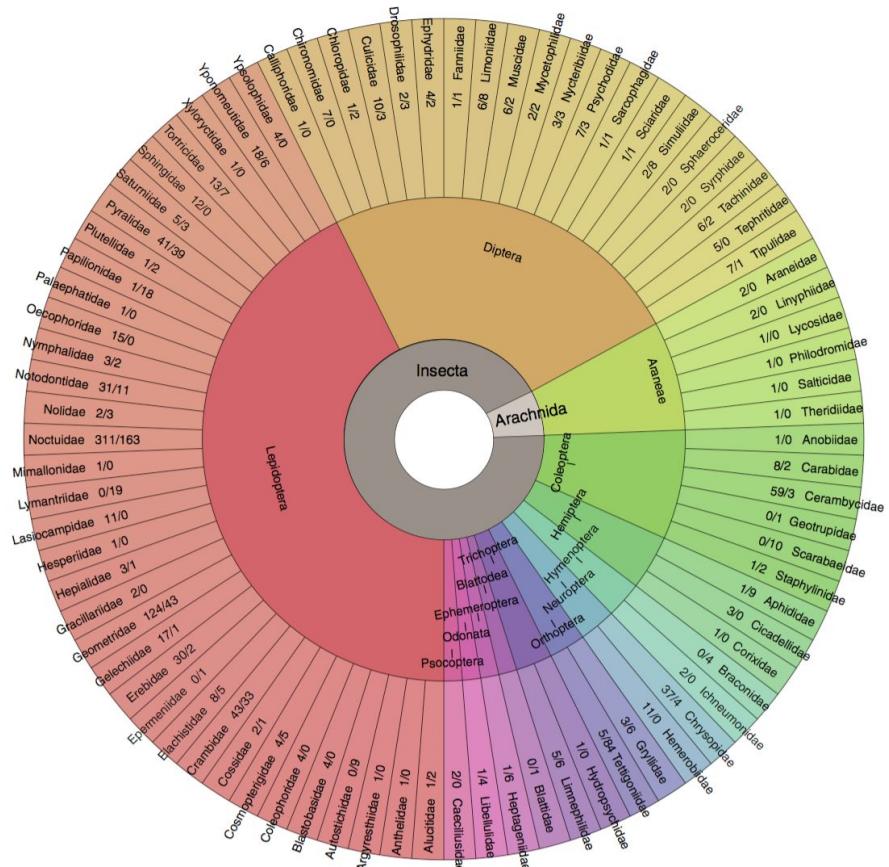
DOI: 10.1111/mec.14474

ORIGINAL ARTICLE

WILEY MOLECULAR ECOLOGY

Agriculture shapes the trophic niche of a bat preying on multiple pest arthropods across Europe: Evidence from DNA metabarcoding

Ostaizka Aizpurua¹ | Ivana Budinski² | Panagiotis Georgiakakis³ |
Shyam Gopalakrishnan¹ | Carlos Ibañez⁴ | Vanessa Mata⁵ | Hugo Rebelo⁵ |
Danilo Russo⁶ | Farkas Szodoray-Parádi⁷ | Violeta Zhelyazkova⁸ | Vida Zrncic⁹ |
M. Thomas P. Gilbert^{1,10} | Antton Alberdi¹ 



1 Introduction to eDNA research

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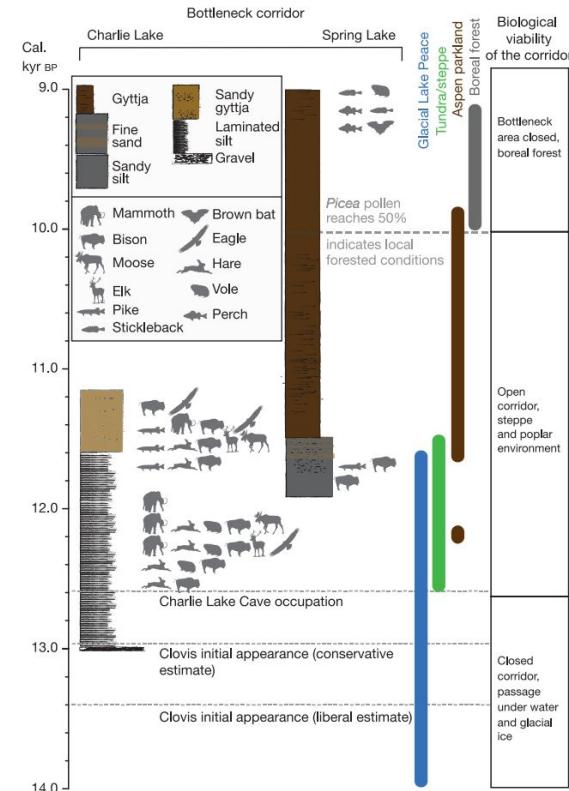
Biodiversity detection

ARTICLE

doi:10.1038/nature19085

Postglacial viability and colonization in North America's ice-free corridor

Mikkel W. Pedersen¹, Anthony Ruter¹, Charles Schweger², Harvey Friebe², Richard A. Staff³, Kristian K. Kjeldsen^{1,4},
Marie L. Z. Mendoza⁵, Alwynne B. Beaudoin⁶, Cynthia Zutter⁶, Nicolaj K. Larsen^{1,7}, Ben A. Potter⁸, Rasmus Nielsen^{1,9,10},
Rebecca A. Rainville¹¹, Ludovic Orlando¹, David J. Meltzer^{1,12}, Kurt H. Kjær¹ & Eske Willerslev^{1,13,14}



1 Introduction to eDNA research



Microbiome research

SCIENTIFIC REPORTS

OPEN

Evaluating the impact of domestication and captivity on the horse gut microbiome

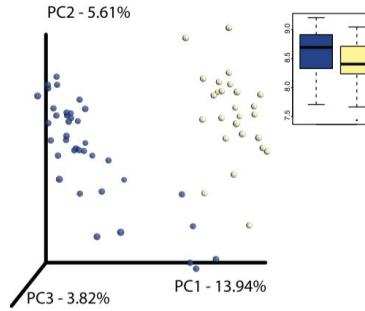
ed: 15 June 2017
ed: 26 October 2017
ied online: 14 November 2017

Jessica L. Metcalf¹, Se Jin Song^{2,3}, James T. Morton^{2,4}, Sophie Weiss⁵, Andaine Seguin-Orlando^{6,7}, Frédéric Joly⁸, Claudia Feh⁹, Pierre Taberlet⁹, Eric Coissac⁹, Amnon Amir¹⁰, Eske Willerslev⁷, Rob Knight¹⁰, Valerie McKenzie³ & Ludovic Orlando^{7,11}

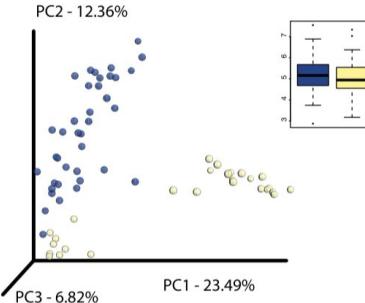
Przewalski's horse

Domestic horse

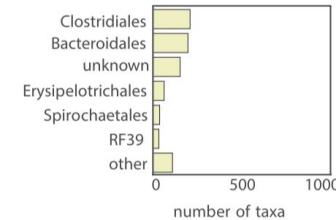
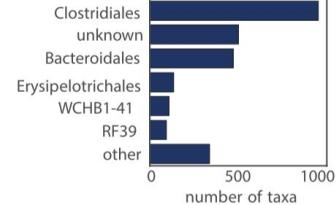
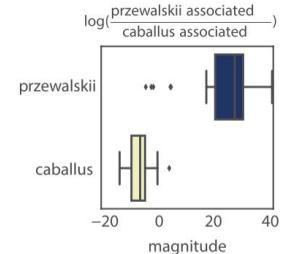
(a) Fecal Microbiome



