NGS: an Introduction

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Chang Gung University 2018
2018.03.13

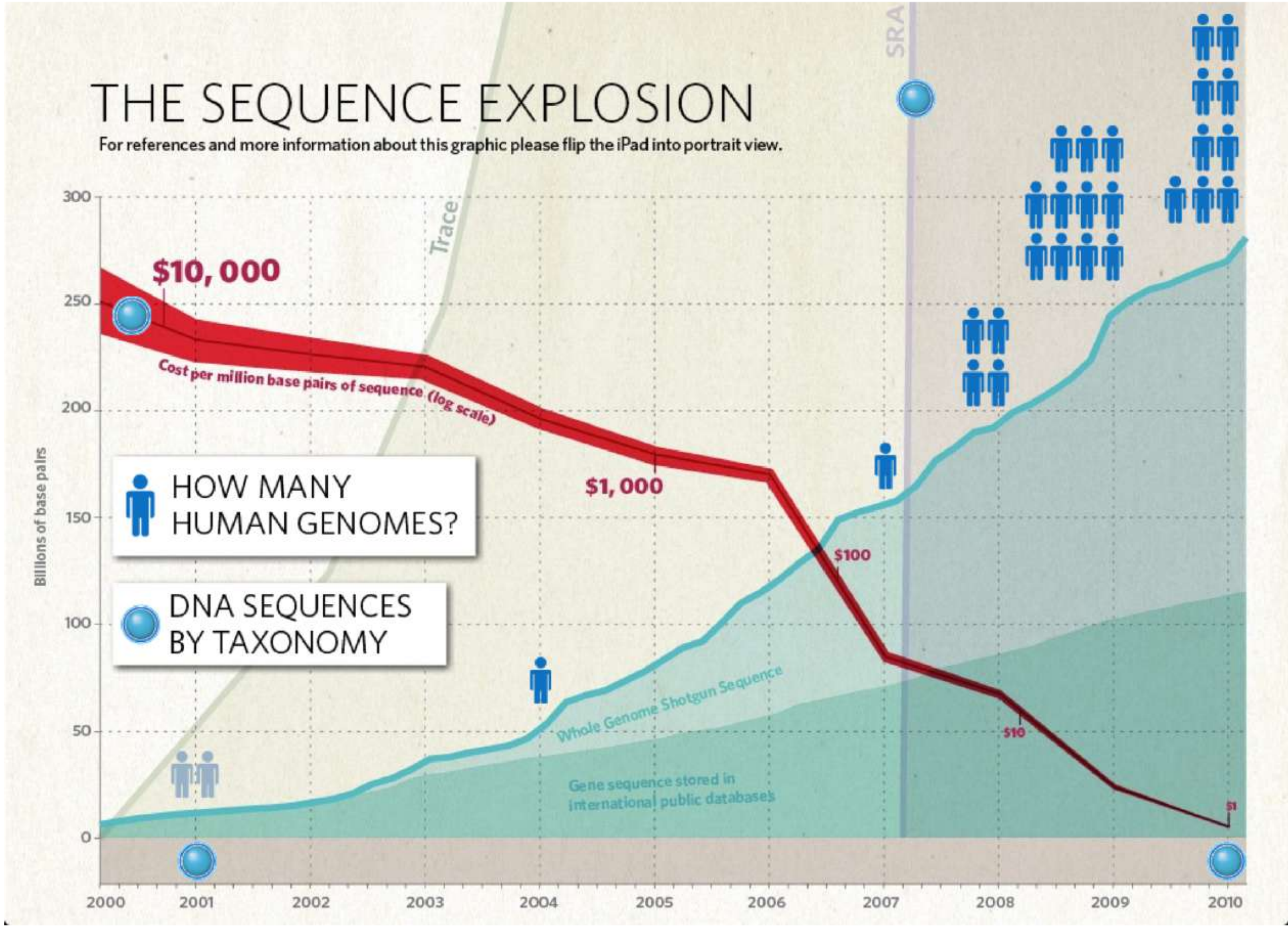
This lecture is called “NGS”

Actually

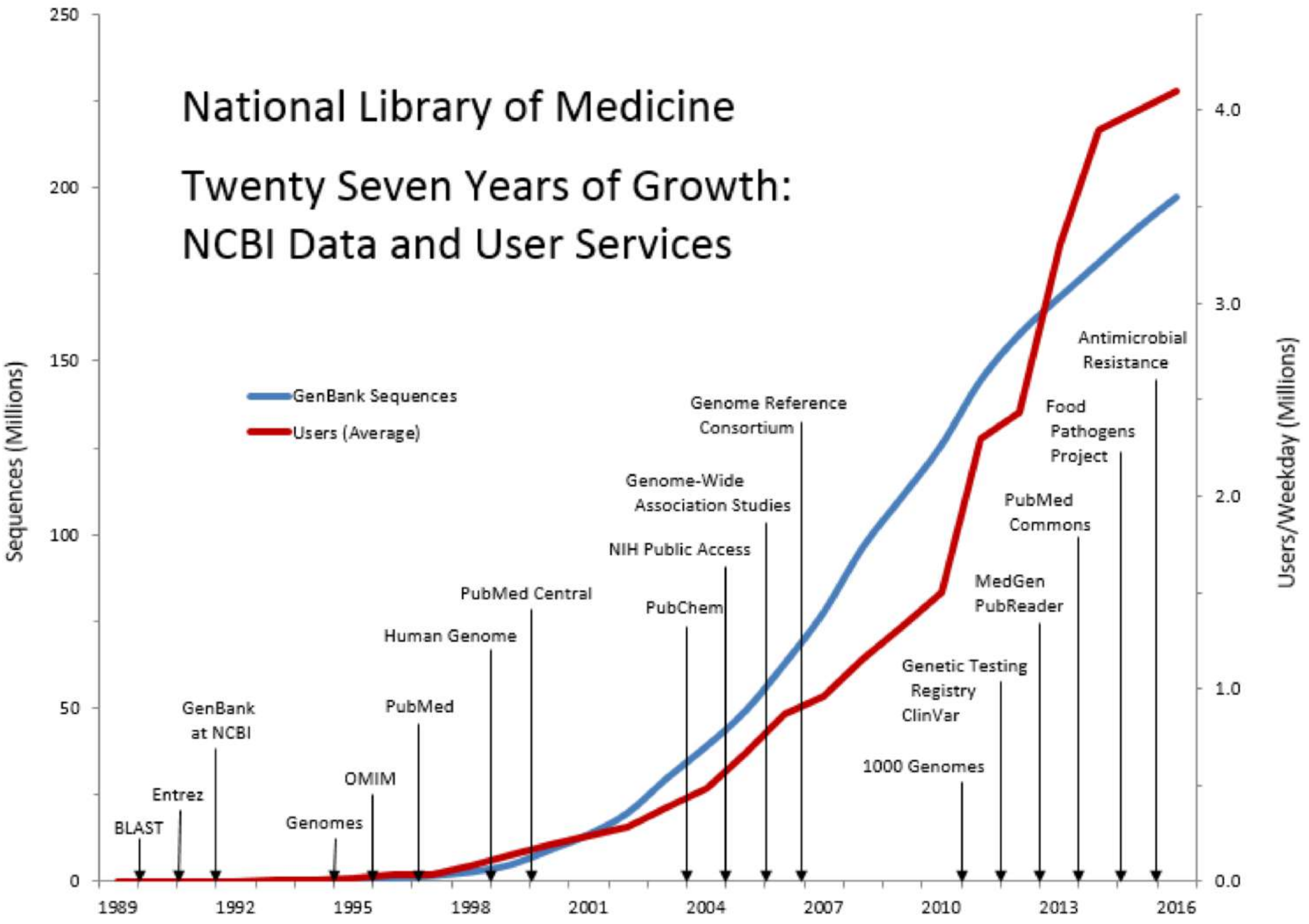
- Next Generation Sequencing is really “now” sequencing
- It won’t be so easy to tell you everything about NGS
  (it’s a bit like saying what can we do with PCR?)

What is NGS?

= Next generation sequencing,
= deep sequencing
= High Throughput Sequencing,
= Massively parallel sequencing
= 次世代定序
= 高速高量定序



http://www.nature.com/news/2010/100331/full/464670a.html



https://www.nlm.nih.gov/about/https://www.nlm.nih.gov/about/2018CJ.html

NGS = sequencing made cheaper, faster and higher throughput

What we will cover today

NGS: Some basics
Sequencing platforms
Data types
Analysis:

- RNAseq
- 16S
- Metagenomics

Previous questions:

- microbiota的paper要怎麼approach
- 16S sequencing region primer choice
- microbiota醫院有fecal transplantation計劃?

My background

Skills

Fundamentals

Topics

Undergraduate:
Biochemistry and Genetics

2005-08 ; MSc & PhD:
Bioinformatics & Population genetics

2009-14 ; Postdoc:
Genomics & parasitology

2015 - ; Academia Sinica:
Microbial diversity & Bioinformatics

Evolutionary biology

Molecular biology

Statistics

Programming

Population genetics

Yeast genomics

Comparative genomics

Genome annotation

Parasite genomics

Phylogenetics

Genome assembly

RNAseq

Microbial ecology

Ecological genomics

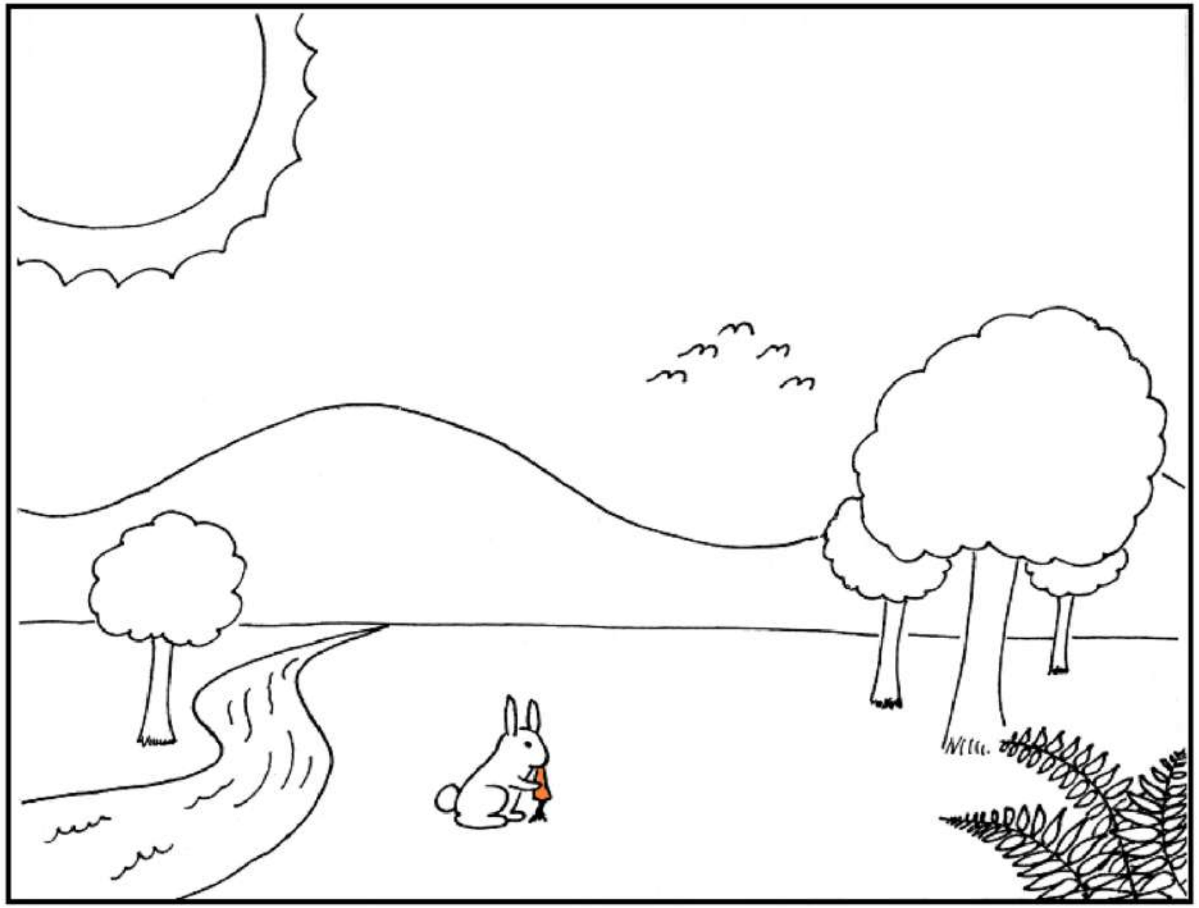
Insect genomes

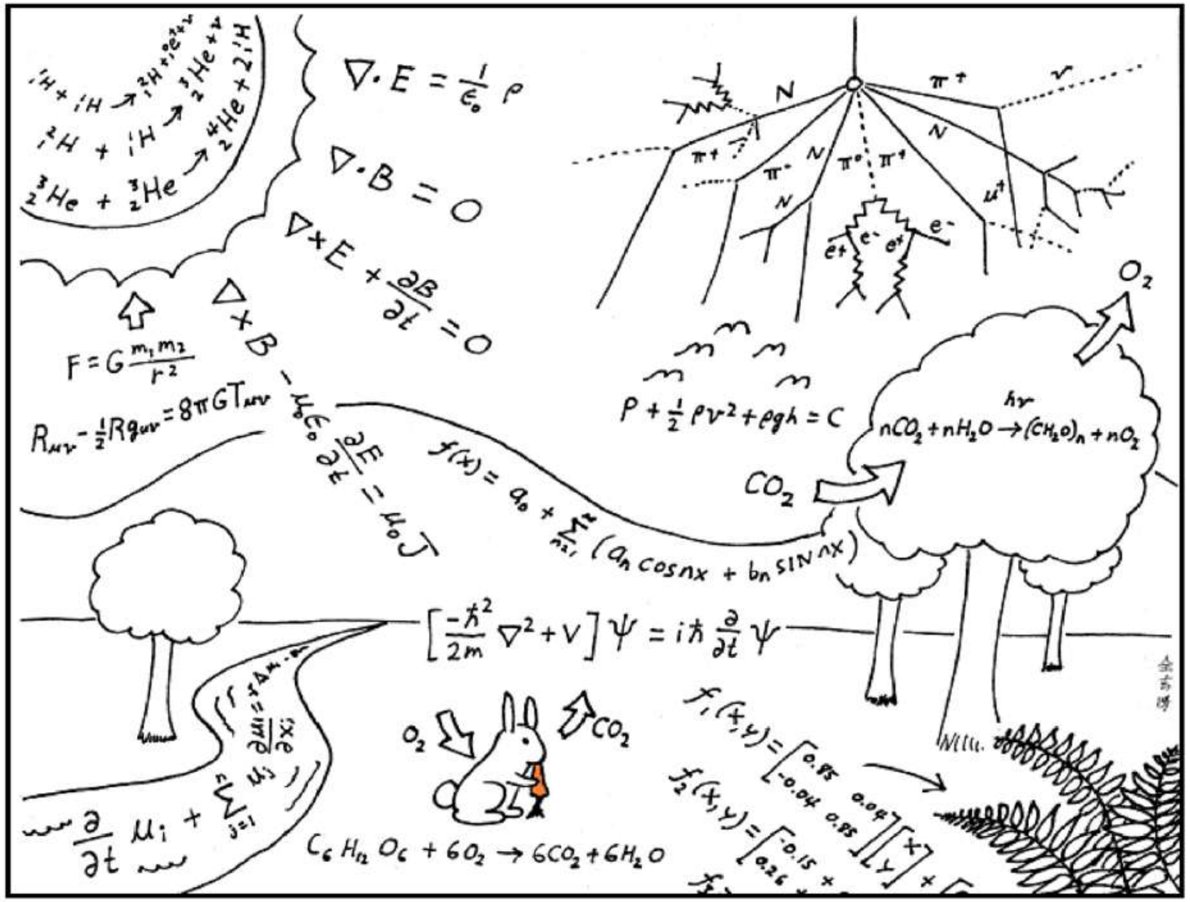
Plant genomes

Bacterial genomes

- **39 publications**
  (2 Nature, 1 Science, 2 Nature Genetics, 2 PNAS,
  3 Genome Biology, 1 Molecular Ecology)

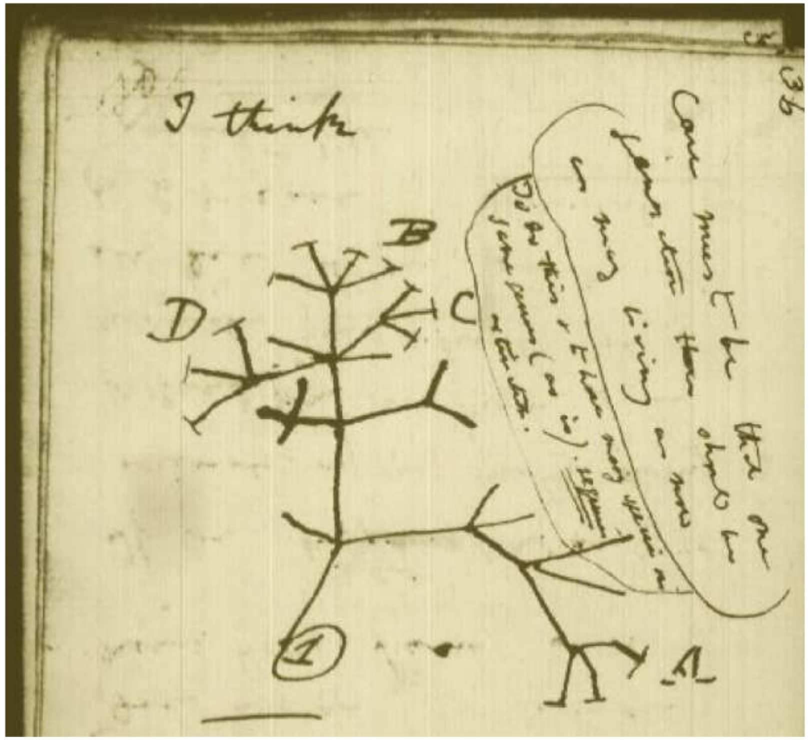
This is how scientists see the world





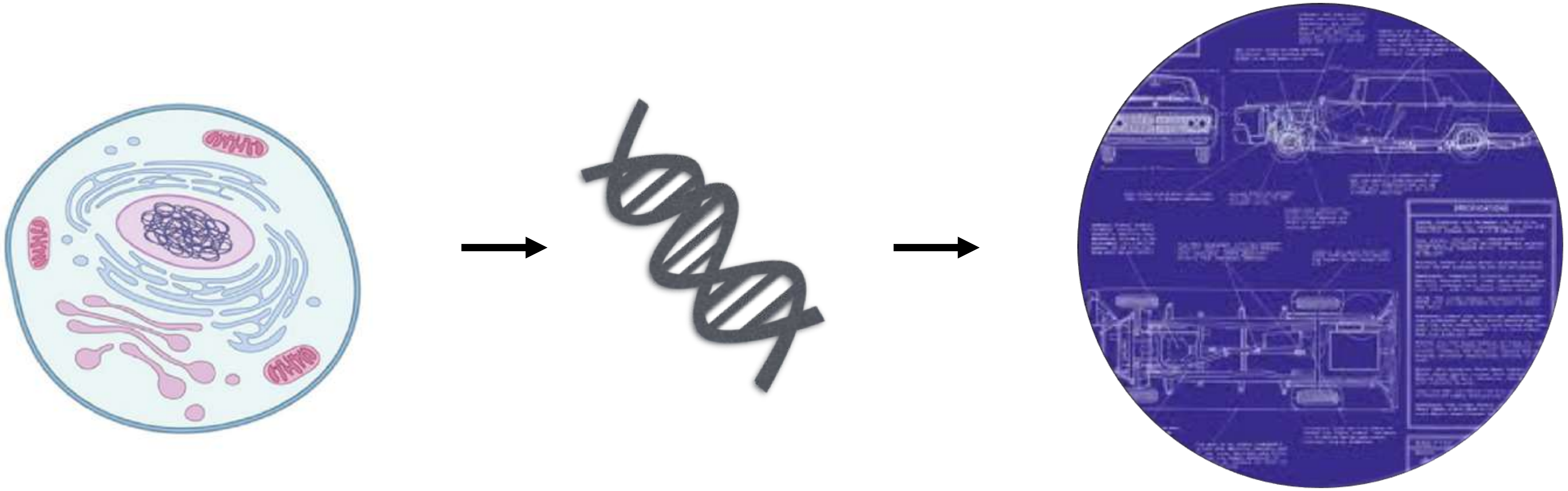
Nothing makes sense in the light of evolution

Theodosius Dobzhansky 1973



What is genomics?

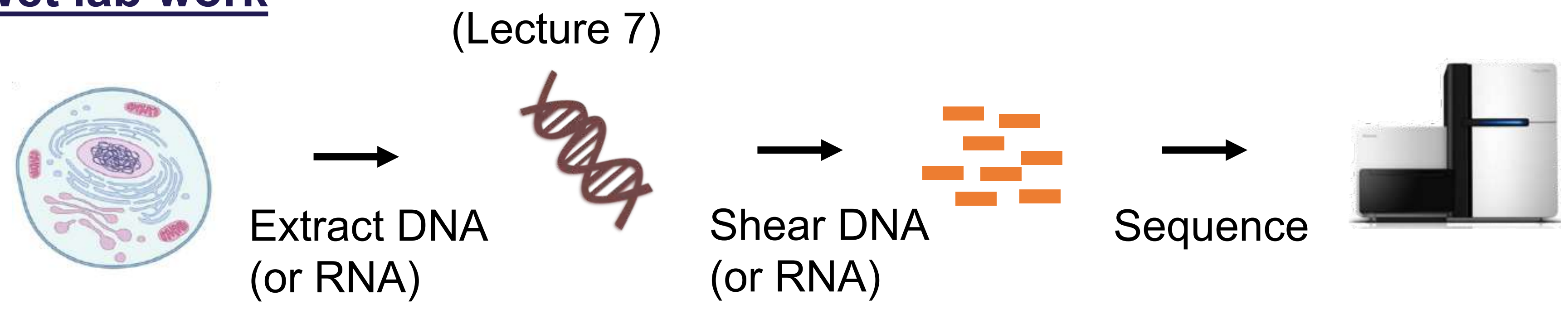
Genome



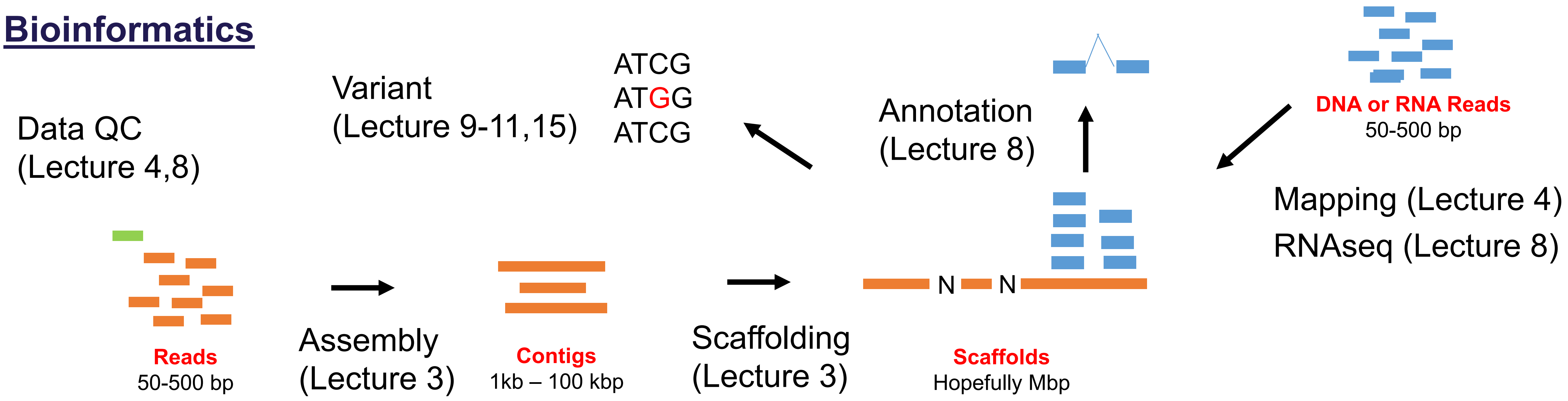
Genome = Parts list of a single genome

A genome project

Wet lab work



Bioinformatics



Three situations you are most likely to encounter

Genome reference is available (for example, humans):
- Re-sequence (DNA, RNA)
- **Map** (align) sequence to the genome

Genome reference is NOT available
- **Assemble** the reads to get the genome

Counting:
- For a given region (gene) we want to know how much.→ gene expression or metagenomics

Many perceptions of NGS / genomics

What my parents
think I do

What less friendly colleagues
think we do

What more friendly colleagues
think we do

What my friends
think I do

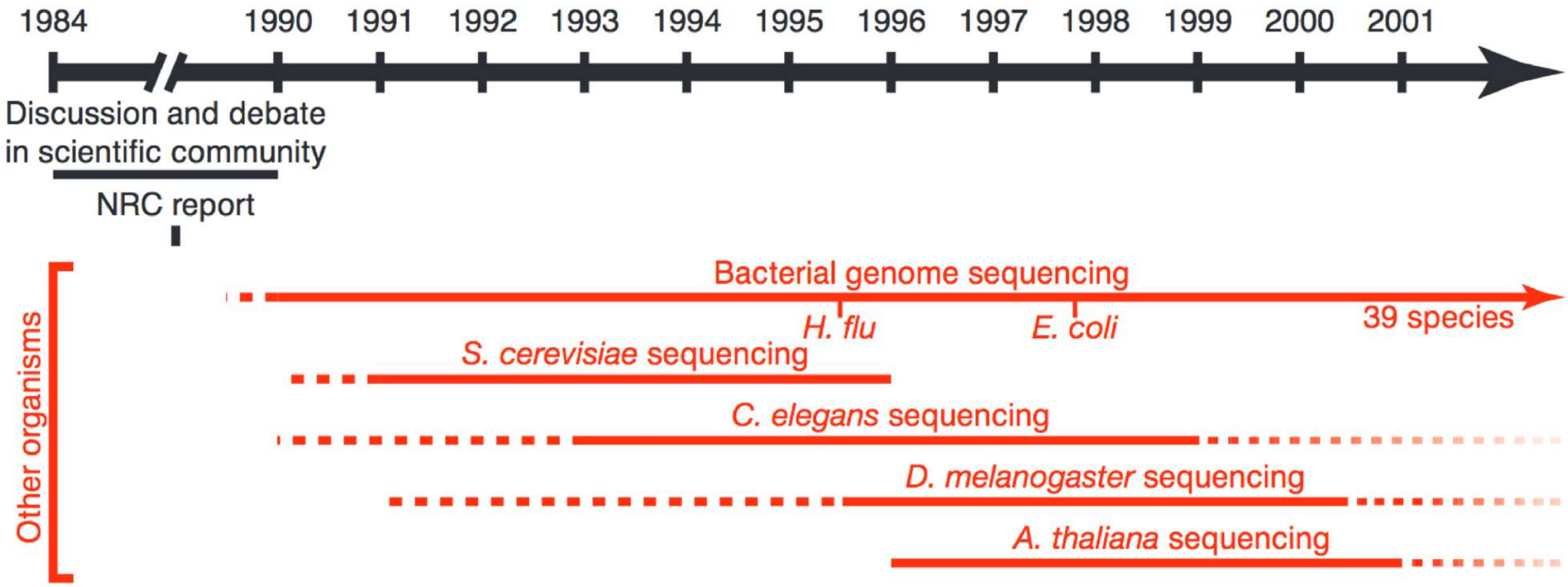
What we
think we do

What we
actually do

Why sequence a genome?

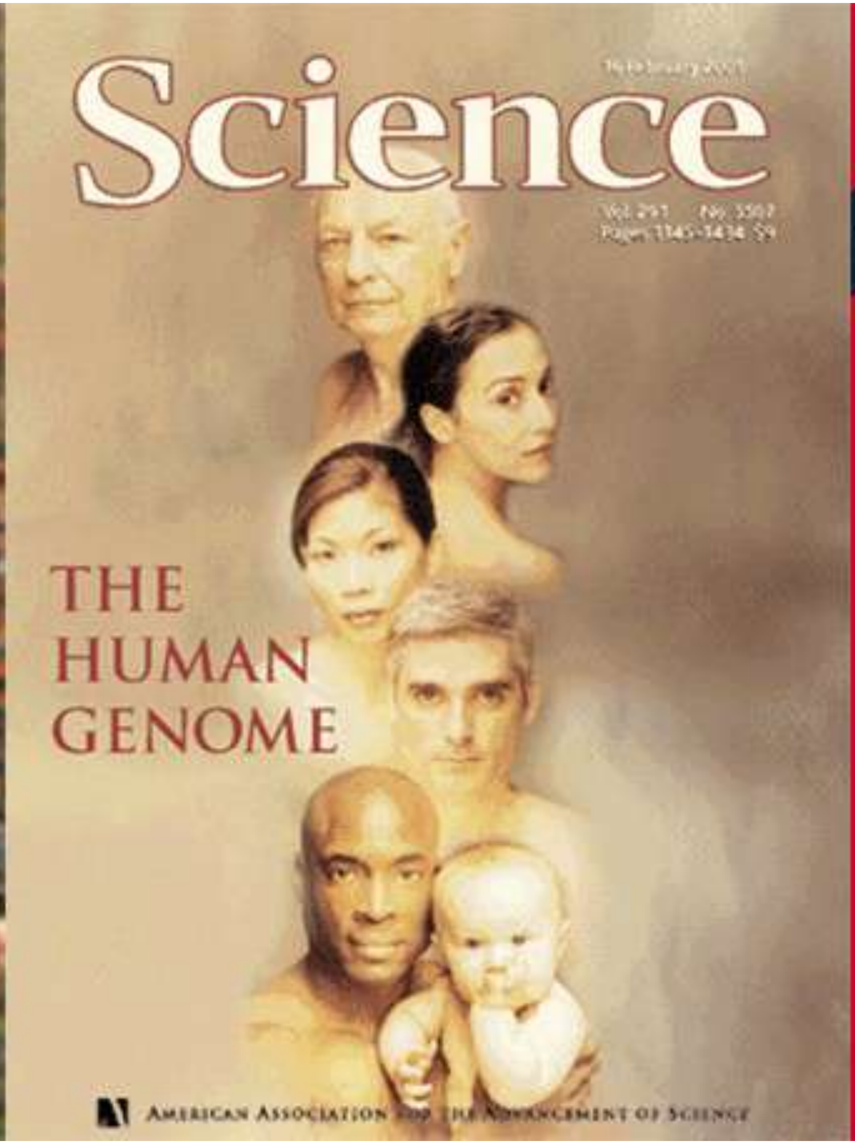
- Phylogenetic position
- Differences between species (comparative genomics)
- Variations between individuals (population genetics)
- Help to understand biology
- Of economic, agricultural, medical, ecology values

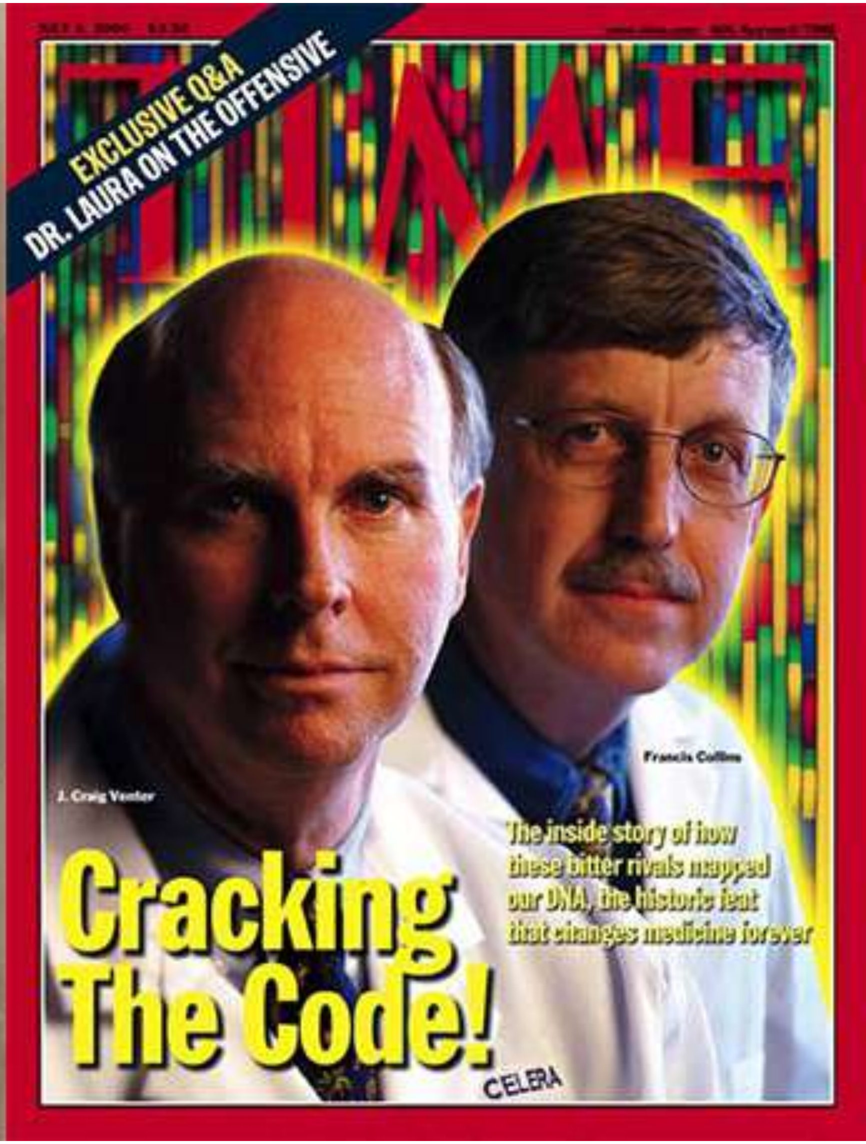
- **Help to understand biology**
- ~~Some lab just had the money~~ ; don't do it



Nature **409**, 860-921(15 February 2001)
doi:10.1038/35057062


nature
the
human
genome


Science
THE
HUMAN
GENOME
AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE


EXCLUSIVE Q&A
DR. LAURA ON THE OFFENSIVE
Francis Collins
J. Craig Venter
The inside story of how these bitter rivals mapped our DNA, the historic feat that changes medicine forever
Cracking The Code!
CELERA

Calculating the economic impact of the Human Genome Project

Public funding of scientific R&D has a significant positive impact on the wider economy, but quantifying the exact impact of research can be difficult to assess. A new report by research firm Battelle Technology Partnership Practice estimates that **between 1988 and 2010, federal investment in genomic research generated an economic impact of $796 billion**, which is impressive considering that Human Genome Project (HGP) spending **between 1990-2003 amounted to $3.8 billion**. This figure equates to a return on investment (ROI) of 141:1 (that is, every $1 invested by the U.S. government generated $141 in economic activity). The report was commissioned by Life Technologies Foundation.

https://www.genome.gov/27544383/calculating-the-economic-impact-of-the-human-genome-project/

FOCUS ON GENOMES OF ICELANDERS

nature genetics

ARTICLES

Large-scale whole-genome sequencing of the Icelandic population

Here we describe the insights gained from sequencing the whole genomes of 2,636 Icelanders to a median depth of 20×.

A collection of Icelandic genealogical records dating back to the 1700s.

The blood of a thousand Icelanders. Photo: Chris Lund

Nature Genetics **volume 47**, pages 435–444 (2015)

UK
10K

RARE GENETIC VARIANTS IN HEALTH AND DISEASE

The project is taking a two-pronged approach to identify rare variants and their effects:

•by **studying and comparing the DNA of 4,000 people whose physical characteristics are well documented**, the project aims to identify those changes that have no discernible effect and those that may be linked to a particular disease;

•by **studying the changes within protein-coding areas of DNA that tell the body how to make proteins of 6,000 people with extreme health problems and comparing them with the first group**, it is hoped to find only those changes in DNA that are responsible for the particular health problems observed.

The project received a **£10.5 million** funding award from Wellcome in March 2010 and sequencing started in late 2010. For more information, please use the links on the right hand side.

https://www.uk10k.org/

Project setup

- Sequencing a species (Comparative genomics)
  - Map, assemble

- **Sequencing multiple individuals of a species (Population genomics)**
  - **Map, count**

- Combination of (1) and (2)

A small project's typical output

Sample Name	Sample ID	Lane ID	Yield (Mb)	# of Reads
F2-1	SG-IB01	1	11,435	75,729,838
F2-2	SG-IB02		12,014	79,561,504
F2-3	SG-IB03		11,577	76,666,714
F3-2	SG-IB05		11,119	73,638,446
F3-4	SG-IB07		10,399	68,870,380
F3-5	SG-IB08		11,671	77,292,976
F3-1	SG-IB09		12,474	82,610,516
F3-3	SG-IB10		11,916	78,915,536
F2-1	SG-IB01	2	11,366	75,271,724
F2-2	SG-IB02		11,920	78,940,010
F2-3	SG-IB03		11,481	76,031,166
F3-2	SG-IB05		11,054	73,203,066
F3-4	SG-IB07		10333	68,429,564
F3-5	SG-IB08		11550	76,488,178
F3-1	SG-IB09		12328	81,640,878
F3-3	SG-IB10		11812	78,225,876

8 exome samples ;
2 Illumina Hiseq lanes with **184GB** of data

~100X of human exome to detect disease causing SNP

Higher yield at lower cost = More samples can be barcoded into one lane

More samples = more replicates (power) in statistical analysis to pick up real biological difference

More data but less people with informatics skills

- Sequencing is the result of many types of experiment

- Everyone wants to make use of this technology
- Not everyone will be able analyse them
    - You can’t just open the file in Microsoft office anymore
- Collaborate or learn yourself

- **Bottleneck is bioinformatics analysis**

Different sequencing platforms /
History of sequencing

Proc. Natl. Acad. Sci. USA
Vol. 74, No. 12, pp. 5463–5467, December 1977
Biochemistry

DNA sequencing with chain-terminating inhibitors

(DNA polymerase/nucleotide sequences/bacteriophage ϕX174)

F. SANGER, S. NICKLEN, AND A. R. COULSON

Medical Research Council Laboratory of Molecular Biology, Cambridge CB2 2QH, England

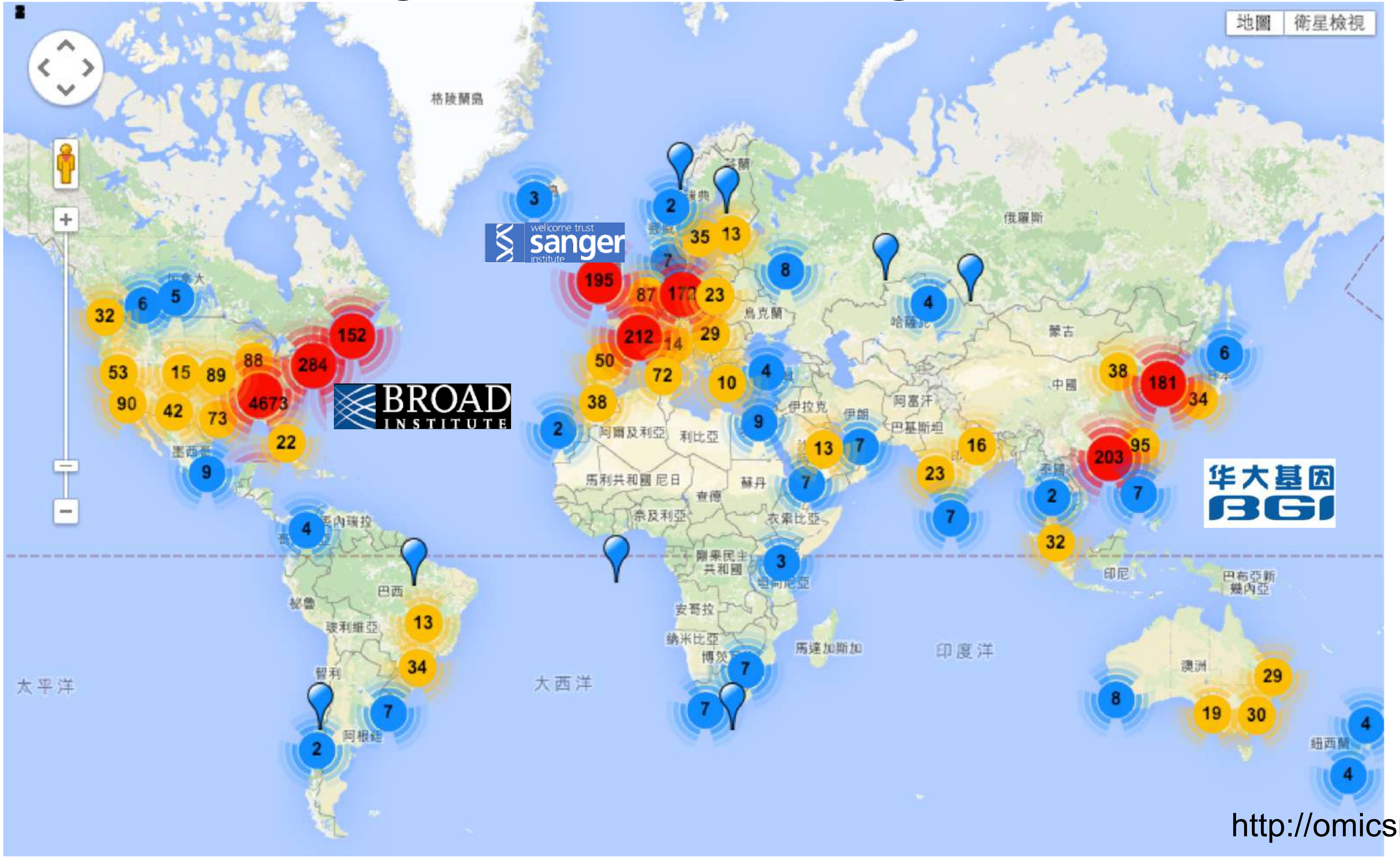
Contributed by F. Sanger, October 3, 1977

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC431765/

ABI 3730xi at TIGR

https://www.flickr.com/photos/jurvetson/57080968

World competing for sequencing power



http://omicsmaps.com/

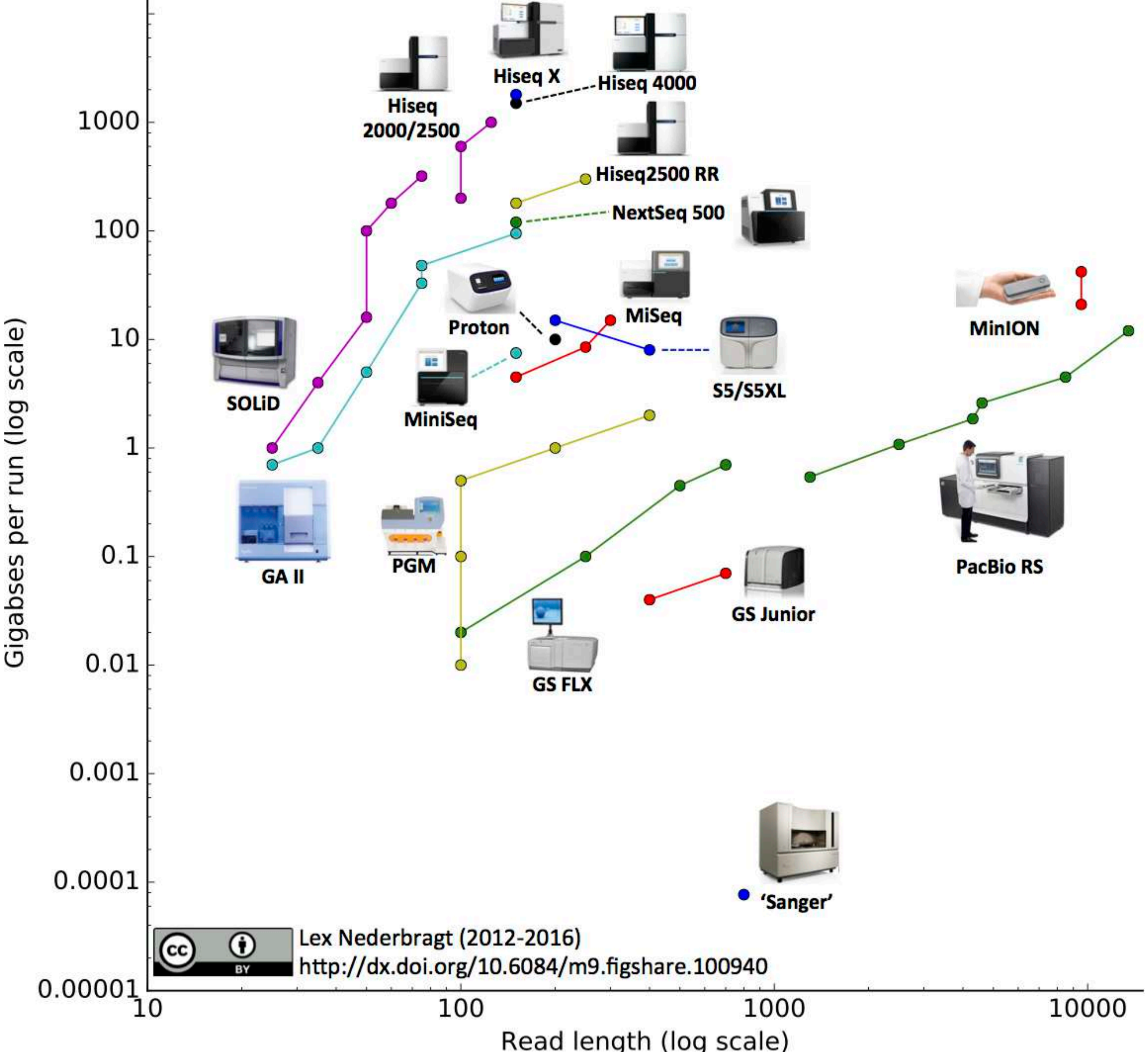
Sequencing Platforms

- Short reads

  1. ~~Genome Analyzer IIx (GAIIx)~~ – Illumina
  2. HiSeq, MiSeq, Novaseq – Illumina

- Long reads

  1. ~~Genome Sequencer FLX System (454) – Roche~~
  2. Pacific Bioscience
  3. Oxford Nanopore


Hiseq X
Hiseq 4000
Hiseq 2000/2500
Hiseq2500 RR
NextSeq 500
Proton
MiSeq
MinION
SOLiD
MiniSeq
S5/S5XL
GA II
PGM
PacBio RS
GS Junior
GS FLX
'Sanger'
Gigabses per run (log scale)
Read length (log scale)
1000
100
10
1
0.1
0.01
0.001
0.0001
0.00001
10
100
1000
10000
Lex Nederbragt (2012-2016)
http://dx.doi.org/10.6084/m9.figshare.100940