(c) Plant Diet



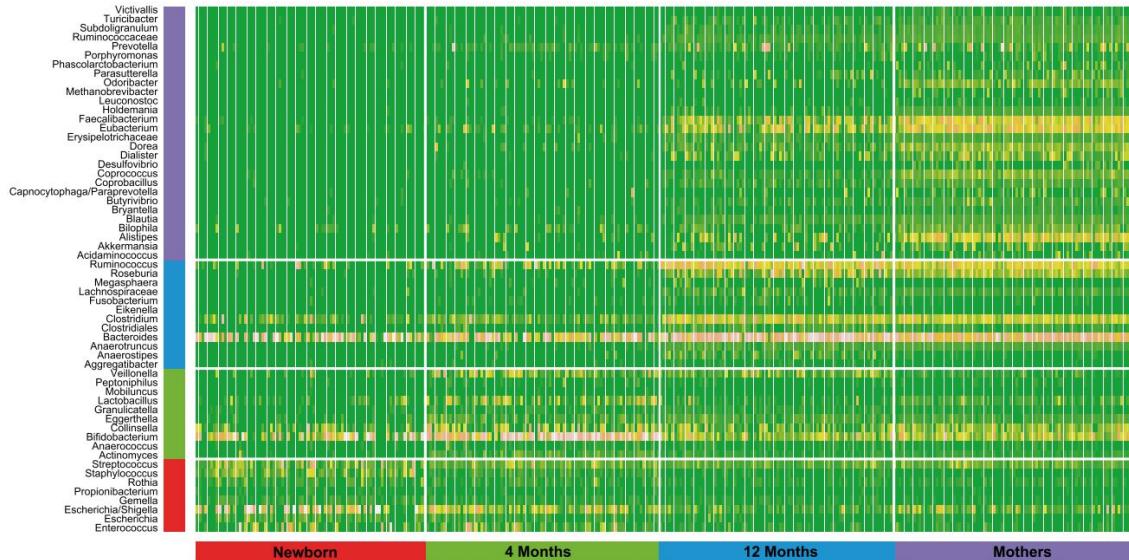
(b)



1 Introduction to eDNA research

Microbiome research

A

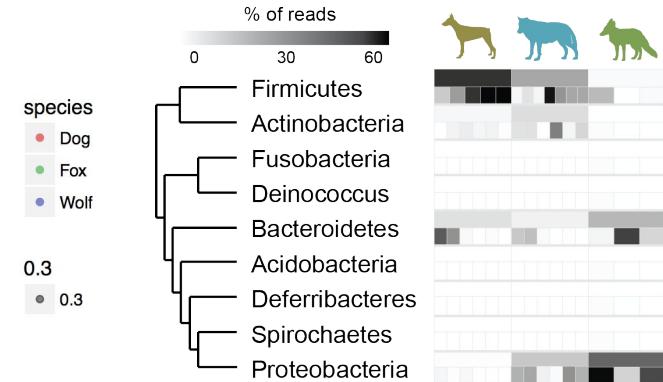
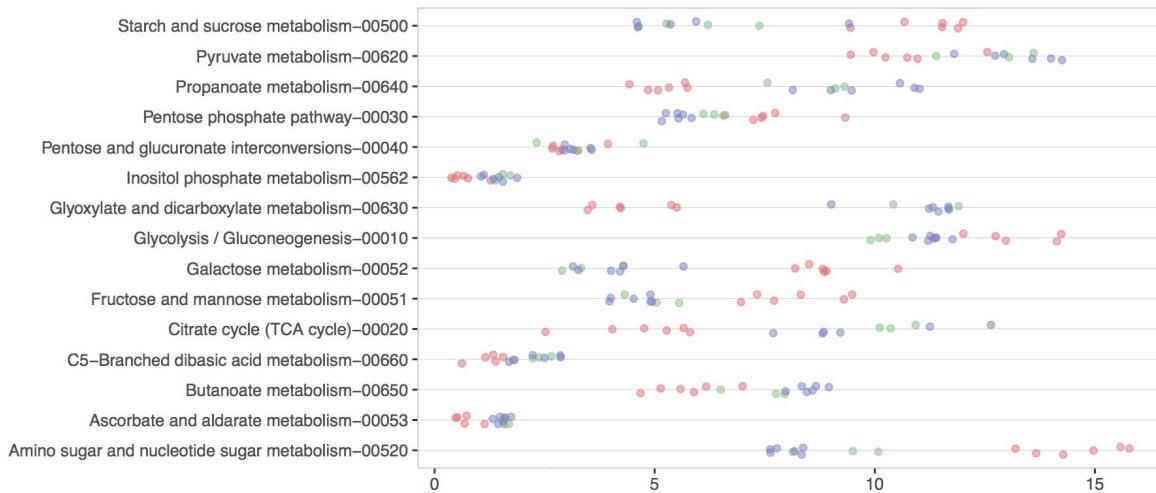


Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life

Frederik Bäckhed,^{1,2,*} Josefine Roswall,^{3,4} Yangqing Peng,⁵ Qiang Feng,^{5,6} Huijue Jia,⁵ Petia Kovatcheva-Datchary,¹ Yin Li,⁵ Yan Xia,³ Hailiang Xie,⁵ Huanzi Zhong,⁷ Muhammad Tanweer Khan,¹ Jianfeng Zhang,⁵ Junhua Li,⁵ Liang Xiao,⁵ Jumana Al-Aama,^{5,7} Dongya Zhang,⁵ Ying Shuan Lee,¹ Dorota Kotowska,⁸ Camilla Colding,⁶ Valentina Tremaroli,¹ Ye Yin,⁵ Stefan Bergman,^{1,8} Xun Xu,⁵ Lise Madsen,^{6,9} Karsten Kristiansen,^{5,6} Jovanna Dahlgren,^{4,12,*} and Jun Wang^{5,6,7,10,11,12,*}

1 Introduction to eDNA research

Microbiome research

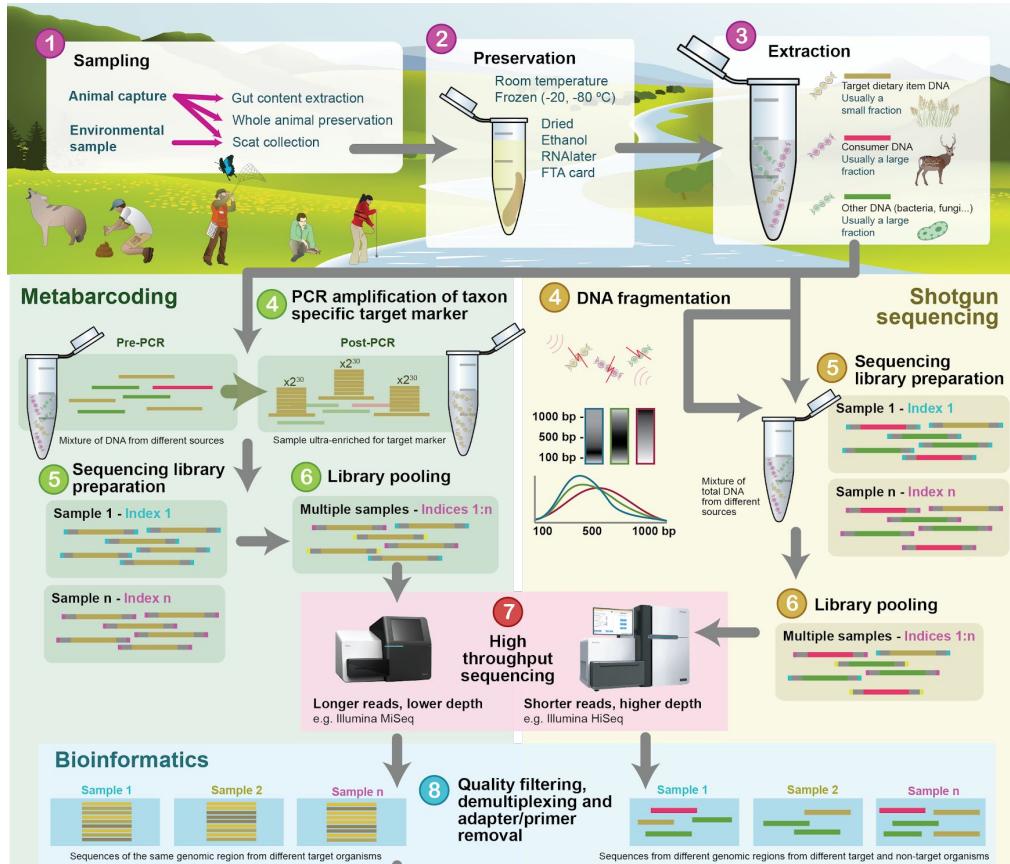


Alberdi et al. *in prep*

Metabarcoding vs Shotgun sequencing

1 Introduction to eDNA research

Shotgun sequencing vs. metabarcoding



Metabarcoding

Analytical procedure whereby **minibarcode primers** are used to **PCR amplify** taxa within a particular taxonomic group after which samples are sequenced **in parallel on a high-throughput sequencing platform** followed by **taxonomical identification** of sequences through comparison to DNA reference databases.

Commonly employed synonyms:

Metabarcoding

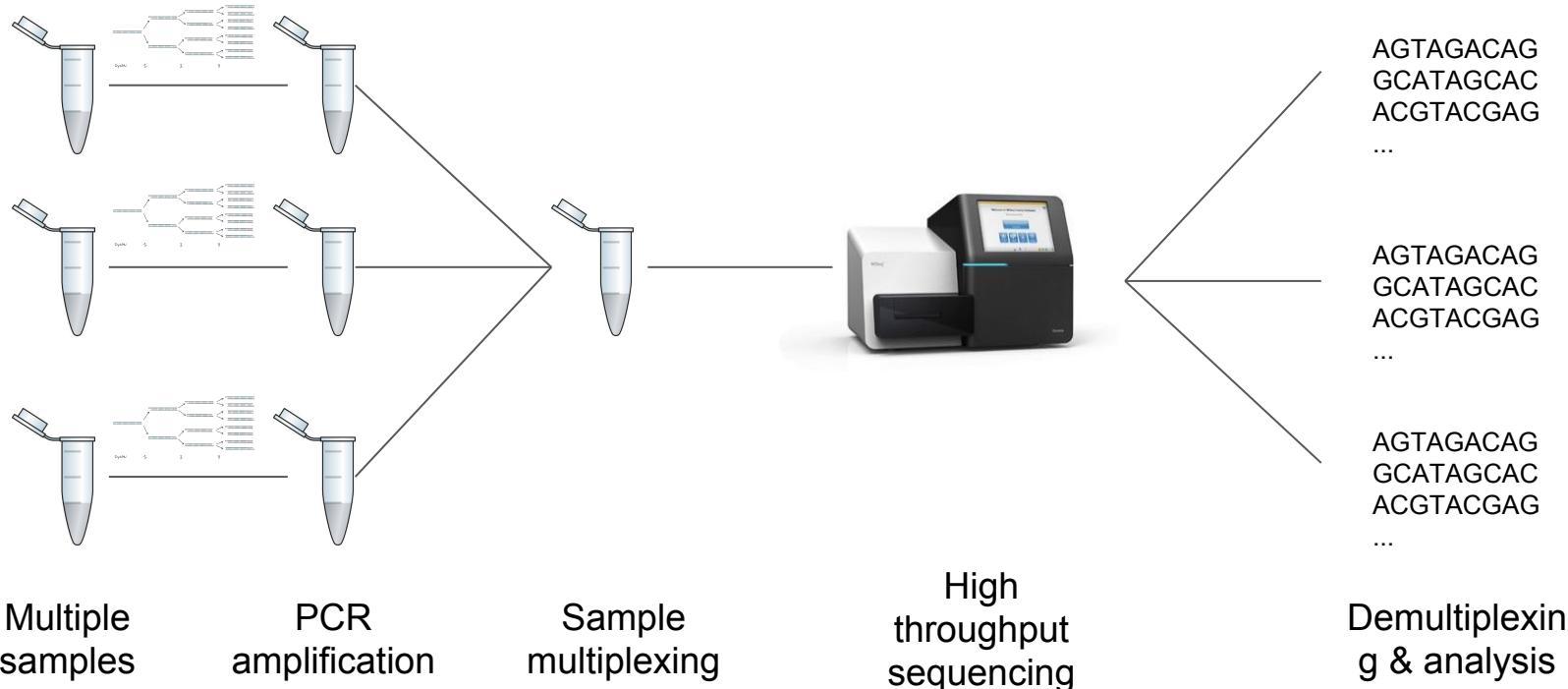
Amplicon sequencing

Targeted sequence metagenomics

...

1 Introduction to eDNA research

Metabarcoding



1 Introduction to eDNA research



Metabarcoding

GTCCAGACTGCACTATGCGAACGGGCTACCACTAATCGGACTACAGCTAACAGACT

1 Introduction to eDNA research



Metabarcoding

GTCCAGACTGCACTATGCGAACGGGCTACCACTAATCGGACTACAGCTAACAGACT

1 Introduction to eDNA research



Metabarcoding



GTCCAGACTGCACTATGCGAACGGGCTACCACTAATCGGACTACAGCTAACAGACT

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Metabarcoding

Forward  AGGTCTGACGTGA
TAATGCCTGATGTCG
Reverse  GTCCAGACTGCACTATGCGAACGGGCTACCACTAATCGGACTACAGCTAACAGACT

1 Introduction to eDNA research

Metabarcoding

$$\begin{aligned} A &= T \\ G &= C \end{aligned}$$

Forward 
AGGTCTGACGTGA

Reverse 
TAATGCCTGATGTCG

GTCCAGACTGCACTATGCGAACGGGCTACCACTAATCGGACTACAGCTAACAGACT

1 Introduction to eDNA research



Metabarcoding

AGGTCTGACGTGAATGCGAACGGGCTACCACTAATGCCTGATGTCG
AGGTCTGACGTGAATGCGAACGGGCTACCACTAATGCCTGATGTCG
AGGTCTGACGTGAATGCGAACGGGCTACCACTAATGCCTGATGTCG
AGGTCTGACGTGAATGCGAACGGGCTACCACTAATGCCTGATGTCG
AGGTCTGACGTGAATGCGAACGGGCTACCACTAATGCCTGATGTCG
AGGTCTGACGTGAATGCGAACGGGCTACCACTAATGCCTGATGTCG
AGGTCTGACGTGAATGCGAACGGGCTACCACTAATGCCTGATGTCG
GTCCAGACTGCACTATGCGAACGGGCTACCACTAATCGGACTACAGCTAACAGACT