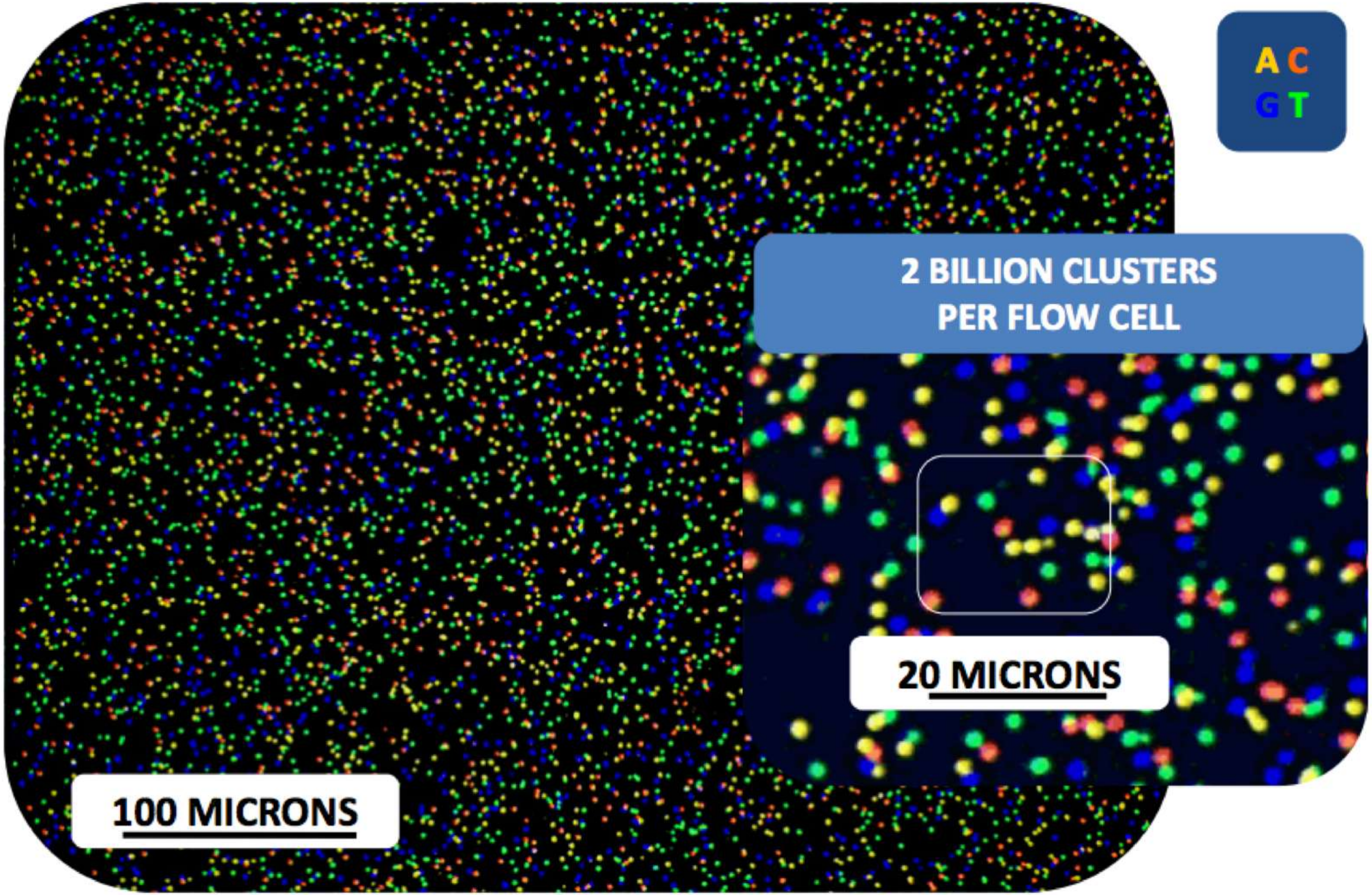
Platform	Reads x run: (M)	Read length: (paired-end*, Half of data in reads**)	Run time: (d)	Yield: (Gb)	Rate: (Gb/d)	Reagents: ($K)	per-Gb: ($)	hg-30x: ($)	Machine: ($)
iSeq 100 1fcell	4	150*	0.77	1.2	1.56	0.625	521	62500	19.9K
MiniSeq 1fcell	25	150*	1	7.5	7.5	1.75	233	28000	49.5K
MiSeq 1fcell	25	300*	2	15	7.5	1	66	8000	99K
NextSeq 550 1fcell	400	150*	1.2	120	100	5	50	5000	250K
HiSeq 2500 RR 2fcells	600	100*	1.125	120	106.6	6.145	51.2	6144	740K
HiSeq 2500 V3 2fcells	3000	100*	11	600	55	23.47	39.1	4692	690K
HiSeq 2500 V4 2fcells	4000	125*	6	1000	166	29.9	31.7	3804	690K
HiSeq 4000 2fcells	5000	150*	3.5	1500	400	--	20.5	2460	900K
HiSeq X 2fcells	6000	150*	3	1800	600	--	7.08	849.6	1M
NovaSeq S1 2018 2fcells	3300	150*	1.66	1000	600	--	18	1800	999K
NovaSeq S2 2fcells	6600	150*	1.66	2000	1200	--	15	1564	999K
NovaSeq S4 2fcells	20000	150*	1.66	6000	3600	64	5.8	700	999K
5500 XL	1400	60	7	180	30	10.5	58.33	7000	595K
Ion S5 510 1chip	2 - 3	200 400	0.21	1	4.8	0.95	950	114000	65K
Ion S5 520 1chip	3 - 6	200 400 600	0.23	1	4.3	1	500	60000	65K
Ion S5 530 1chip	20	200 400 600	0.29	4	13.8	1.2	150	18000	65K
Ion S5 540 1chip	80	200	0.42	15	35.7	1.4	93.3	11196	65k
Ion S5 550 1chip	130	200	0.5	25	50	1.67	66.8	8016	65k
PacBio RSII P6-C4 16cells	0.88	20K**	4.3	12	2.8	2.4	200	24000	695K
PacBio Sequel 16cells 2018	6.4	33K**	6.6	160	24.2	--	80	9600	350K
PacBio R&D end 2018	--	32K**	--	192	--	1	6.6	1000	350K
SmidgION 1fcell	--	--	TBC	TBC	TBC	TBC	TBC	--	--
Flongle 1fcell	--	--	0.7	1-3.3	--	--	90-30	--	--
MinION R9.5.1 1fcell	--	--	2	17-40	--	--	30-12.5	--	--
GridION X5 5fcells	--	--	2	85-200	--	--	17.5-7.5	--	--
PromethION RnD 48fcells	--	--	2	3000/6000-20000	--	--	43136	--	--
QiaGen GeneReader	400	--	--	80	--	0.5	--	--	--
BGISEQ 500	1600	100*	7	260	37.14285714	--	--	600?	500K
BGISEQ 50	1600	50*	0.4	8	20	--	--	--	--
MGISEQ 2000	--	100*	2	600	300	4.8	8	960	310K
MIGSEQ 200	--	100*	--	60	--	--	--	--	150K

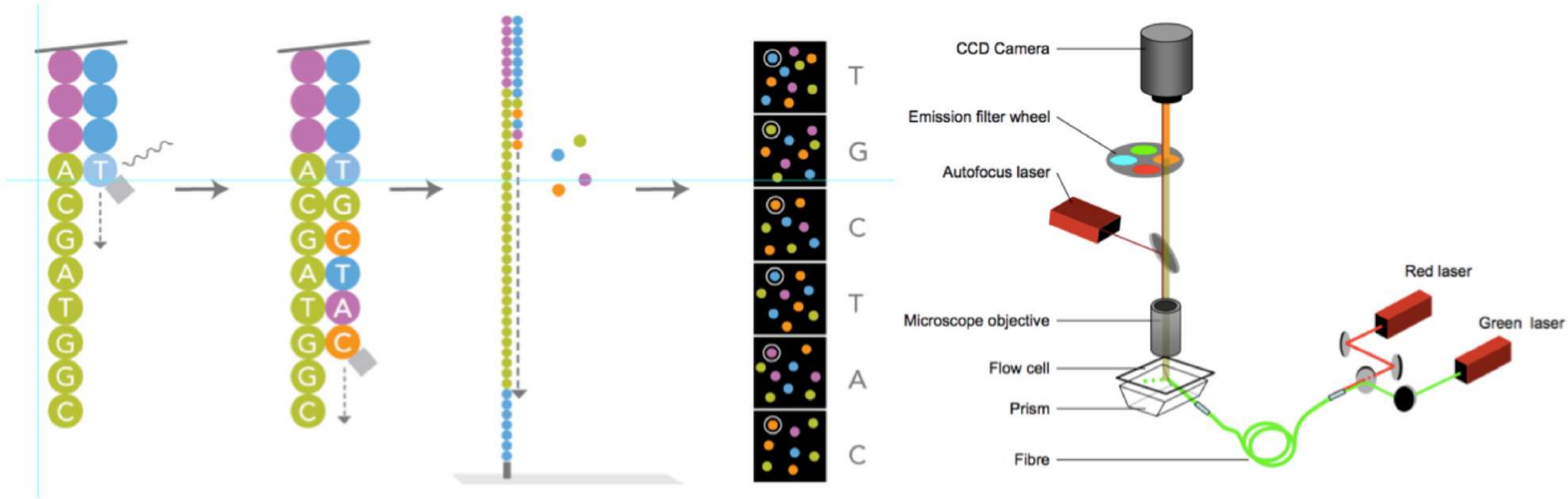
http://twitter.com/albertvilella ; Last updated: 2018.02.20

Illumina HiSeq

Sequencing by synthesis

https://www.youtube.com/watch?v=fCd6B5HRaZ8


A C
G T
2 BILLION CLUSTERS
PER FLOW CELL
20 MICRONS
100 MICRONS



Nature **456**, 53-59 (6 November 2008)
| doi:10.1038/nature07517

HiSeq and MiSeq

HiSeq 2000

Initially capable of up to 600Gb per run in 13 days.
Cost of resequencing one human genome:
30x coverage about $6,000- $9,000

HiSeq 2500
Initially capable of up 100Gb per run in 27hours.
Cost per genome - ???

MiSeq
-Small capacity system. PE 2x250cycles in 24 hours.
-Long insert size possible: 1.5kb, 3kb
-2x400bp in R&D

Novaseq

Illumina platform comparison

Platform	Reads x run: (M)	Read length: (paired-end*, Half of data in reads**)	Run time: (d)	Yield: (Gb)	Rate: (Gb/d)	Reagents: ($K)	per-Gb: ($)	hg-30x: ($)	Machine: ($)
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NovaSeq S4 2fcells	20000	150*	1.66	6000	3600	64	5.8	700	999K

Third generation sequencing

PacBio (Pacific Biosciences)

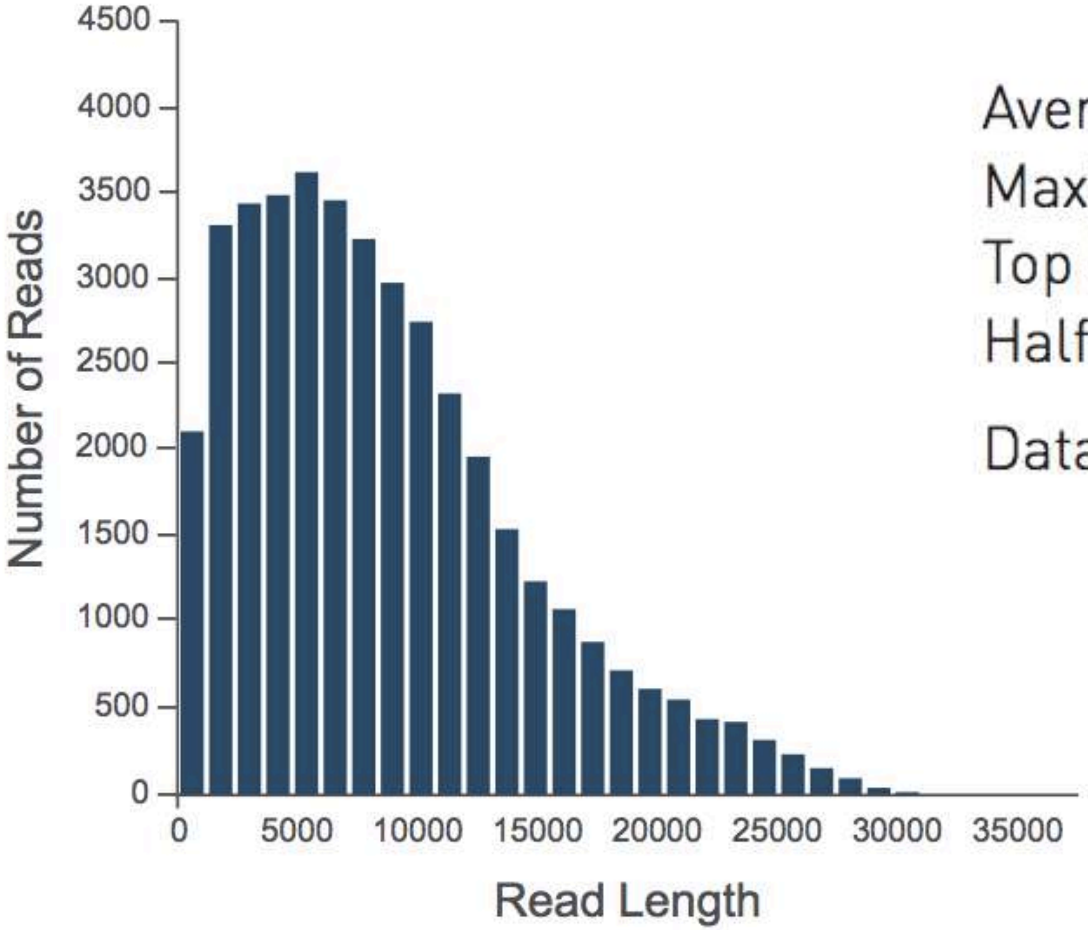
RSII

Sequel

Single molecule sequencing

https://www.youtube.com/watch?v=NHCJ8PtYCFc

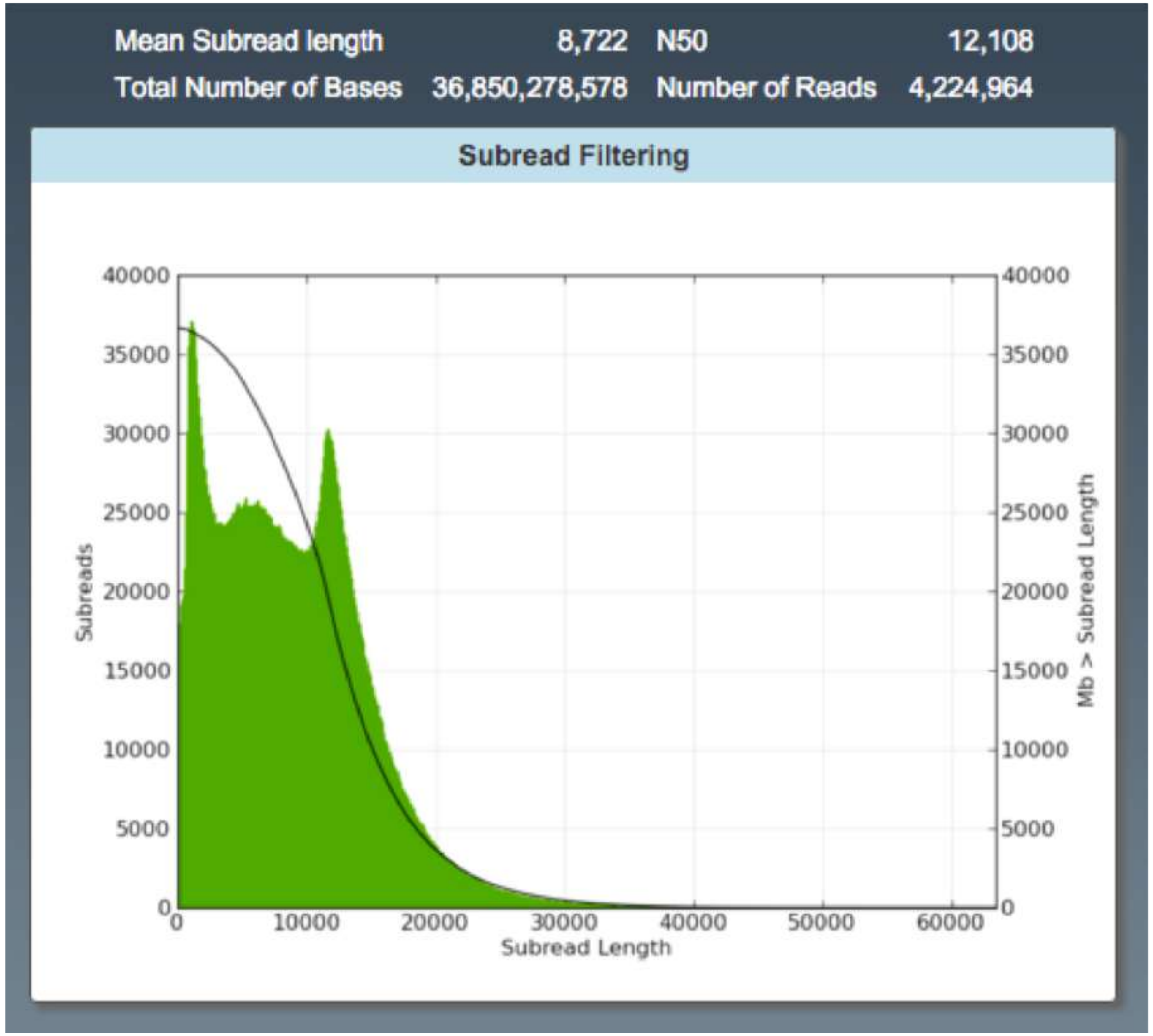
PacBio (Pacific Biosciences)

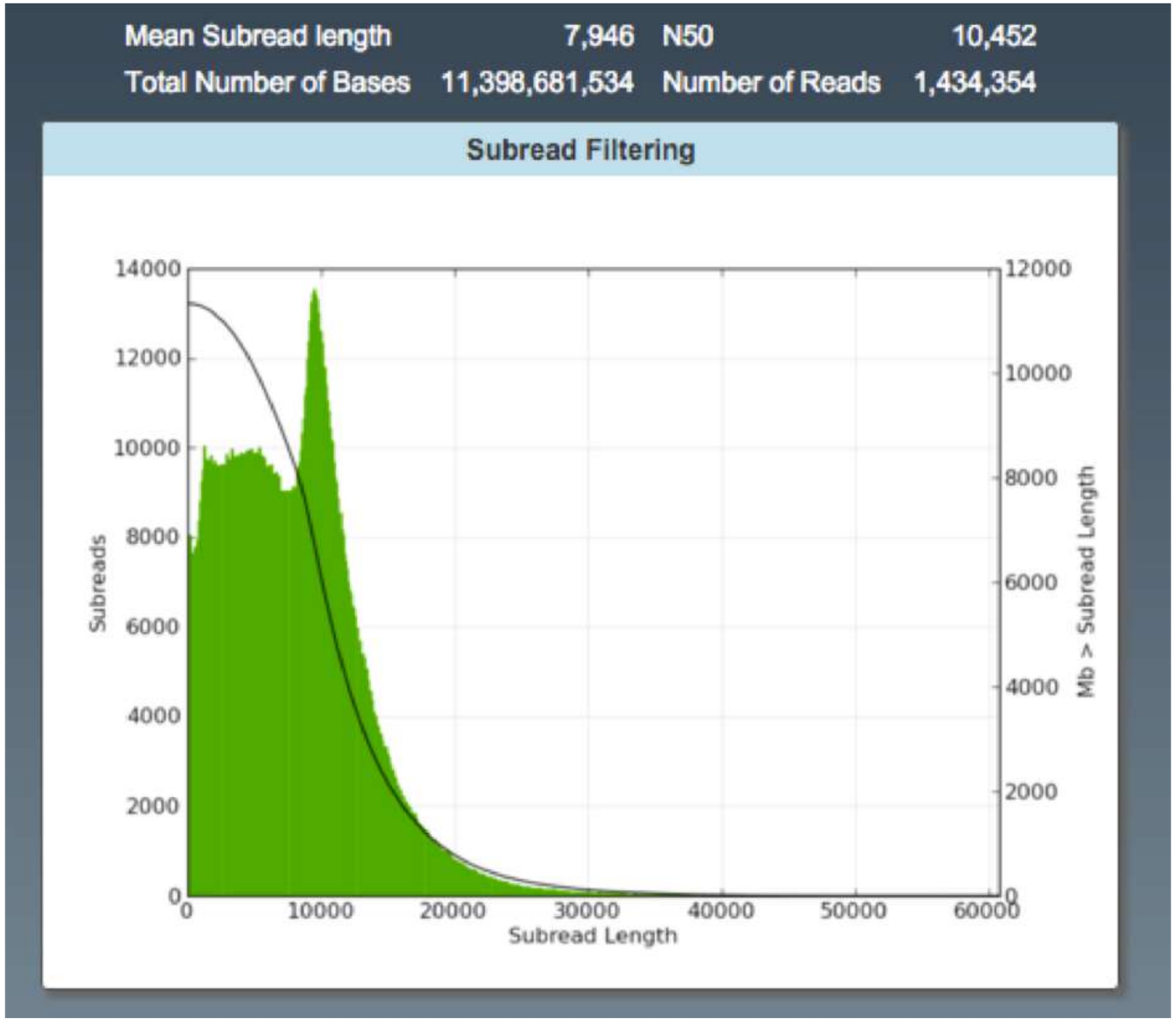


Average:	~ 8.5 kb
Maximum:	> 30 kb
Top 5% of reads:	> 18 kb
Half of data in reads:	> 10 kb
Data per SMRT® Cell:	~ 375 Mb

http://files.pacb.com/pdf/PacBio_RS_II_Brochure.pdf

PacBio (Pacific Biosciences)





Oxford Nanopore

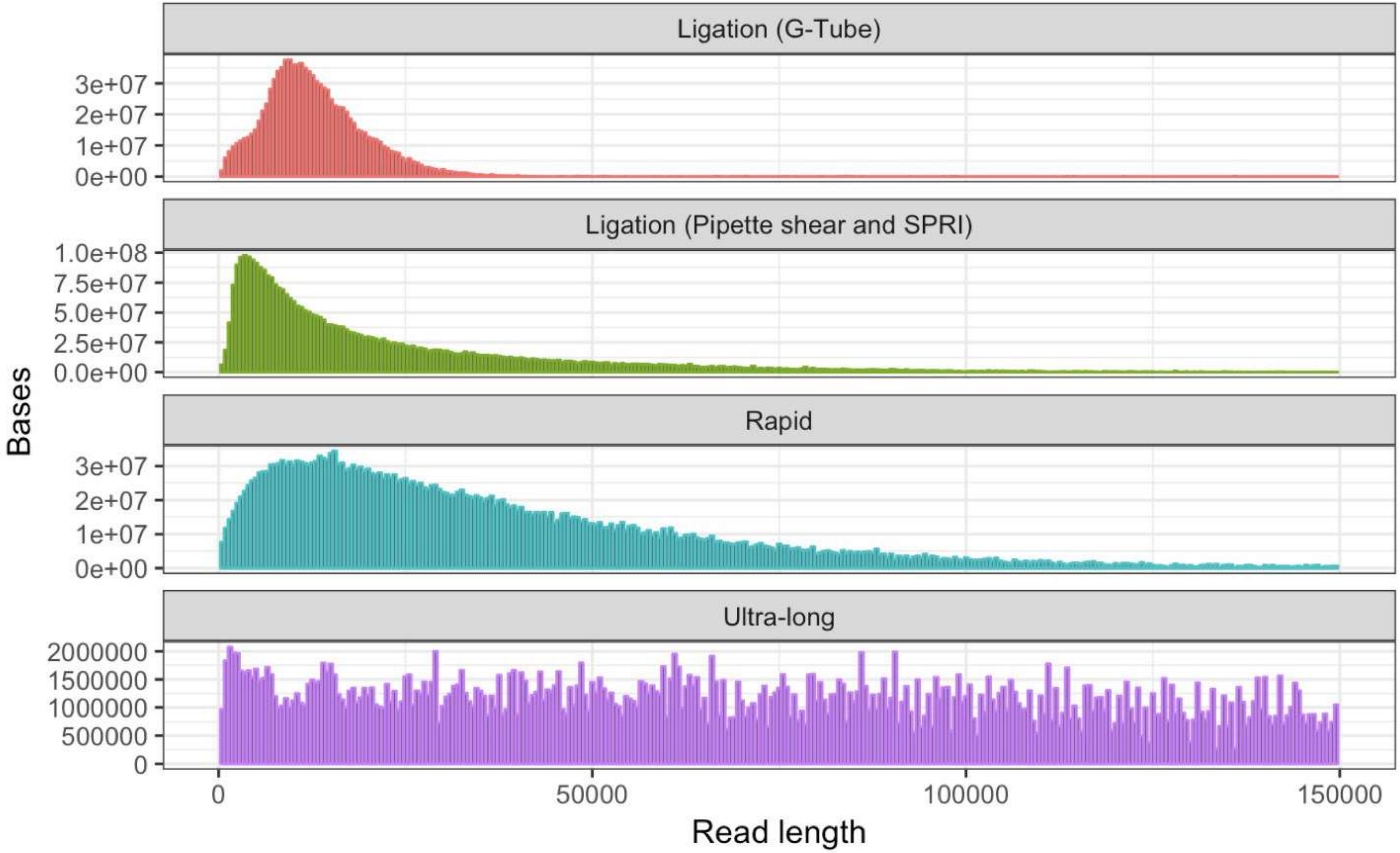
    

Key	SmidgION	Flongle	MinION	GridION	PromethION
System Price	TBC	Included in $5K Starter Pack	Included in $1K Starter Pack	Included in $50K Starter Pack	Included in $135K Starter Pack
Number of channels	200 channels	128 channels	512 channels	5 x 512 = 2,560*	48 x 3,000* = 144,000
Per flow cell Current Data – Max Data	TBC	1 - 3.3 Gb	17 - 40 Gb	17 - 40 Gb	125 - 311 Gb
Per Device Current Data – Max Data				85 - 200 Gb	3/6 - 20 Tb
Price per Gb Current Data – Max Data	**TBC**	**$90 - $30**	**$30 - $12.5**	**$17.5 - $7.5**	**$5 - $2**

Oxford Nanopore – how it works

https://nanoporetech.com/how-it-works

Read length go beyond



https://www.slideshare.net/scalene/ultralong-read-methods-for-nanopore-single-moleculesequencing-88022069

Break here

Three situations you are most likely to encounter

Genome reference is available (for example, humans):

- Re-sequence (DNA, RNA)
- **Map** sequence to the genome

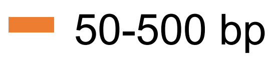
Genome reference is NOT available

- **Assemble** the reads to get the genome

Counting:

- For a given region (gene) we want to know how much.→ gene expression or metagenomics

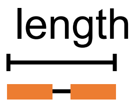
More Definition



Read — A sequenced piece of DNA

Paired-end read — Sequencing both ends of a short DNA fragment

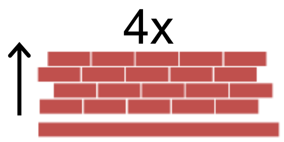
Mate-pair read — Sequencing both ends of a long DNA fragment



Insert size — The length of the DNA fragment

Contig — A set of overlapping DNA segments that represents a consensus region of DNA

Scaffold — Contigs separated by gaps of known length



Coverage — The number of times a specific position in the genome is covered by reads

What is an alignment? (mapping)

Align the following two sequences:

```
ATTGAAAGCTA
GAAATGAAAAGG
1:
--ATTGAAA-GCTA
  | ||||| |
GAAATGAAAAGG--

2:
ATTGAAA-GCTA---
   |||| |  |
---GAAATGAAAAGG
```

Scoring scheme is needed:

1 for match

-1 for mismatch

-2 for gap

insertions / deletions (indels) mismatches

Which alignment is better?

Assembly

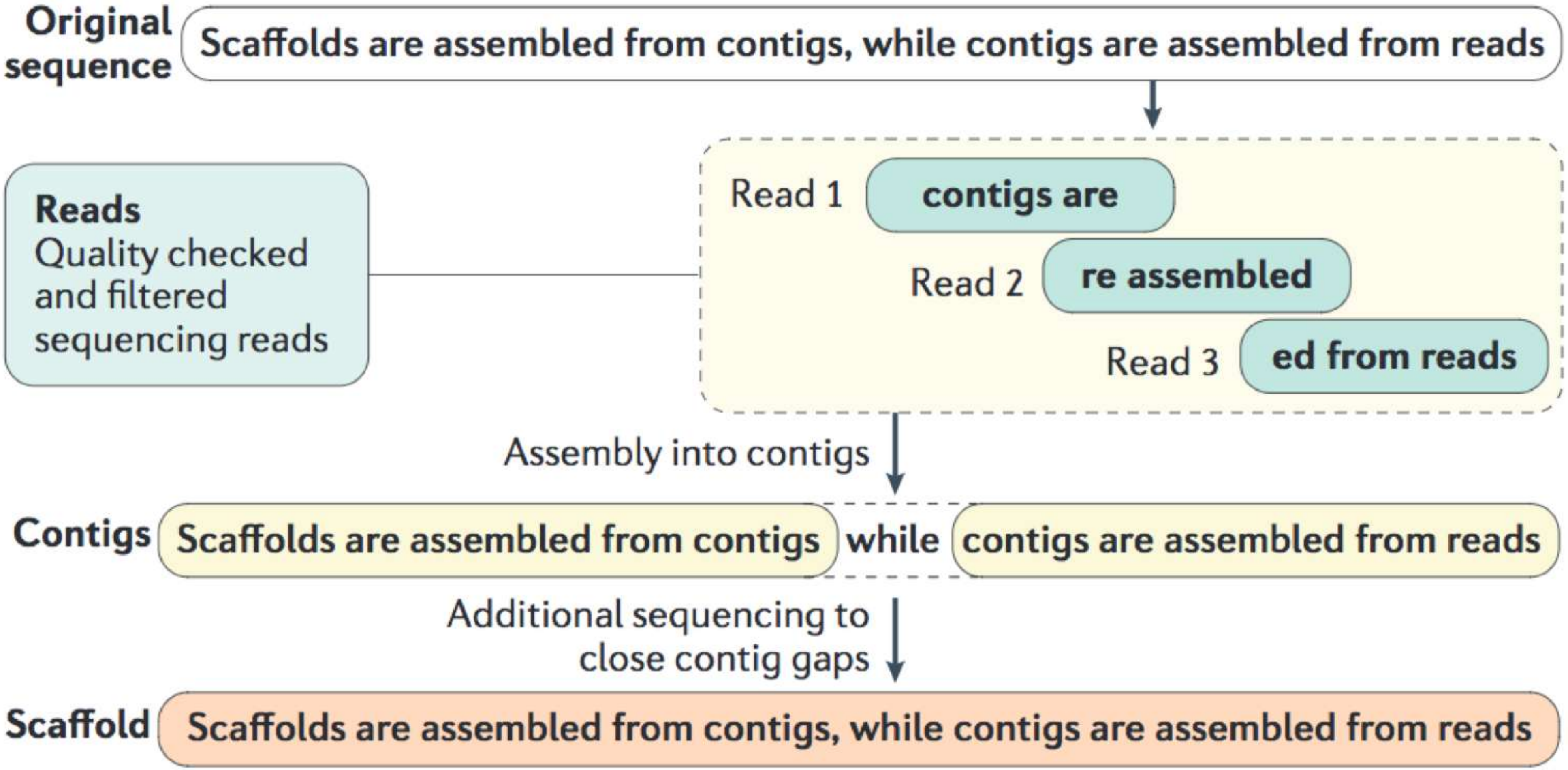


Figure 2 | **Sequence read assembly.** A mock example explaining bioinformatic sequence assembly along with the terms sequence, reads, contigs and scaffolds.

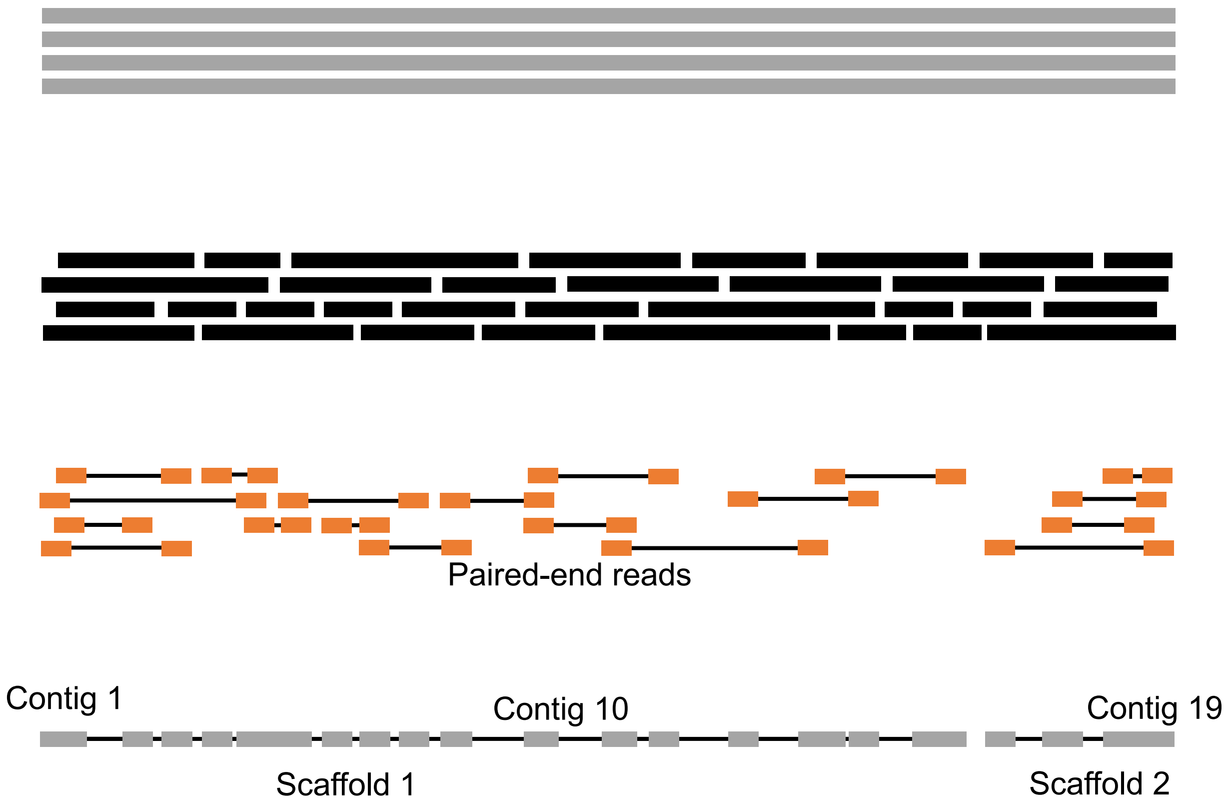
Assembly

Genome

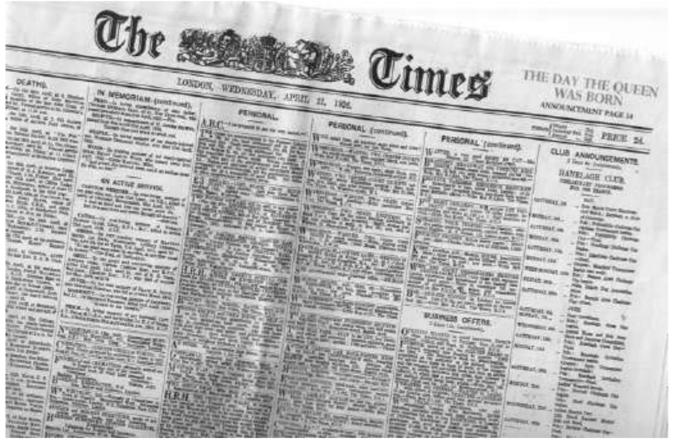
Fragment

Sequence

Assemble



Assembly



Genome
(3.000.000 letters)

Sequencing →

Reads
(50-500 letters each)

Assembly →

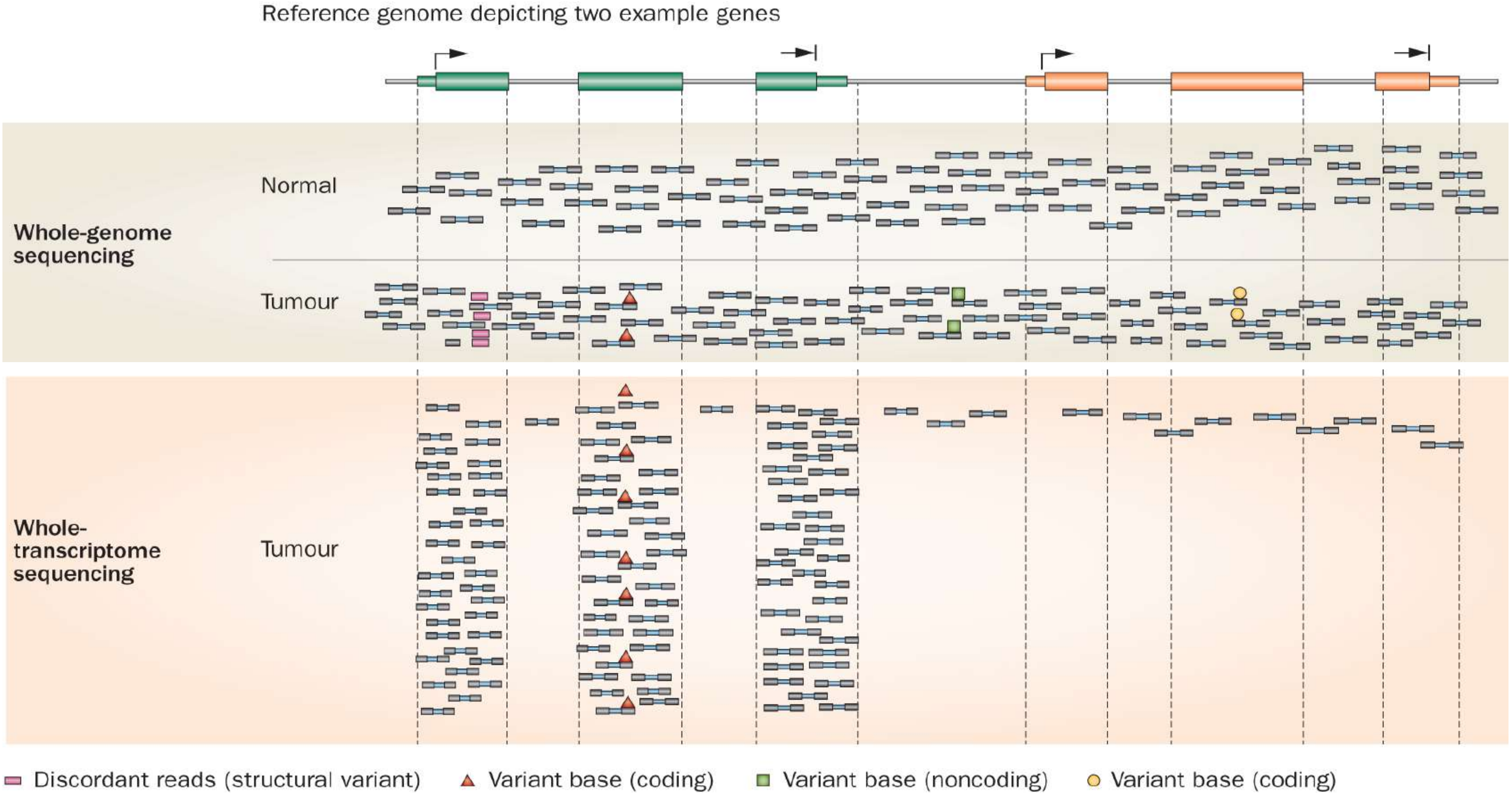
Genome
(3.000.000 letters)

After assembly

- Say you have an assembly with 200 contigs and 34 scaffolds. What do you do next?

- How accurate is it?
- Have you tried different assemblers?
- Can you improve with additional data or diminishing returns?
- Is there contamination?
- How does it compare to other species?

Mapping



doi:10.1038/nrgastro.2012.126

Read length matters in sequencing

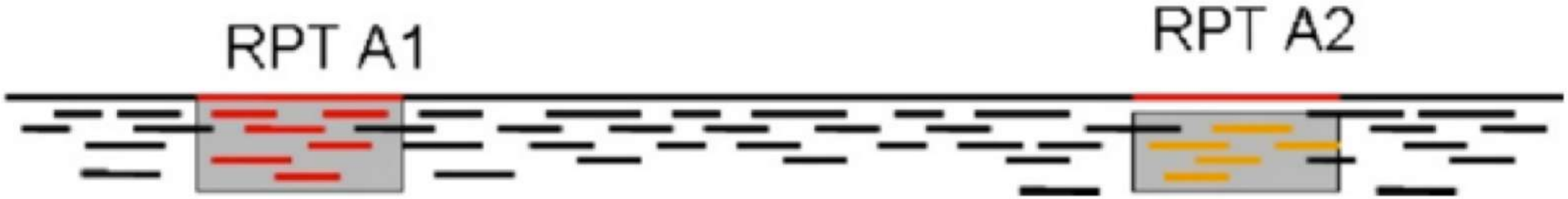


Figure 5. Two copies of a repeat along a genome. The reads colored in red and those colored in yellow appear identical to the assembly program.

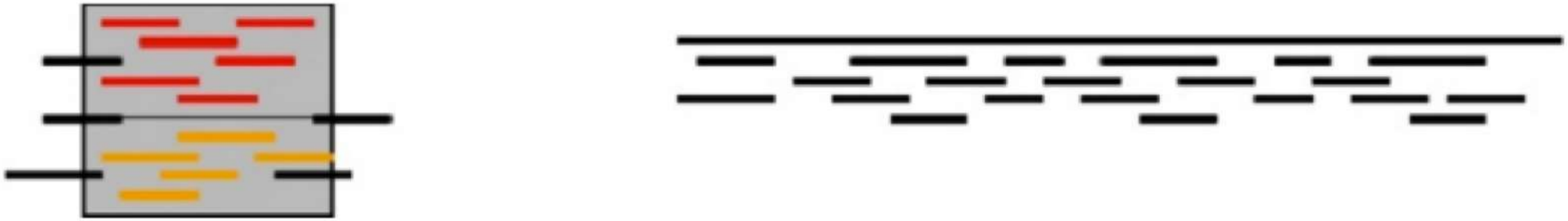
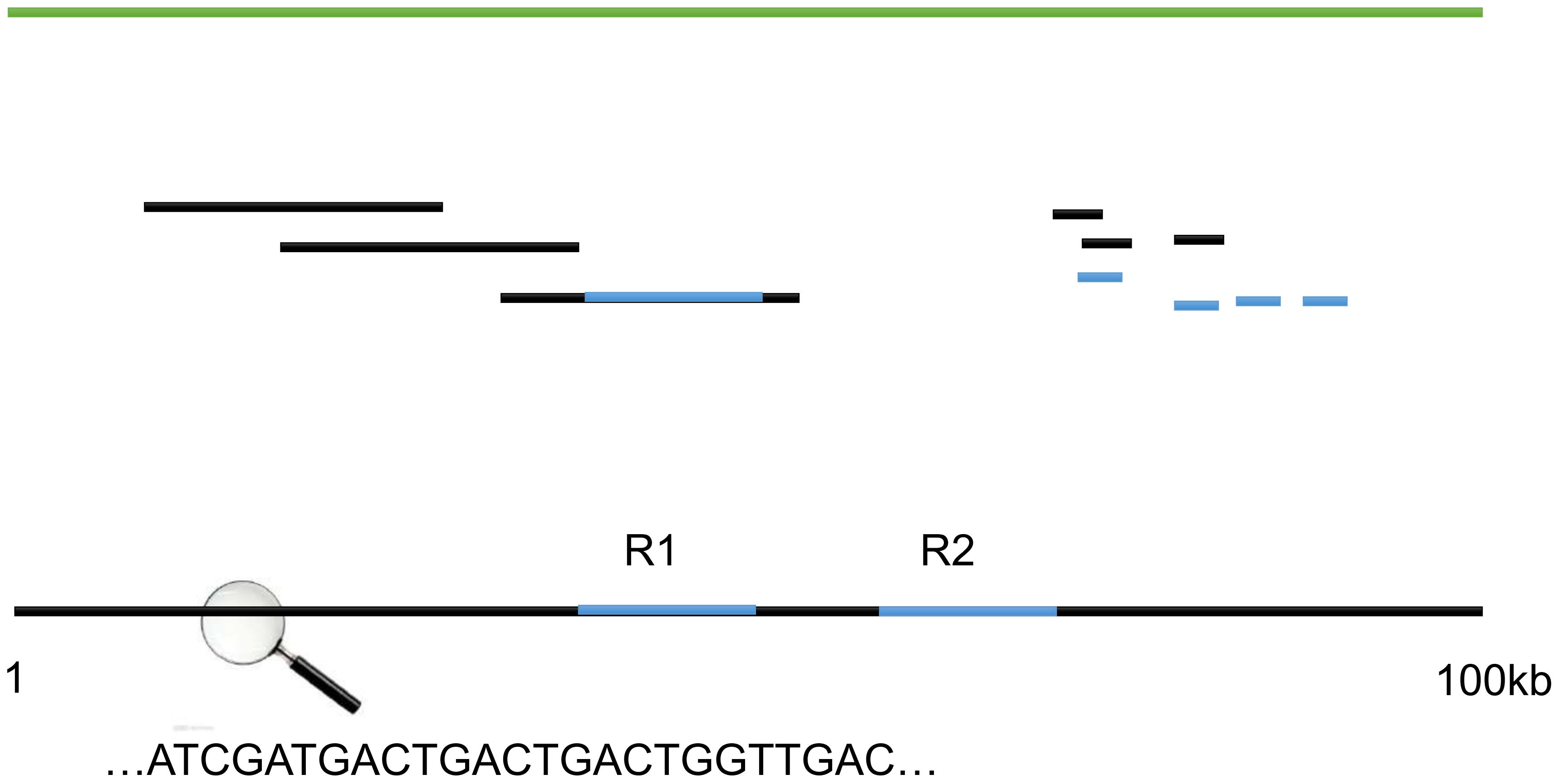


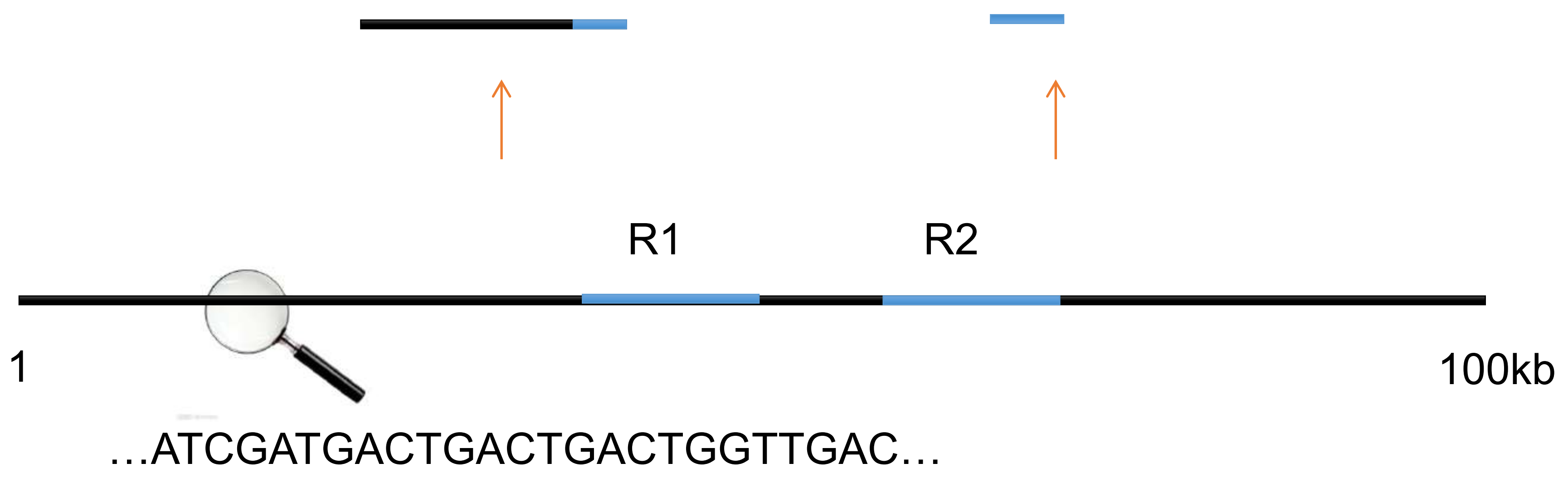
Figure 6. Genome mis-assembled due to a repeat. The assembly program incorrectly combined the reads from the two copies of the repeat leading to the creation of two separate contigs

https://www.cbcb.umd.edu/research/assembly_primer

Read length matters in sequencing



Paired end and insert size matter in sequencing



Depth matters in sequencing

ATCGATGACTGACTGAATGGTTGAC
ATCGATGACTGACTGAATGGTTGAC
ATCCATGACTGACTGAATGGTTGAC
ATCGATGACTGACTGAATGGTTGAC
ATCGATGACTGACTGAATGGTTGAC
ATCGATGACTGAGTGAATGGTTGAC
ATCGATGACTGAGTGAATGGTTGAC
ATCGATGACTGAGTGAATGGTTGAC
ATCGATGACTGAGTGAATGGTTGAC
10X ATCGATGACTGAGTGAATGGTTGAC

Homozygous? Heterozygous?