1 Introduction to eDNA research



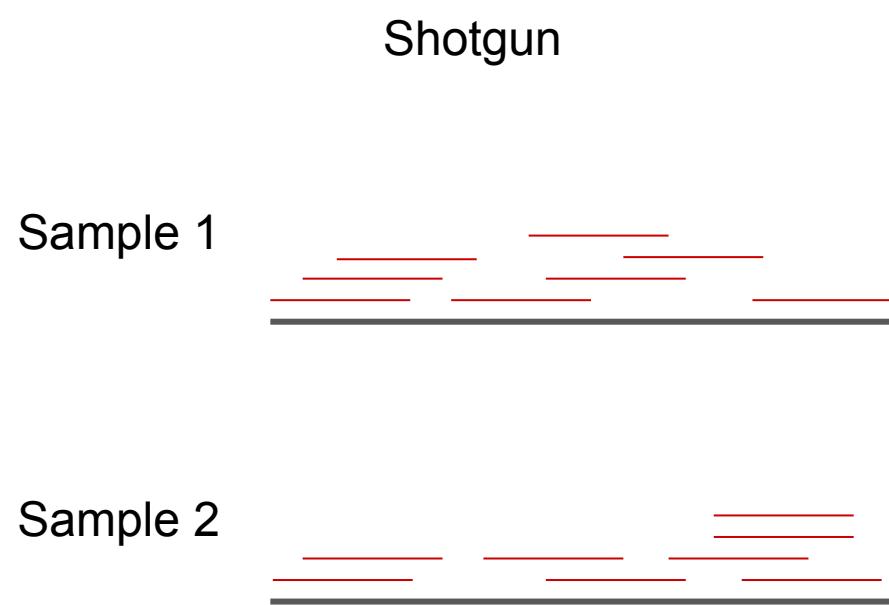
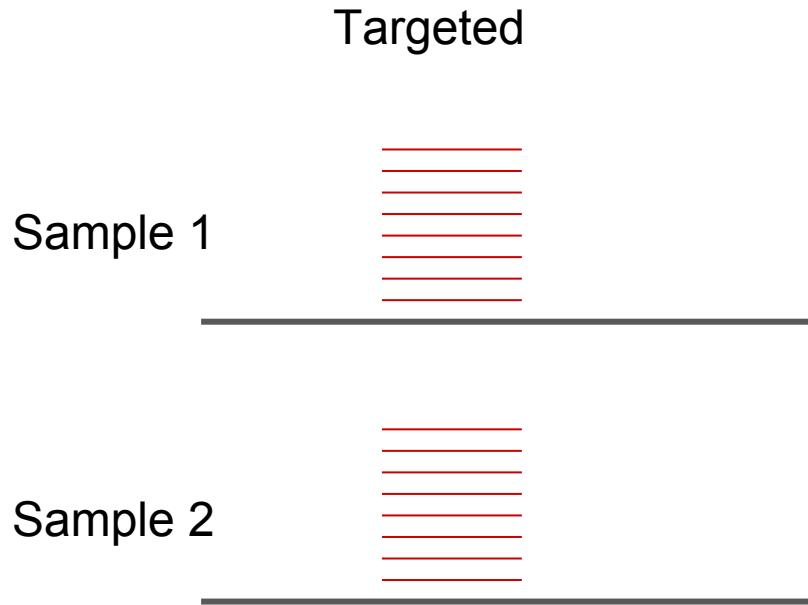
Metabarcoding

AGGTCTGACGTGAATGCGAACGGGCTACCACTAATGCCTGATGTCG
AGGTCTGACGTGAATGCGAACGGGCTACCACTAATGCCTGATGTCG
AGGTCTGACGTGAATGCGAACGGGCTACCACTAATGCCTGATGTCG
AGGTCTGACGTGAATGCGAACGGGCTACCACTAATGCCTGATGTCG
AGGTCTGACGTGAATGCGAACGGGCTACCACTAATGCCTGATGTCG
AGGTCTGACGTGAATGCGAACGGGCTACCACTAATGCCTGATGTCG
AGGTCTGACGTGAATGCGAACGGGCTACCACTAATGCCTGATGTCG
GTCCAGACTGCACTATGCGAACGGGCTACCACTAATCGGACTACAGCTAACAGACT