1X ATCGATCACTGACTGACTGGTTGAC

…ATCGATGACTGACTGACTGGTTGAC…

reference

Case studies

Classical genetics

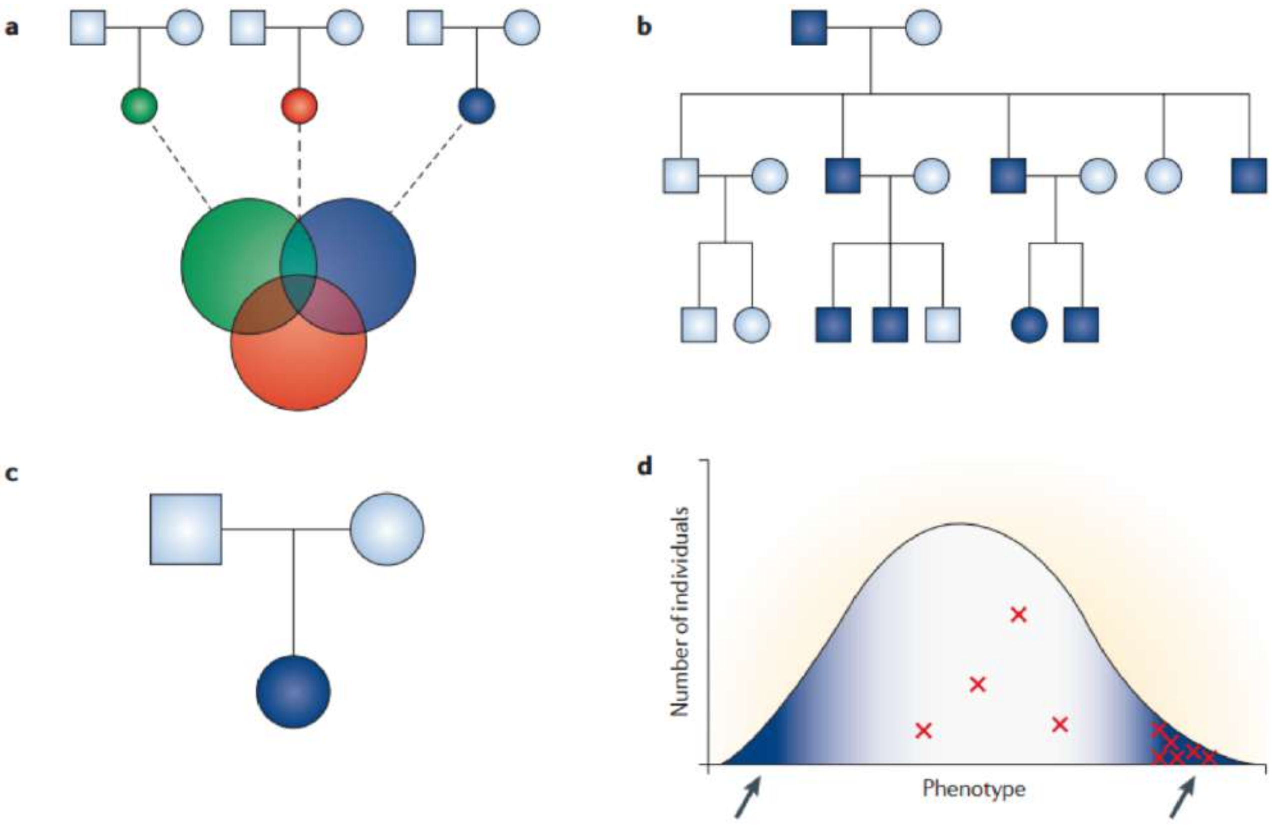
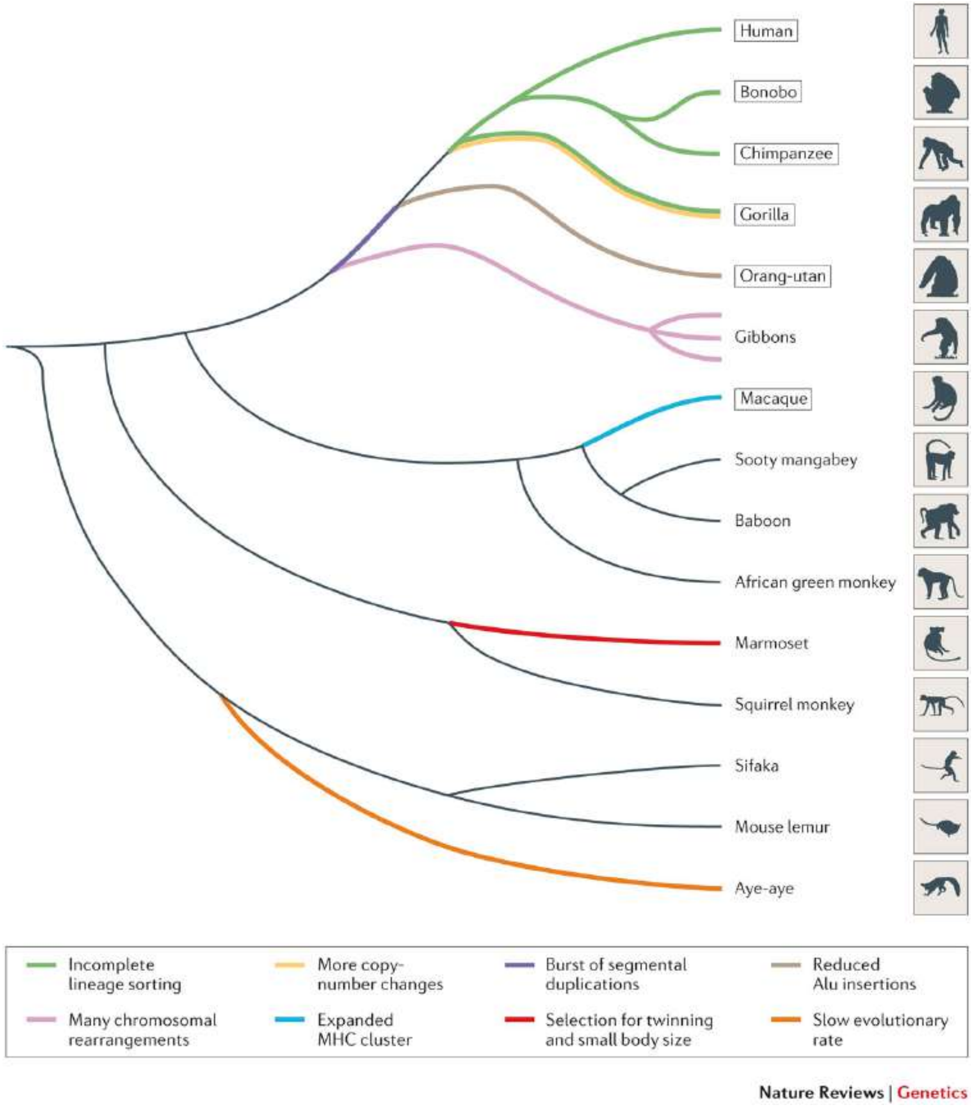
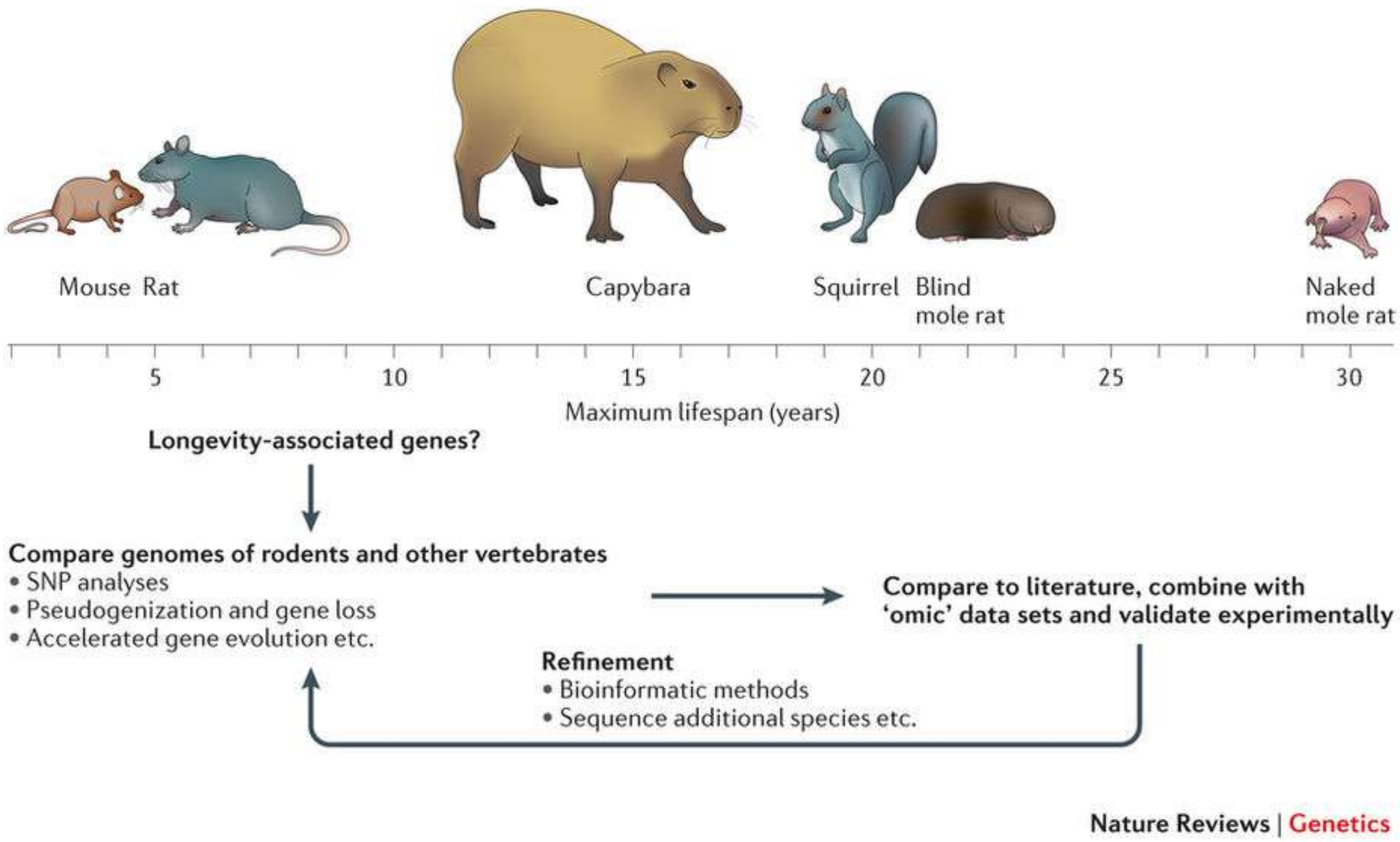


Figure 2 | **Strategies for finding disease-causing rare variants using exome sequencing.** Four main strategies are illustrated. **a** | Sequencing and filtering across multiple unrelated, affected individuals (indicated by the three coloured circles). This approach is used to identify novel variants in the same gene (or genes), as indicated by the shaded region that is shared by the three individuals in this example. **b** | Sequencing and filtering among multiple affected individuals from within a pedigree (shaded circles and squares) to identify a gene (or genes) with a novel variant in a shared region of the genome. **c** | Sequencing parent–child trios for identifying *de novo* mutations. **d** | Sampling and comparing the extremes of the distribution (arrows) for a quantitative phenotype. As shown in panel **d**, individuals with rare variants in the same gene (red crosses) are concentrated in one extreme of the distribution.

http://www.nature.com/nrg/journal/v12/n11/pdf/nrg3031.pdf

Comparative genomics





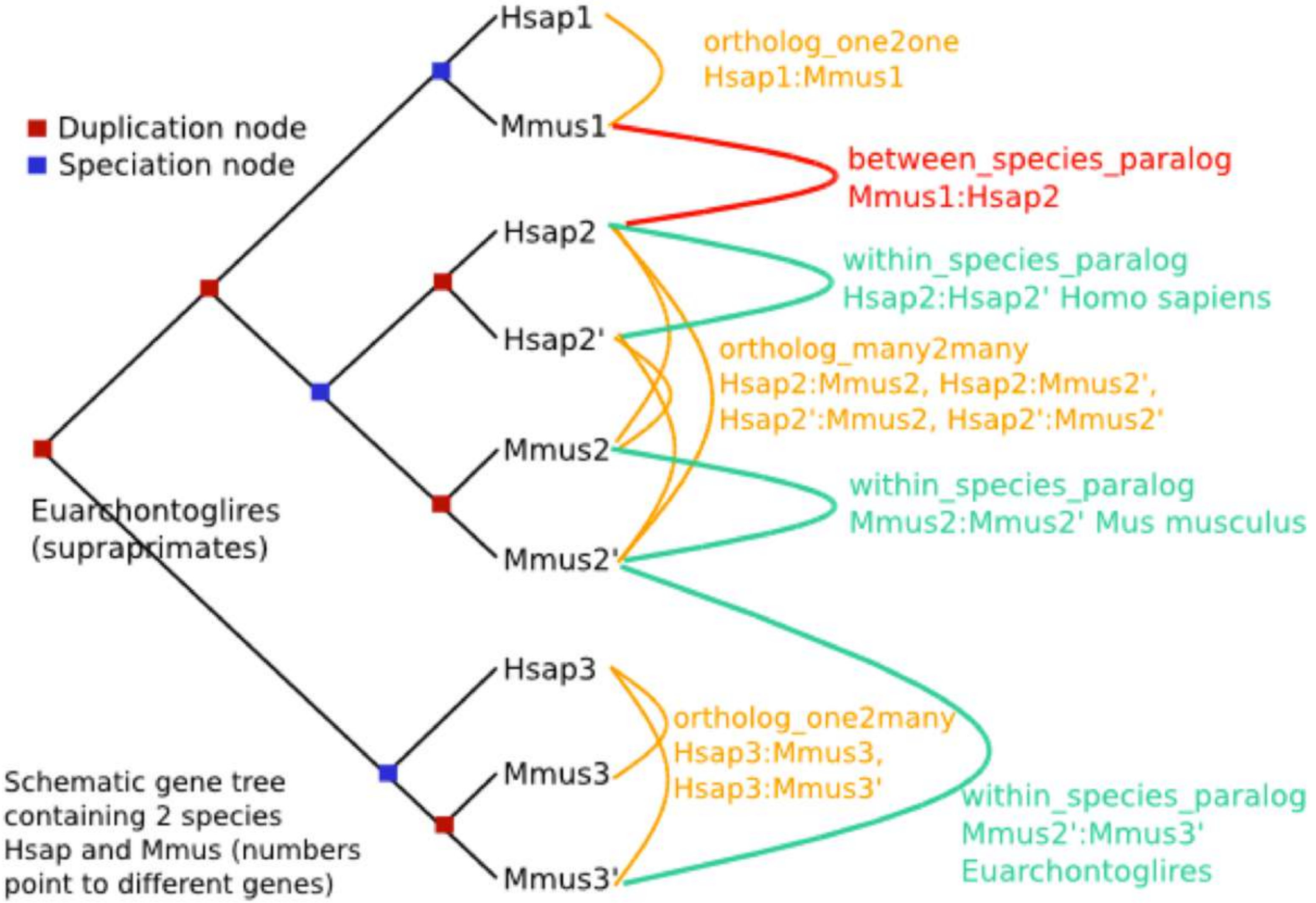
Roger & Gibbs Nature Reviews Genetics (2014) doi:10.1038/nrg3728

Homologs: Orthologs and paralogs

Genes in different species and related by a speciation event are defined as **orthologs**.

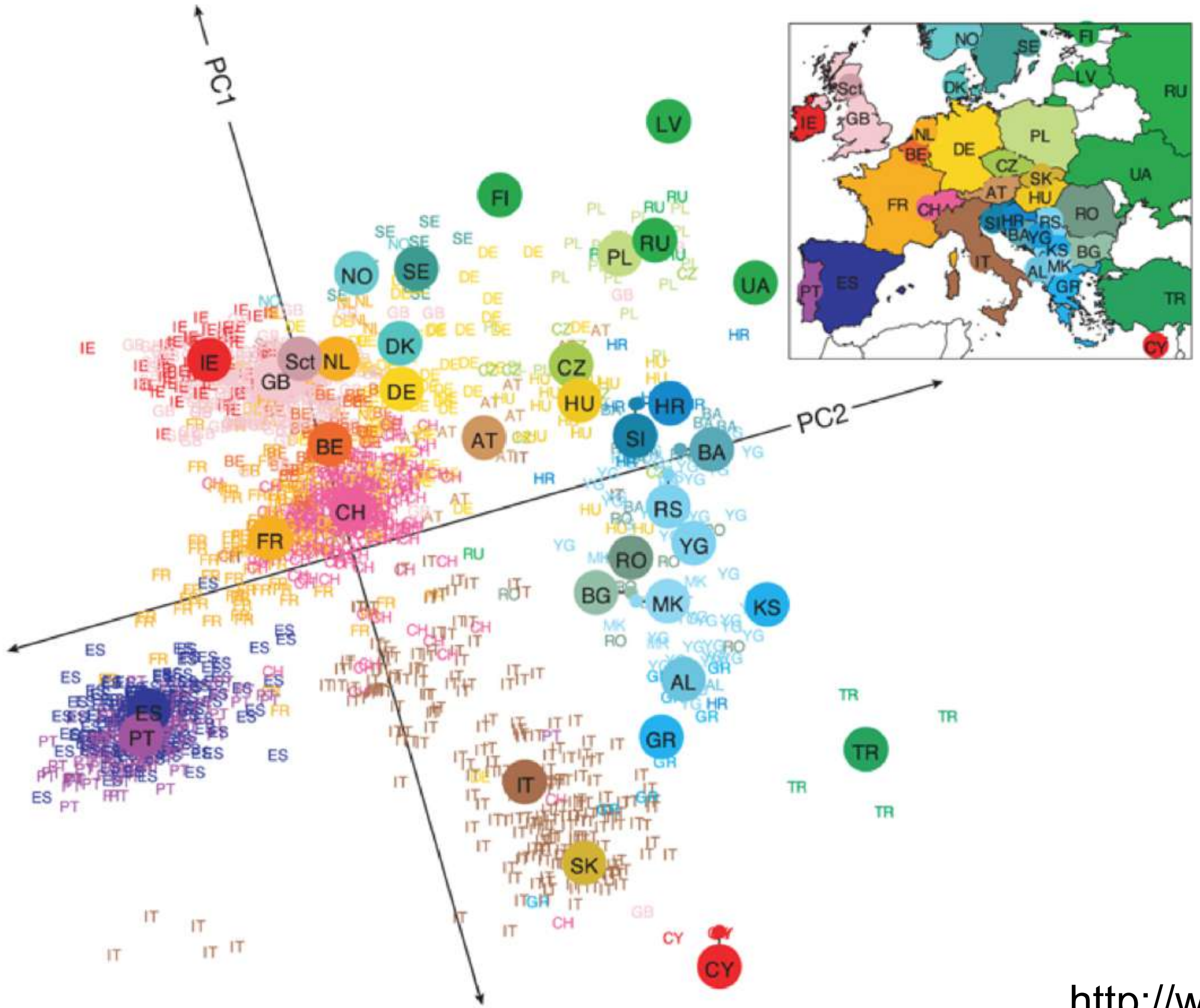
Depending on the number of genes found in each species, we differentiate among 1:1, 1:many and many:many relationships.

Genes of the same species and related by a duplication event are defined as **paralogs**.

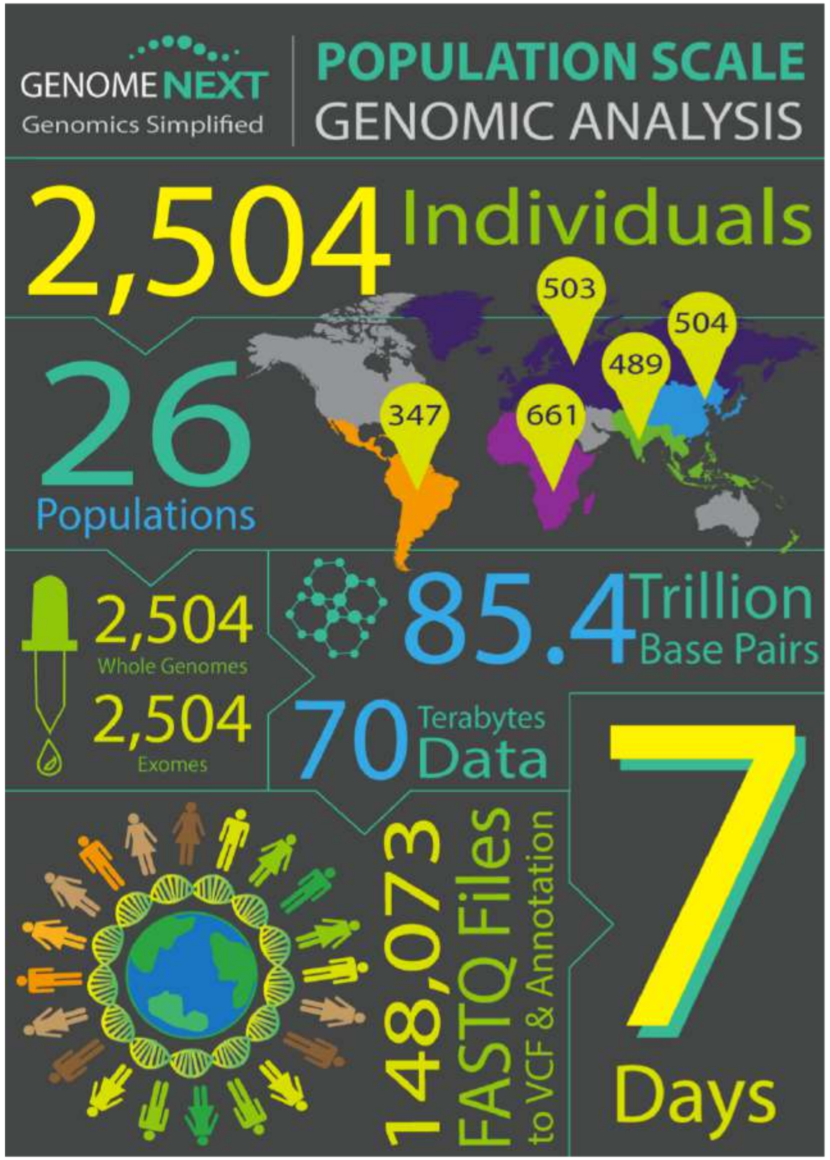


http://asia.ensembl.org/info/genome/compara/homology_method.html?redirect=no

Population genomics

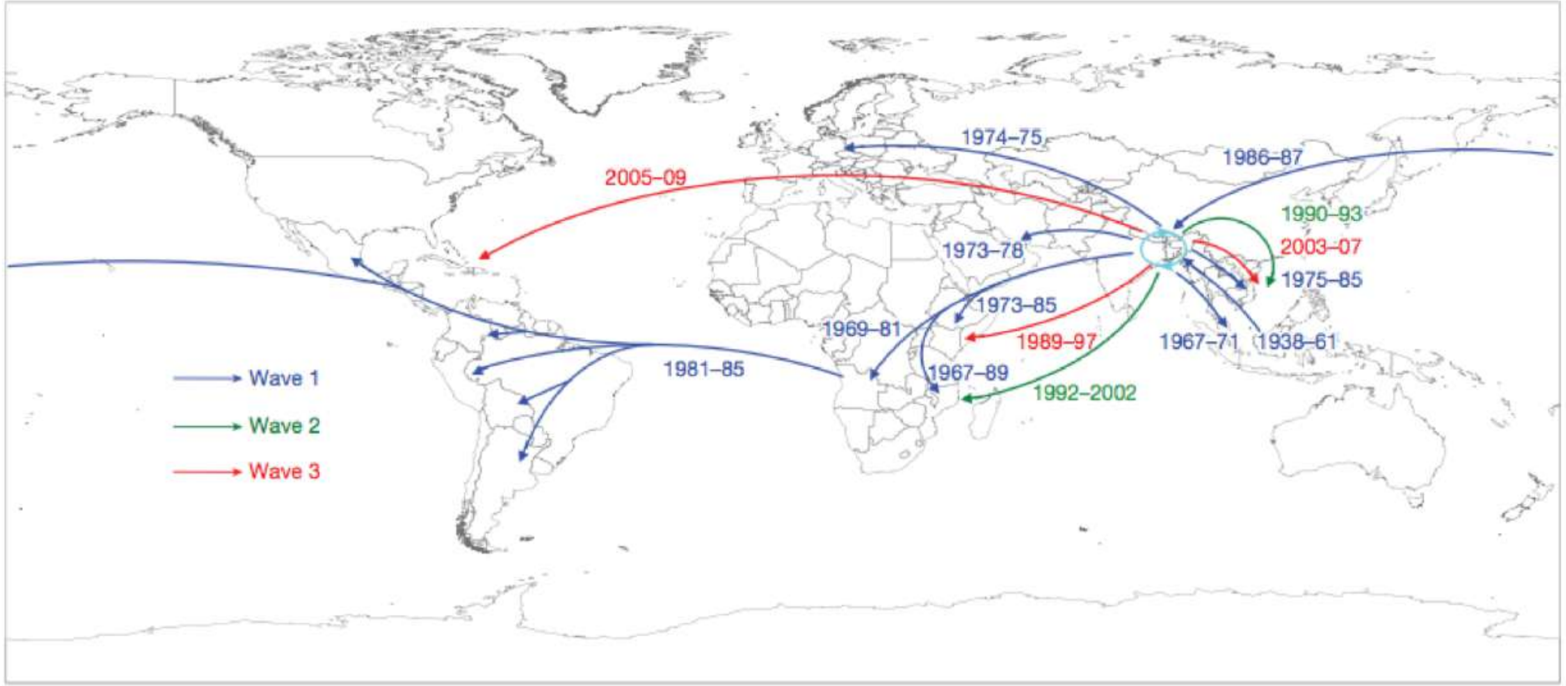


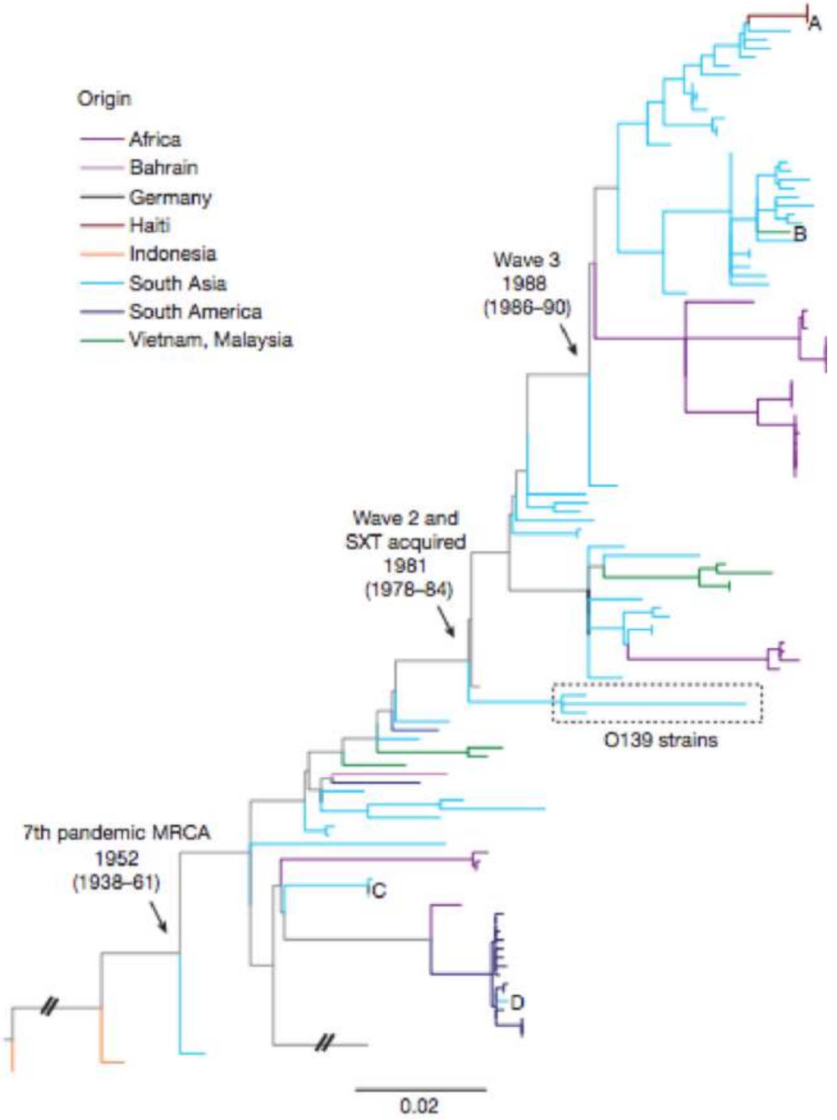
Novembre et al Nature (2008)



http://www.genomenext.com/casestudies_post/population-scale-analysis-genomic-samples-analyzed-from-2504-individuals-in-1-week/

Population genomics

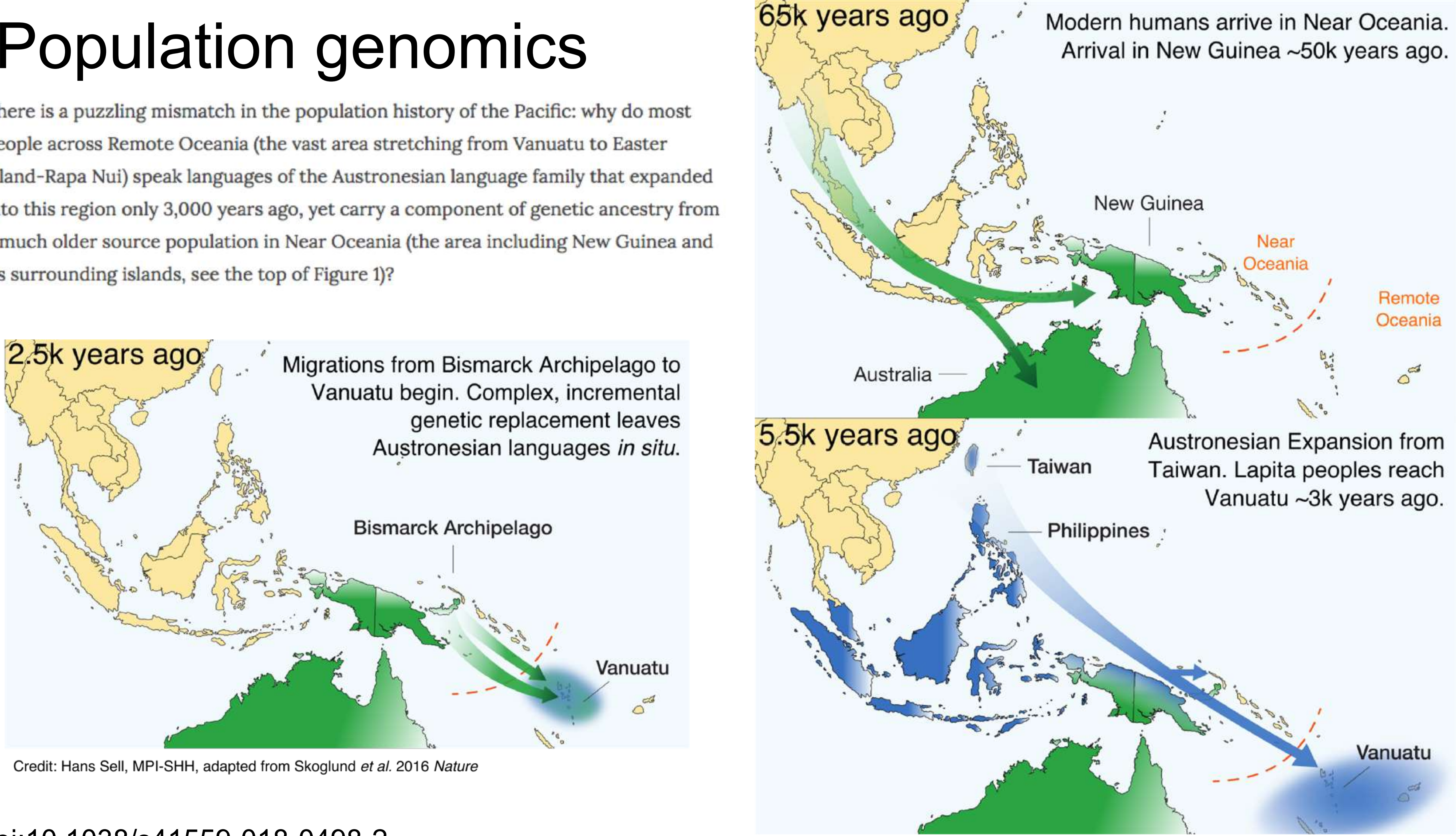




Mutreja *et al.*, Nature Genetics (2011)

Population genomics

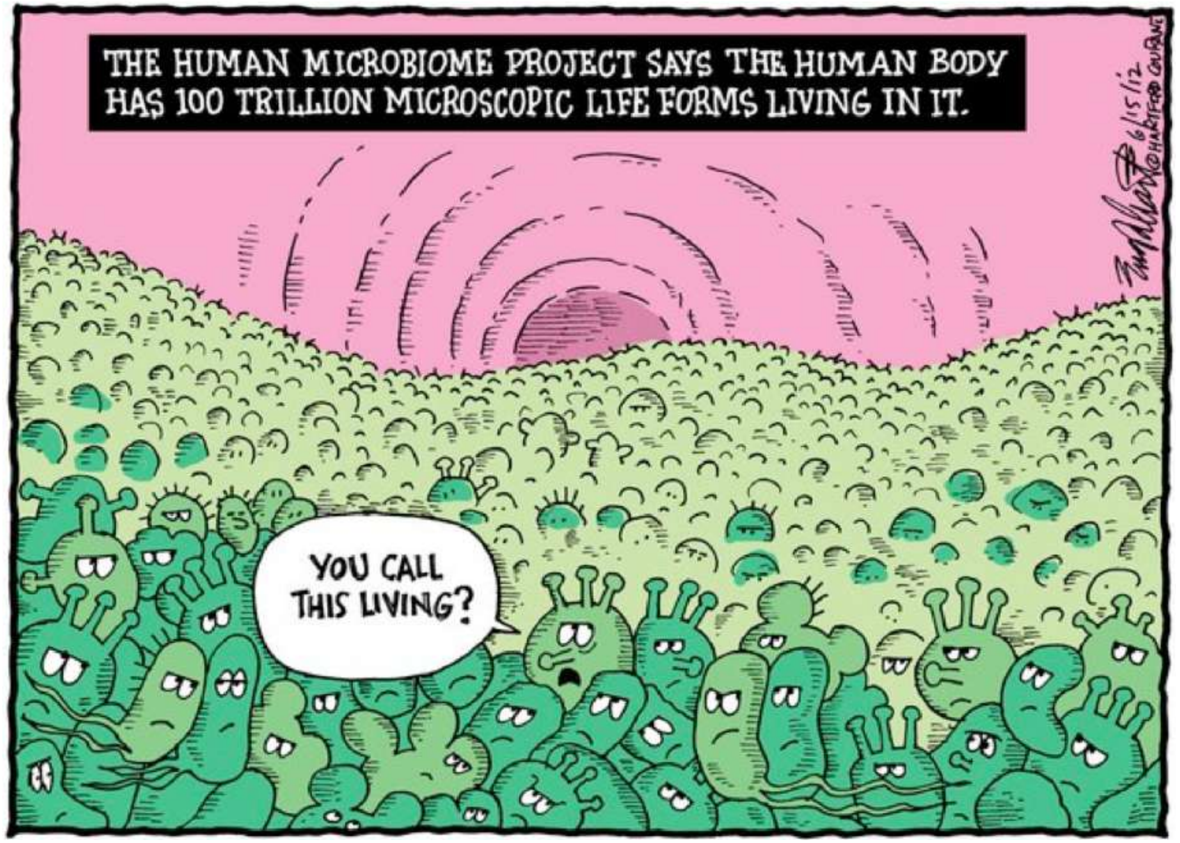
There is a puzzling mismatch in the population history of the Pacific: why do most people across Remote Oceania (the vast area stretching from Vanuatu to Easter Island-Rapa Nui) speak languages of the Austronesian language family that expanded into this region only 3,000 years ago, yet carry a component of genetic ancestry from a much older source population in Near Oceania (the area including New Guinea and its surrounding islands, see the top of Figure 1)?

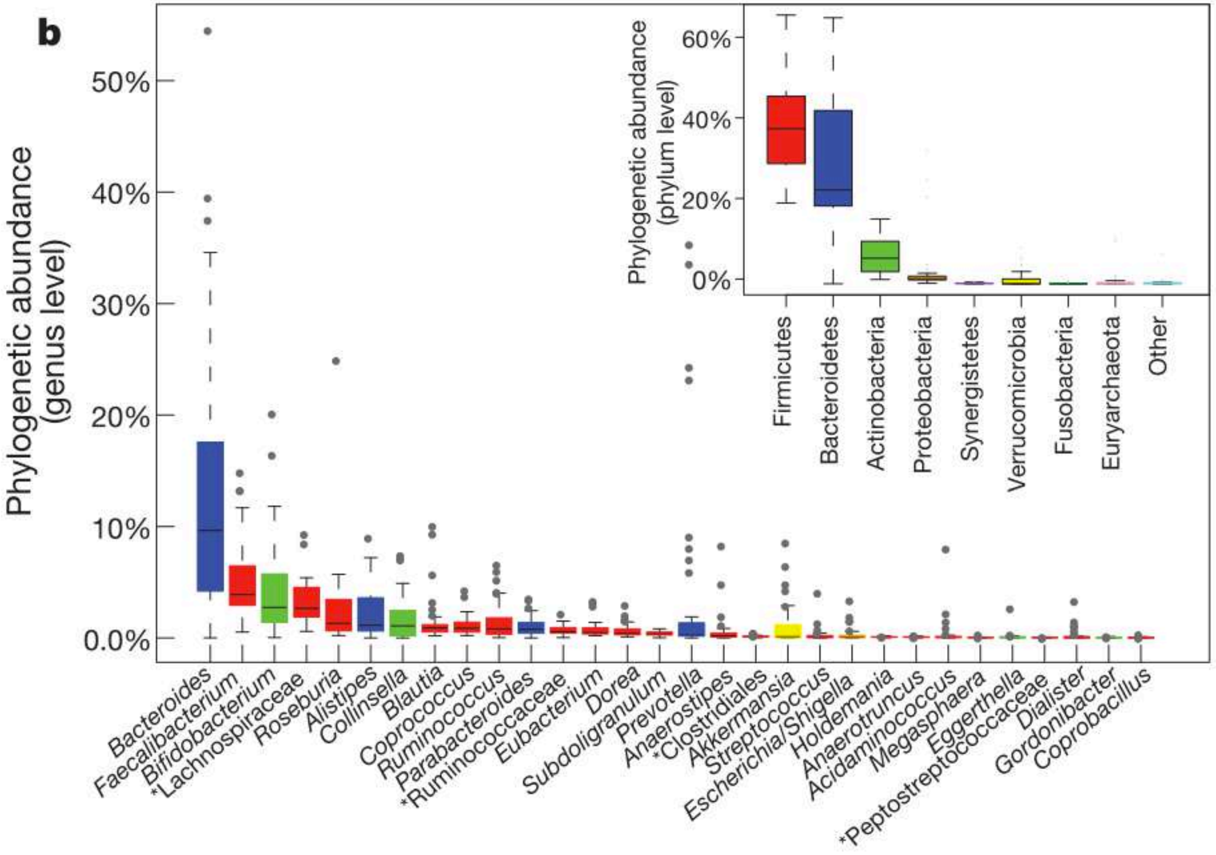


Credit: Hans Sell, MPI-SHH, adapted from Skoglund *et al.* 2016 *Nature*

doi:10.1038/s41559-018-0498-2

Metagenomics





doi:10.1038/nature09944

Transcriptomics / RNAseq

Applications of RNAseq

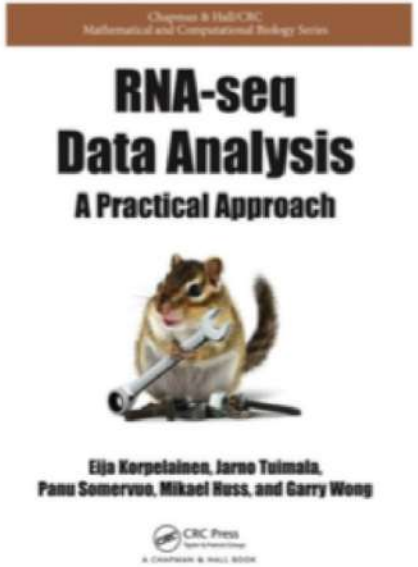
Discovery / Annotation

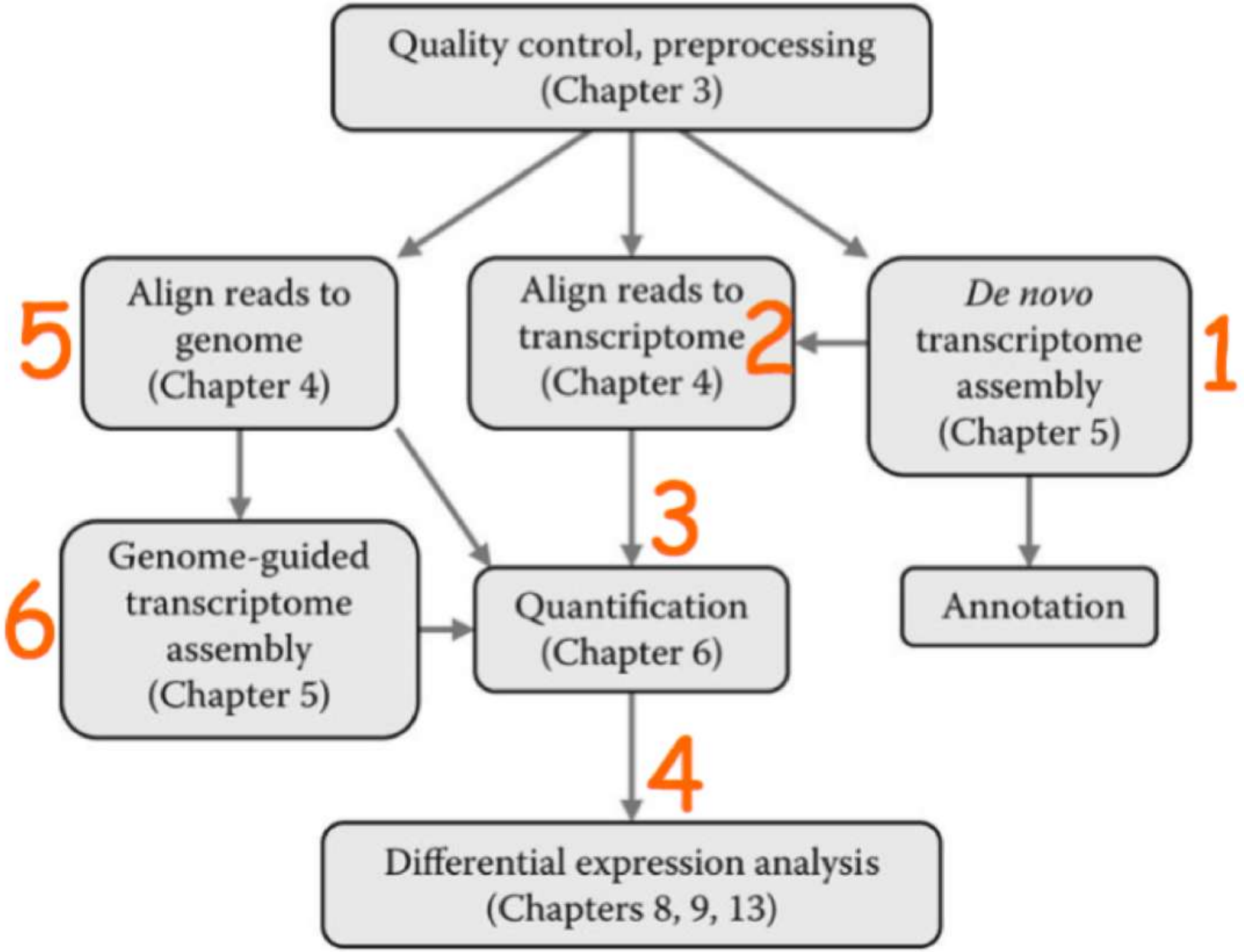
- Find new genes
- Find new transcripts
- Find new ncRNAs, xxx, xxx ….
- Gene fusion

Comparison / Quantification : **given X conditions, find the effect of Y on**

- **expression**
- **Isoform abundance, splice patterns, transcript boundaries**

Expression quantification and transcript assembly



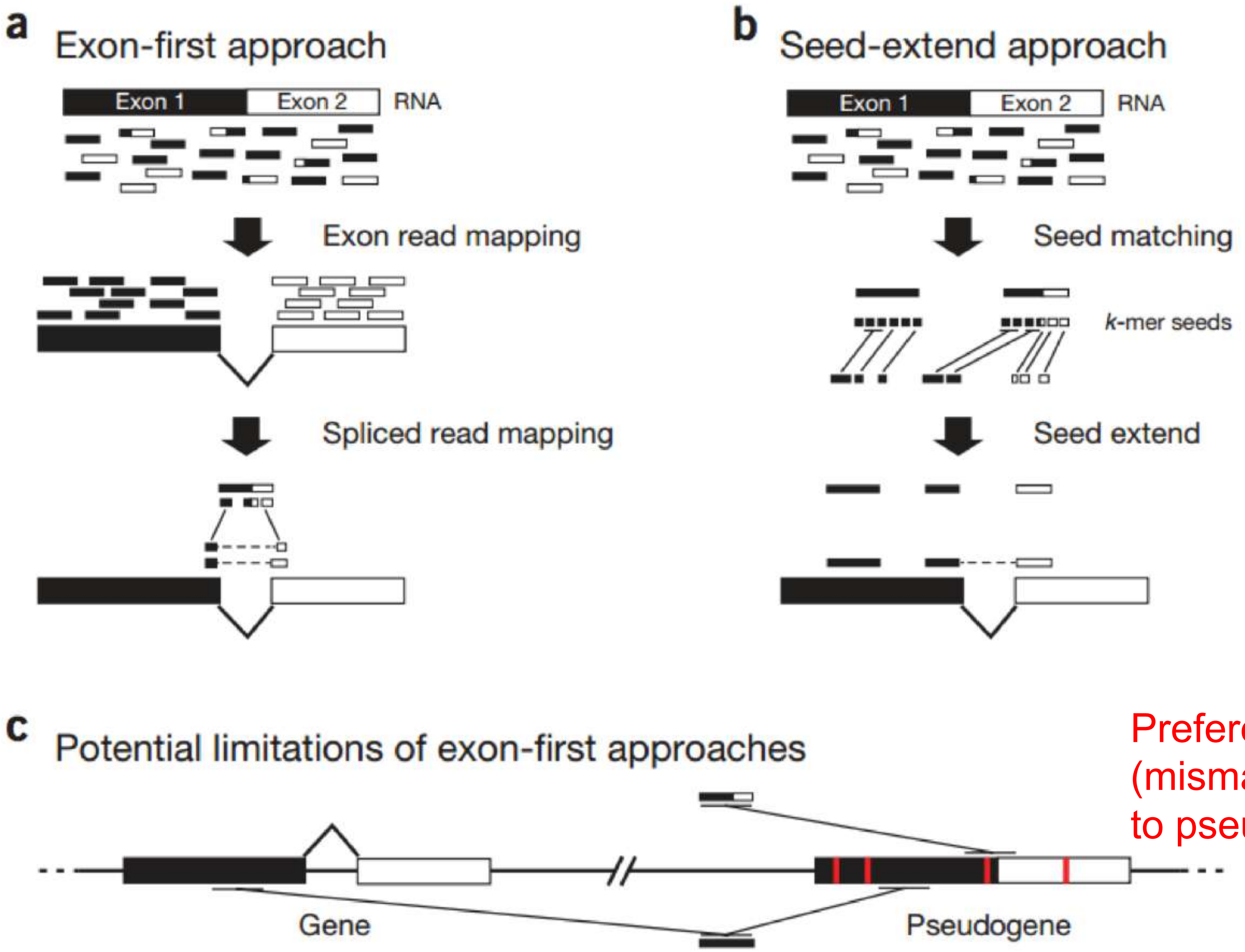


September 9, 2014
E-Book - ISBN 9781482262353

FIGURE 2.1 Possible paths in RNA-seq data analysis.