Standardised sequence = Barcode

1 Introduction to eDNA research

Shotgun sequencing



Metabarcoding vs. Shotgun sequencing



Metabarcoding

Small fraction of total DNA

Taxonomic identification

Biases

No quantitative

No functional profiling

Metagenomics

(Almost) no biases

Quantitative

Functional identification

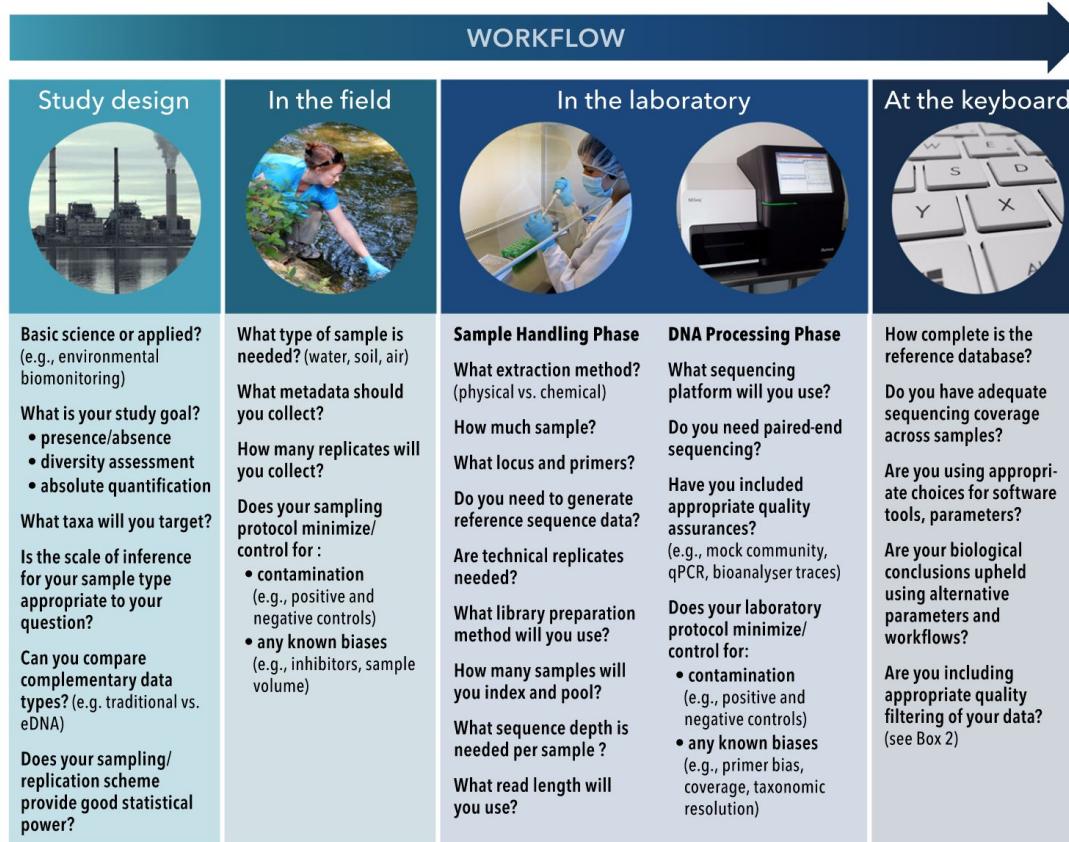
Large fraction of total DNA

No quantitative

1 Introduction to eDNA research

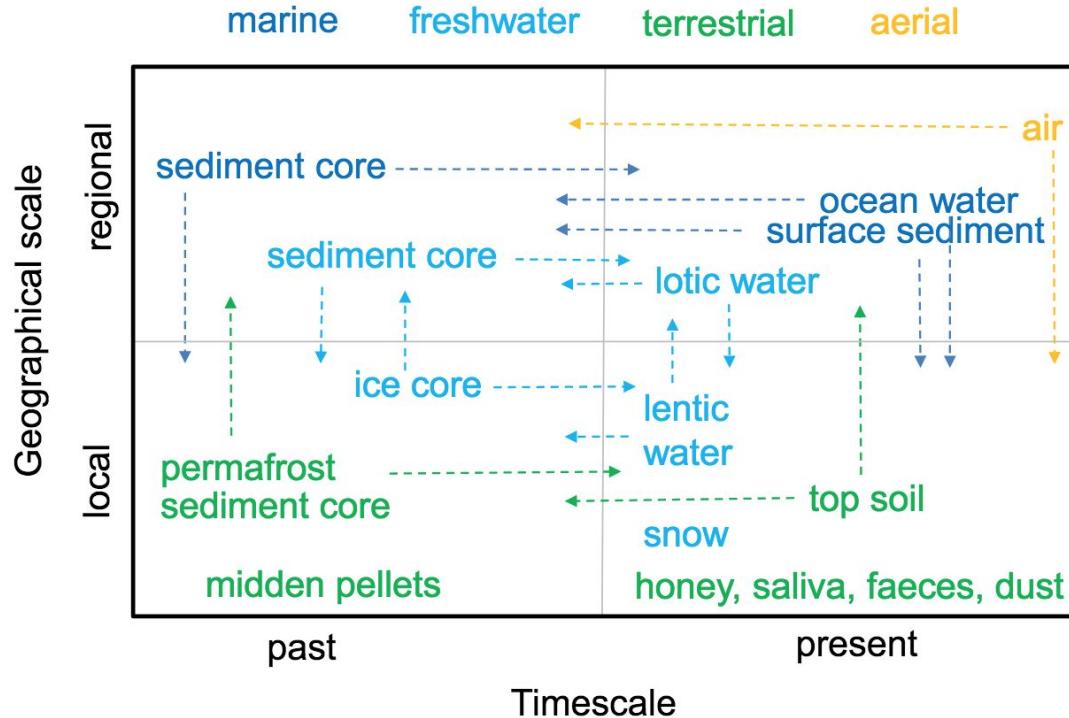


Study design



Deiner et al. 2017 Mol Ecol

1 Introduction to eDNA research



1 Introduction to eDNA research

Sample type: Open



1 Introduction to eDNA research

Sample type: Discrete

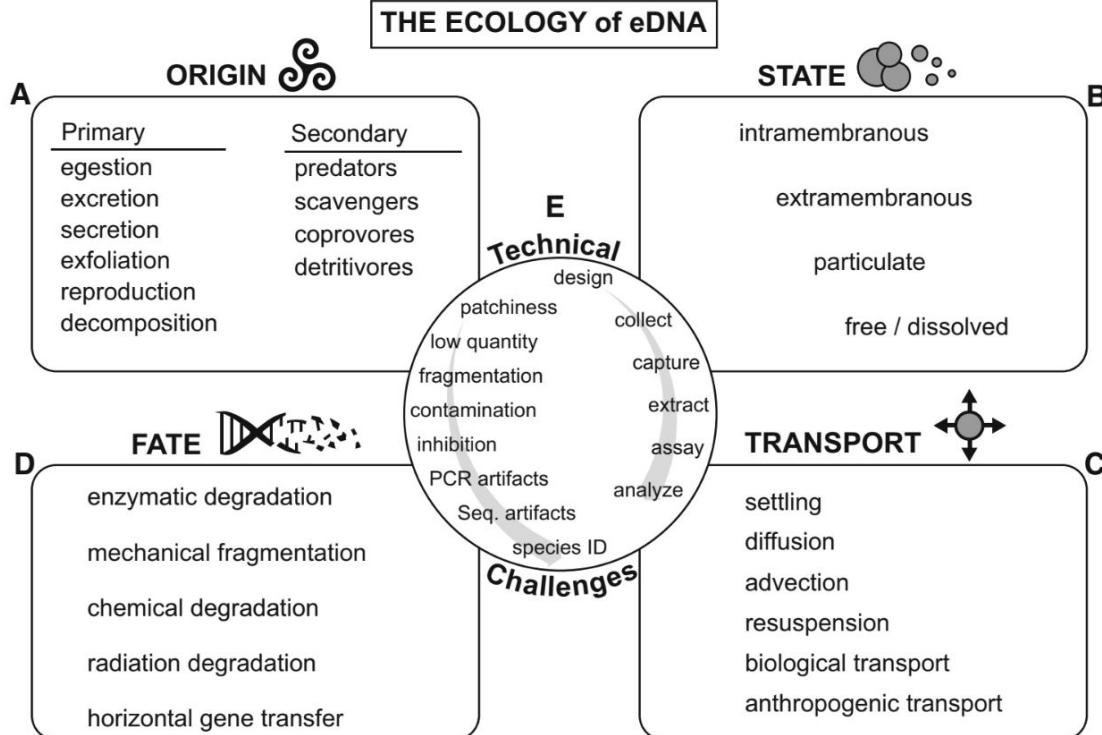


1 Introduction to eDNA research

Sample age: Modern vs. Ancient



1 Introduction to eDNA research



1 Introduction to eDNA research

Alberdi et al. 2018 Mol Ecol Res (under review)

A) Study design

Which system is the best to address my research question?



Which individuals should I target or avoid?
Adults, juveniles, breeding...



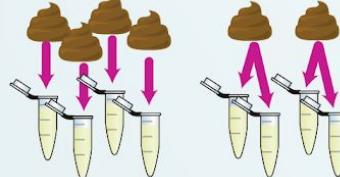
What spatial and temporal scale do I need to cover?



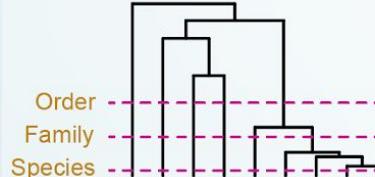
Should I pool the samples or treat them individually?



How many samples and replicates should I use?



What taxonomic resolution do I need?



Which sample type is the most appropriate?
Faeces, stomach contents...



Which type of error should I minimise (Type I/Type II)?

Reality		
	Taxon present	Taxon absent
Taxon present	 Type I	 Type II
Taxon absent	 Type II	 Type I

1 Introduction to eDNA research

Permits and accreditations

Environmental samples



No permit /
Permit

Animal manipulation



Permit
Accreditation

Directive 2010/63/EU

1 Introduction to exercises



Introduction to exercises

1 Introduction to exercises



Trends in Ecology & Evolution

CellPress

Opinion

Do Vertebrate Gut Metagenomes Confer Rapid Ecological Adaptation?

Antton Alberdi,^{1,*} Ostaizka Aizpurua,¹ Kristine Bohmann,^{1,2}
Marie Lisandra Zepeda-Mendoza,¹ and
M. Thomas P. Gilbert^{1,3,4,*}

During times of rapid environmental change, survival of most vertebrate populations depends on their phenomic plasticity. Although differential gene-expression and post-transcriptional processes of the host genome receive focus as the main molecular mechanisms, growing evidence points to the gut microbiota as a key driver defining hosts' phenotypes. We propose that the plasticity of the gut microbiota might be an essential factor determining phenomic plasticity of vertebrates, and that it might play a pivotal role when

Trends

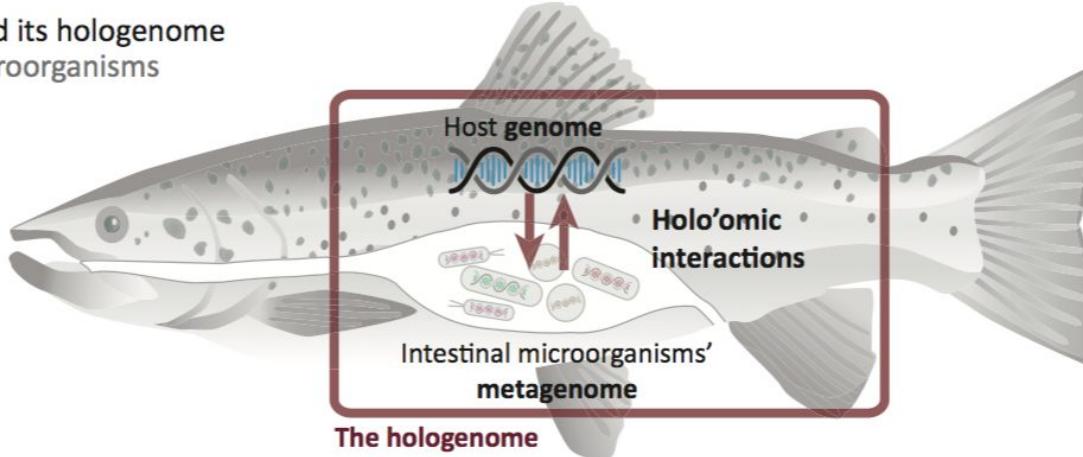
Recent studies highlight the large impact that gut microbiota has on host biology.