Once you have the raw data: **map** the RNAseq reads

Strategies for gapped alignments of RNAseq reads



Preferential alignment (mismatch rather than split) to pseudogene

Garber et al (2010)

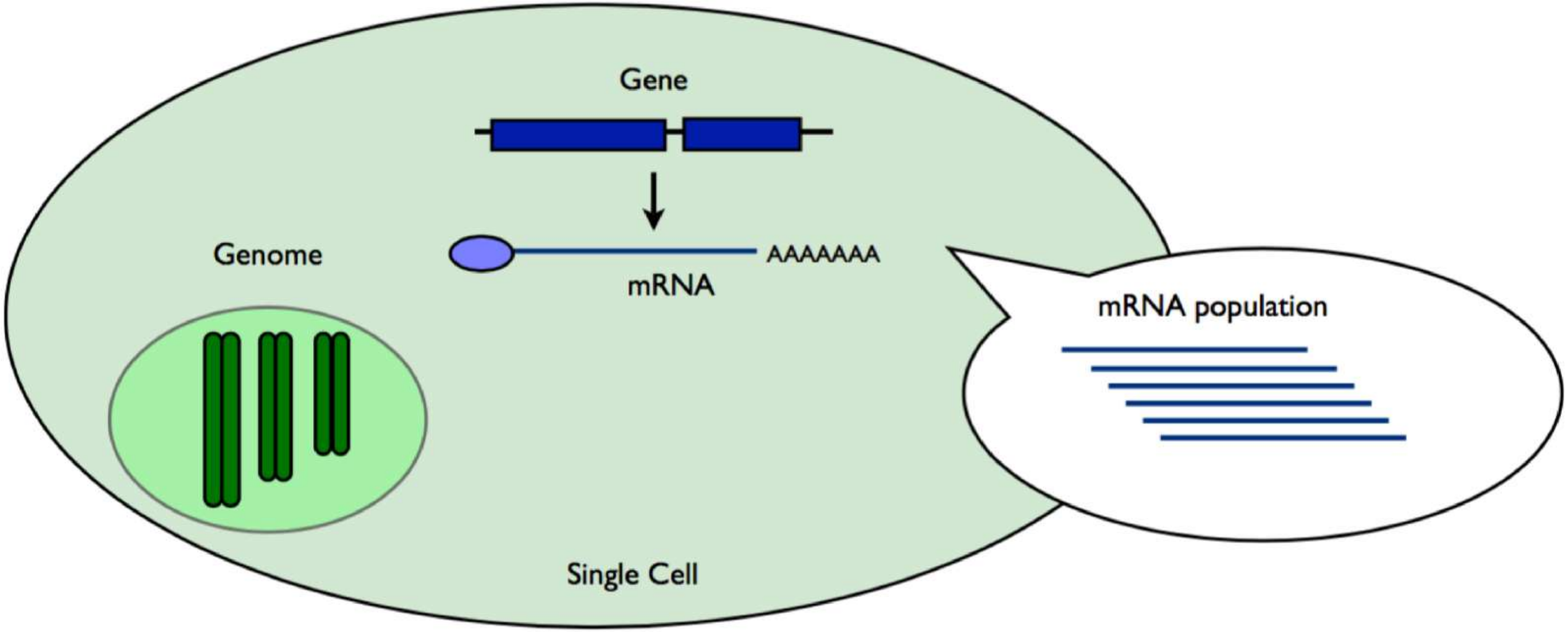
Differential expression

Types of experiments

Transcriptome Complexity:

Simple System:

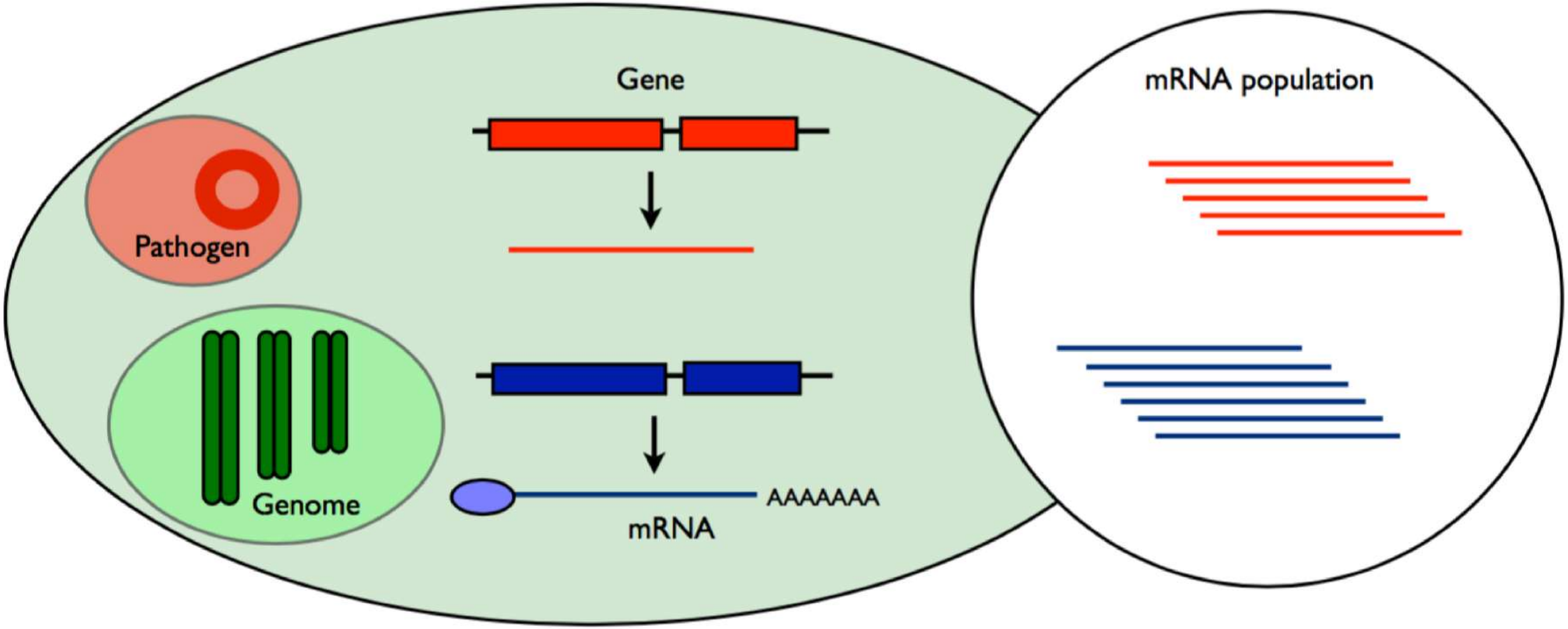
One Genome => Gene 1 copy => Single mRNA



How many species we are analyzing ?

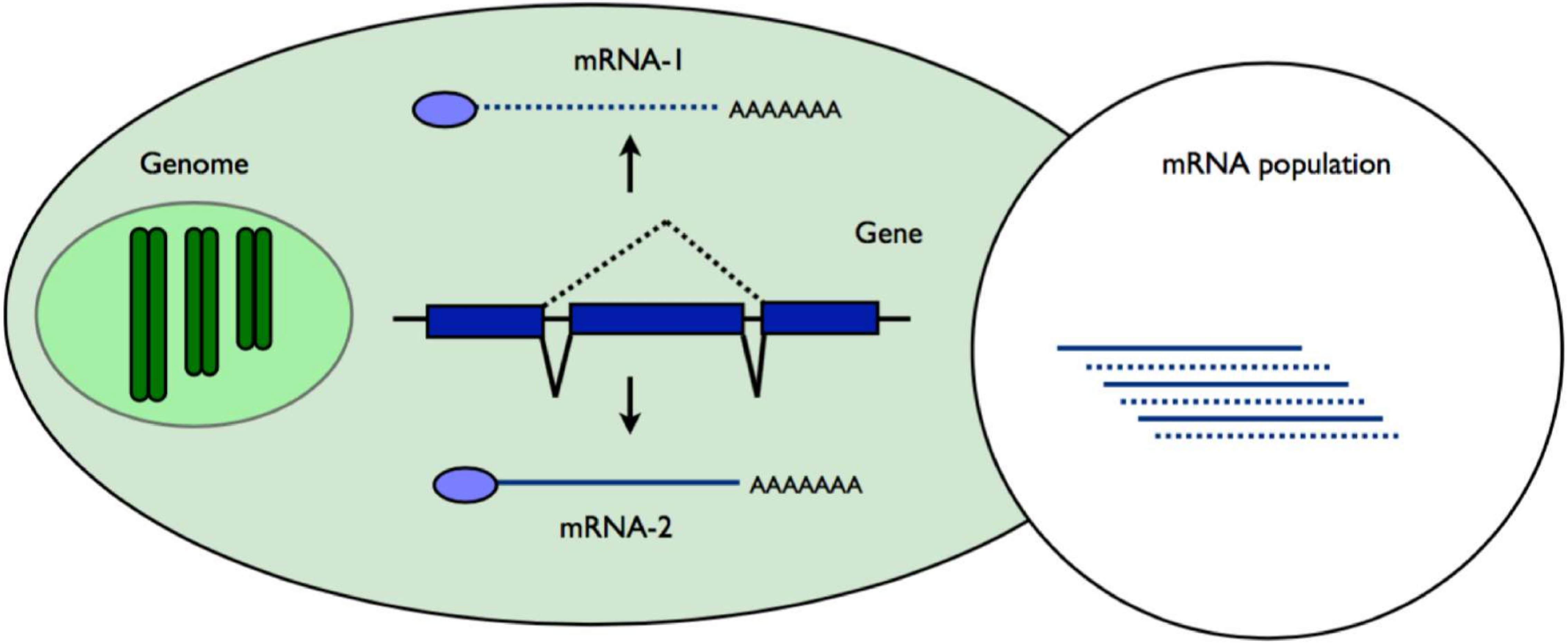
1) Problems to isolate a single species (rhizosphere)

2) Species interaction study (plant-pathogen)



How many isoforms we expect for each allele ?

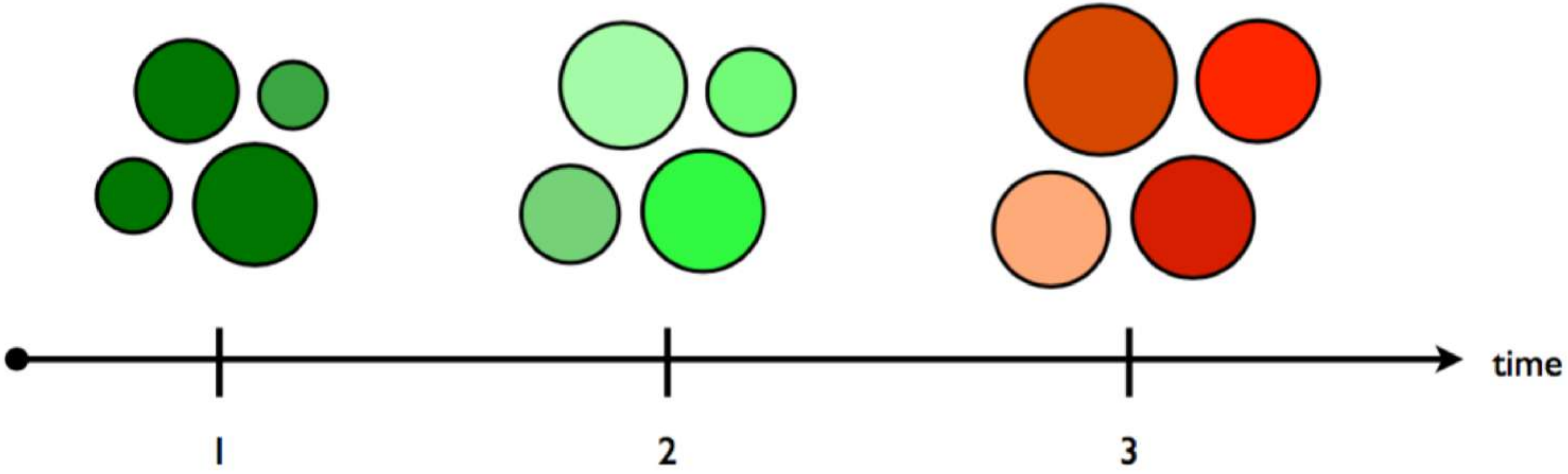
1) Alternative splicings



Is the study performed at different time points?

1) Developmental stages (difficult to select the same)

2) Response to a treatment

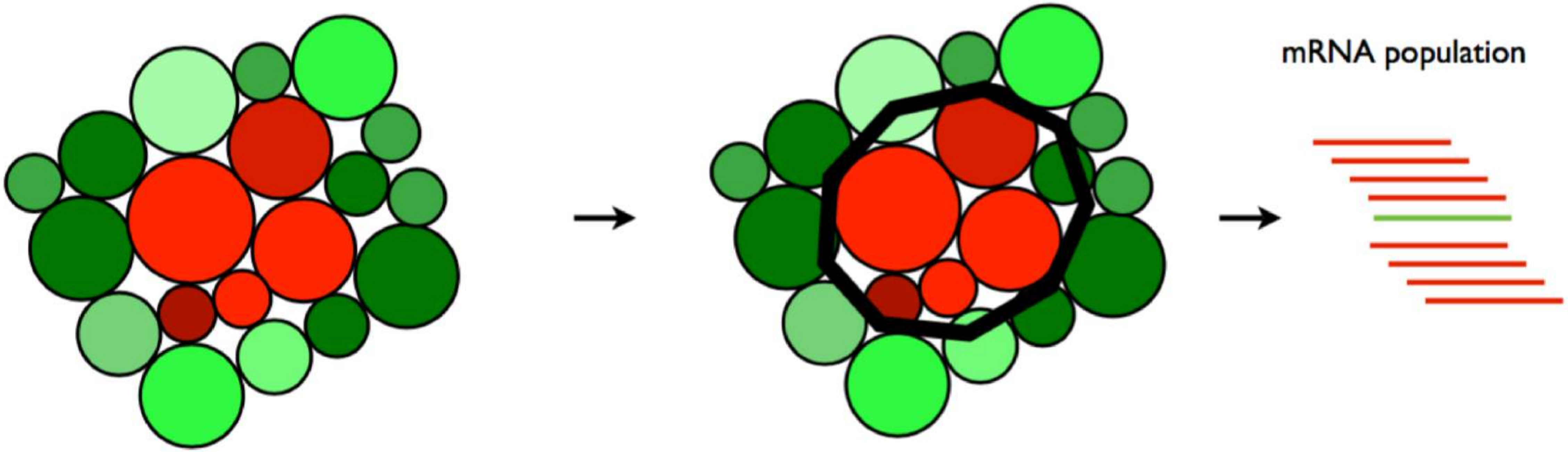


Is the study performed with different parts?

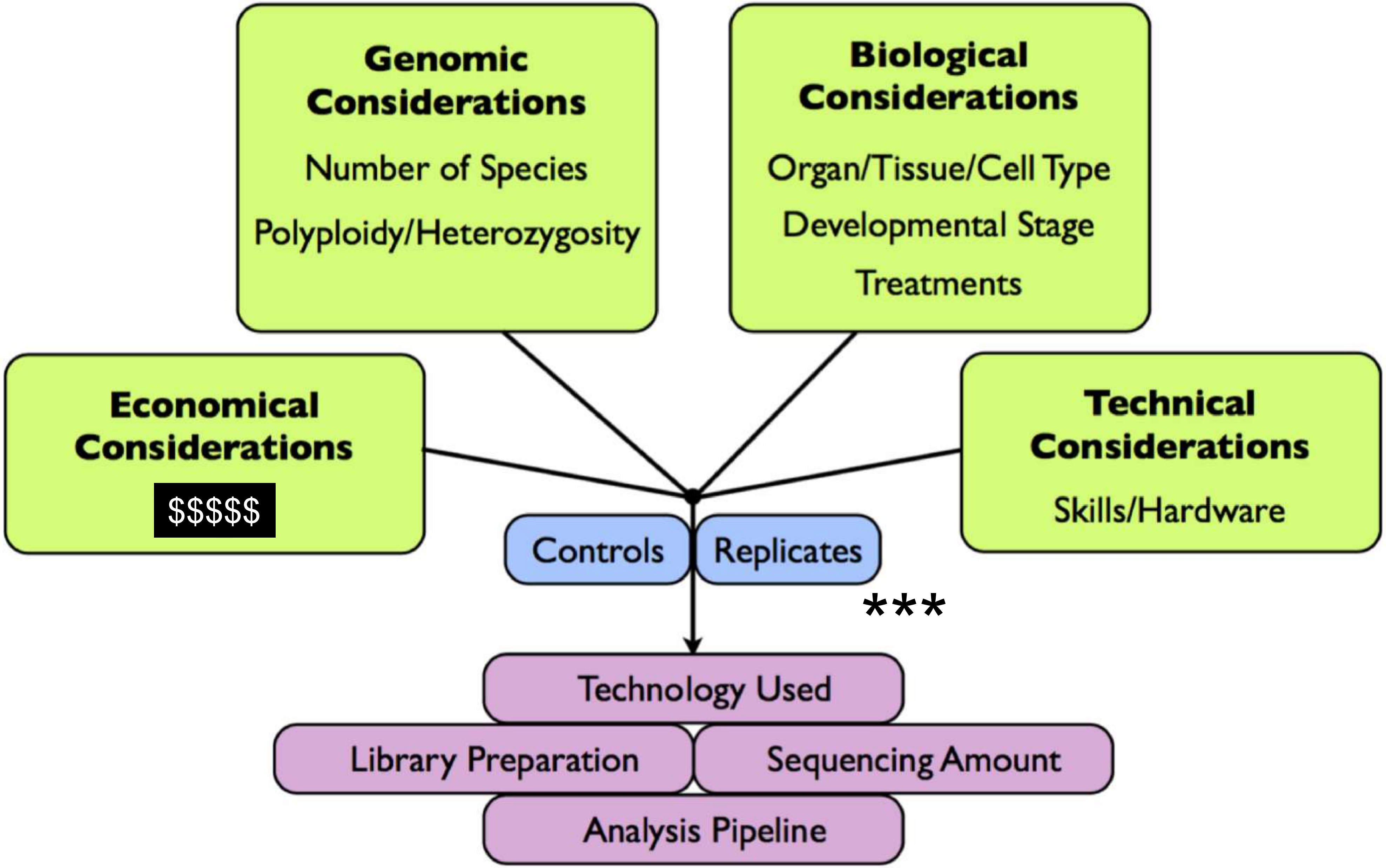
1) Organ specific

2) Tissue/Cell type specific

(Laser Capture Microdissection, LCM)



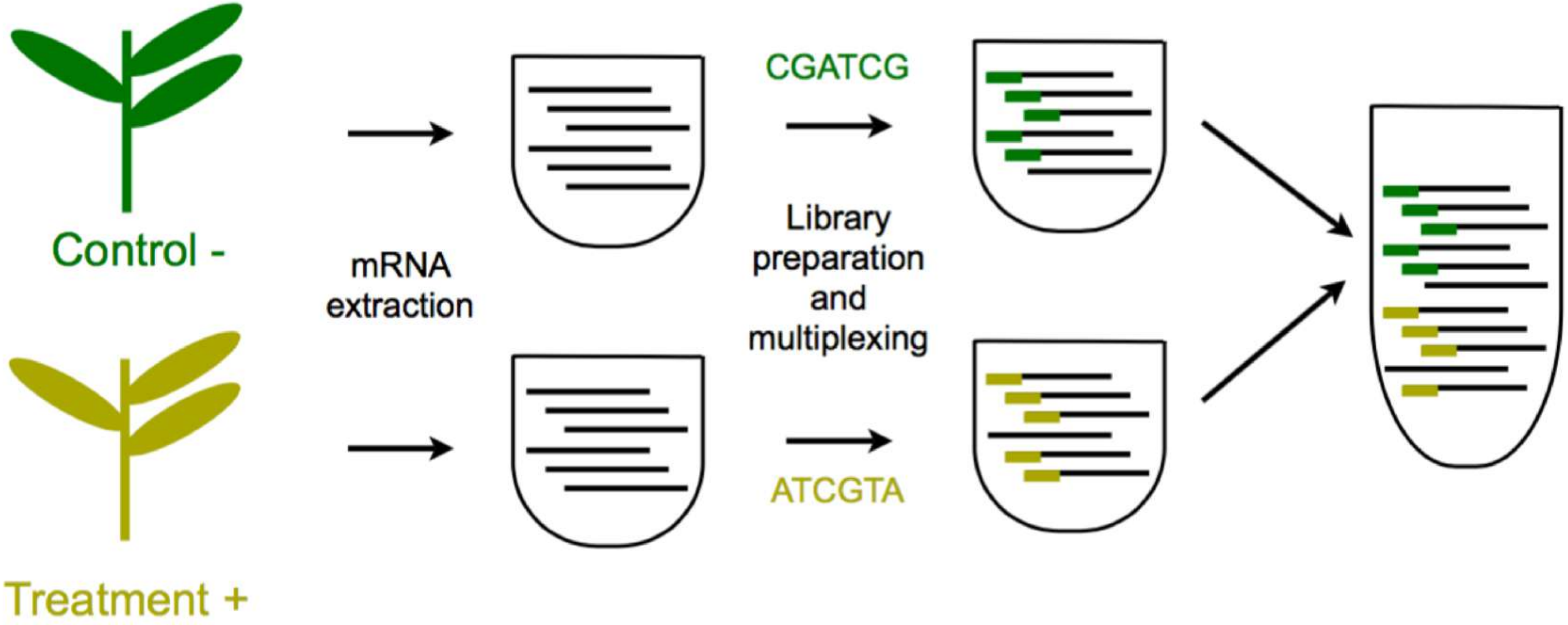
Experimental design



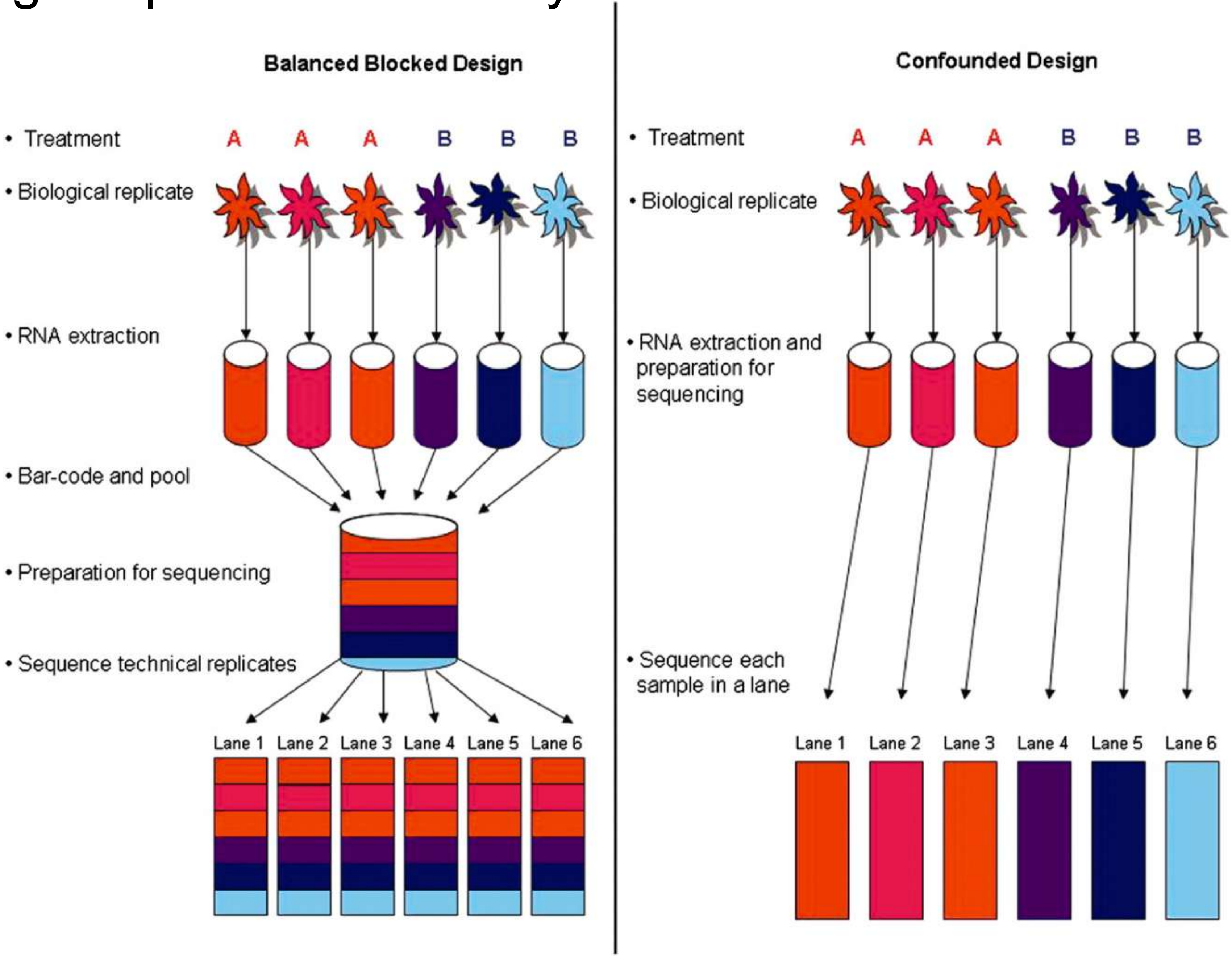
Prep and treatment

Sequencing of multiple samples can be performed using **multiplexing.**

The multiplexing add a tag/**barcode** of 4-6 nucleotides during the library preparation to identify the sample. Common kits can add up to 96 different tags.

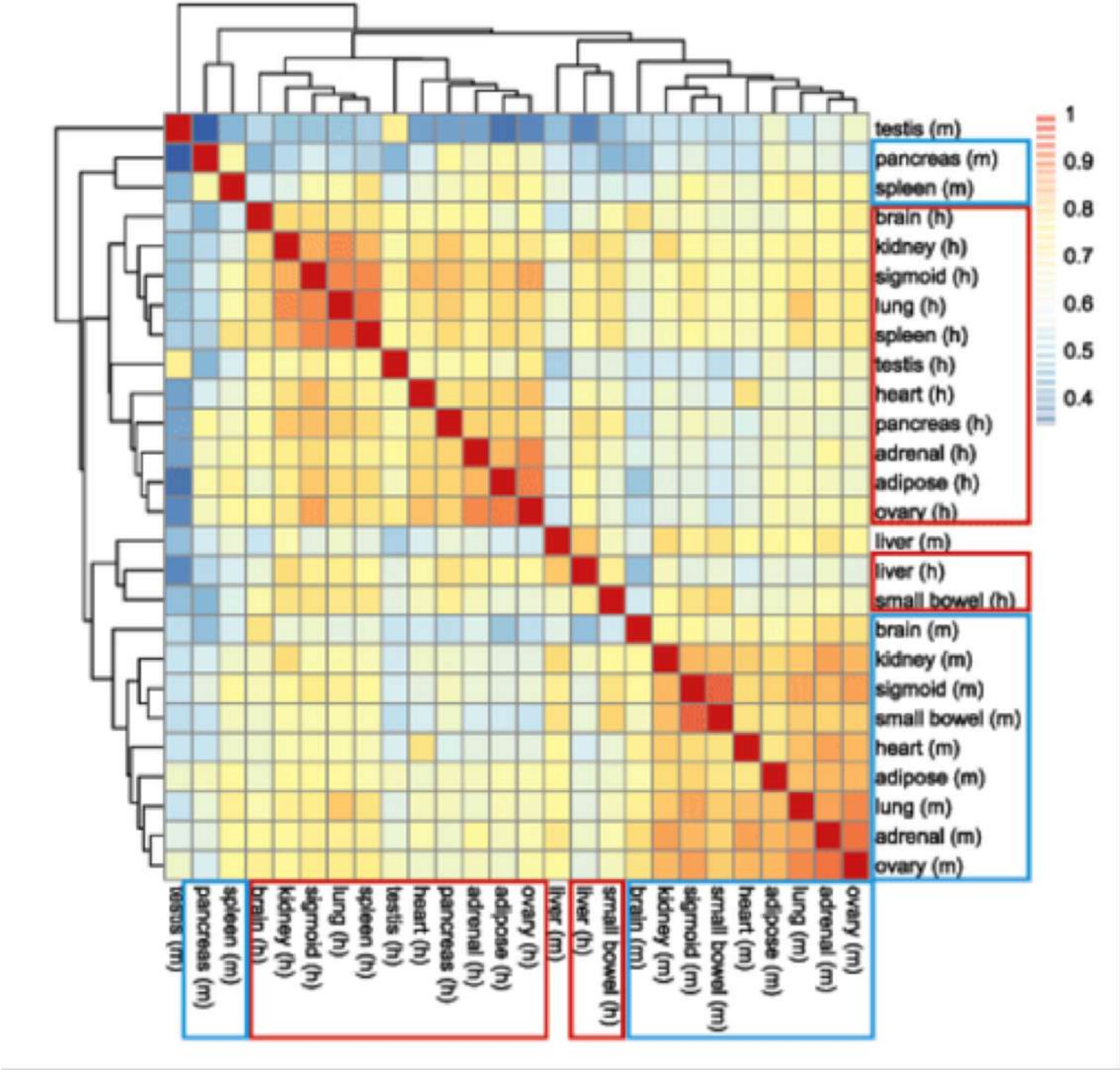


You need to design experiment carefully



Auer *et al.,* Genetics (2010)

Example of batch effect:



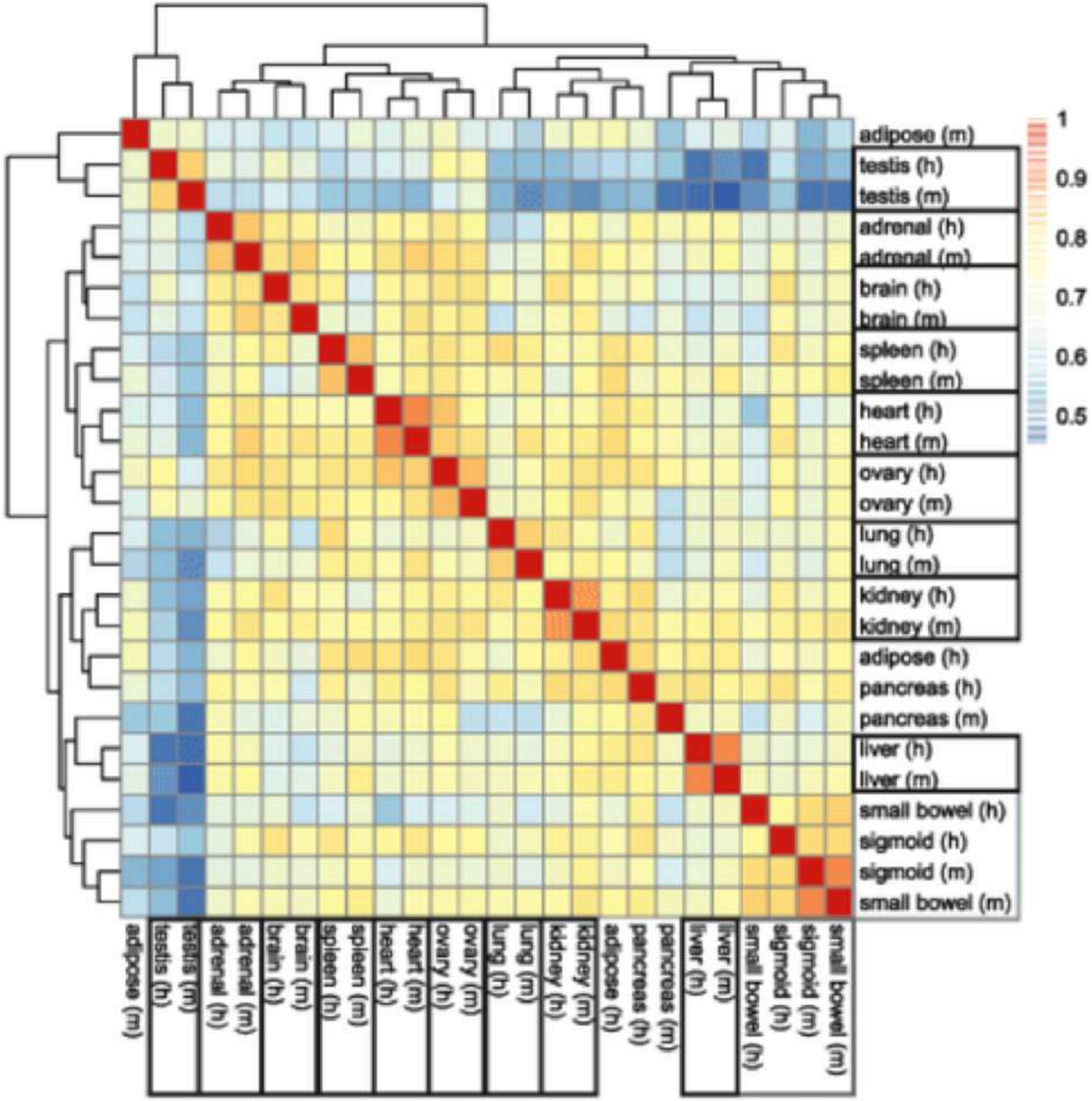
Lin *et al.,* (PNAS) 2014

Example of batch effect:

Yoav Gilad
@Y_Gilad

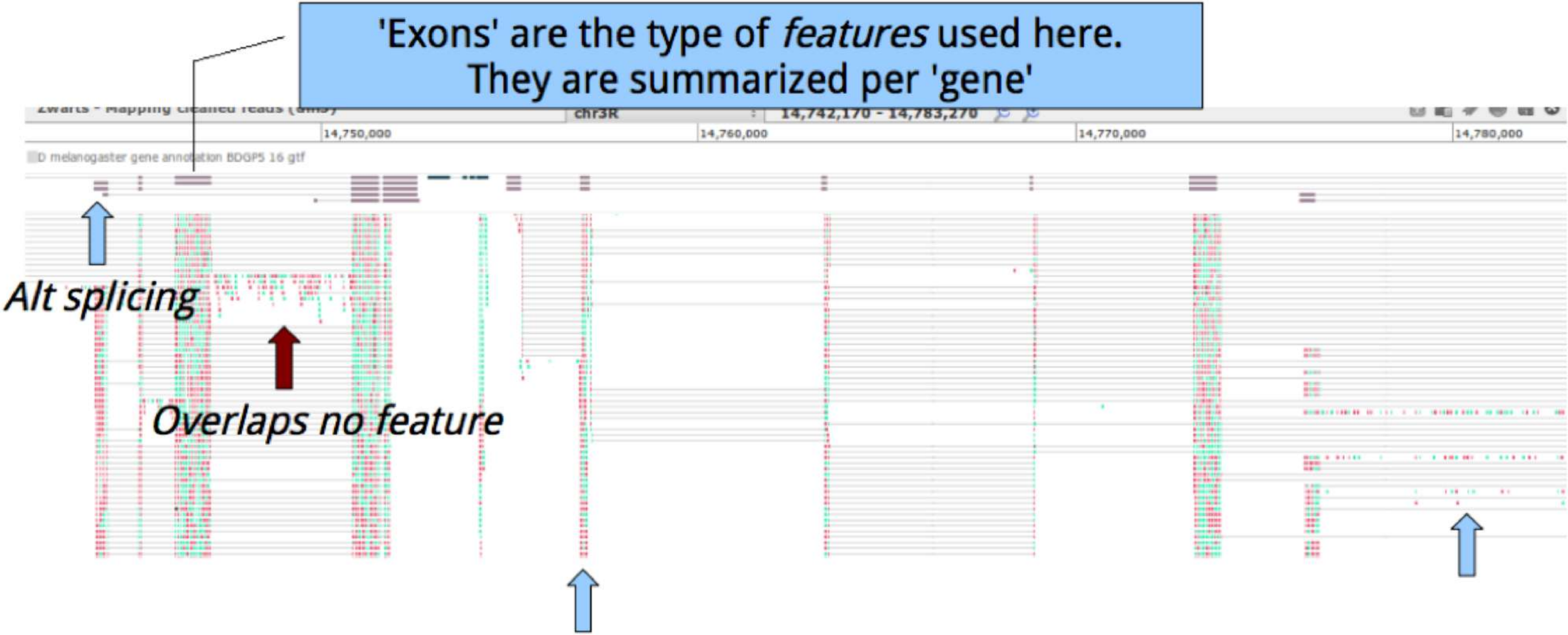
Following

We reanalyzed the data from pnas.org/content/111/48… and found the following:



http://f1000research.com/articles/4-121/v1

Once you have mappings, you can start counting



Concept:

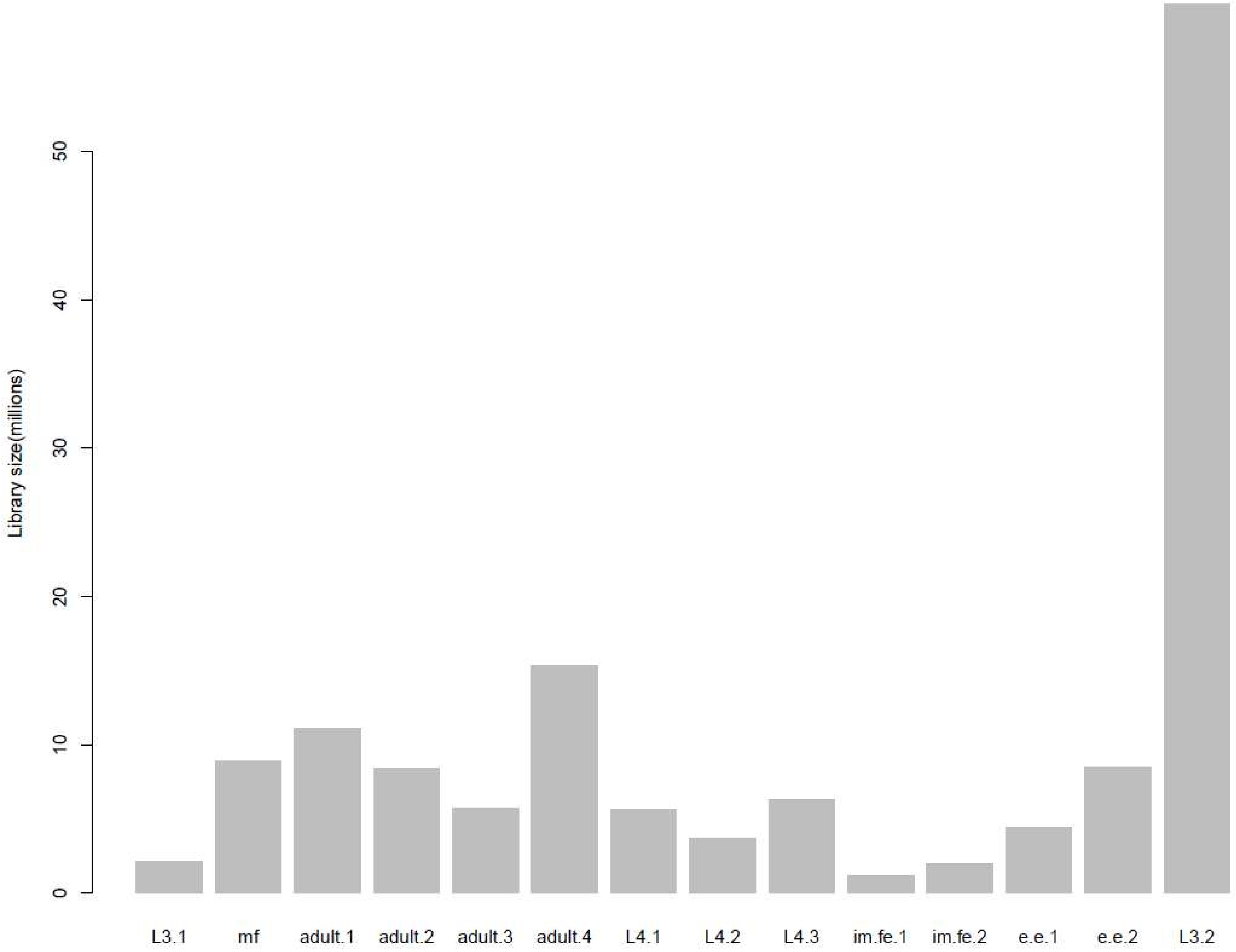
GeneA = exon 1 + exon 2 + exon 3 + exon 4 = 215 reads

GeneB = exon 1 + exon 2 + exon 3 = 180 reads

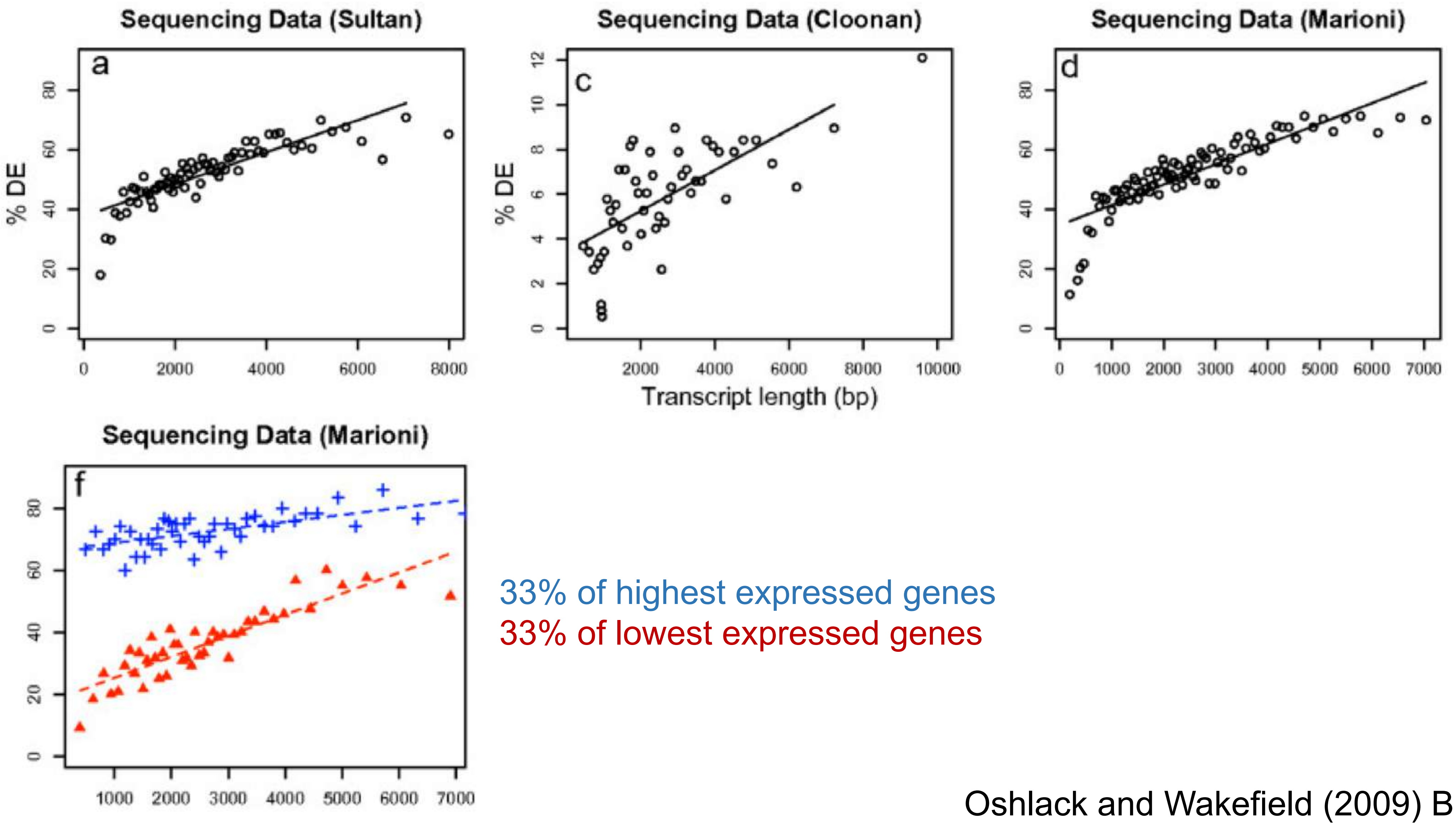
This is the bit we care about!