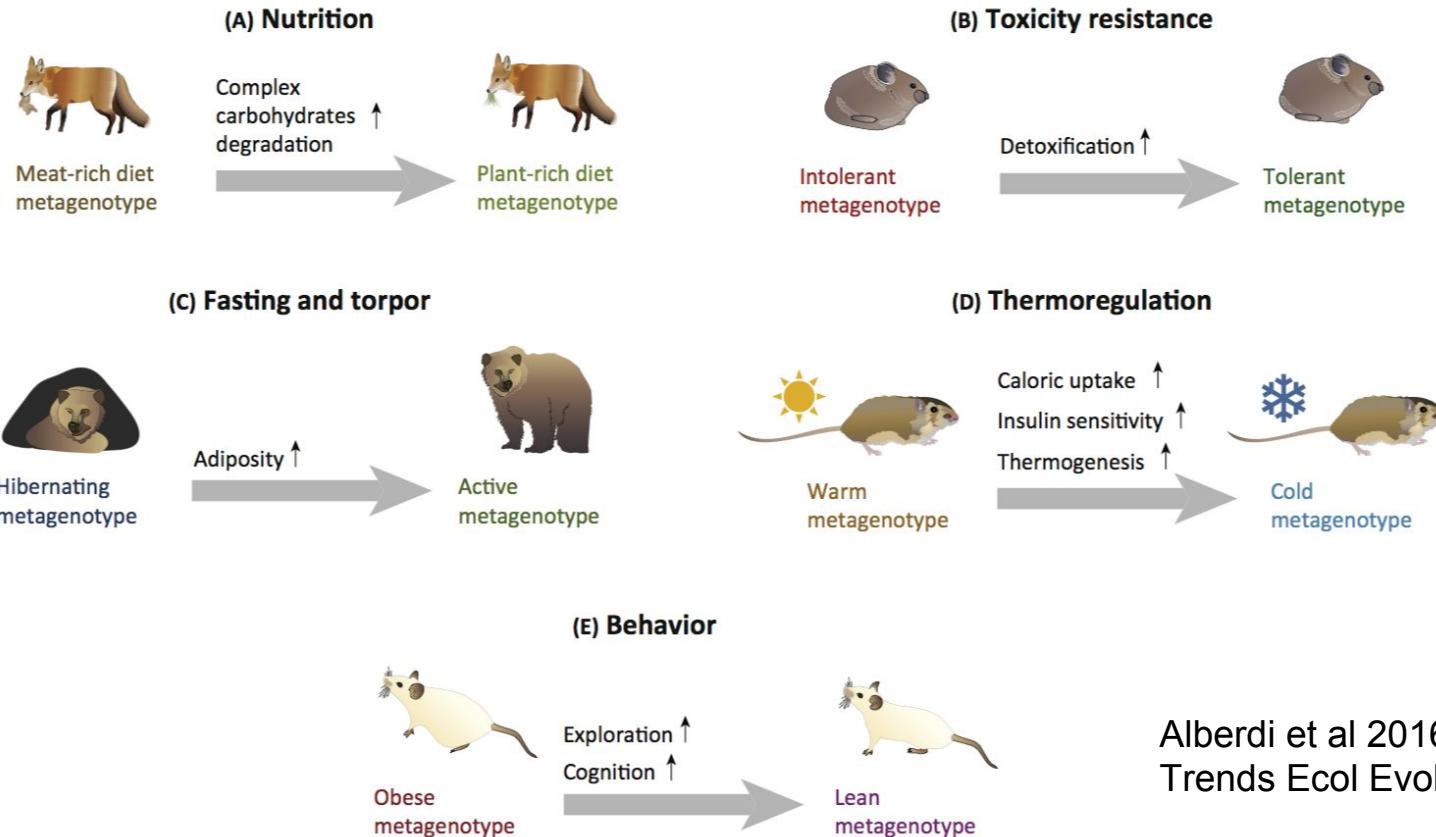
The vertebrate gut microbiota is a plastic element that can vary considerably as a response to environmental change. This variation can influence host phenomes,

1 Introduction to exercises

(A) The holobiont and its hologenome

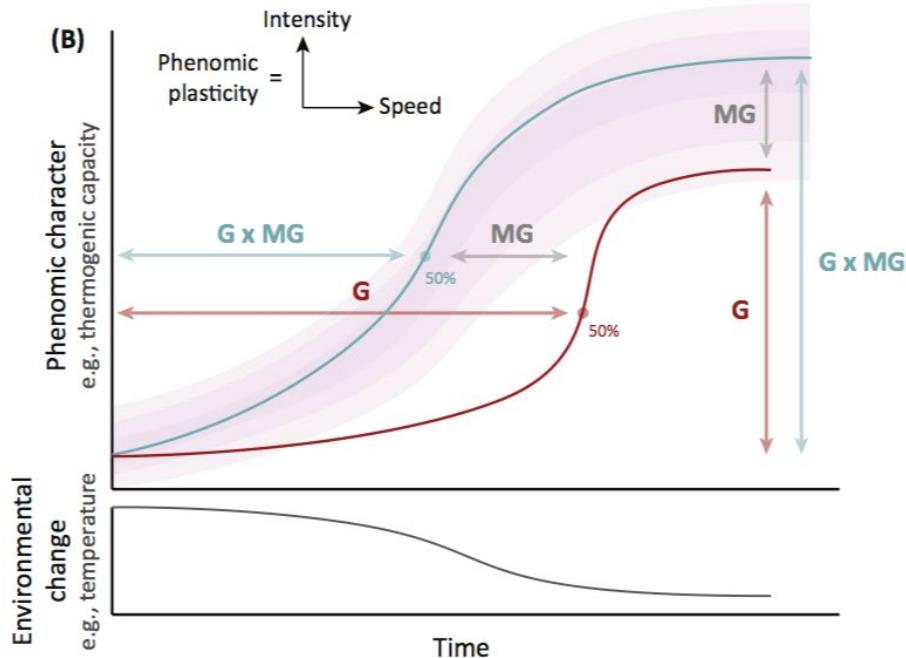
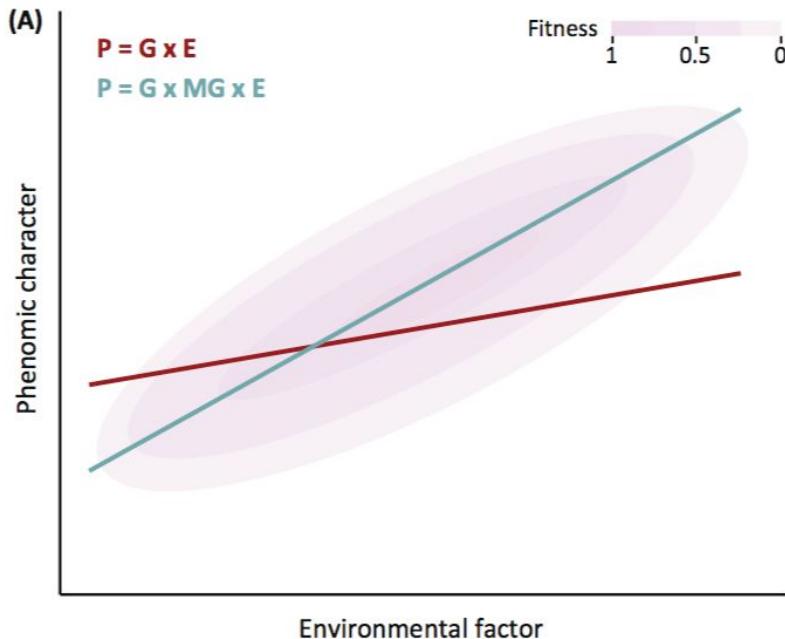
Host + intestinal microorganisms