Counts of the gene depends on **expression** ,transcript length ,sequencing depth and simply chance

Higher sequencing depth equals more counts



Counts are proportional to the transcript length x mRNA expression level



33% of highest expressed genes

33% of lowest expressed genes

Oshlack and Wakefield (2009) Biology Direct.

Normalization: different goals

- **R/FPKM:** (Mortazavi et al. 2008)
  - Correct for: differences in sequencing depth and transcript length
  - Aiming to: compare a gene across samples and diff genes within sample

- **TMM**: (Robinson and Oshlack 2010)
  - Correct for: differences in transcript pool composition; extreme outliers
  - Aiming to: provide better across-sample comparability

- **TPM**: (Li et al 2010, Wagner et al 2012)
  - Correct for: transcript length distribution in RNA pool
  - Aiming to: provide better across-sample comparability

- **Limma voom (logCPM)**: (Lawet al 2013)
  - Aiming to: stabilize variance; remove dependence of variance on the mean

Optimal Scaling of Digital Transcriptomes

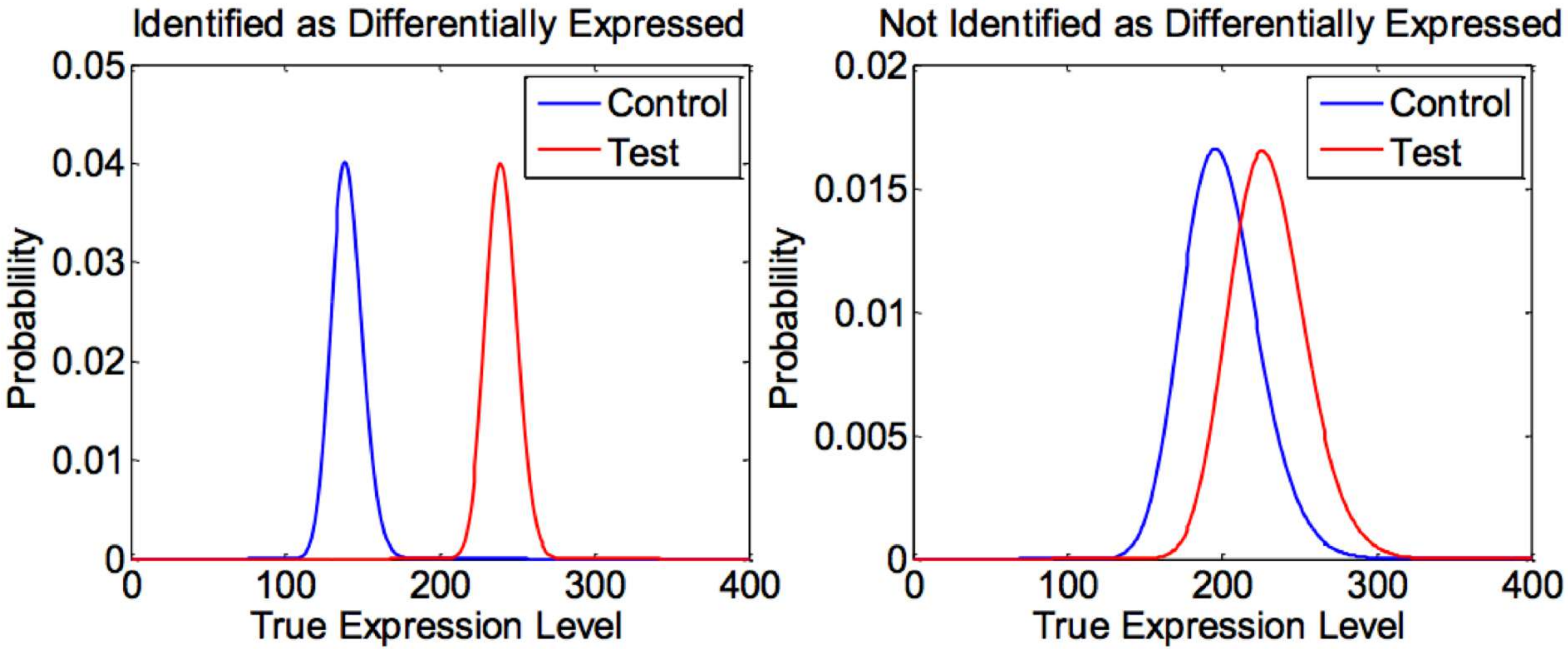
Gustavo Glusman, Juan Caballero, Max Robinson, Burak Kutlu, Leroy Hood

Published: Nov 06, 2013 • DOI: 10.1371/journal.pone.0077885

But how do you know your count = 2 is really 2?

- Differentially expressed genes = counts of genes change between conditions **more systematically** than expected by chance
- Need **biological and technical replicates** to detect differential expression

Fitting a distribution for every gene for **DE**

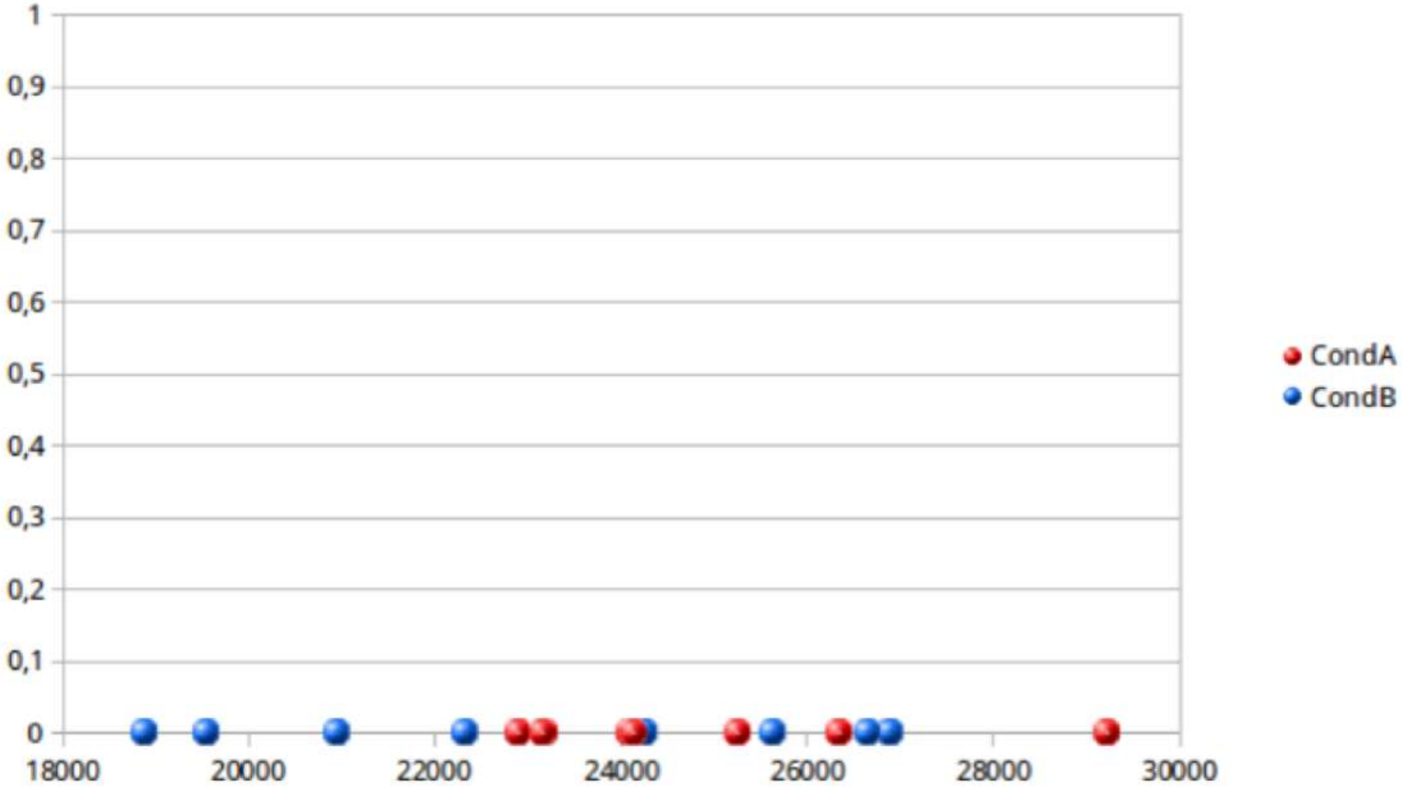


. Busby *et al.*, Bioinformatics (2013)

Scenario

gene_id CAF0006876

Condition A	sample1 23171	sample2 22903	sample3 29227	sample4 24072	sample5 23151	sample6 26336	sample7 25252	sample8 24122
Condition B	Sample9 19527	sample10 26898	sample11 18880	sample12 24237	sample13 26640	sample14 22315	sample15 20952	sample16 25629



JAKONIX
VIB

Scenario

gene_id CAF0006876

Condition A	sample1 23171	sample2 22903	sample3 29227	sample4 24072	sample5 23151	sample6 26336	sample7 25252	sample8 24122
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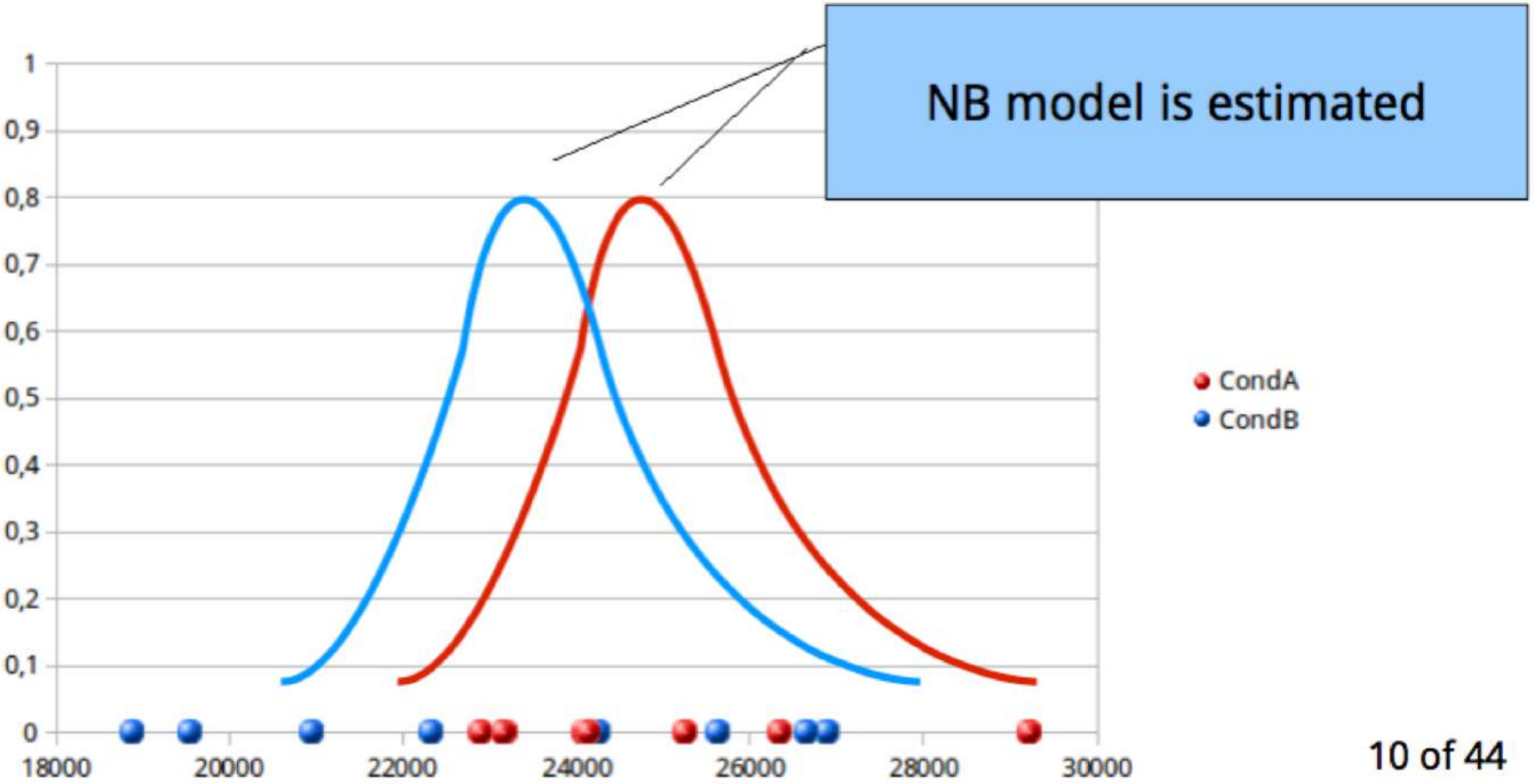

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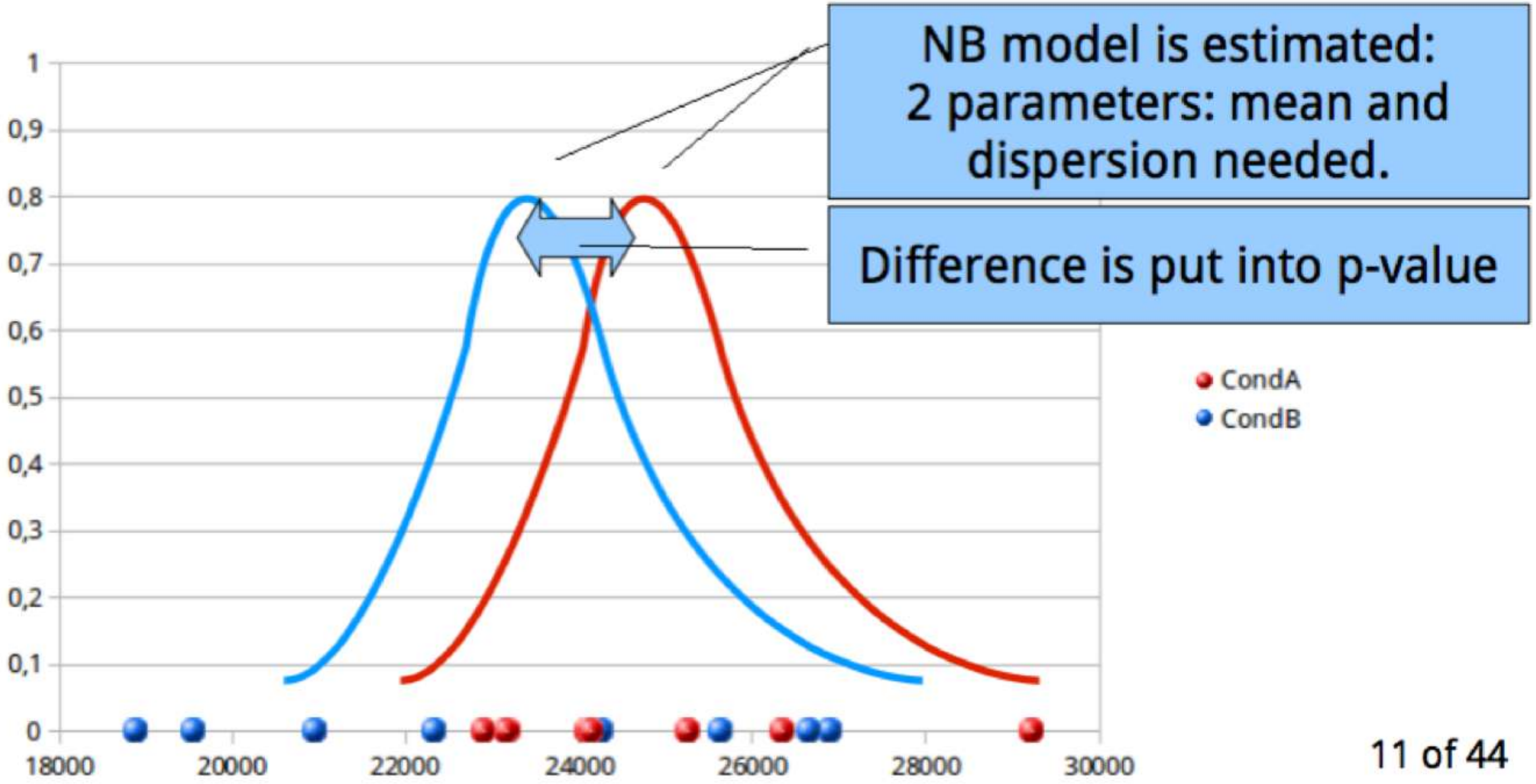
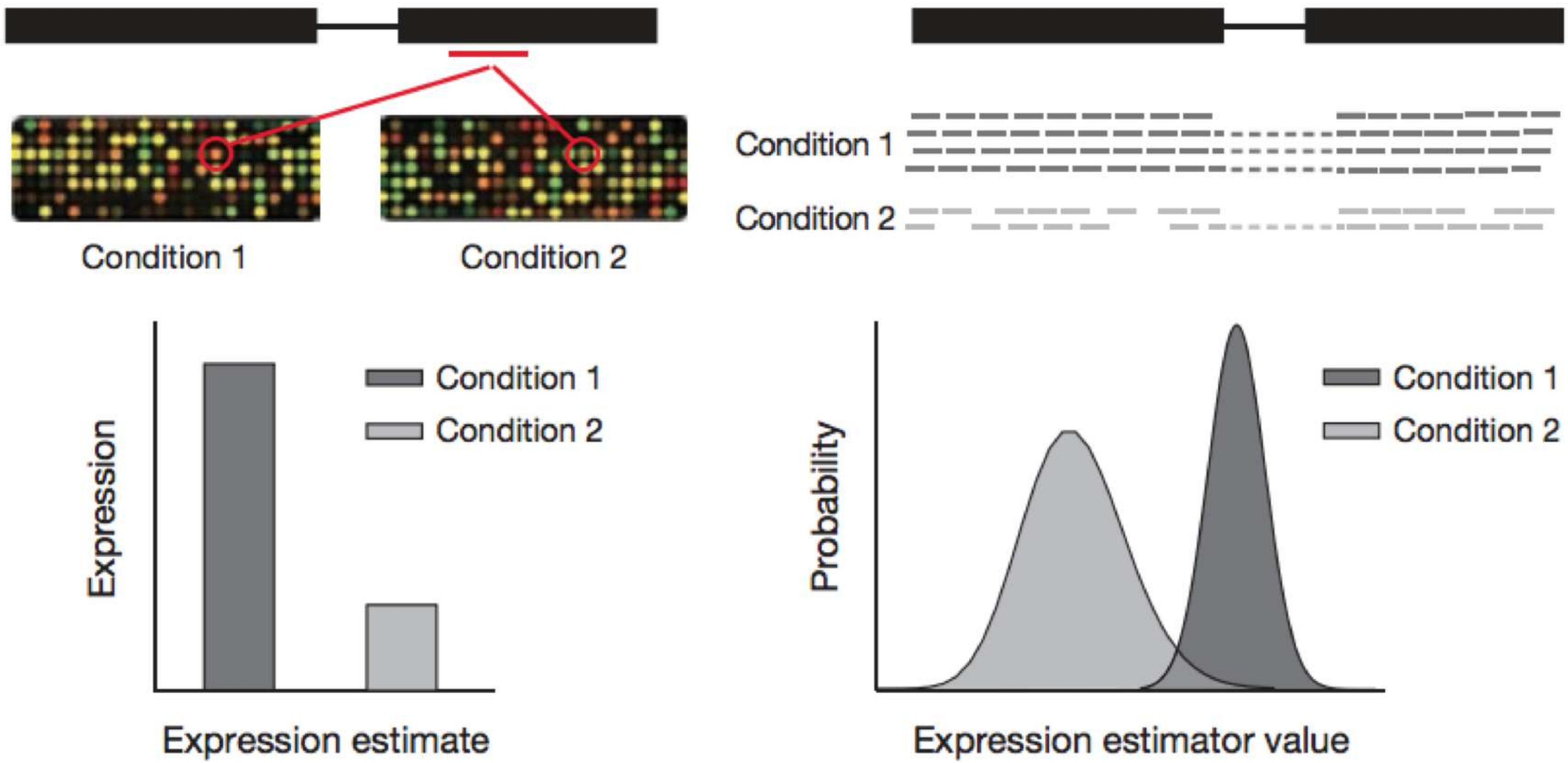
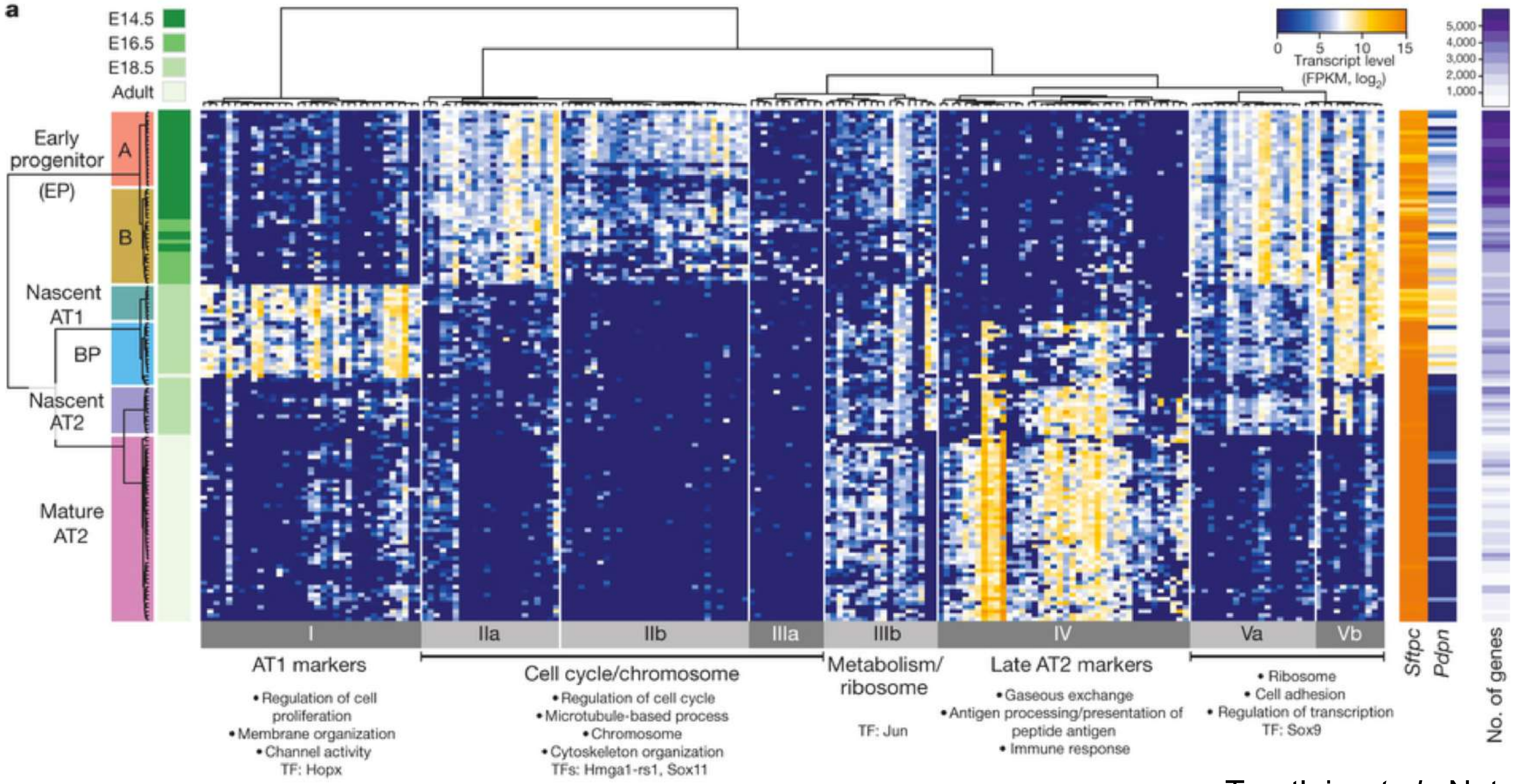


RNAseq vs Microarray



Once you have set of differentially expressed genes

Summarization visualizing the expression data through heatmap ; Classification using Gene Ontology terms and metabolic annotations



Treutlein *et al.*, Nature (2014)

Amplicon / Metagenomics: An Intro

TECHNIQUES, TECHNOLOGY & ANALYSIS

A clinician's guide to microbiome analysis

Marcus J. Claesson[1,2], Adam G. Clooney[1–3]* and Paul W. O'Toole[1,2]*

Abstract | Microbiome analysis involves determining the composition and function of a community of microorganisms in a particular location. For the gastroenterologist, this technology opens up a rapidly evolving set of challenges and opportunities for generating novel insights into the health of patients on the basis of microbiota characterizations from intestinal, hepatic or extraintestinal samples. Alterations in gut microbiota composition correlate with intestinal and extraintestinal disease and, although only a few mechanisms are known, the microbiota are still an attractive target for developing biomarkers for disease detection and management as well as potential therapeutic applications. In this Review, we summarize the major decision points confronting new entrants to the field or for those designing new projects in microbiome research. We provide recommendations based on current technology options and our experience of sequencing platform choices. We also offer perspectives on future applications of microbiome research, which we hope convey the promise of this technology for clinical applications.

https://www.nature.com/articles/nrgastro.2017.97.pdf

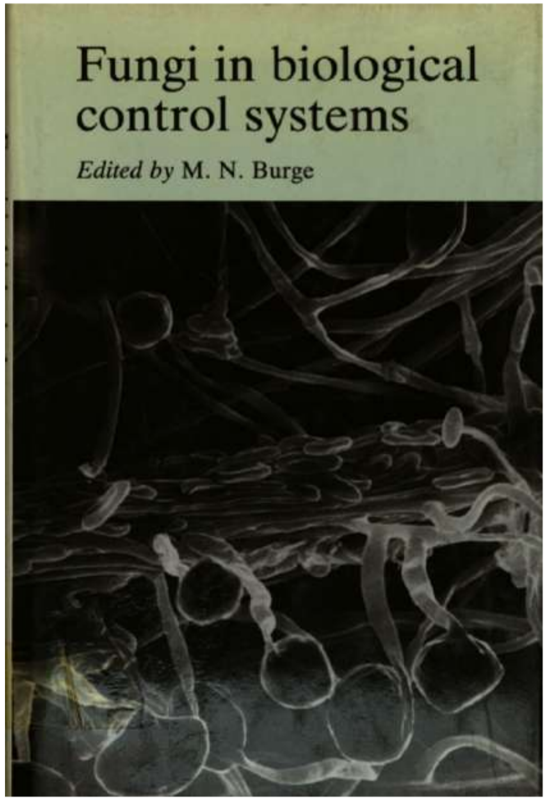
Key points

- Complex communities of microorganisms live on and in the human body, and variations in the composition and function of these communities are increasingly linked to various conditions and diseases
- Although it is not known if microbiome changes are causative or consequential in most pathophysiologies, they might provide biomarkers for disease detection or management
- Microbiome analysis is likely to become a routine component of secondary health care and is emerging as a modifiable environmental risk factor in multifactorial diseases that could be targeted by novel therapeutics
- Technology advancements are leading to a range of powerful methods for microbiome analysis becoming available and affordable for clinical studies
- Judicious choice of sample type and sequencing platform are required to maximize the clinical utility of microbiome data

https://www.nature.com/articles/nrgastro.2017.97.pdf

What is the microbiome?

Fungi in Biological Control Systems (1988)



A convenient ecological framework in which to examine biocontrol systems is that of the microbiome. This may be defined as a characteristic microbial community occupying a reasonably well defined habitat which has distinct physico-chemical properties. The term thus not only refers to the microorganisms involved but also encompasses their theatres of activity. In relation to fungal diseases of crops and their control, major microbiomes are the phylloplane, spermosphere, rhizosphere and rhizoplane, and numerous kinds of plant residues persisting on or in the soil. Mention should also be made of the wood of standing or felled trees as microbiomes where biocontrol of forest diseases using fungi has been achieved. However, in most cases competitive interactions other than mycoparasitism seem to be of greater importance.

http://microbe.net/2015/04/08/what-does-the-term-microbiome-mean-and-where-did-it-come-from-a-bit-of-a-surprise/

And then what is the metagenome?

Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products

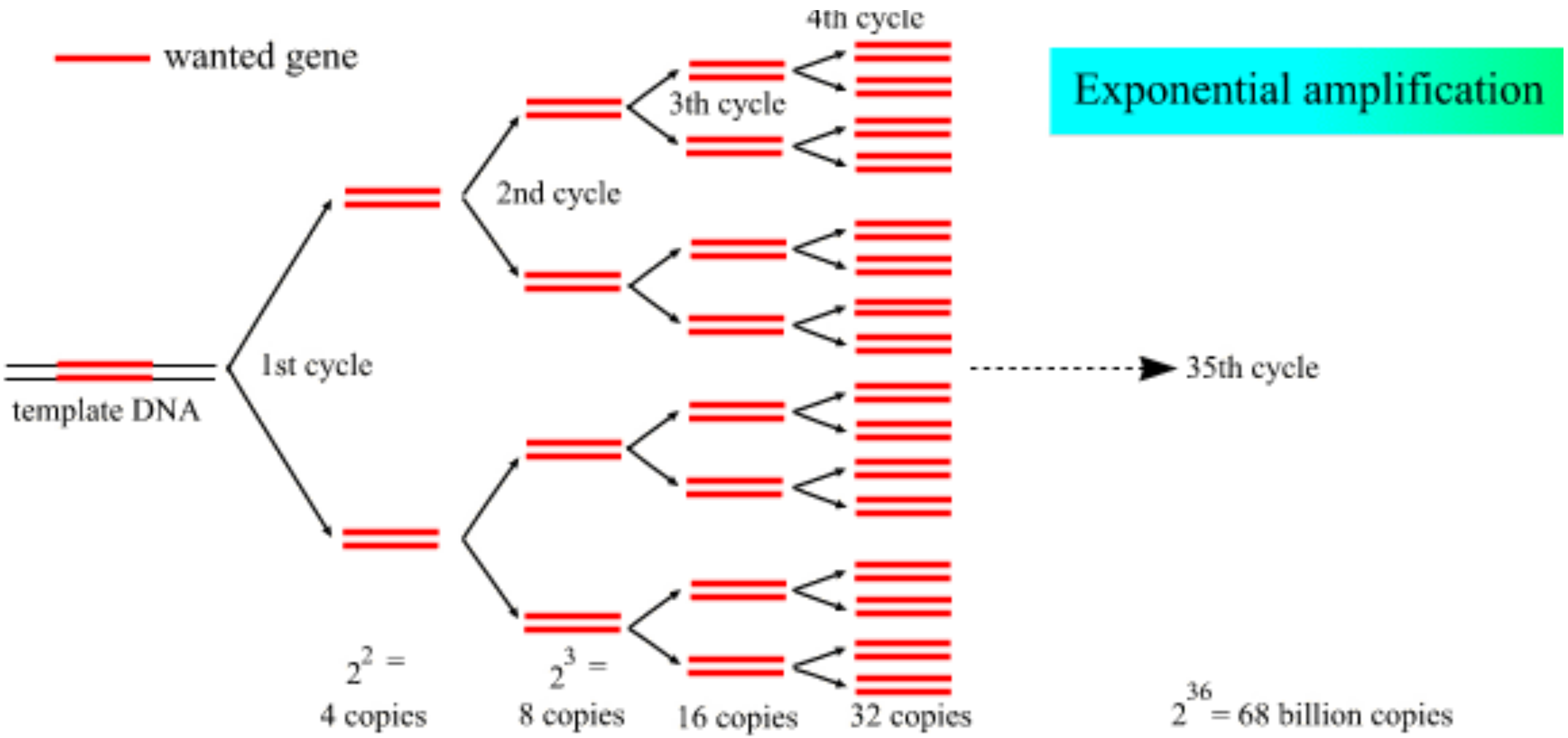
Jo Handelsman[1], Michelle R Rondon[1], Sean F Brady[2], Jon Clardy[2] and Robert M Goodman[1]

Chemistry & Biology October 1998, **5**:R245–249
http://biomednet.com/elecref/10745521005R0245

… This approach involves directly accessing the genomes of soil organisms that cannot be, or have not been, cultured by isolating their DNA

What is amplicon sequencing?

Anything that requires PCR-based amplification of a specific target gene (locus)



And then what is the metagenome?

OPEN ACCESS Freely available online

PLoS COMPUTATIONAL BIOLOGY

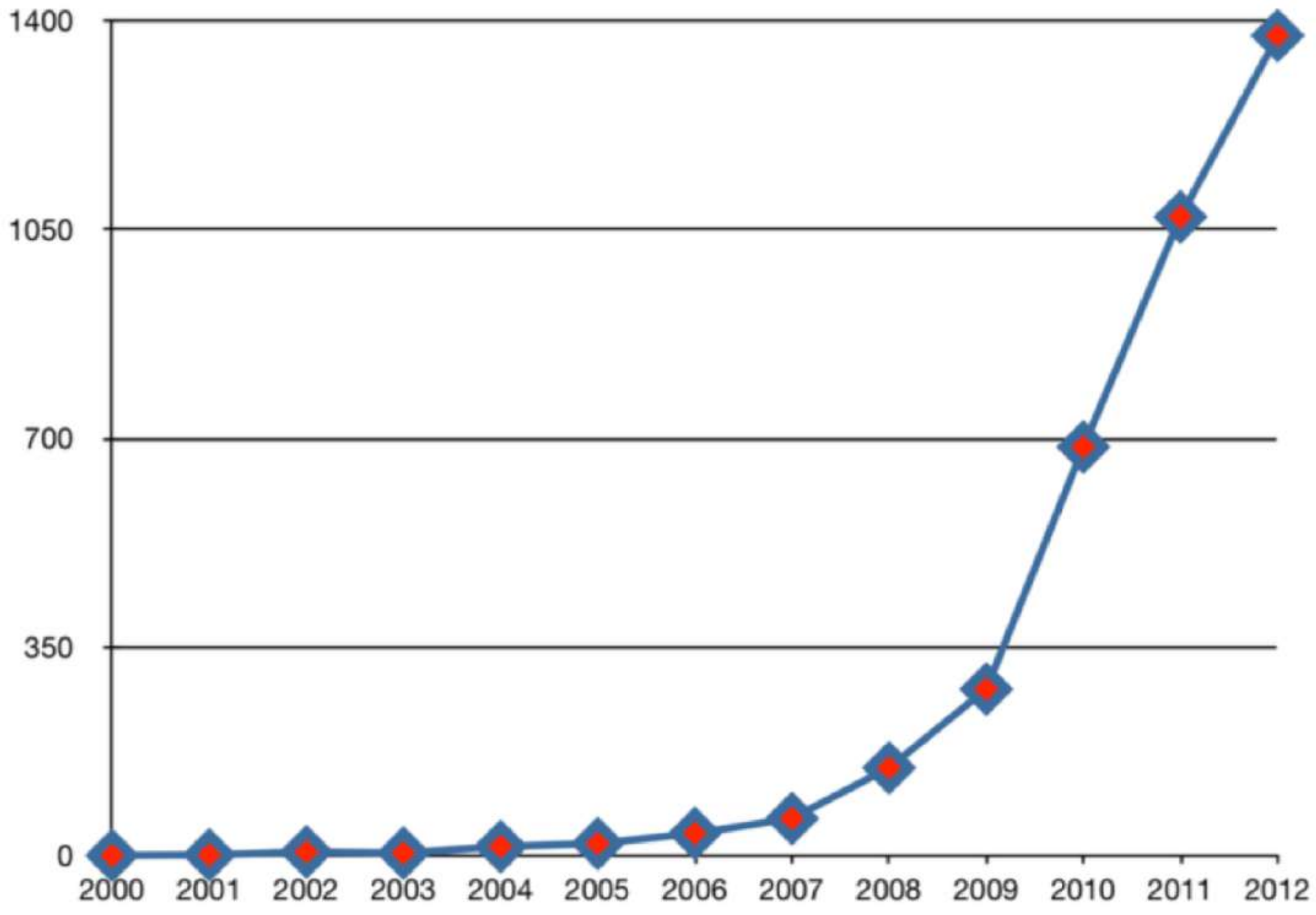
Review

Bioinformatics for Whole-Genome Shotgun Sequencing of Microbial Communities

Kevin Chen*, Lior Pachter*

Metagenomics is the application of modern genomics techniques to the study of communities of microbial organisms directly in their natural environments, bypassing the need for isolation and lab cultivation of individual species. The field has its roots in the culture-independent retrieval of **16S rRNA** genes, pioneered by Pace and colleagues two decades ago.

Pubmed hits for “Microbiome”



Jonathan Eisen, Slideshare

Metagenomics $\neq$ Amplicon sequencing

Metagenomics is undergoing a crisis

Please don’t make things worse ☺

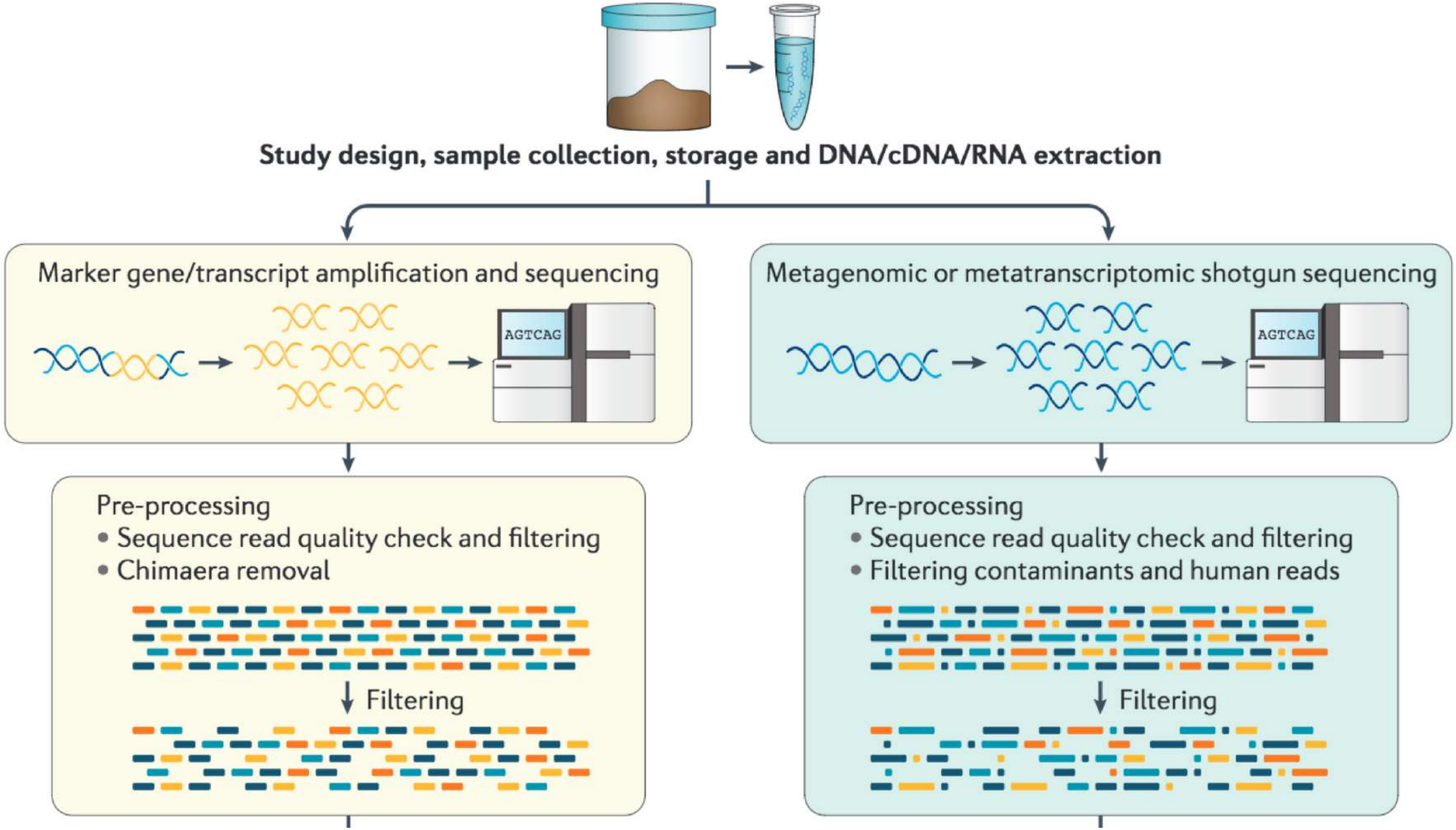
- Crisis 1
  - **The correlation/causation fallacy**. For example….
  - Patients with type II diabetes have a different gut microbiome compared to healthy patients
  - Does the microbiome cause diabetes?
  - Or do they have a different microbiome because they have diabetes? (therefore different diet)
- Crisis 2
  - A lot of people want to do it, but don’t know how
  - Errors, bad experimental design, incorrect conclusions

Mick Watson, Slideshare

Basic Purpose

Characteristics of (microbial) community

Who are they?
Where do they come from?
Are their similarities (at what level)
between communities
of different conditions
of similar conditions?
within a community?
What are they doing?
How are they doing?



Study design, sample collection, storage and DNA/cDNA/RNA extraction
Marker gene/transcript amplification and sequencing
AGTCAG
Metagenomic or metatranscriptomic shotgun sequencing
AGTCAG
Pre-processing
• Sequence read quality check and filtering
• Chimaera removal
Filtering
Pre-processing
• Sequence read quality check and filtering
• Filtering contaminants and human reads
Filtering

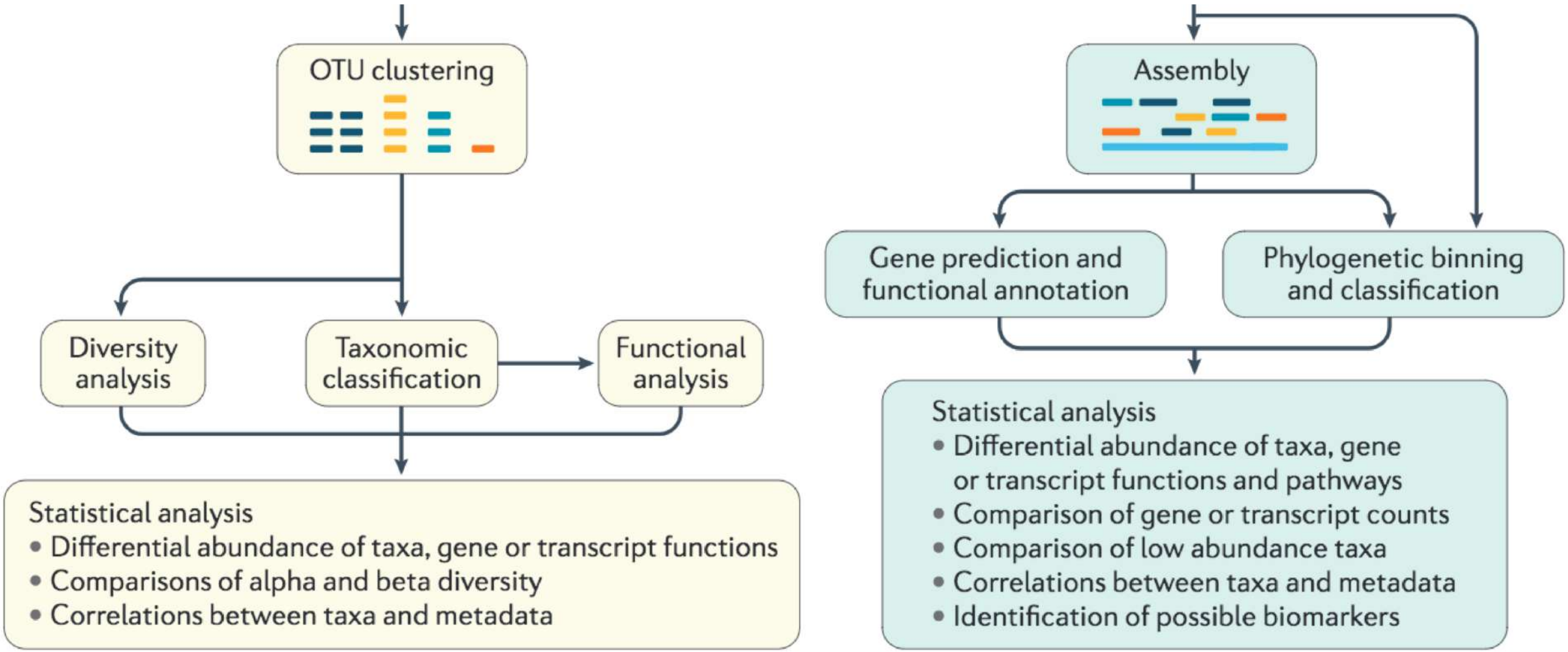


Figure 1 | **Flowchart of the major steps involved in bioinformatic analysis of the microbiome.** The analysis is divided into two sections depending on the type of sequencing. This schematic describes the basic steps and might vary depending on the aim of the analysis. OTU, operational taxonomic unit.

Applications

What have metagenomics been used for?

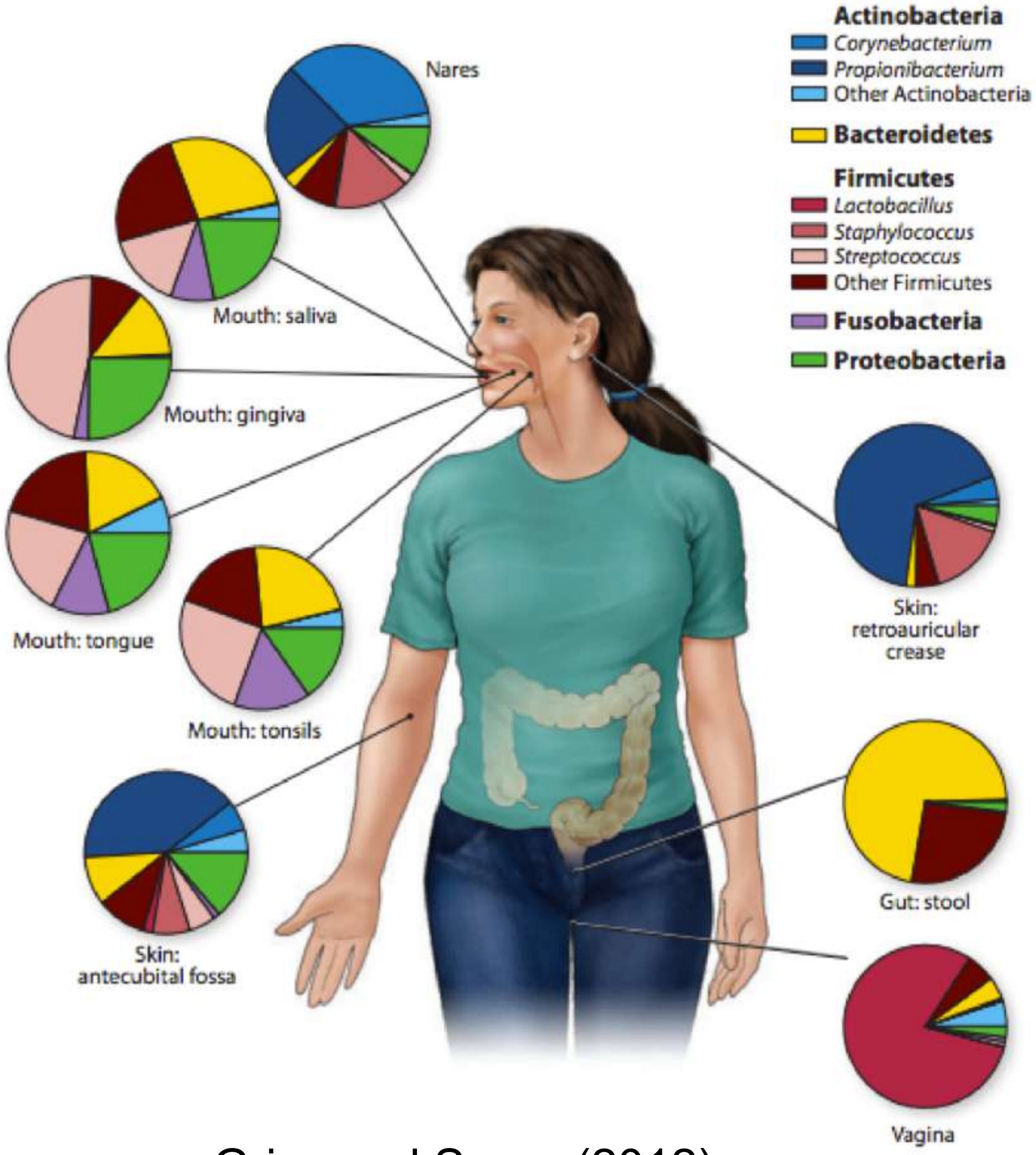
Exploration and categorisation

Rusch *et al*., 2007 **Plos Biology**

- 6.3 Gbp of sequence (2x Human genomes, 2000 x Bacterial genomes)
- Most sequences were novel compared to the databases

Qin *et al*., 2010 **Nature**

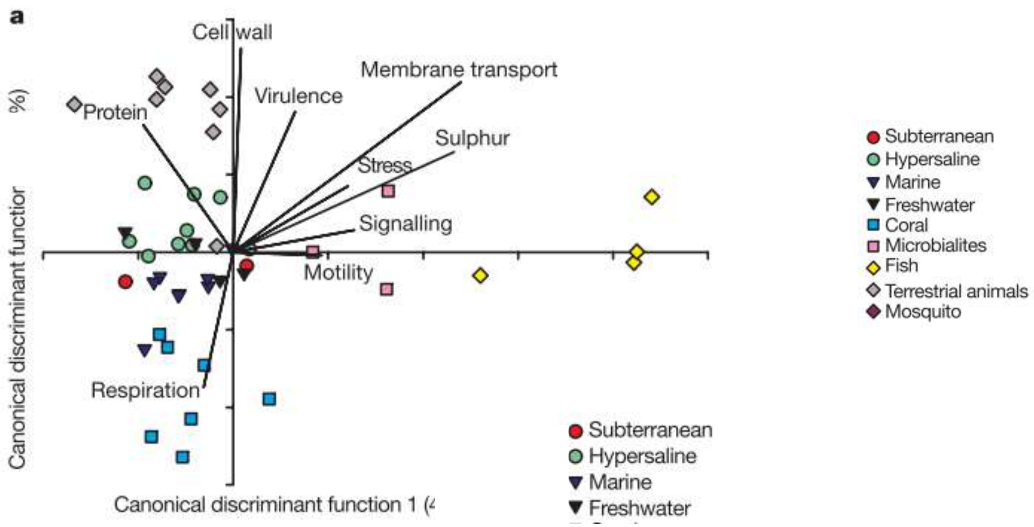
- 127 Human gut metagenomes
- 600 Gbp sequence (200 x Human genomes)
- 3.3 million genes identified
- Minimal gut metagenome definded



Grice and Segre (2012)

What have metagenomics been used for?

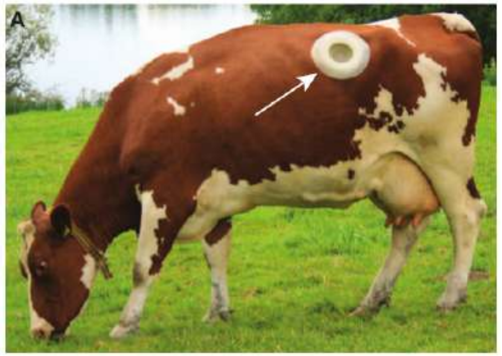
Comparative



Dinsdale *et al*., 2008 **Nature**

- A characteristic microbial fingerprint for each of the nine different ecosystem types

Specific functions

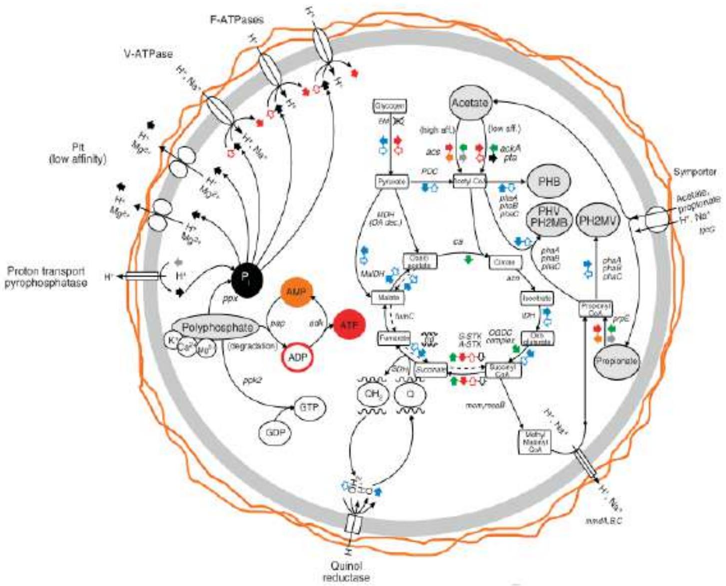


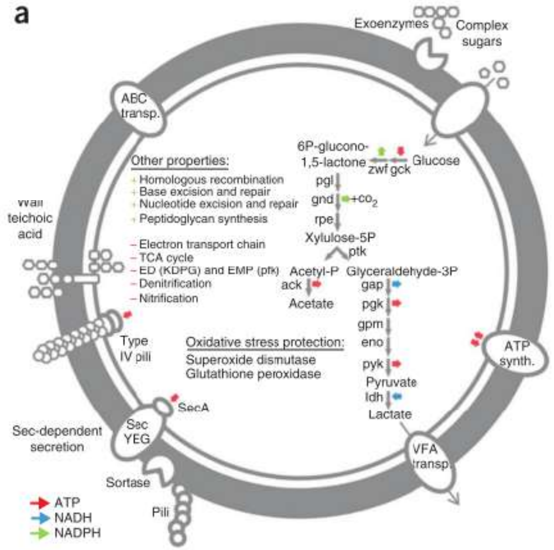
Hess *et al*., 2011 **Science**

- Identified 27.755 putative carbohydrate-active genes from a cow rumen metagenome
- Expressed 90 candidates of which 57% had enzymatic activity against cellulosic substrates

What have metagenomics been used for?

Extracting genomes





Garcia Martin *et al*., 2006 **Nat. Biotechnol.**

- Genome extraction from low complexity metagenome
- *Candidatus* Accumulibacter phosphatis
- The first genome of a polyphosphate accumulating organism (PAO) with a major role en enhanced biological phosphorus removal

Albertsen *et al*., 2013 **Nat. Biotechnol.**

- Genome extraction of low abundant species (< 0.1%) from metagenomes
- First complete TM7 genome
- Access to genomes of the ”uncultured majority”

Concept: OTU (Operational Taxonomic Unit)

OTU for Ecology

Operational Taxonomic Unit: a grouping of similar sequences that can be treated as a single “species”

Strengths

- Conceptually simple
- Mask effect of poor quality data
    - Sequencing error
    - in vitro recombination

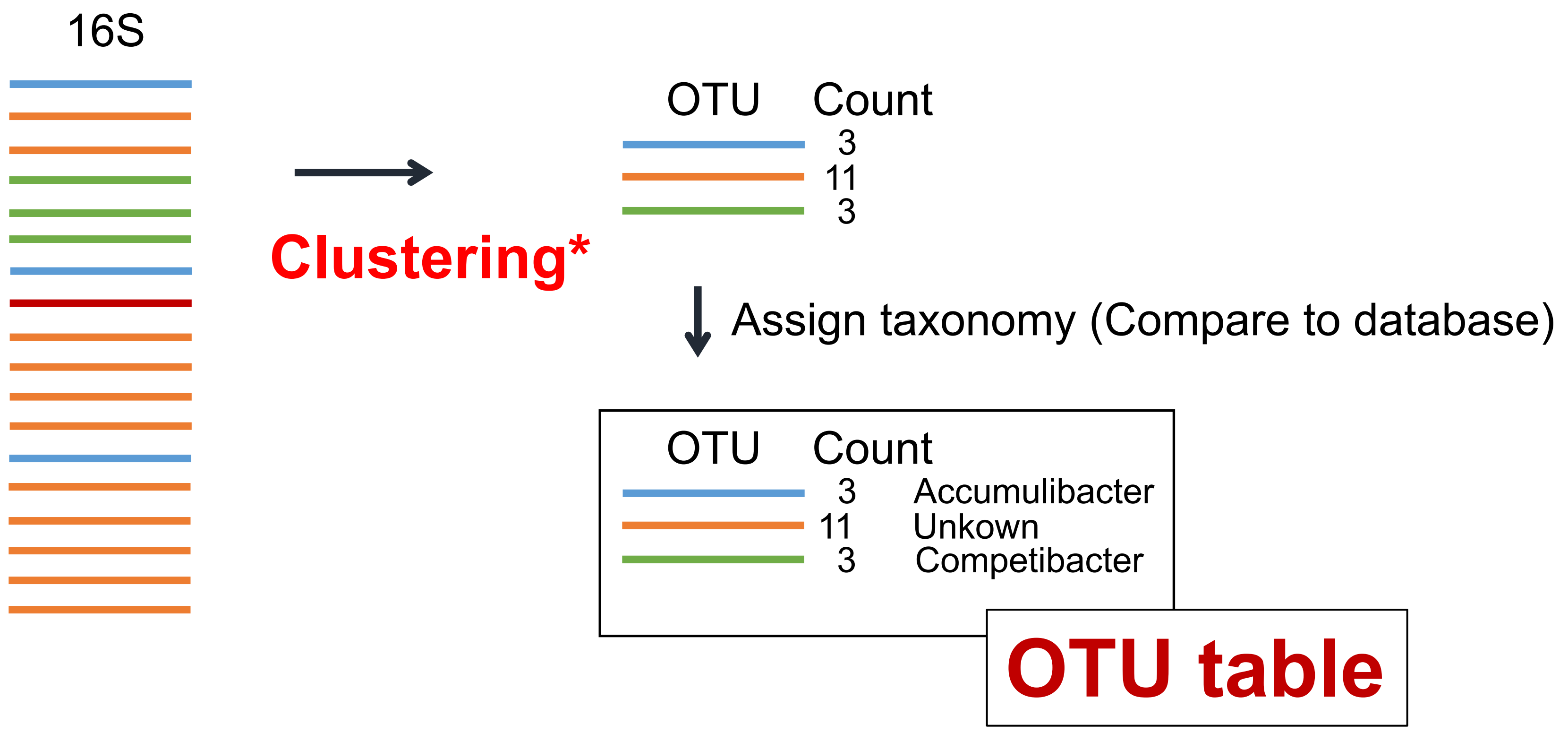
Weaknesses

- Limited resolution
- Logically inconsistent definition

Slide of Aaron Darlin

Assign OTU

- Cluster by their similarity to other sequences in the sample (operations taxonomic units → OTU)
- 95% genus level, **97% species level**, 99% strain level



OTU “picking”

The process of bin sequences into clusters of OTUs.

De Novo

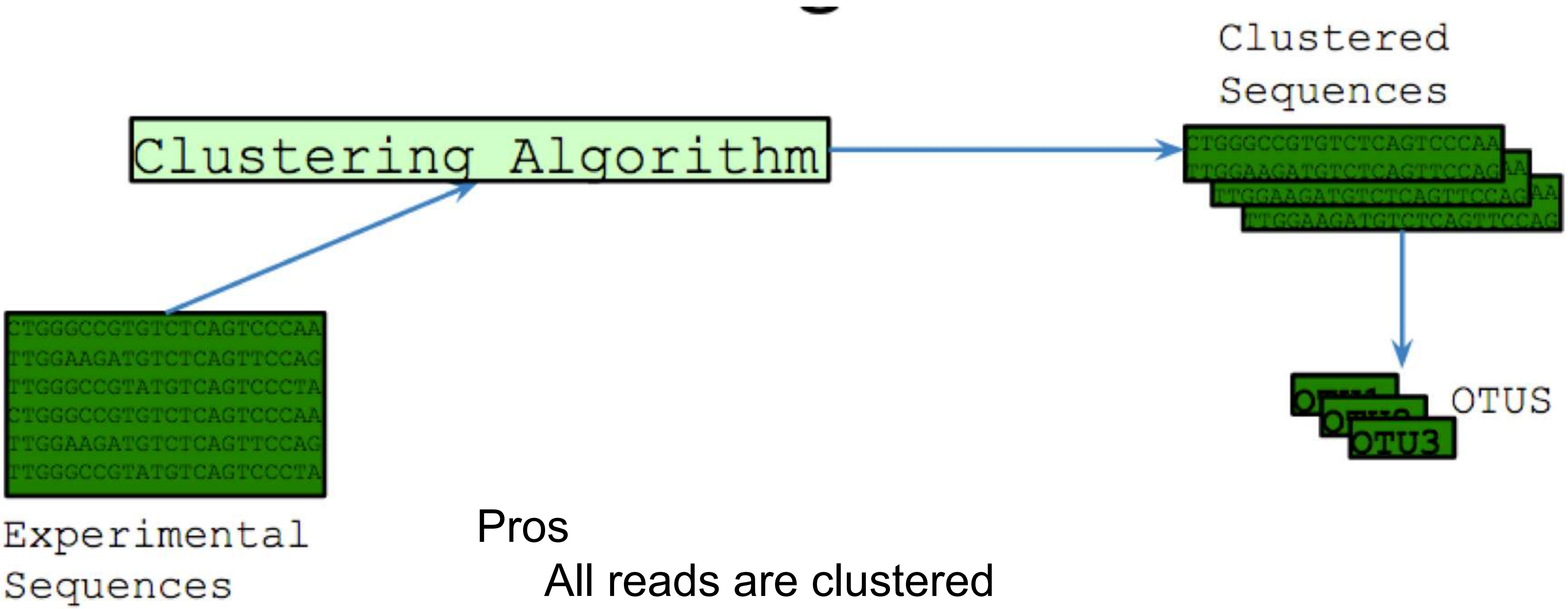
- Reads are clustered based on similarity to one another.

Reference-based

- Closed reference: any reads which don’t hit a reference sequence are discarded
- Open reference: any reads which don’t hit a reference sequence are clustered de novo

http://qiime.org/tutorials/otu_picking.html

De novo OTU picking



Pros

    All reads are clustered

Cons

    Not parallelizable

    OTUs may be defined by erroneous reads

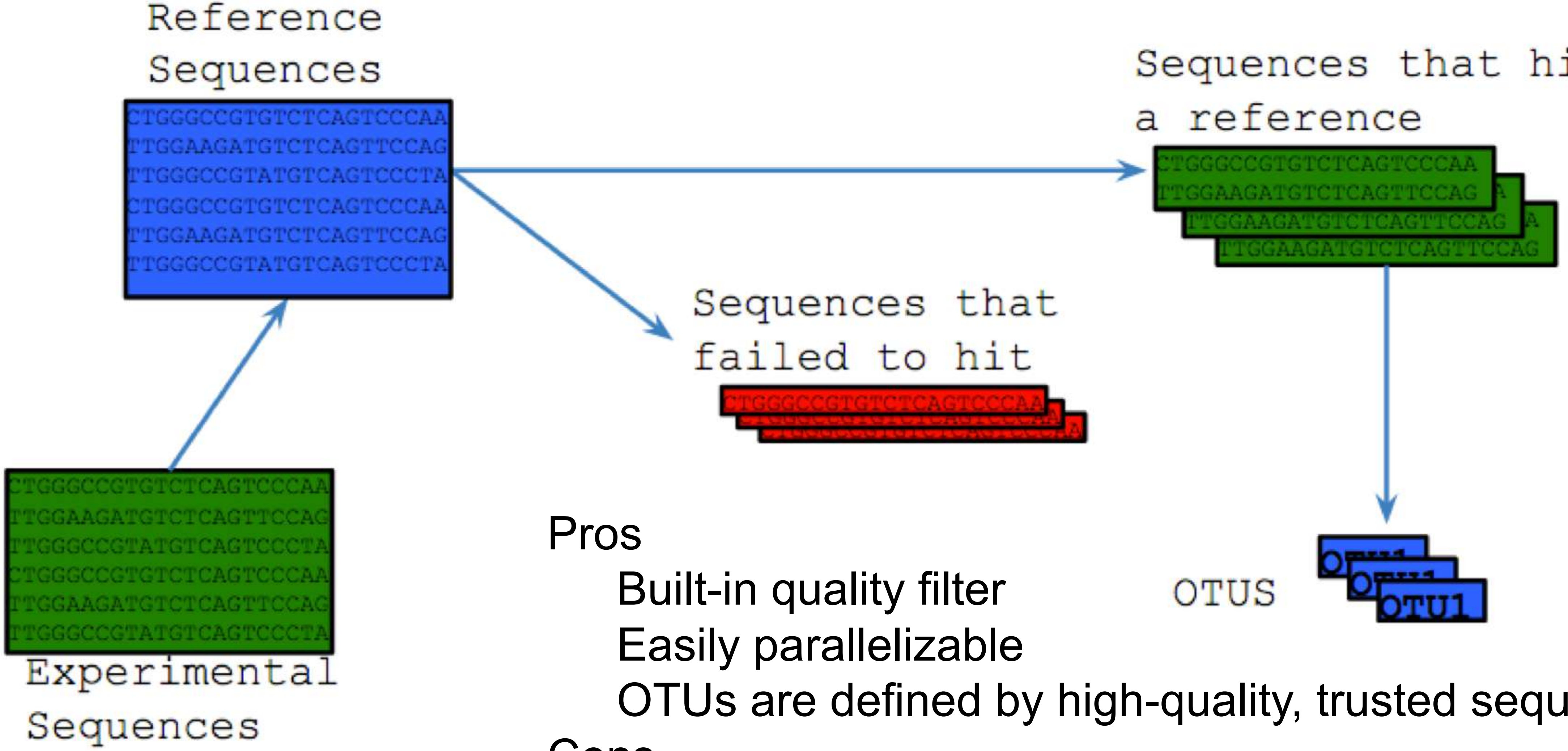
Slide of John Chase

De novo OTU picking

- You **must** use if:
  - You do not have a reference sequence collection to cluster against, for example because you're working with an infrequently used marker gene.
- You **cannot** use if:
  - You are comparing non-overlapping amplicons, such as the V2 and the V4 regions of the 16S rRNA.
  - You working with very large data sets, like a full HiSeq 2000 run. (Technically you can, but it will be *really* slow.)

pick_de_novo_otus.py
http://qiime.org/tutorials/tutorial.html

Closed-reference OTU picking



Pros
- Built-in quality filter
- Easily parallelizable
- OTUs are defined by high-quality, trusted sequences

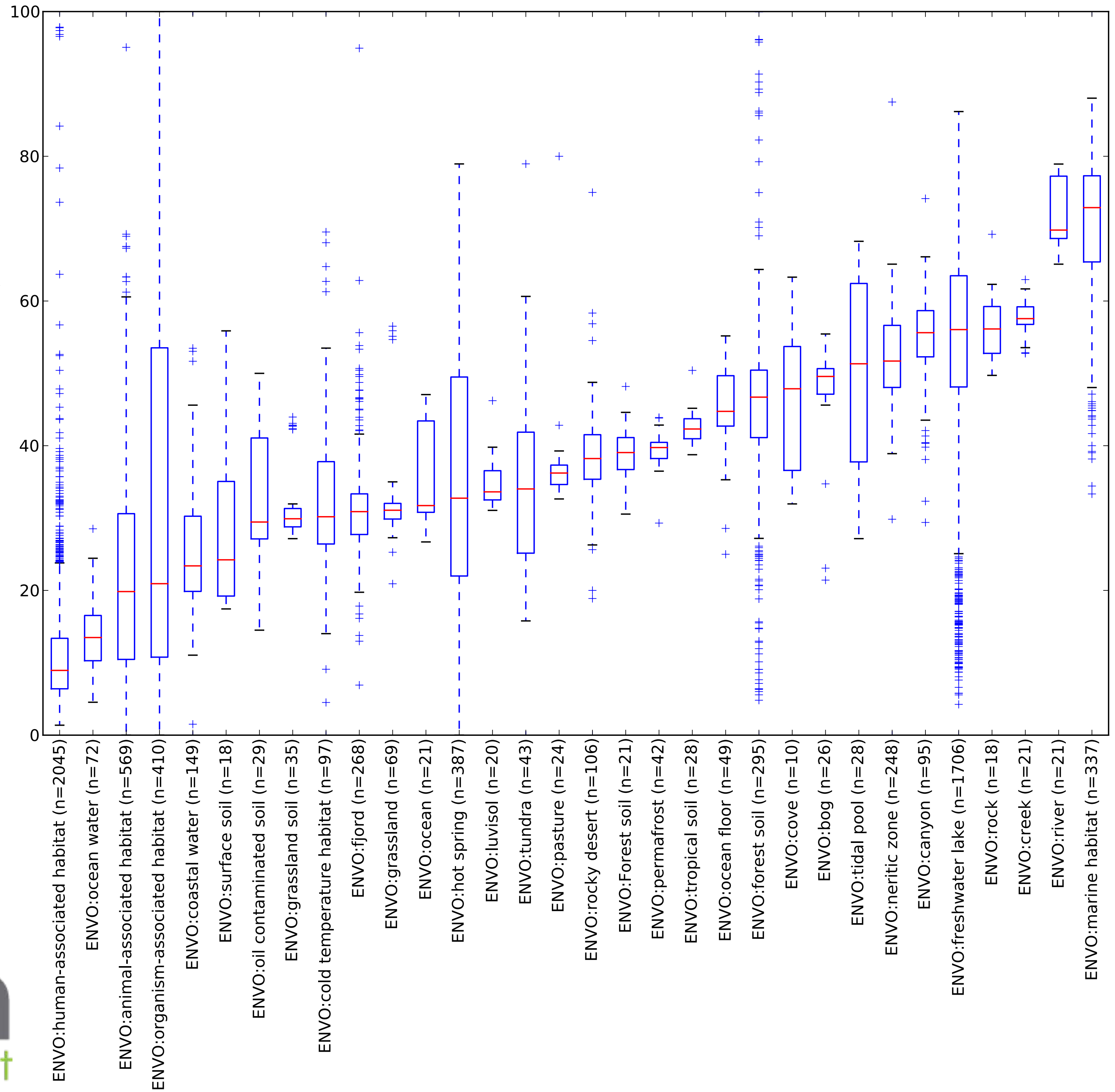
Cons
- Reads that don’t hit reference dataset are excluded, so you can never observe new OTUs

Slide of John Chase

Closed-reference OTU picking

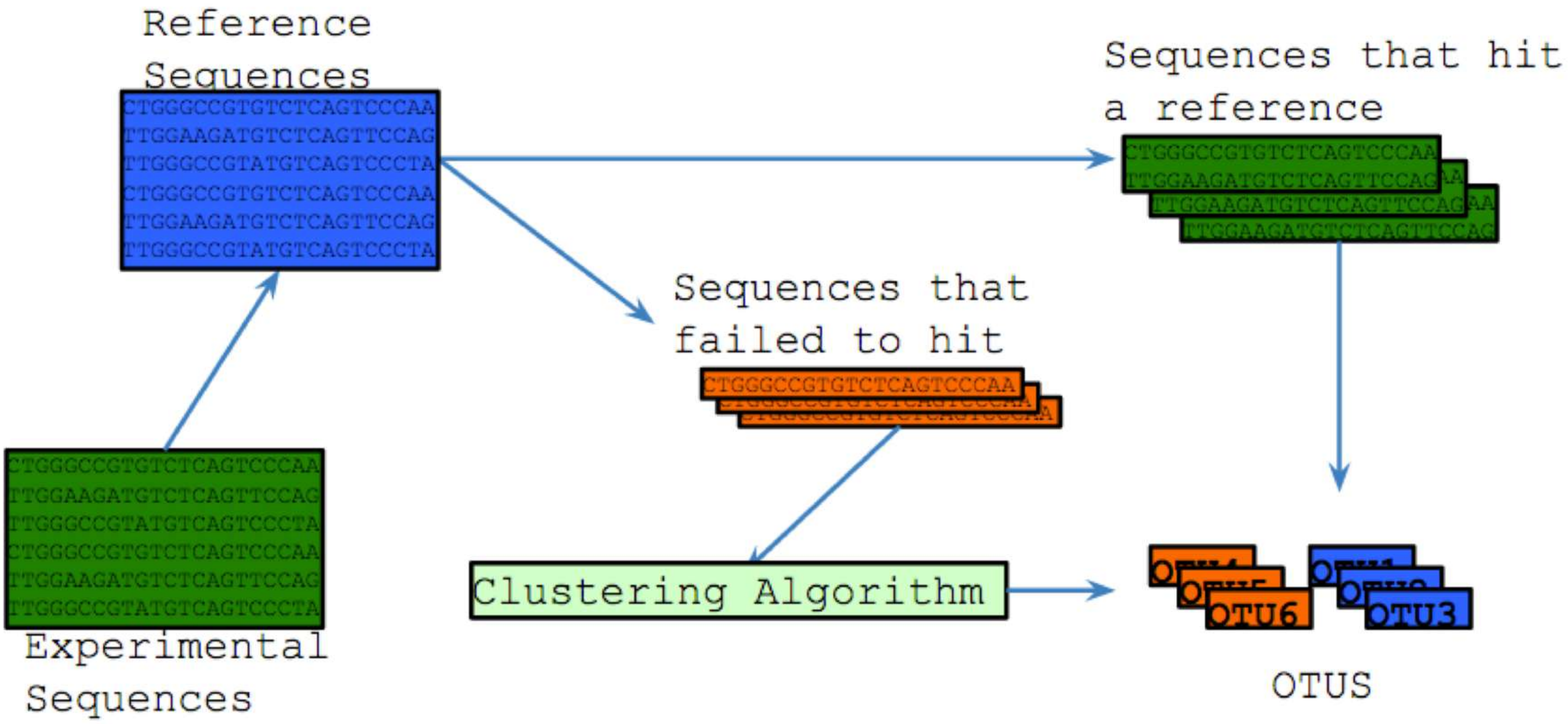
- You **must** use if:
  - You are comparing non-overlapping amplicons, such as the V2 and the V4 regions of the 16S rRNA. Your reference sequences must span both of the regions being sequenced.
- You **cannot** use if:
  - You do not have a reference sequence collection to cluster against, for example because you're working with an infrequently used marker gene.

Percentage of reads that do not hit the reference collection, by environment type.



earth microbiomeproject

Open-reference OTU picking

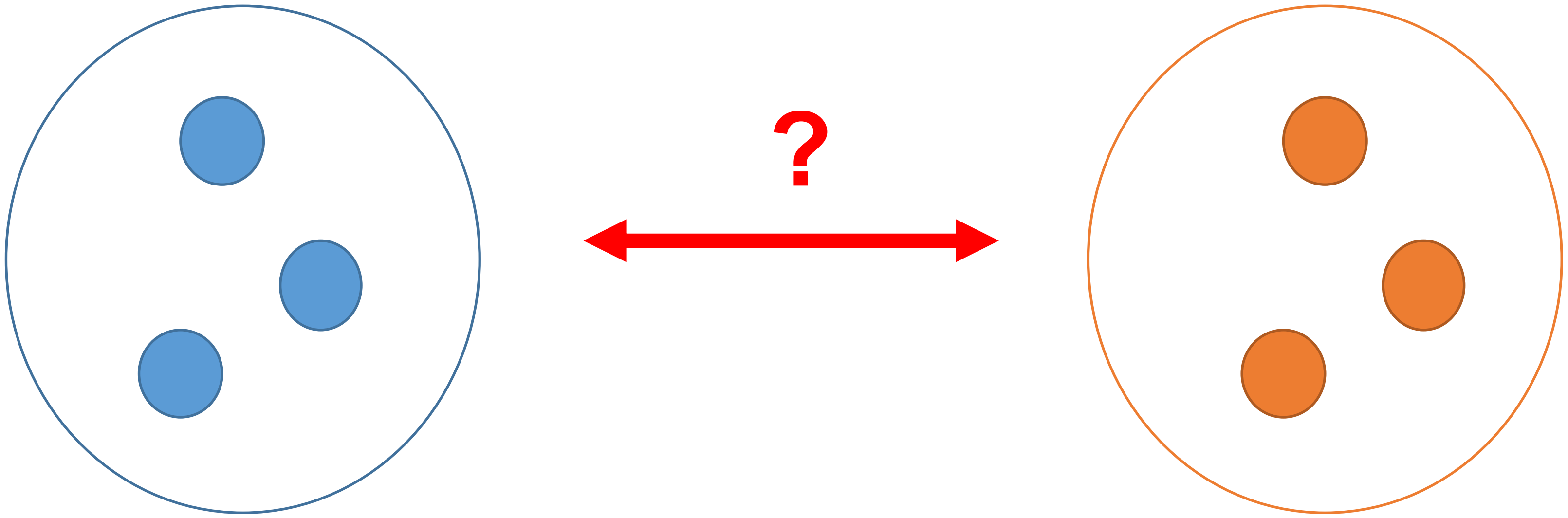


Slide of John Chase

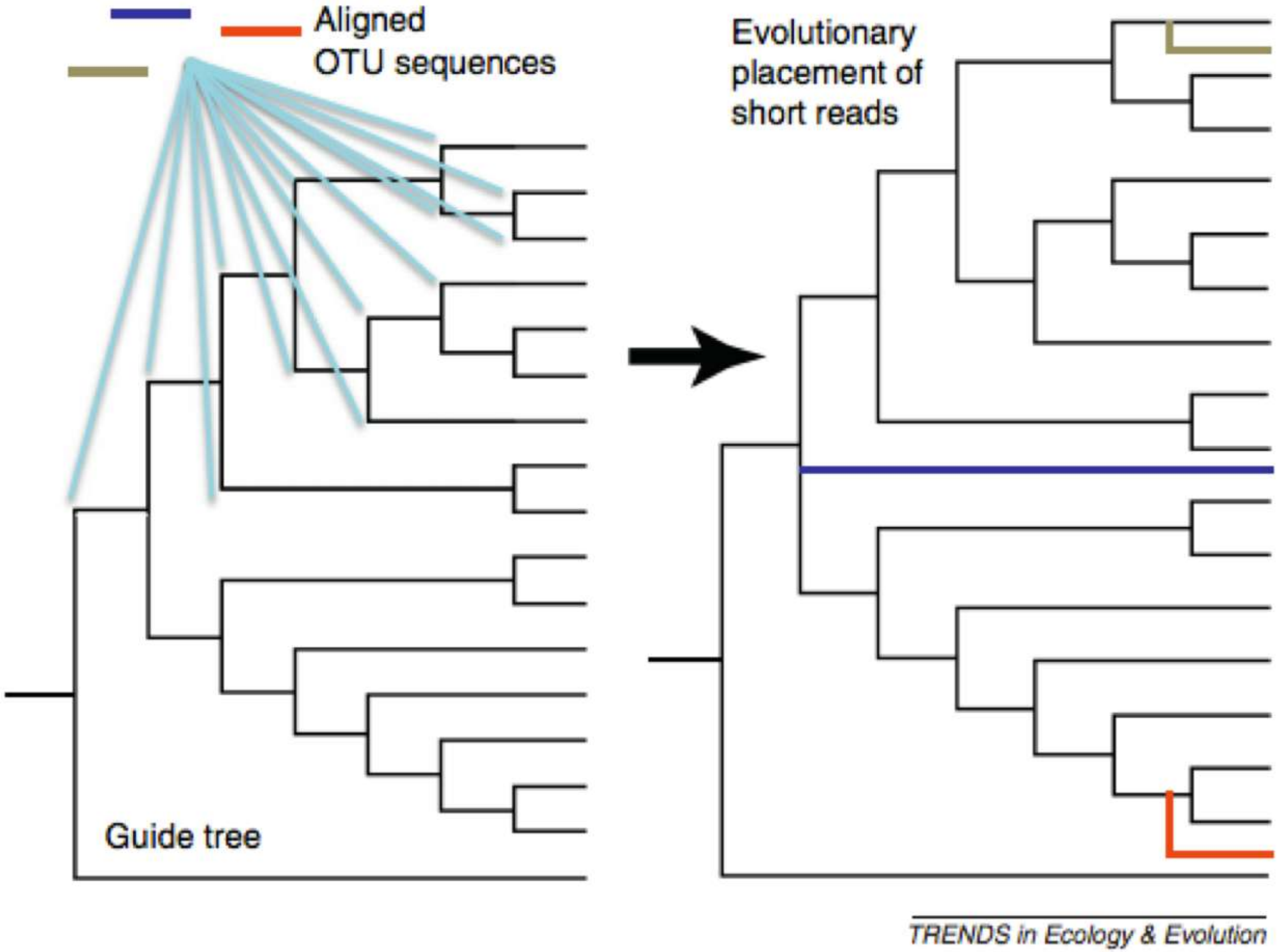
Open-reference OTU picking

- Pros
  - All reads are clustered
  - Partially parallelizable
- Cons
  - Only *partially* parallelizable
  - Mix of high quality sequences defining OTUs (i.e., the database sequences) and possible low quality sequences defining OTUs (i.e., the sequencing reads)

Assigned OTUs -> Loss of information



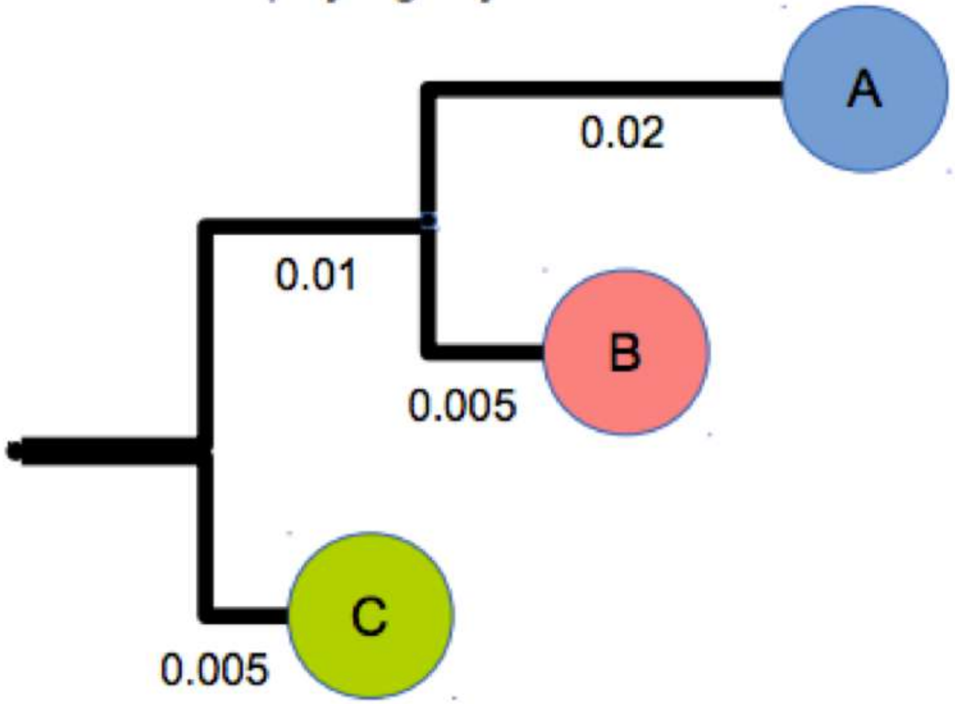
OTU relationship using phylogenetics



Bik et al (2011)

Logical inconsistency: OTUs at 97% ID

Assume the true phylogeny:



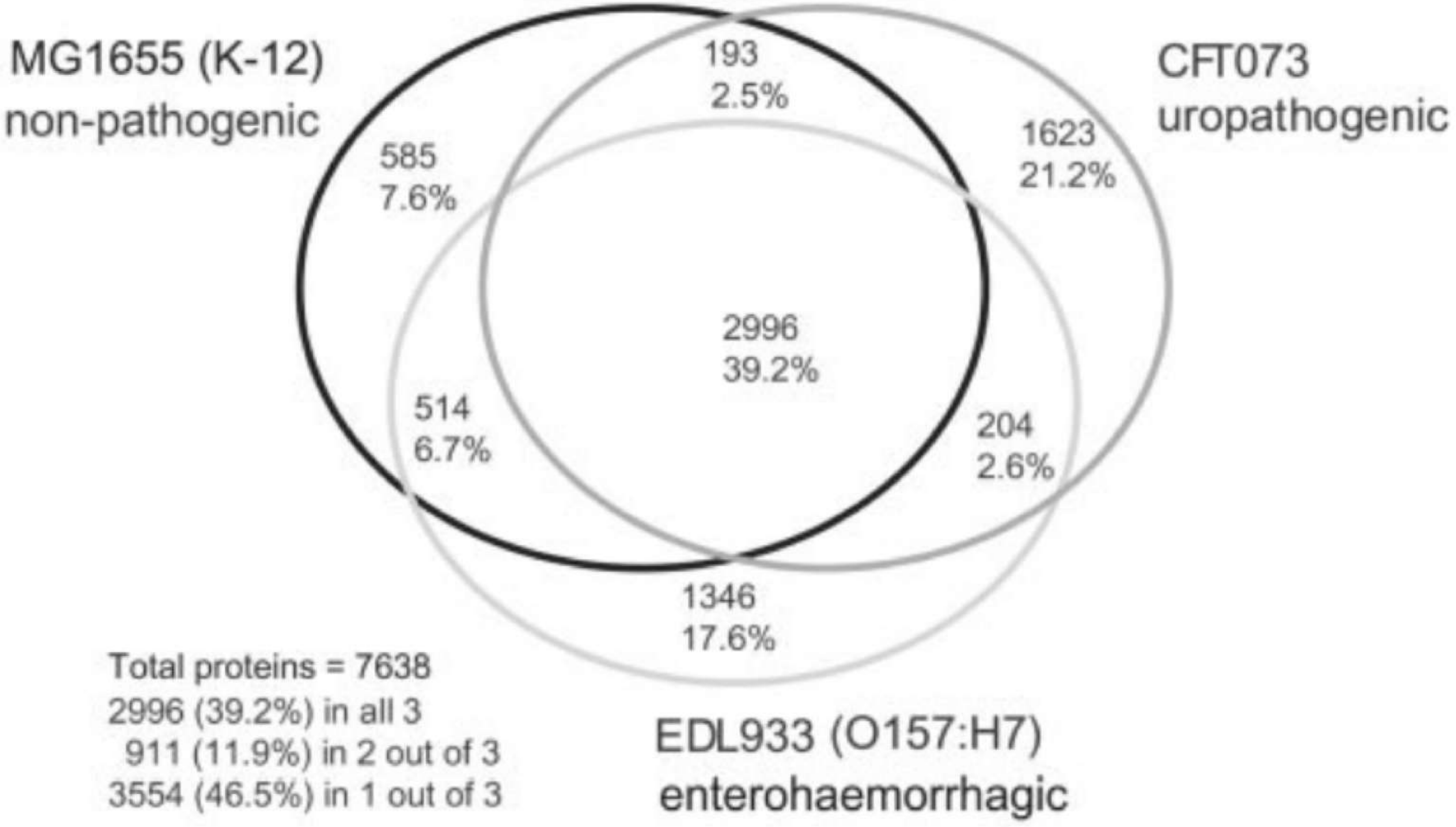
A, B > 97% identity
B, C > 97% identity
A and C not > 97% ID

Possible valid OTUs:
AB, C (with A & C centroids)
A, BC (with A & C centroids)
ABC (with B centroid)

OTU pipelines will arbitrarily pick one of the three solutions.
Is this actually a problem??