1

Introduction to exercises



1 Introduction to exercises



Aphanius iberus
Spanish toothcarp



Valencia hispanica
Valencia toothcarp



Gambusia holbrooki
Eastern mosquitofish

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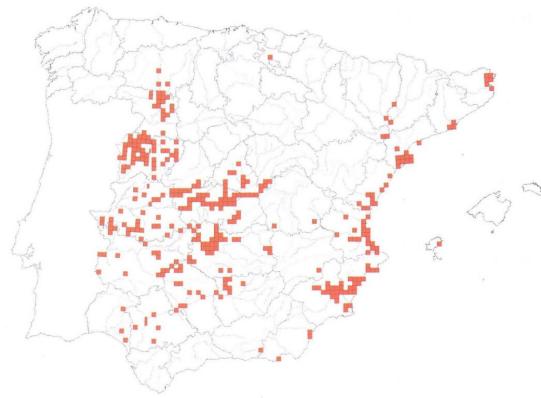
Aphanius iberus
Spanish toothcarp



Valencia hispanica
Valencia toothcarp



Gambusia holbrooki
Eastern mosquitofish



1 Introduction to exercises

Aphanius iberus
Spanish toothcarp



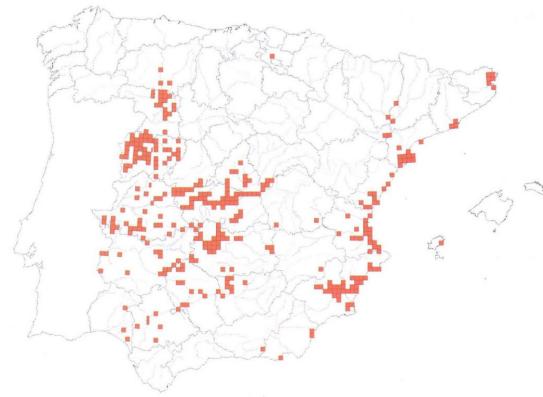
Endangered

Valencia hispanica
Valencia toothcarp



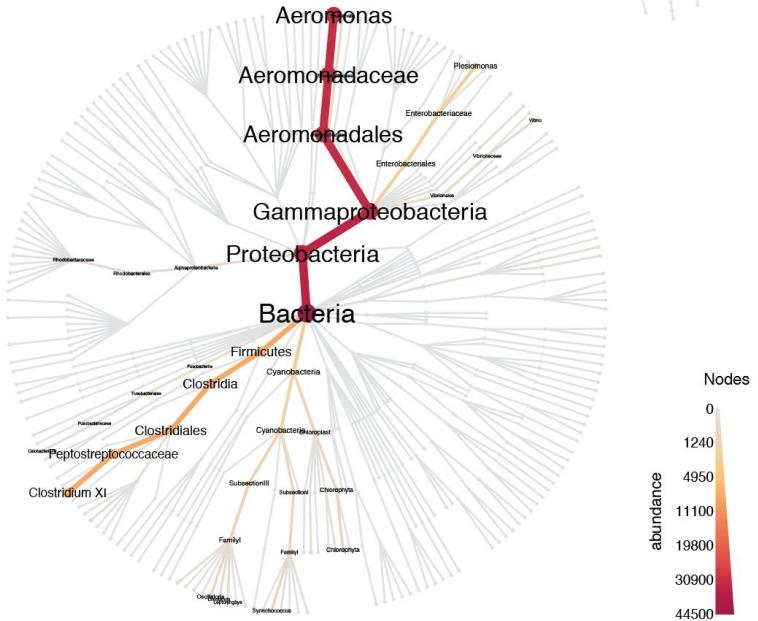
Endangered

Gambusia holbrooki
Eastern mosquitofish

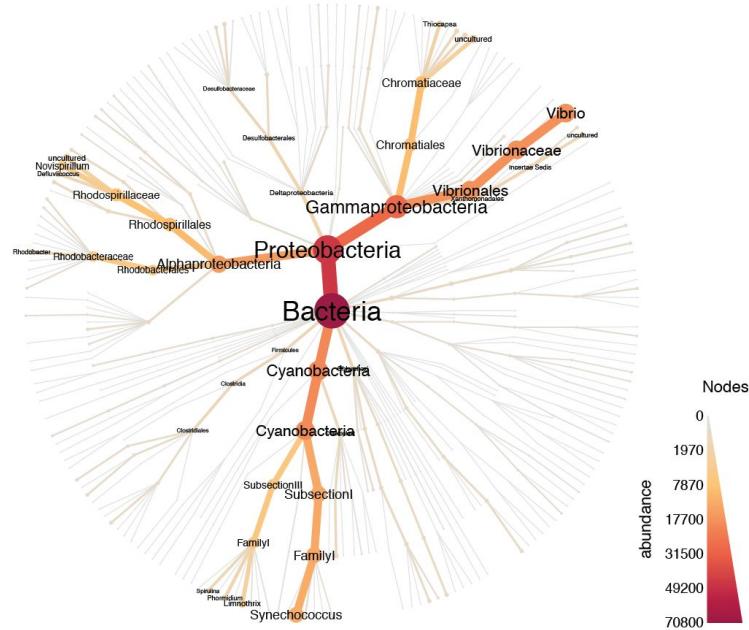


Invasive

1 Introduction to exercises

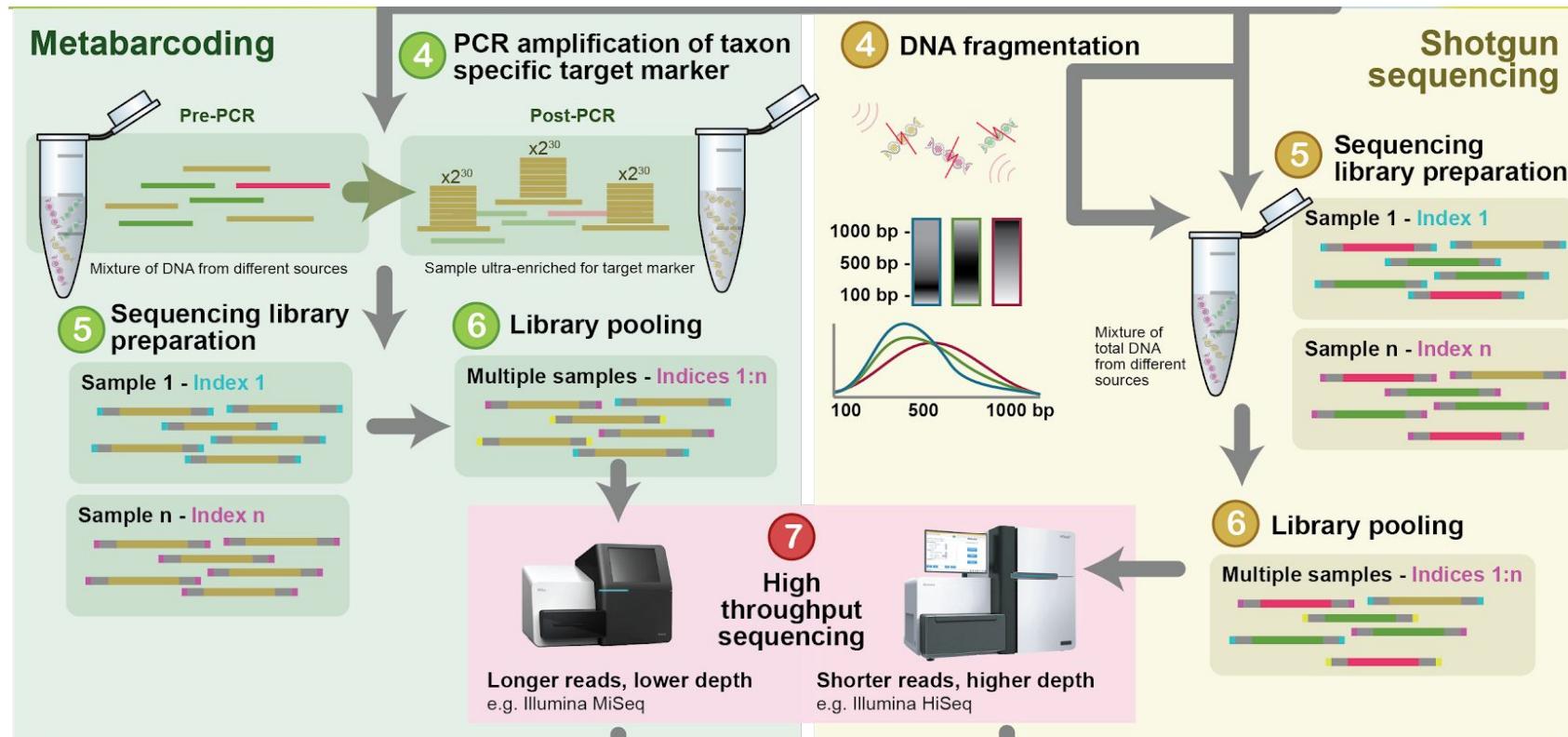


Valencia hispanica
Valencia toothcarp



Aphanius iberus
Spanish toothcarp

1 Introduction to exercises



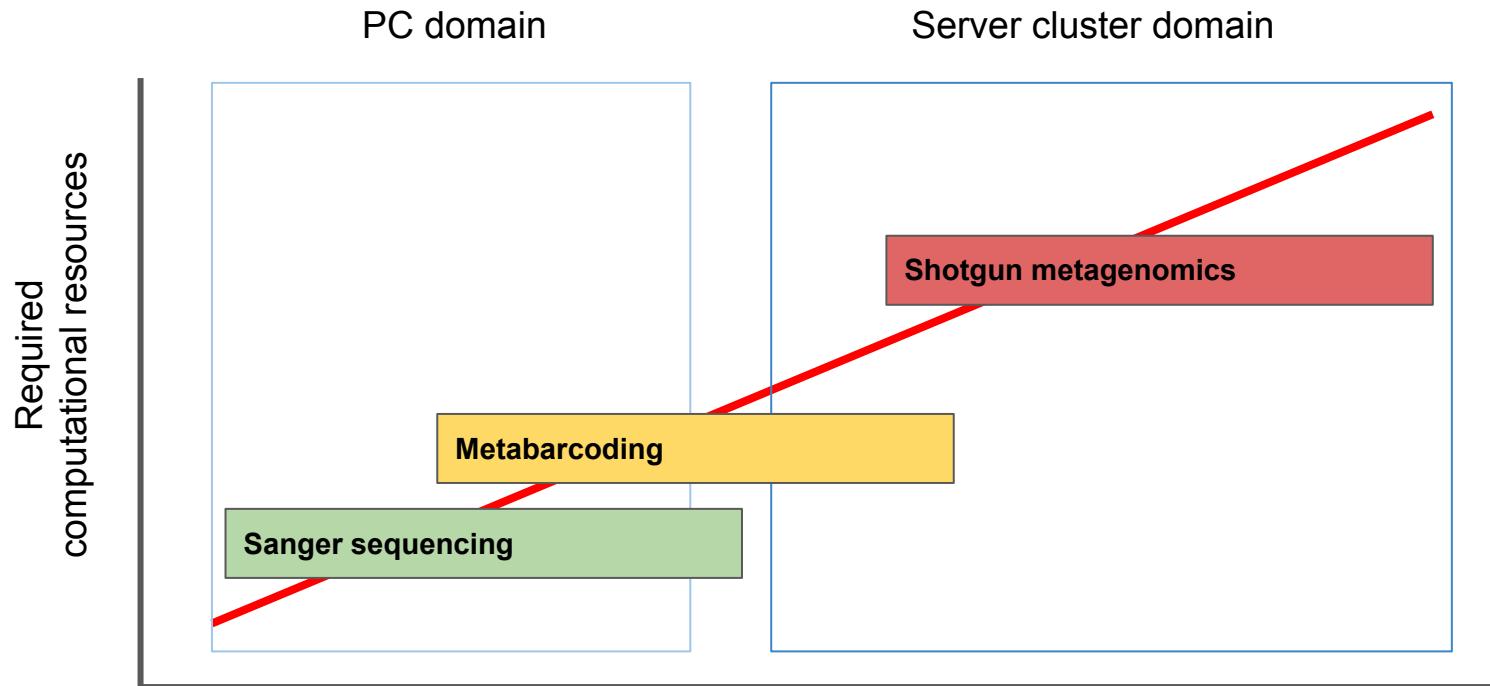
1 Introduction to exercises



Personal
computers

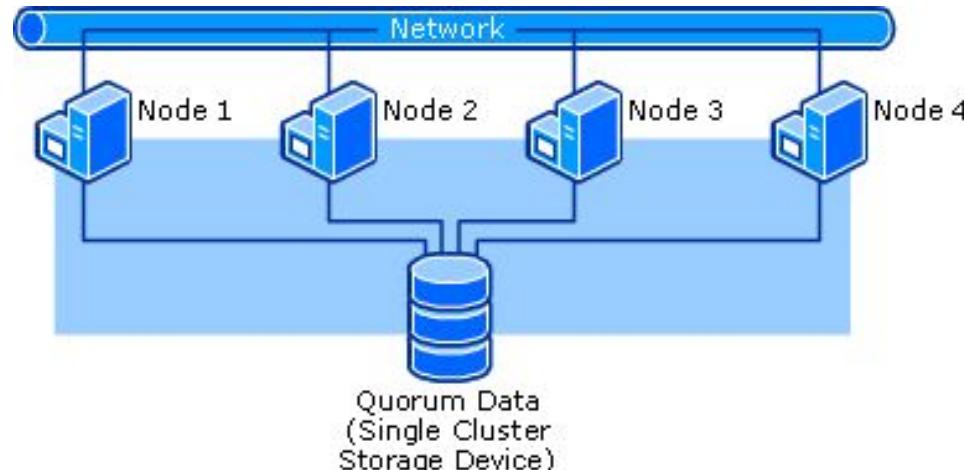


1 Introduction to exercises



1 Introduction to exercises

- **Personal computer:** multi-use computer with user interface