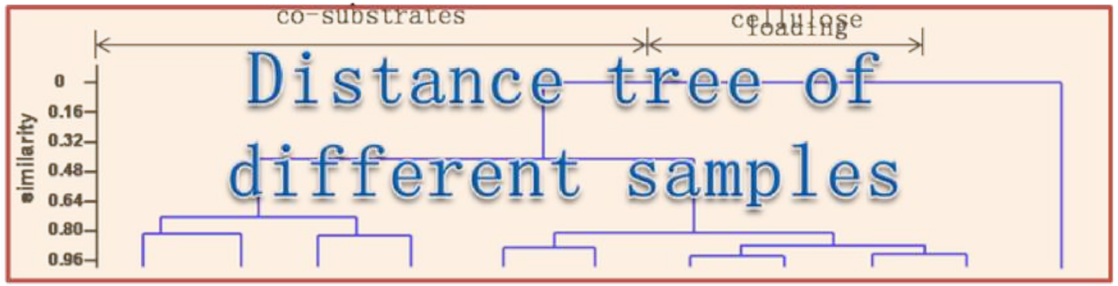
Slide of Aaron Darlin

Same species (16S): Different genomes



Welch et al (2002)

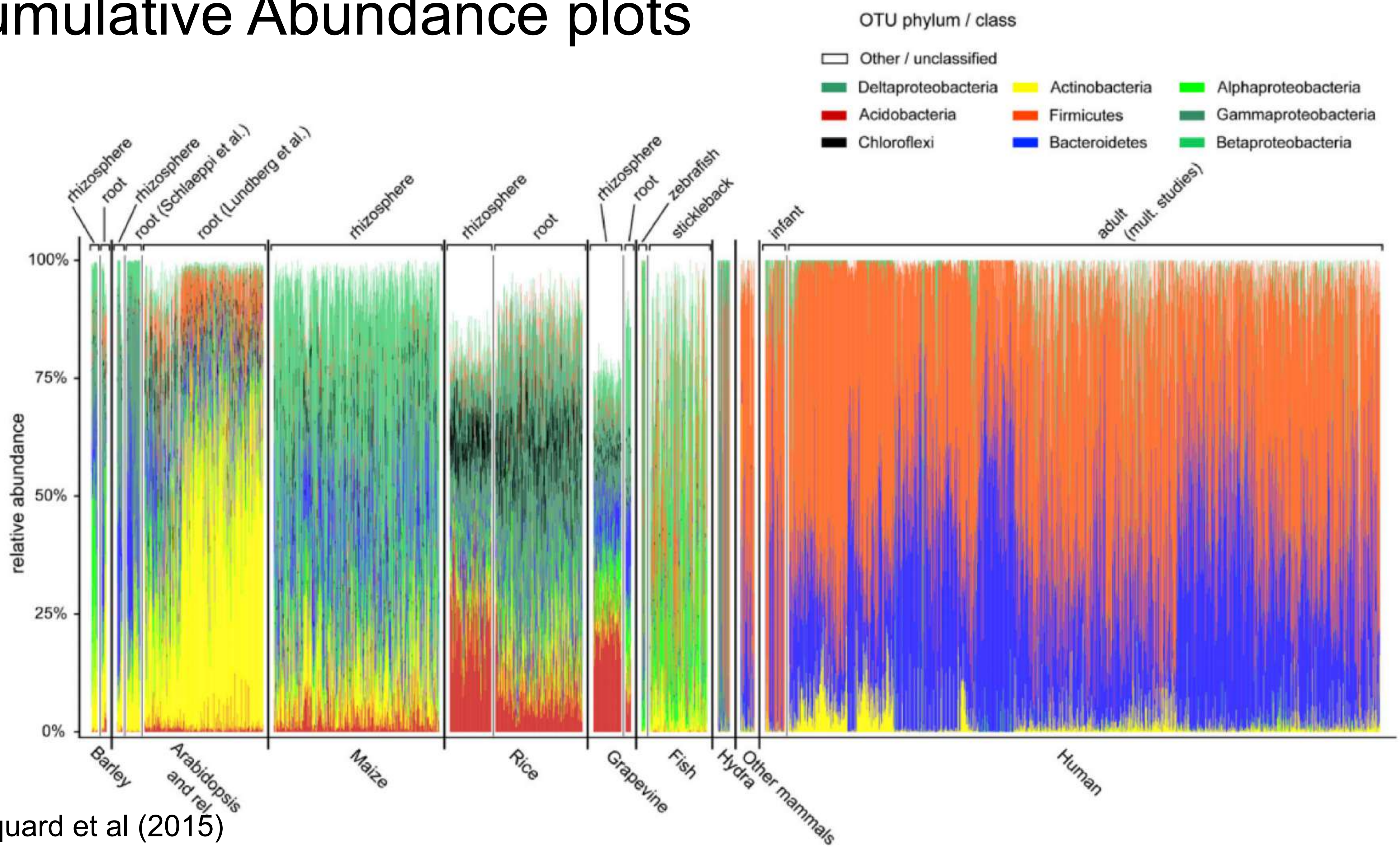
Tree way plot with top OTUs abundance and classification



OUT NO.	G1	X1	G2	X2	S1	S2	High	Medium	Low	Inoculum	Seed	
1	16.394%	18.740%	9.072%	10.154%	31.778%	26.680%	32.332%	32.312%	40.620%	39.410%	0.040%	*Clostridium sp.* 4-2a
2	7.282%	6.904%	4.352%	4.994%	12.884%	13.464%	13.726%	13.334%	15.630%	13.790%	0.006%	*Clostridium sp.* 4-2a
3	7.108%	7.174%	4.070%	4.610%	13.604%	12.230%	13.612%	13.276%	15.874%	14.348%	0.006%	*Clostridium sp.* 4-2a
4	5.264%	5.418%	2.988%	3.376%	10.168%	9.328%	10.632%	11.062%	11.600%	10.104%	0.004%	*Clostridium sp.* 4-2a
5	0.002%	0.002%	1.136%	0.000%	0.666%	2.178%	0.000%	0.000%	0.006%	0.000%	0.000%	*Anoxybacillus sp.* HT8
6	0.338%	0.296%	0.382%	0.244%	0.564%	0.866%	0.006%	0.036%	0.070%	0.816%	0.000%	*Clostridiaceae bacterium* 37-7-2Cl
7	2.460%	3.044%	1.586%	2.646%	4.904%	5.052%	2.068%	2.434%	0.708%	1.532%	0.002%	*Clostridium sp.* YN5
8	1.508%	1.246%	0.980%	1.338%	2.340%	3.154%	1.282%	1.542%	0.460%	0.814%	0.002%	*Clostridium sp.* YN6
9	1.192%	1.142%	0.784%	1.022%	2.026%	2.516%	1.066%	1.258%	0.386%	0.750%	0.000%	*Clostridium sp.* YN7
10	1.098%	[illegible]	0.626%	[illegible]	[illegible]	[illegible]	0.870%	1.192%	0.336%	0.582%	0.000%	*Clostridium sp.* YN8
11	3.686%	[illegible]	[illegible]	[illegible]	0.218%	[illegible]	[illegible]	[illegible]	[illegible]	0.000%	0.000%	*Thermoanaerobacterium xylanolyticum* LX-11
12	1.862%	0.904%	[illegible]	0.864%	0.042%	[illegible]	0.000%	0.002%	0.004%	0.002%	0.002%	*Thermoanaerobacterium xylanolyticum* LX-11
13	1.756%	0.946%	1.302%	0.698%	0.068%	0.078%	0.002%	0.002%	0.000%	0.000%	0.000%	[illegible]
14	5.978%	12.532%	16.236%	[illegible]	[illegible]	[illegible]	0.008%	0.006%	0.004%	0.008%	0.010%	*Thermoanaerobacterium aotearoense* SCUT27
15	10.494%	6.218%	8.710%	4.958%	[illegible]	[illegible]	0.002%	0.002%	0.010%	0.000%	0.002%	*Thermoanaerobacterium xylanolyticum* LX-11
16	3.152%	5.022%	7.460%	7.656%	0.404%	1.214%	0.004%	0.004%	0.006%	0.004%	0.010%	*Thermoanaerobacterium aotearoense* SCUT27
17	8.504%	4.474%	6.390%	3.668%	0.256%	0.332%	0.000%	0.002%	0.008%	0.004%	0.002%	*Thermoanaerobacterium xylanolyticum* LX-11
18	2.764%	5.346%	6.940%	7.068%	0.446%	1.230%	0.006%	0.002%	0.008%	0.000%	0.004%	*Thermoanaerobacterium aotearoense* SCUT27
19	4.642%	2.480%	3.866%	2.190%	0.162%	0.210%	0.000%	0.002%	0.002%	0.002%	0.002%	*Thermoanaerobacterium xylanolyticum* LX-11
20	2.098%	3.808%	4.594%	4.858%	0.384%	0.986%	0.000%	0.008%	0.002%	0.000%	0.004%	*Thermoanaerobacterium aotearoense* SCUT27
21	1.486%	0.276%	4.892%	0.530%	0.106%	0.396%	0.000%	0.002%	0.002%	0.000%	0.000%	*Thermoanaerobacterium saccharolyticum* D22
22	0.018%	0.300%	0.042%	3.894%	0.016%	0.064%	0.000%	0.006%	0.000%	0.002%	0.002%	*Thermoanaerobacterium thermosaccharolyticum* CECT5853T
23	0.014%	0.362%	0.064%	5.142%	0.018%	0.134%	0.002%	0.002%	0.000%	0.000%	0.004%	*Thermohydrogenium kirishiense* DSM11055T
24	0.146%	0.216%	0.084%	0.112%	0.040%	0.018%	1.244%	1.048%	0.662%	1.742%	0.004%	Uncultured *Bacillus sp.* clone 93
25	0.138%	0.140%	0.062%	0.054%	0.002%	0.006%	0.922%	0.838%	0.536%	1.036%	0.000%	Uncultured *Bacillus sp.* clone 94
26	0.012%	0.016%	0.002%	0.002%	0.038%	0.020%	0.004%	0.006%	0.004%	0.050%	3.950%	*Mycobacterium gilvum* B4
27	0.000%	0.002%	0.000%	0.004%	0.004%	0.004%	0.002%	0.008%	0.002%	0.000%	4.204%	*Nocardioides terrae* VA15
28	0.108%	0.122%	0.018%	0.044%	1.392%	0.316%	0.614%	1.012%	0.880%	0.440%	0.000%	*Anaerolinea thermolimosa* IMO-1
29	0.166%	0.078%	0.014%	0.026%	0.210%	0.102%	1.744%	0.870%	0.914%	0.152%	0.000%	Uncultured bacterium clone 49c
30	0.002%	0.010%	0.008%	0.004%	0.072%	0.016%	0.000%	5.428%	0.128%	0.478%	0.006%	*Clostridium cellobioparum* JCM 1422
Coverage	89.7%	90.4%	91.1%	89.0%	86.9%	87.6%	80.1%	85.7%	88.9%	86.1%	8.3%	

http://www.shuixia100.com/my-blog/mothur-tutorial-1

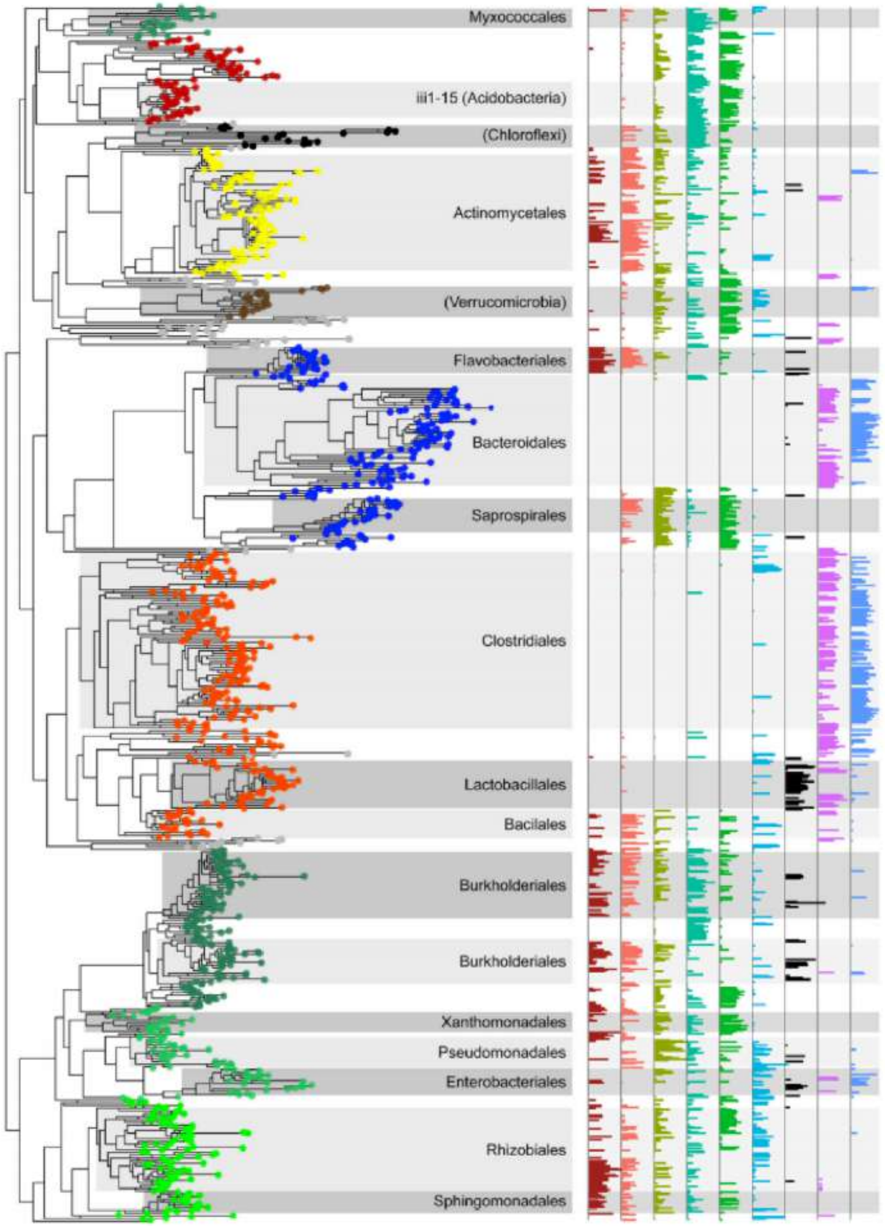
Cumulative Abundance plots



Hacquard et al (2015)

Phylogenetic Analysis of OTU abundances

Relationship between OTUs



How do we compare between different samples?

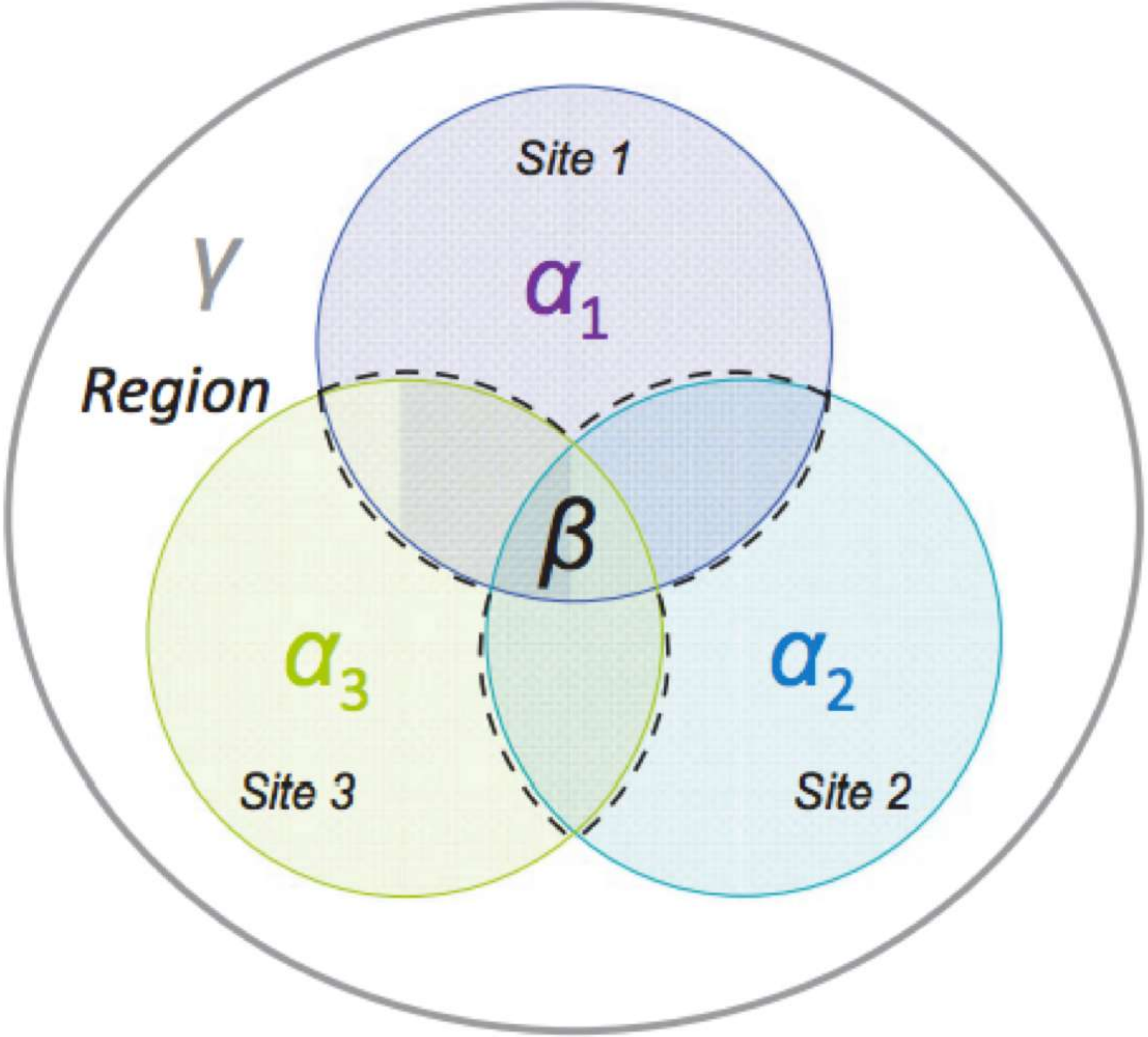
Hacquard et al (2015)

Concept: Diverisity measures

Measures of biodiversity

Zinger et al (2012)

"… measuring biodiversity consists of characterizing the **number**, **composition** and **variation** in taxonomic or functional units **(OTU)** over a wide range of biological organizations"



Measures of biodiversity

Zinger et al (2012)

Alpha diversity refers to the diversity within one location or sample. It is often measured as species richness (i.e. number of species), seldom as species evenness (extent of species dominance). Species richness is strongly sensitive to sampling effort, and requires standardized samples, or the use of estimators that corrects undersampling biases, such as Chao1 or ACE. Evenness is less affected by undersampling biases and is usually assessed with Simpson's or Pielou's indices or rank abundance curves (review in Magurran 2004).

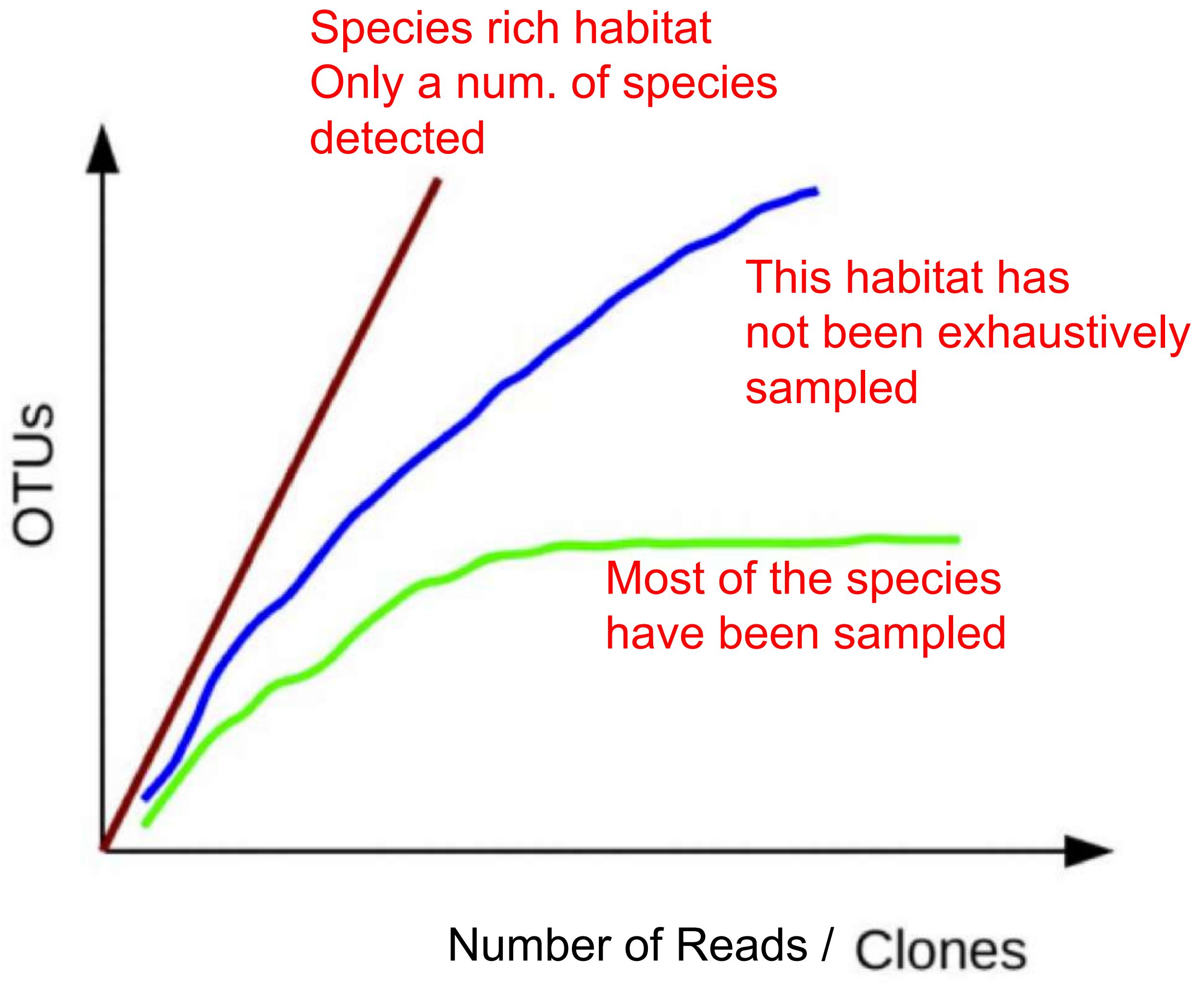
Beta diversity consists in determining the difference in diversity or community composition between two or more locations or samples (i) by considering species composition only, and use incidence data with associated metrics such as Jaccard or Sorensen similarity indices or (ii) by taking species relative abundances into account, and use Bray–Curtis or Morisita–Horn dissimilarity measures (Anderson *et al*. 2011). Using abundance data is, however, strongly discussed among microbiologists when dealing with rRNA gene data because of variations in gene copy number among strains (Acinas *et al*. 2004b; Zhu *et al*. 2005) as well as PCR artefacts.

Gamma diversity, or regional diversity, is similar to alpha diversity but applies for a larger area that encompasses the units under study.

Finally, the spatial scale of investigation can produce very different results and should be consistent in cross-study comparisons (Magurran 2004).

Species sampling and Rarefaction

Rarefaction allows the calculation of **species richness** for a given number of individual samples, based on the construction of so-called **rarefaction curves**. This curve is a plot of the number of species as a function of the number of samples



Wooley et al (2010)

Alpha diversity

a measure of the diversity within a single sample

Types of alpha diversity

Total # of species = **richness**

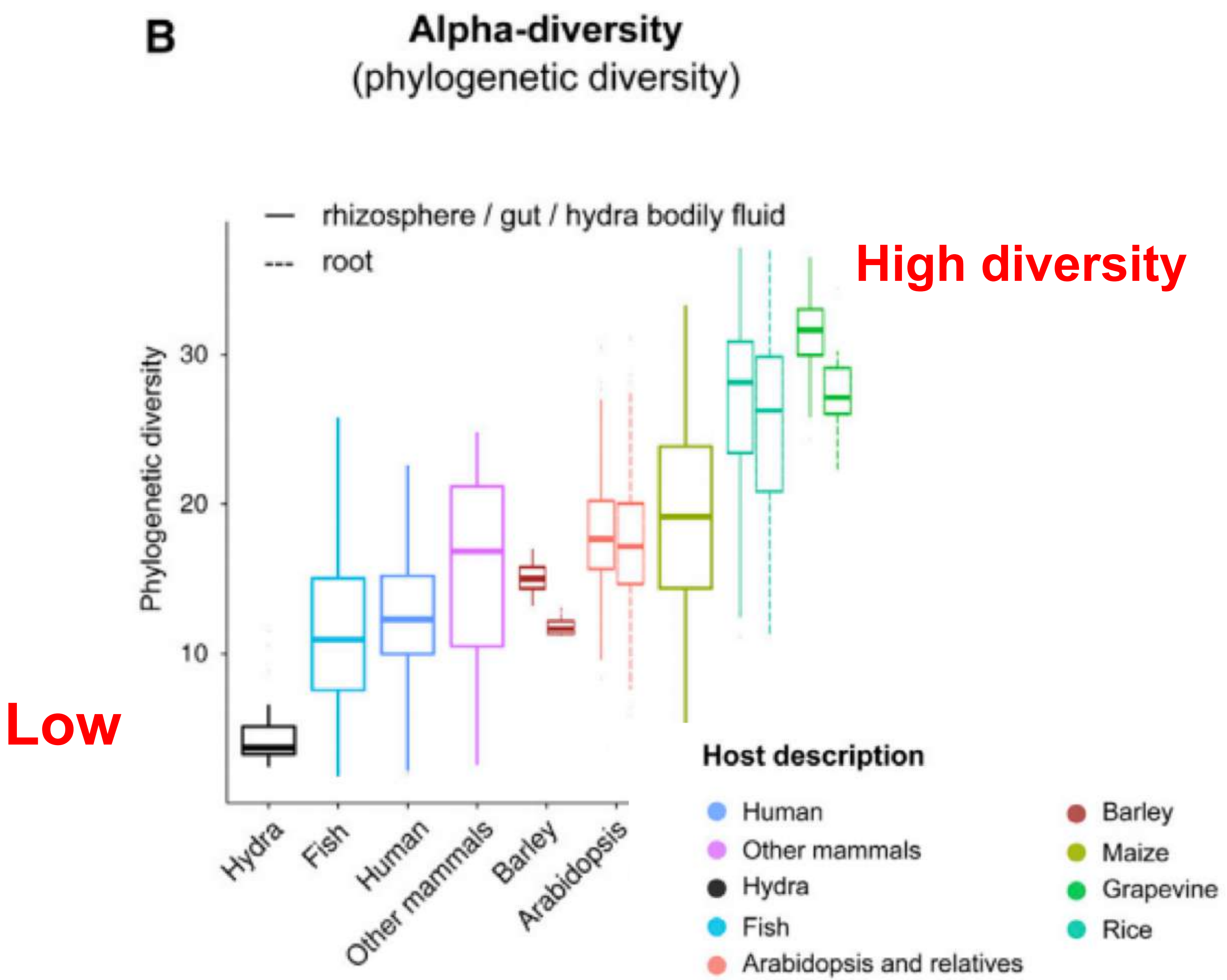
How many OTUs?

Total # of genes = genetic richness

Phylogenetic diversity of genes = genetic PD

Eveness = What is the distribution of abundance in the community?

How many OTUs at high abundance and how many OTU at low abundance?



Hacquard et al (2015)

Beta diversity

a measure of **the similarity in diversity between samples**

Types of beta diversity

- Species presence/absence
- Shared phylogenetic diversity
- Gene presence / absence
- Shared phylogenetic diversity of genes

Frequently used as values for PCA of PCoA analysis

Beta diversity

A. Membership:
shared OTU occurences across communities
1 = present, 0 = below detection

List of observed OTUs

	Occurences in community **A**	Occurences in community **B**	Shared occurences **A & B**
OTU 1	1	0	
OTU 2	0	1	
OTU 3	1	1	X
OTU 4	1	1	X
OTU 5	1	1	X

B. Composition:
similar OTU abundances across communities

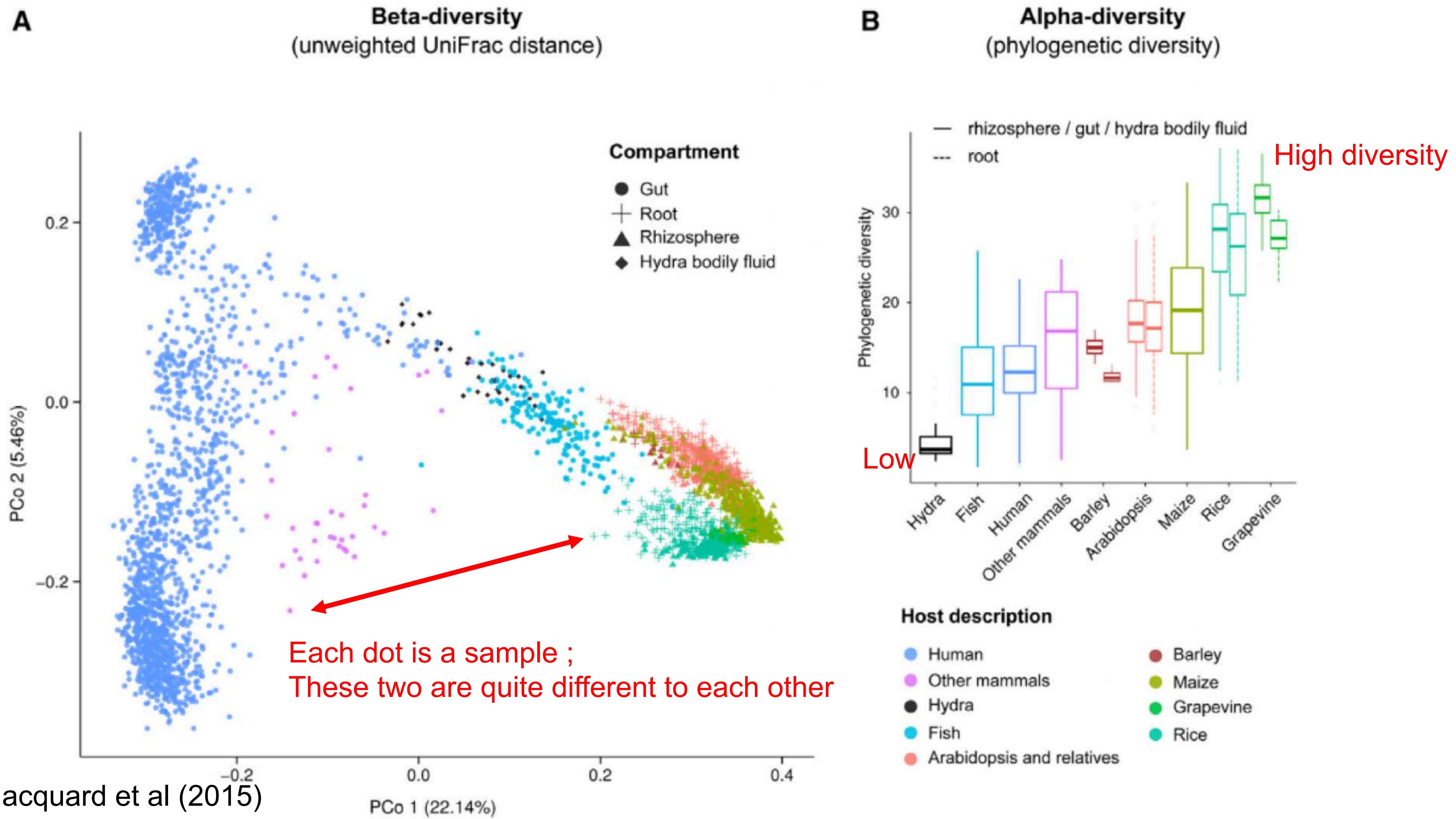
List of observed OTUs

	Abundances community **A**	Abundances community **B**	Similar abundances **A & B**
OTU 1	0.4	0	
OTU 2	0	0.1	
OTU 3	0.1	0.1	X
OTU 4	0.2	0.5	
OTU 5	0.3	0.3	X

Phylogeny:
shared OTU lineages across communities

	Abundances community **A**	Abundances community **B**	Similar abundances **A & B**
OTU 1	0.4	0	X (OTU 1–2)
OTU 2	0	0.1	
OTU 3	0.1	0.1	X (OTU 3–4)
OTU 4	0	0.8	
OTU 5	0.5	0	

Shade and Handelsman (2012)



Hacquard et al (2015)

Amplicon sequencing or metagenomes?

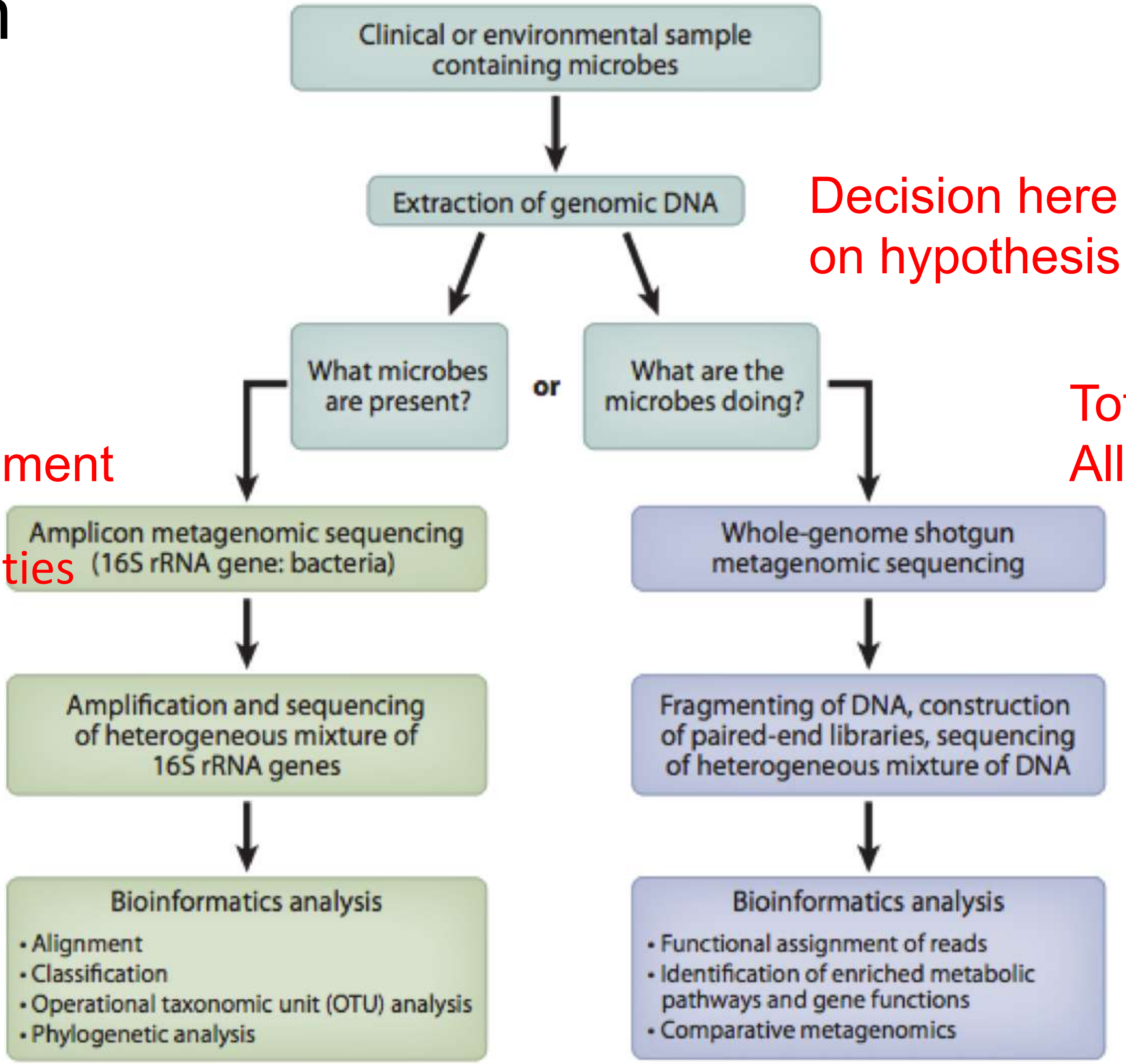
Workflow decision

Decision here based
on hypothesis and budget

PCR product
One gene fragment

Total DNA
All genes

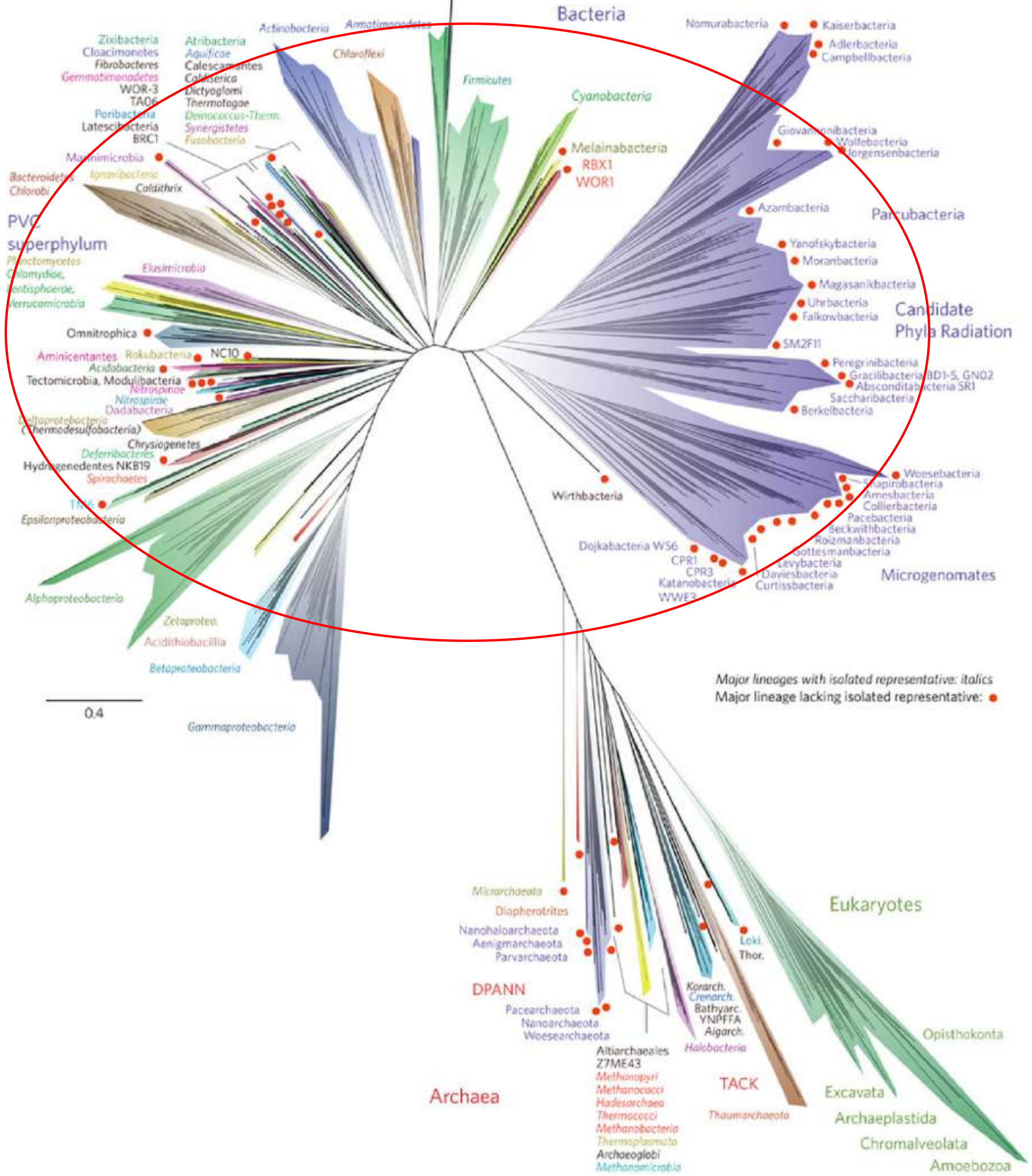
Fast survey of large communities
Good for first round survey



Grice and Segre (2012)

Amplicon sequencing

New Tree of life

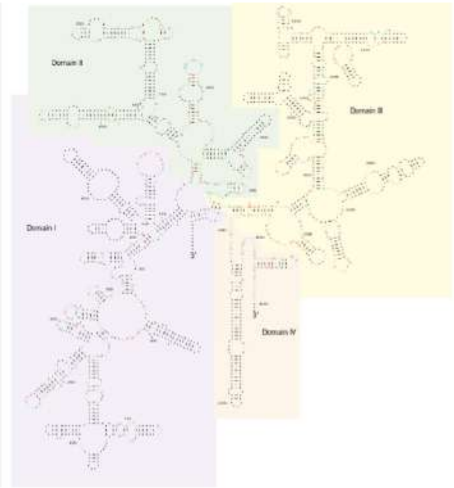


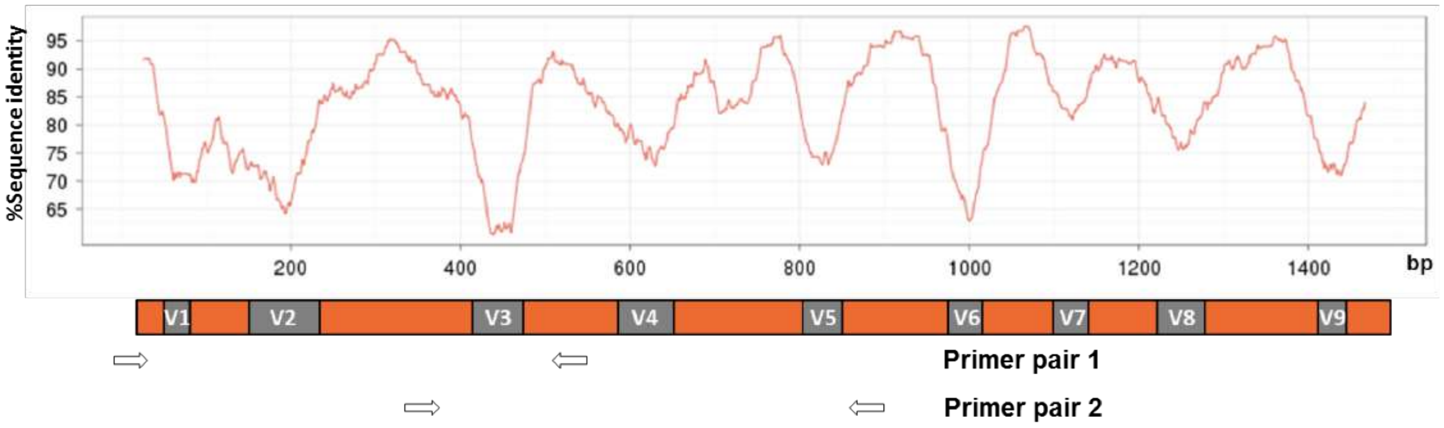
What do they have in common?

Hug et al (2016)

http://www.nytimes.com/2016/04/12/science/scientists-unveil-new-tree-of-life.html?_r=0

16S



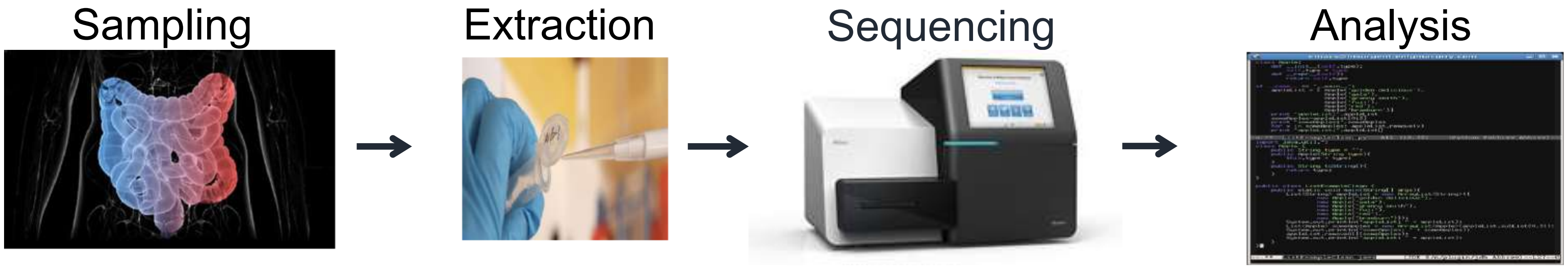


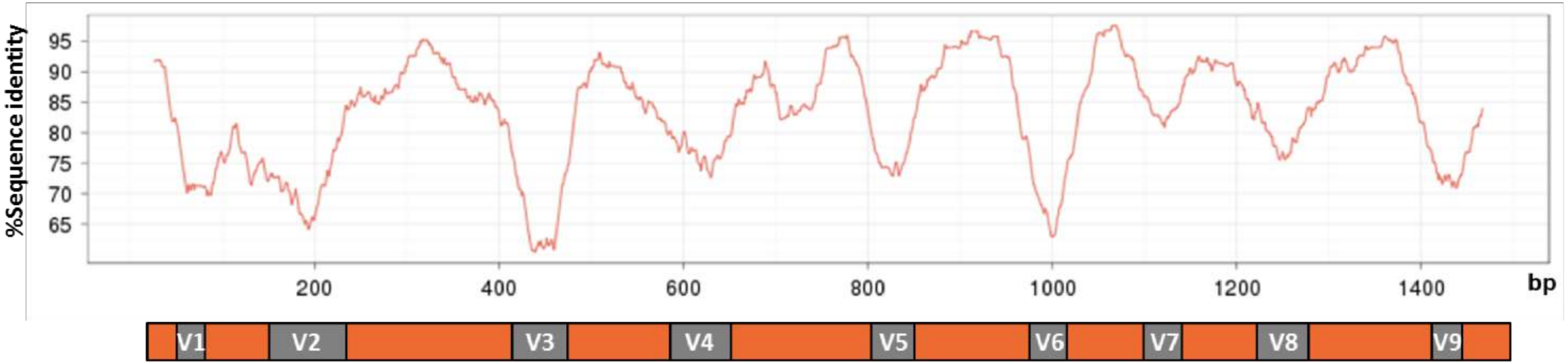
- **Advantages**:
  - Universal: Every bacterial and archea species has this gene
  - Conserved regions (for primer design)
  - **Variable regions (to distinguish different species)**
  - Great databases and alignments (for human related species)
  - Mainly used for taxonomical classification

- Problems:
  - Variable copy number in each species
  - No universal (unbiased) primers
  - (Not directly correlated with activity)
  - (Lack of functional information)

http://en.wikipedia.org/wiki/16S_ribosomal_RNA

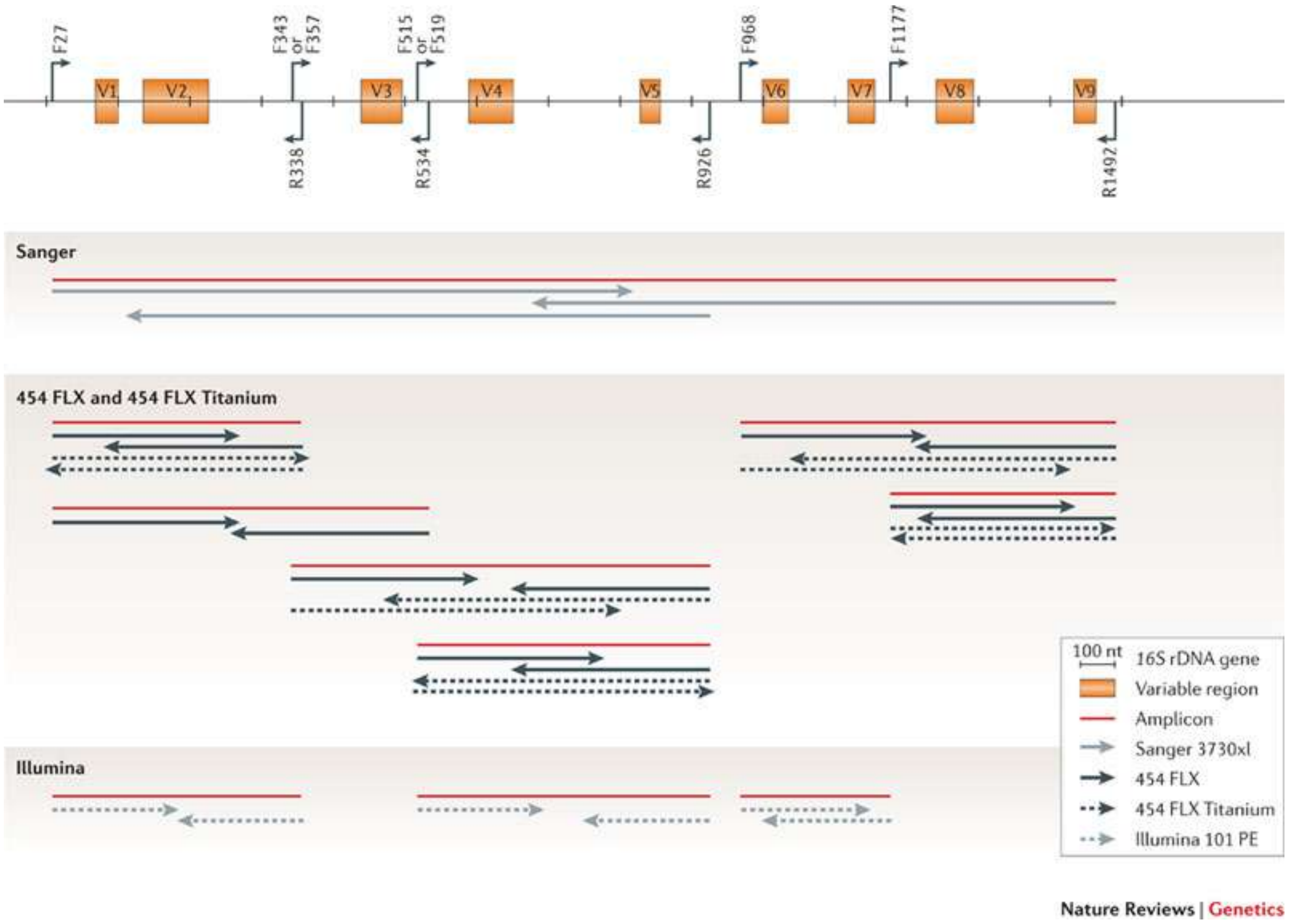
Typical workflow





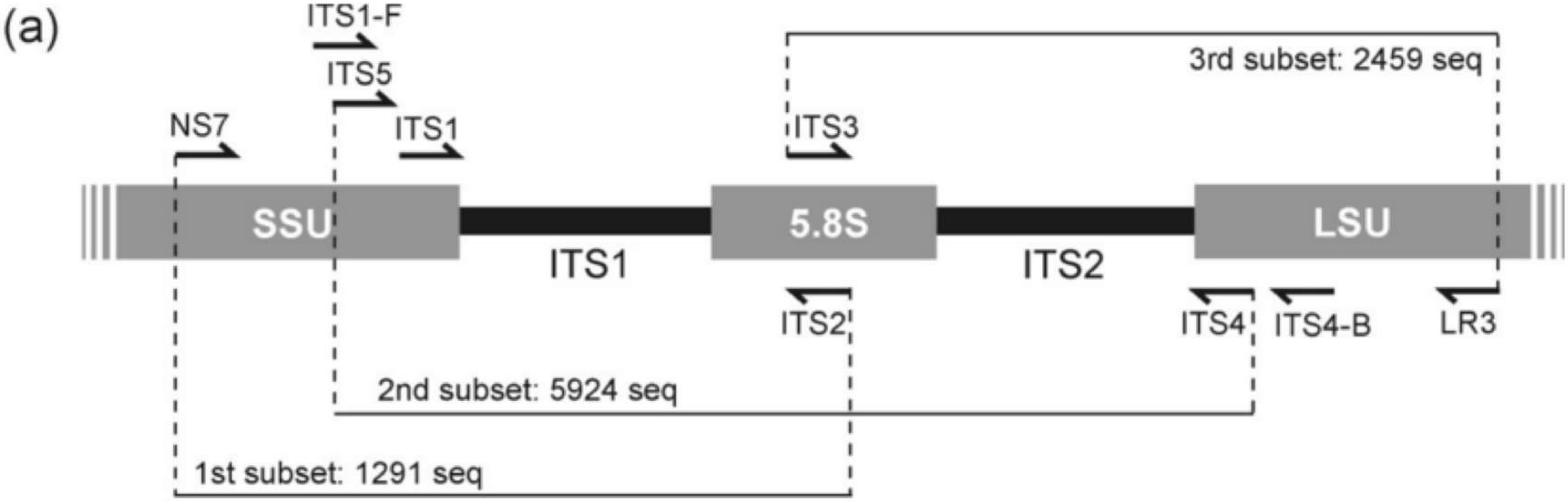
Which region to sequence?