- **Server:** dedicated computer without user interface
- **Cluster:** interconnected server (node) collection
 - **Node:** computing unit
 - **Server:** user-accession unit



1 Introduction to exercises

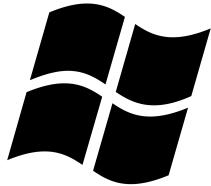
Amount	Name	Type	Proc	Cores	Speed	RAM (GB)	Disk (GB)	Label
1	Guinness	Server	2	8	2.3 GHz	12	—	—
1	Katramila	Server	2	8	2.6 GHz	128	—	—
1	cn3	Node	2	8	2.3 GHz	96	250	xeon,xeon8
17	cn4-20	Node	2	8	2.3 GHz	24	250	xeon,xeon8
36	cn21-56	Node	2	8	2.3 GHz	48	250	xeon,xeon8
4	cn57-60	GPU node	2	8	2.3 GHz	24	250	xeon,xeon8,gpu
18	cn61-78	Node	2	12	2.4 GHz	48	250	xeon,xeon12
1	cn79	Node	2	12	2.4 GHz	96	250	xeon,xeon12
35	cn80- cn114	Node	2	12	2.4 GHz	24	250	xeon,xeon12
30	n1-n30	Node	2	20	2.8 GHz	128	128	xeon,xeon20
1	n31	Node	2	32	2.6 GHz	128	1000	xeon,xeon20
1	n32	GPU node	2	20	2.8 GHz	128	128	xeon,xeon20,gpu

Arina Cluster (UPV/EHU)



1 Introduction to exercises

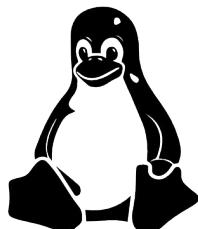
- Establish the connection (SSH) using the terminal/console/command line



PuTTY



Applications > Utilities > Terminal



Dash > type “terminal”
Type Ctrl+Alt+T