16S amplified region



Kuczynski et al (2011)

ITS for characterization of fungi species



Bellemain et al (2010)

Workflow

Filter for contaminants and low quality reads

↓

Assemble overlapping reads

↓

Reduce datasets (clustering)

↓

Perform taxonomic classification and compute diversity metrics

- Quality plots and read trimming
  - FastQC
    http://www.bioinformatics.babraham.ac.uk/projects/fastqc/
  - FASTX
    http://hannonlab.cshl.edu/fastx_toolkit/
- Chimera removal
  - AmpliconNoise
    http://code.google.com/p/ampliconnoise/
  - UCHIME
    http://www.drive5.com/uchime/

Slide of Surya Saha (slideshare)

Workflow

Because the read length (2x250 or 2x300) will be longer than the actual DNA fragment

Filter for contaminants and low quality reads

Assemble overlapping reads

Reduce datasets (clustering)

Perform taxonomic classification and compute diversity metrics

- Merge overlapping paired end reads
  - FLASH

    http://www.genomics.jhu.edu/software/FLASH/index.shtml
  - FastqJoin

    http://code.google.com/p/ea-utils/wiki/FastqJoin
  - CD-HIT read-linker

    http://weizhong-lab.ucsd.edu/cd-hit/wiki/doku.php?id=cd-hit-auxtools-manual

Slide of Surya Saha (slideshare)

Workflow

Filter for contaminants and low quality reads

Assemble overlapping reads

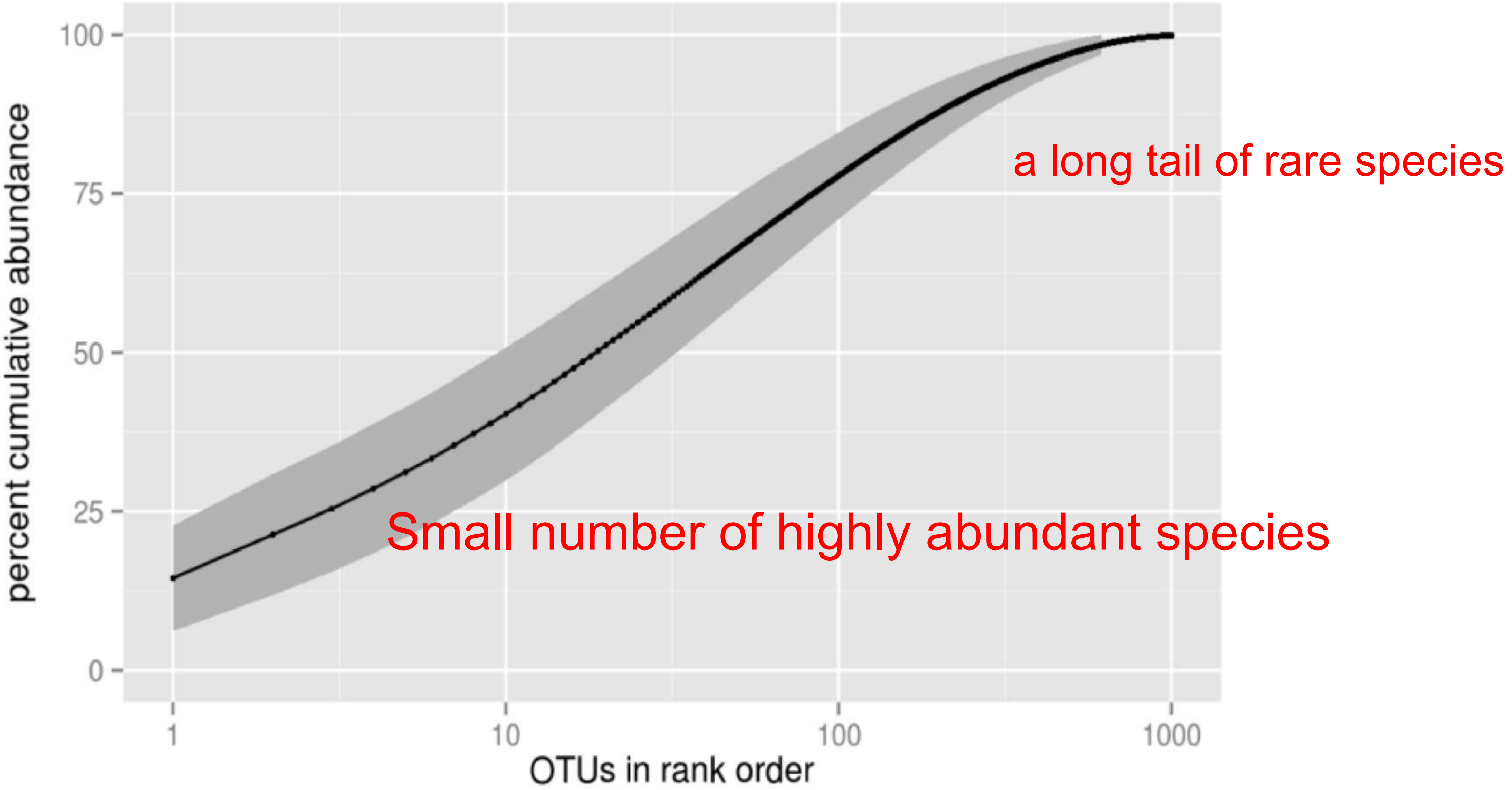
Reduce datasets (clustering)

Perform taxonomic classification and compute diversity metrics

- Clustering with high stringency
  - UCLUST/USEARCH (16S only)
    http://www.drive5.com/usearch/
  - CD-HIT-OTU (16S only)
    http://weizhong-lab.ucsd.edu/cd-hit-otu/
  - phylOTU (16S only)
    https://github.com/sharpton/PhylOTU

Slide of Surya Saha (slideshare)

Typical number of OTUs



Workflow

Filter for contaminants and low quality reads

Assemble overlapping reads

Reduce datasets (clustering)

Perform taxonomic classification and compute diversity metrics

- Composition based classifiers
  - RDP database + classifier

    http://rdp.cme.msu.edu/classifier/classifier.jsp
- Homology based classifiers
  - ARB + Silva database (16S only)

    http://www.arb-home.de/
  - GreenGenes database (16S only)

    http://greengenes.lbl.gov/cgi-bin/nph-index.cgi
  - UNITE database (ITS only)

    http://unite.ut.ee/
  - FungalITSPipeline (ITS only)

    http://www.emerencia.org/fungalitspipeline.html

Slide of Surya Saha (slideshare)

Using a “Classifier” to annotate OTUs

Uses an existing phylogeny
Find best unambiguous match to references

green genes
16S rRNA gene database and workbench compatible with ARB
greengenes.lbl.gov

silva
high quality ribosomal RNA databases

rdp

http://greengenes.lbl.gov/cgi-bin/nph-index.cgi http://www.arb-silva.de/ https://rdp.cme.msu.edu/

Analysis Packages

Qiime2 (https://qiime2.org/)

Mothur (mothur.org)

R

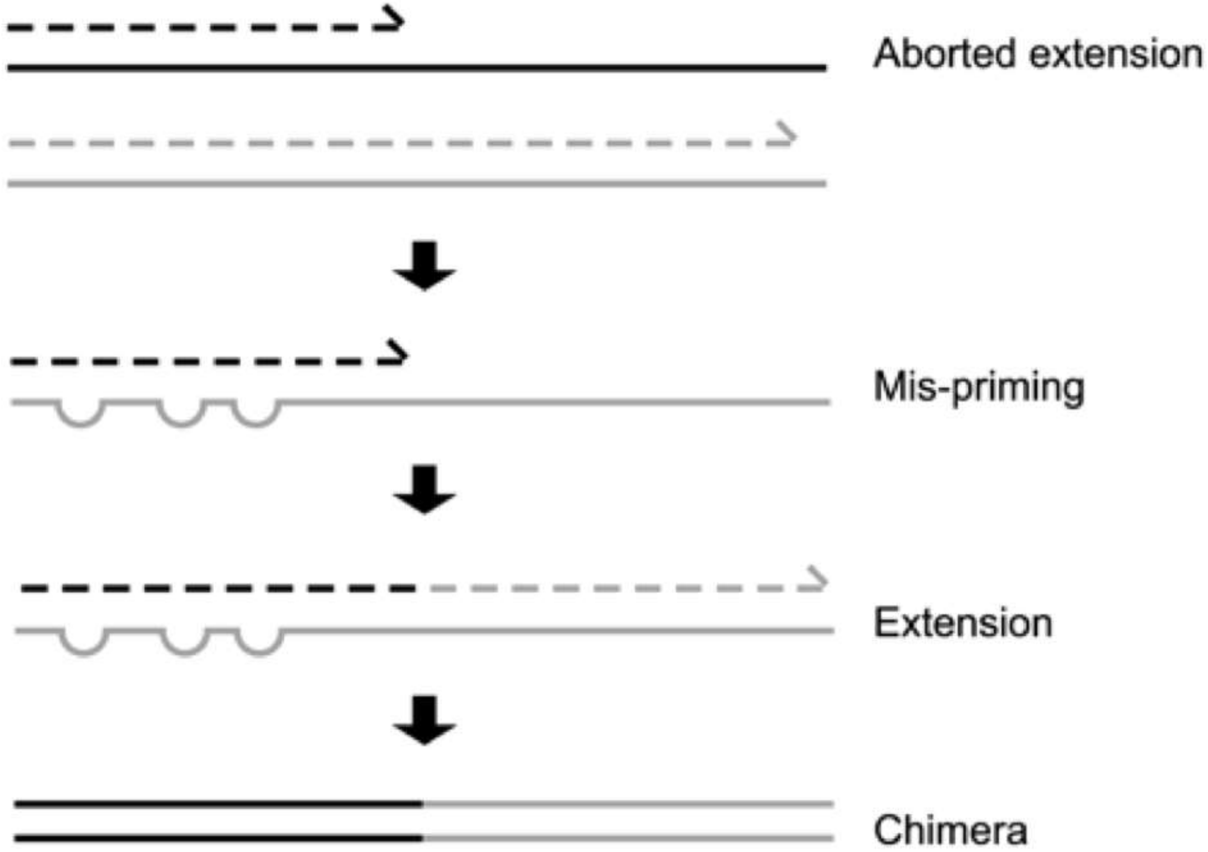
Phyloseq (https://joey711.github.io/phyloseq/)

Potential problem

- Lack of tools for processing ITS/Fungal microbiome data sets
- Amplification bias effects accuracy and replication
- Use of short reads prevents disambiguation of similar strains
- 16S or ITS may not differentiate between similar strains –
  - Clustering is done at 97%
  - Regions may be >99% similar
- Sequencing error inflates number of OTUs
- Chloroplast 16S sequences can get amplified in plant metagenomes

Slide of Surya Saha

Chimeric 16S (Artificial sequences formed during PCR amplification



"Chimeras were found to reproducibly form among independent amplifications and contributed to false perceptions of sample diversity and the false identification of novel taxa, **with less-abundant species exhibiting chimera rates exceeding 70%"**

Haas et al (2011)

Metagenomics

Advantage of metagenomics approach

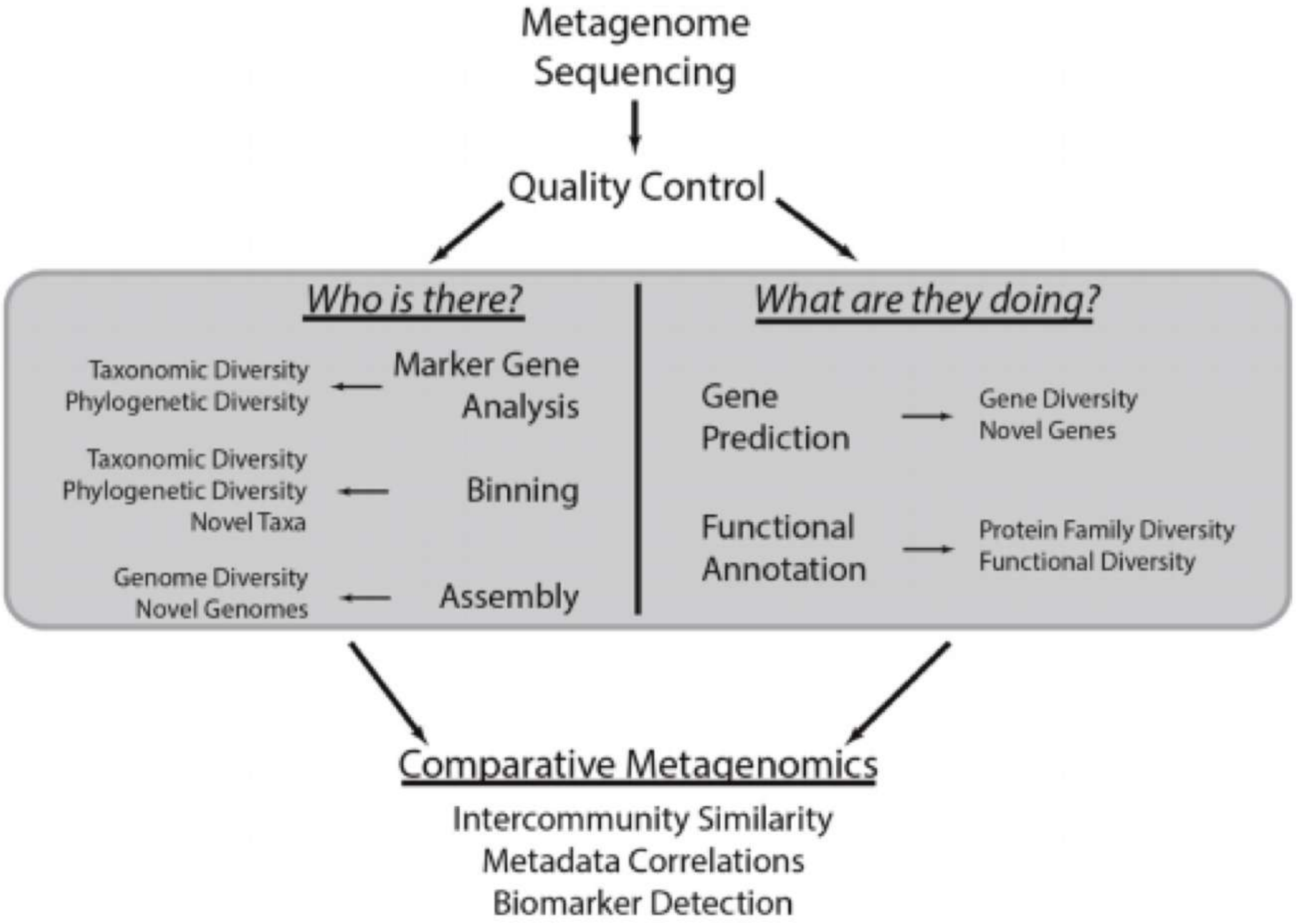
Better classification with Increasing number of complete genomes
Focus on whole genome based phylogeny (whole genome phylotyping)

•Advantages
No amplification bias like in 16S/ITS

Issues
Poor sampling beyond eukaryotic diversity
Assembly of metagenomes is **challenging** due to uneven coverage
Requires **high** depth of coverage

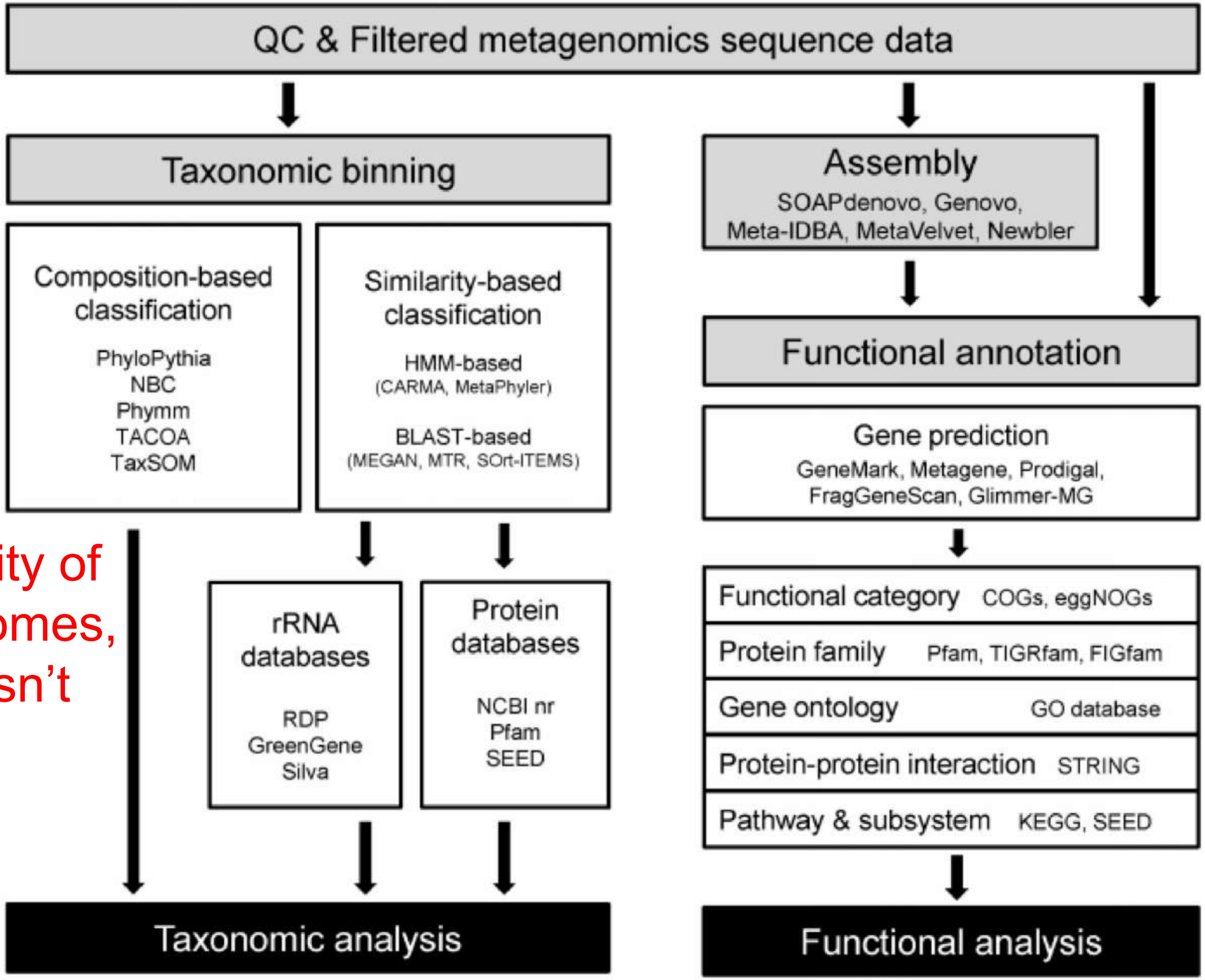
Slide of Surya Saha (slideshare)

Overall workflow



Sharpton (2014)

Overall workflow

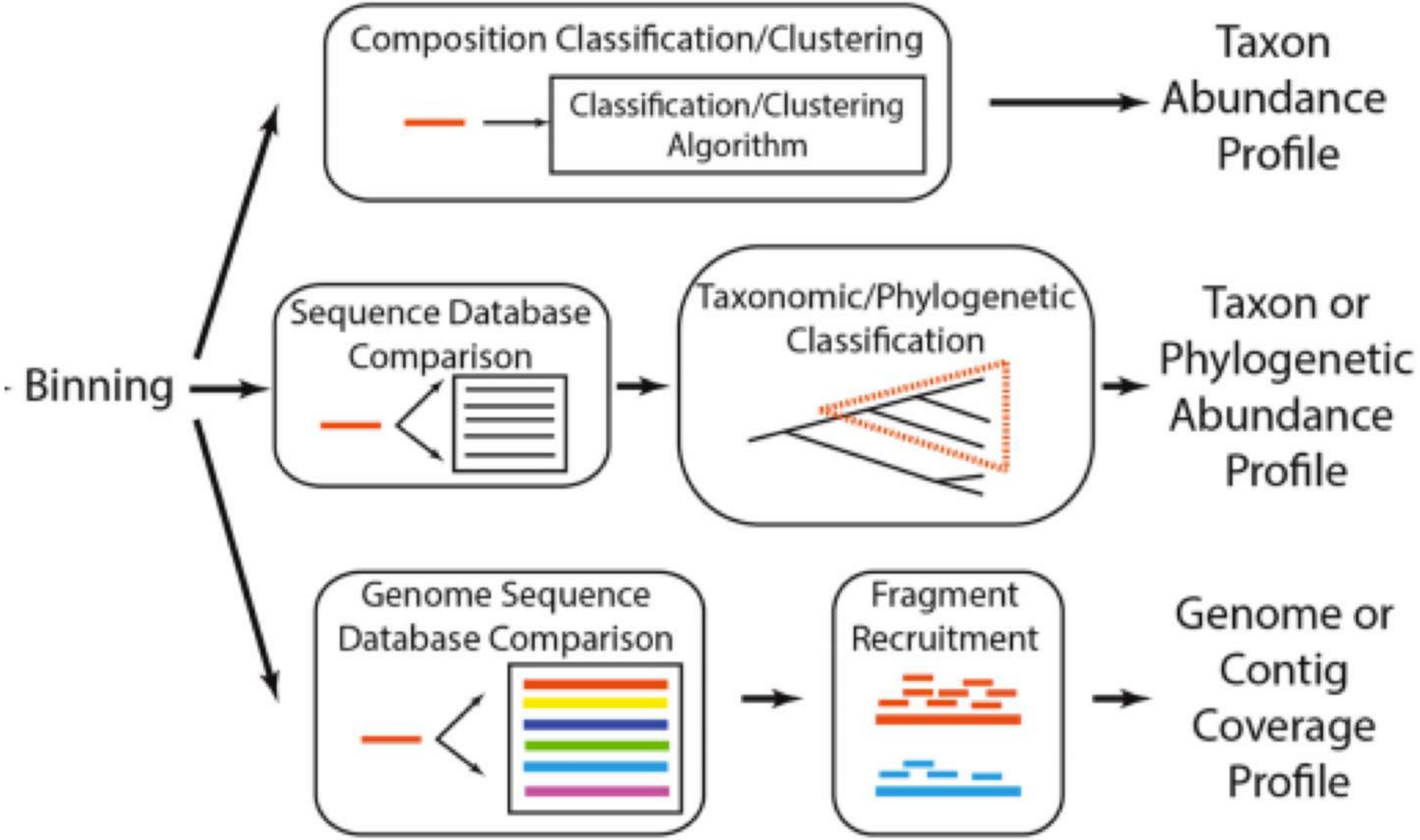


With the increase availability of reference sequenced genomes, probably one day one doesn't require assembly of metagenomes

Kim et al (2013)

Binning methods

E.g., GC, Tetranucleotide



Phylosift
MetaPhAln2

GroopM

Sharpton (2014)

Binning methods: A combination of

Classification based on **sequence composition:**

Advantage : all reads can be categorised into bins

Disadvantage: no taxonomy / function of the bins.

Classification based on **sequence similarity (of known genes)**

Advantage: One can determine taxonomy and function of reads.

Disadvantage: reads with similarity can not be classified .

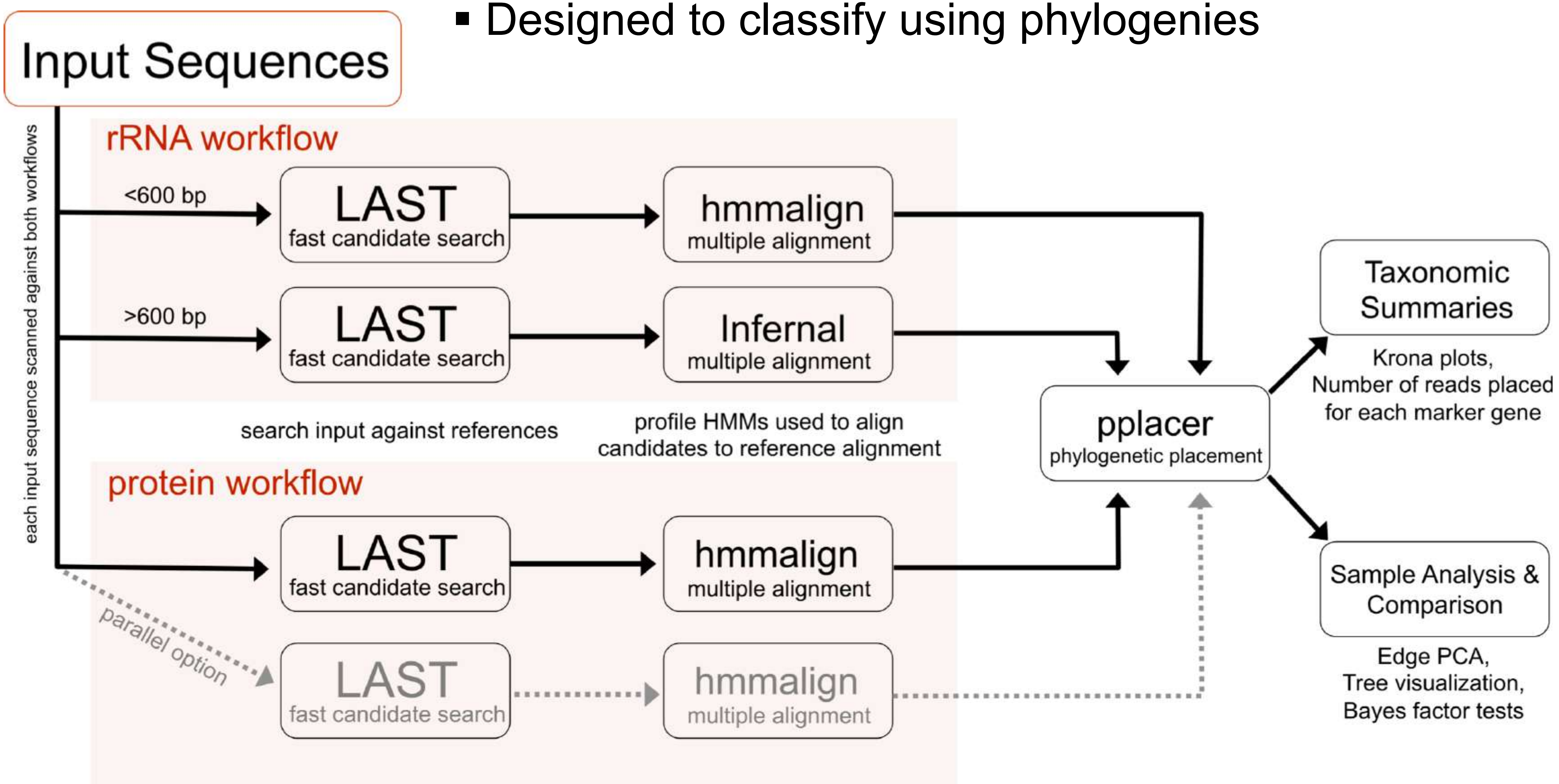
Sharpton (2014)

https://phylosift.wordpress.com/

PhyloSift

mining the global metagenome

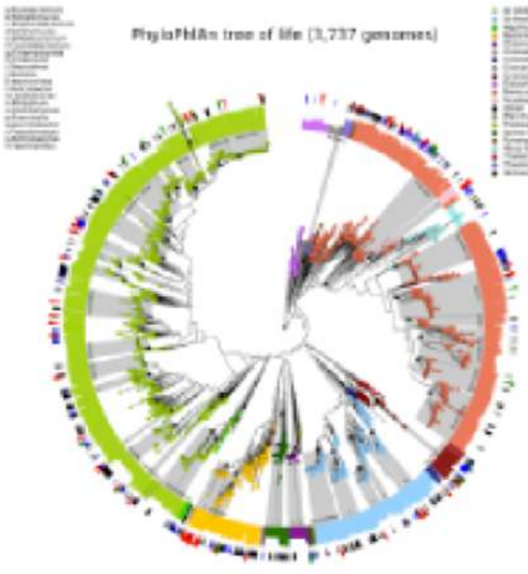
- Uses a database of 37 universal proteins & rRNA genes.
- Designed to classify using phylogenies

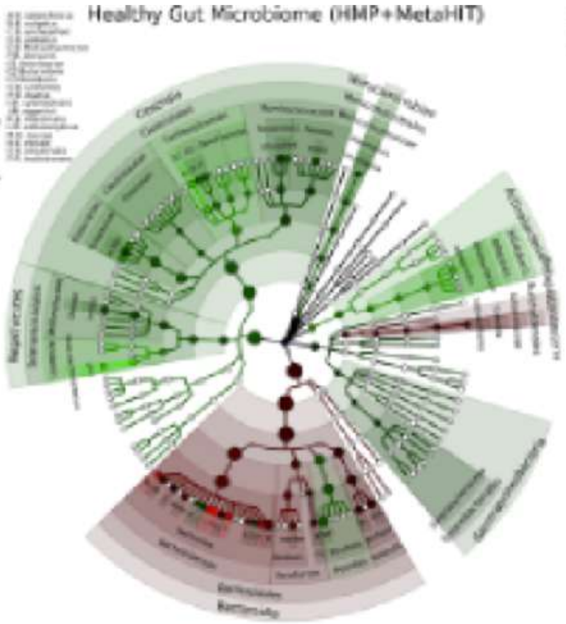


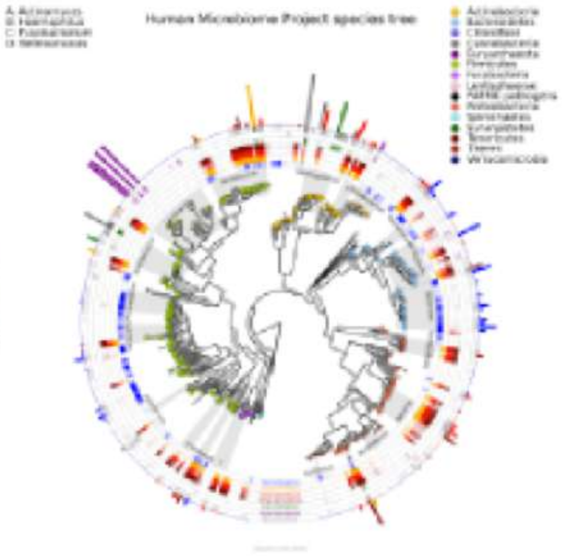
MetaPhAln2 – enhanced metagenomic taxonomic profiling

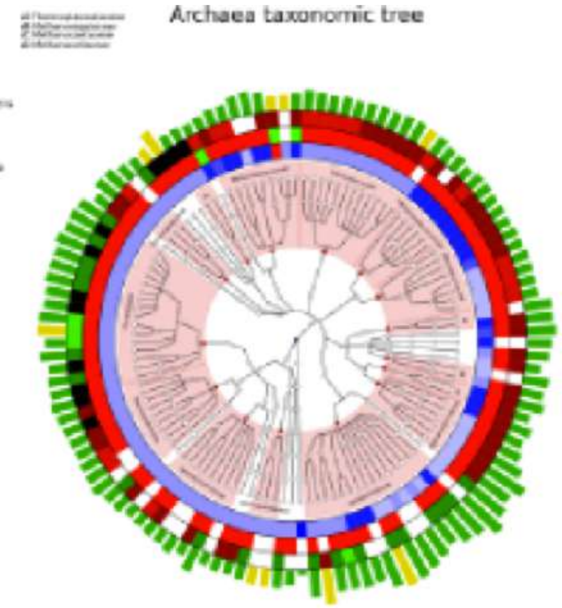
relies **on ~1M unique clade-specific marker genes** identified from ~17,000 reference genomes (~13,500 bacterial and archaeal, ~3,500 viral, and ~110 eukaryotic), allowing:

Species level resolution
Good visualisation with **GraphAln**
(So it's useful with known ecosystems)

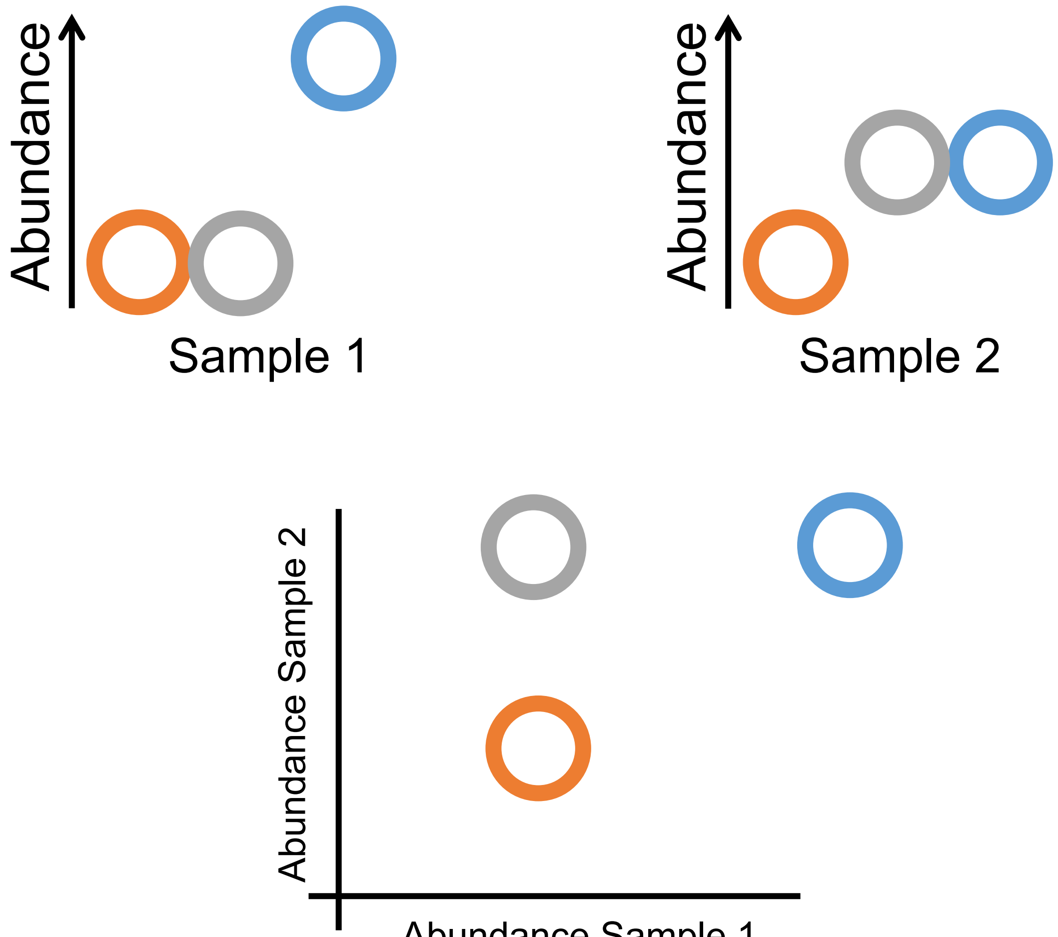


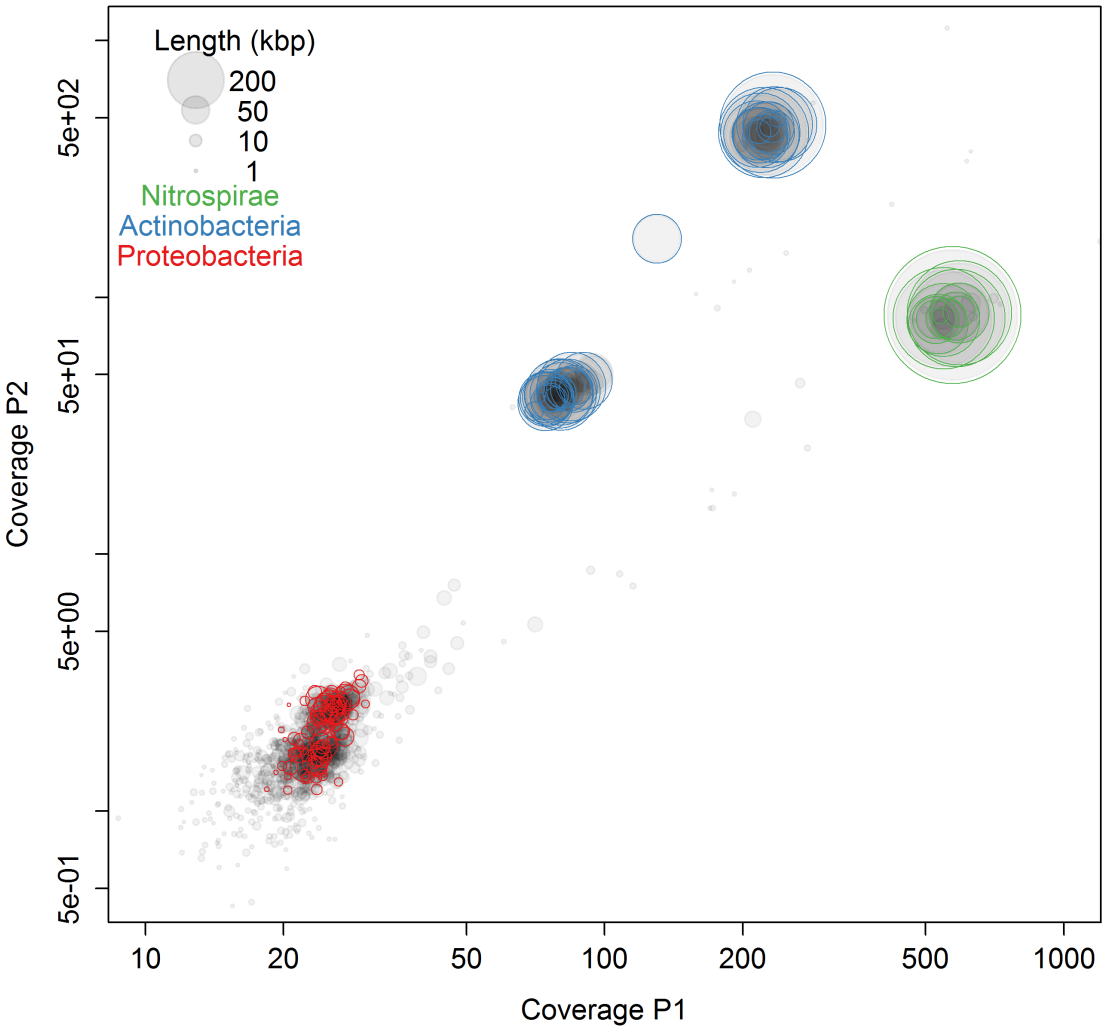






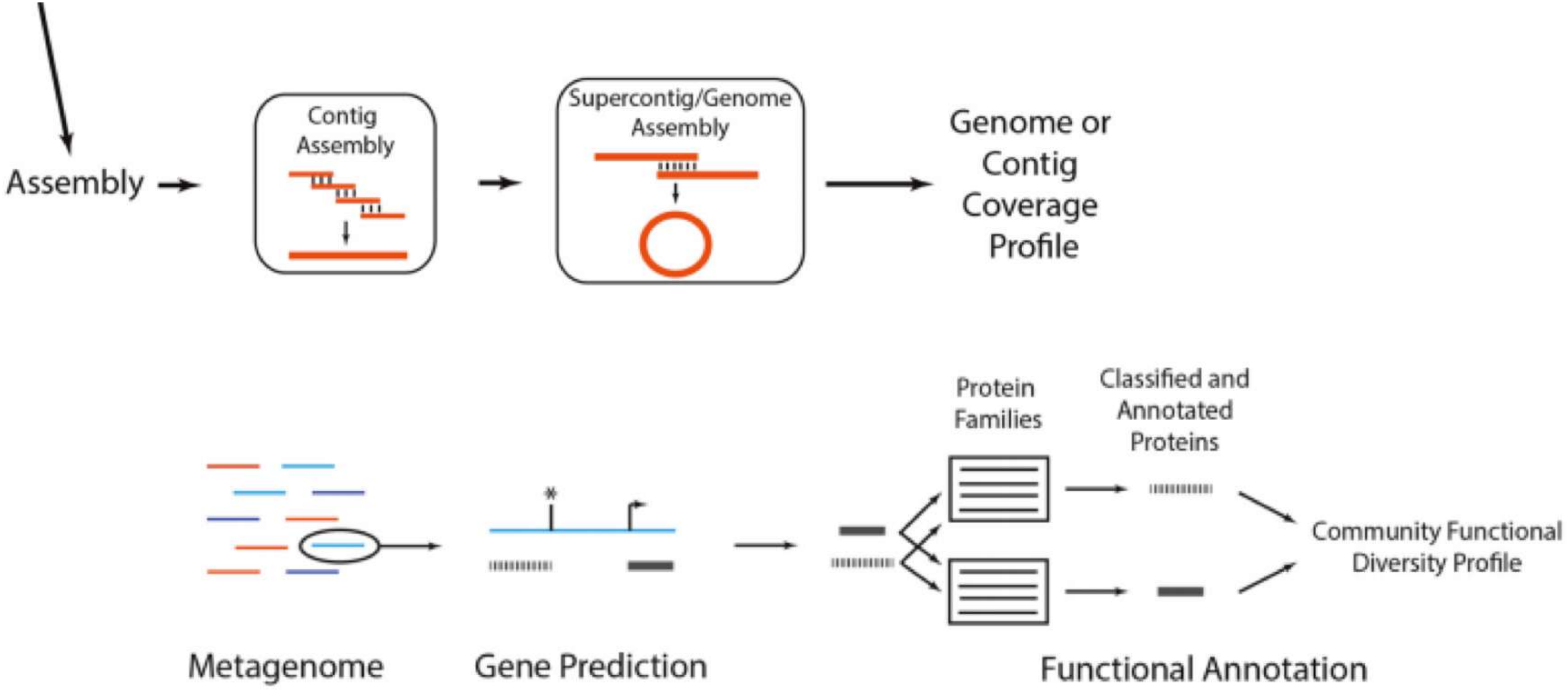
Example of binning based on differential coverage





H. Daims & C. Dorninger, DOME, University of Vienna

Actual assembly



Sharpton (2014)

Algorithm advancements lead to recovery of genomes

nature microbiology

ARTICLES

DOI: 10.1038/s41564-017-0012-7

OPEN

Corrected: Author correction

Recovery of nearly 8,000 metagenome-assembled genomes substantially expands the tree of life

Donovan H. Parks, Christian Rinke, Maria Chuvochina, Pierre-Alain Chaumeil, Ben J. Woodcroft, Paul N. Evans, Philip Hugenholtz* and Gene W. Tyson*

Biases

Extraction protocol matters

ELSEVIER

Soil Biology & Biochemistry 36 (2004) 1607–1614

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Impact of DNA extraction method on bacterial community composition measured by denaturing gradient gel electrophoresis

Julia R. de Lipthay[a,b], Christiane Enzinger[b,1], Kaare Johnsen[a,2], Jens Aamand[a], Søren J. Sørensen[b,*]

[a]Department of Geochemistry, Geological Survey of Denmark and Greenland, Øster Voldgade 10, DK-1350 Copenhagen K, Denmark
[b]Department of Microbiology, University of Copenhagen, Sølvgade 83H, DK-1307 Copenhagen K, Denmark

Received 1 September 2003; received in revised form 6 March 2004; accepted 15 March 2004

Abstract

The impact of DNA extraction protocol on soil DNA yield and bacterial community composition was evaluated. Three different procedures to physically disrupt cells were compared: sonication, grinding–freezing–thawing, and bead beating. The three protocols were applied to three different topsoils. For all soils, we found that each DNA extraction method resulted in unique community patterns as measured by denaturing gradient gel electrophoresis. This indicates the importance of the DNA extraction protocol on data for evaluating soil bacterial diversity. Consistently, the bead-beating procedure gave rise to the highest number of DNA bands, indicating the highest number of bacterial species. Supplementing the bead-beating procedure with additional cell-rupture steps generally did not change the bacterial community profile. The same consistency was not observed when evaluating the efficiency of the different methods on soil DNA yield. This parameter depended on soil type. The DNA size was of highest molecular weight with the sonication and grinding–freezing–thawing procedures (approx. 20 kb). In contrast, the inclusion of bead beating resulted in more sheared DNA (approx. 6–20 kb), and the longer the bead-beating time, the higher the fraction of low-molecular weight DNA. Clearly, the choice of DNA extraction protocol depends on soil type. We found, however, that for the analysis of indigenous soil bacterial communities the bead-beating procedure was appropriate because it is fast, reproducible, and gives very pure DNA of relatively high molecular weight. And very importantly, with this protocol the highest soil bacterial diversity was obtained. We believe that the choice of DNA extraction protocol will influence not only the determined phylogenetic diversity of indigenous microbial communities, but also the obtained functional diversity. This means that the detected presence of a

Wesolowska-Andersen et al. Microbiome 2014, 2:19
http://www.microbiomejournal.com/content/2/1/19

Microbiome

RESEARCH Open Access

Choice of bacterial DNA extraction method from fecal material influences community structure as evaluated by metagenomic analysis

Agata Wesolowska-Andersen[1], Martin Iain Bahl[2], Vera Carvalho[2], Karsten Kristiansen[3], Thomas Sicheritz-Pontén[1], Ramneek Gupta[1*] and Tine Rask Licht[2*]

Abstract

Background: In recent years, studies on the human intestinal microbiota have attracted tremendous attention. Application of next generation sequencing for mapping of bacterial phylogeny and function has opened new doors to this field of research. However, little attention has been given to the effects of choice of methodology on the output resulting from such studies.

Results: In this study we conducted a systematic comparison of the DNA extraction methods used by the two major collaborative efforts: The European MetaHIT and the American Human Microbiome Project (HMP). Additionally, effects of homogenizing the samples before extraction were addressed. We observed significant differences in distribution of bacterial taxa depending on the method. While eukaryotic DNA was most efficiently extracted by the MetaHIT protocol, DNA from bacteria within the Bacteroidetes phylum was most efficiently extracted by the HMP protocol.

Conclusions: Whereas it is comforting that the inter-individual variation clearly exceeded the variation resulting from choice of extraction method, our data highlight the challenge of comparing data across studies applying different methodologies.

"we found that each DNA extraction method resulted in unique community patterns"

"We observed significant differences in distribution of bacterial taxa depending on the method."

Alpha diversity is always overestimated

Table 1. Effect of quality filtering and clustering on diversity estimates (OTU number), error rate and data loss of pyrotags amplified from two regions of E. coli MG1655 16S rRNA genes.

	Number of OTUs at percentage identity thresholds							
Read filtering	100	99	98	97	95	90	% errorless reads	% reads used
5′ forward (V1 and V2)								
Theoretical number	5	4	3	1	1	1		
No quality filtering	643	95	31	16	5	3	68.7	77.9
Reads with N's removed	600	85	29	14	4	3	69.8	76.7
Quality score-based filtering (% per-base error probability)								
3	638	92	31	13	3	3	68.9	77.7
2	632	90	30	14	3	3	69.0	77.6
1	609	79	24	9	3	3	69.1	77.3
0.5	562	66	15	7	3	3	70.7	75.3
0.2	469	30	6	**3**	3	3	73.2	70.8
0.1	372	26	5	3	3	3	77.8	57.8
3′ reverse (V8)								
Theoretical number	1	1	1	1	1	1		
No quality filtering	385	43	13	7	5	4	84.6	94.4
Reads with N's removed	361	40	12	6	4	3	85.3	93.6
Quality score-based filtering (% per-base error probability)								
3	378	40	12	7	5	4	84.8	94.2
2	368	32	10	6	5	4	85.1	93.8
1	342	25	9	6	5	4	85.3	93.3
0.5	310	20	8	6	5	4	87.5	89.5
0.2	236	7	2	**2**	2	2	89.6	82.1
0.1	196	4	2	2	2	2	90.7	70.6

Diversity estimates should be considered relative to the theoretical number of OTUs from E. coli.

Kunin et al (2010)

Reagent and laboratory contamination

RESEARCH ARTICLE — Open Access

Reagent and laboratory contamination can critically impact sequence-based microbiome analyses

Susannah J Salter[1*], Michael J Cox[2], Elena M Turek[2], Szymon T Calus[3], William O Cookson[2], Miriam F Moffatt[2], Paul Turner[4,5], Julian Parkhill[1], Nicholas J Loman[3] and Alan W Walker[1,6*]

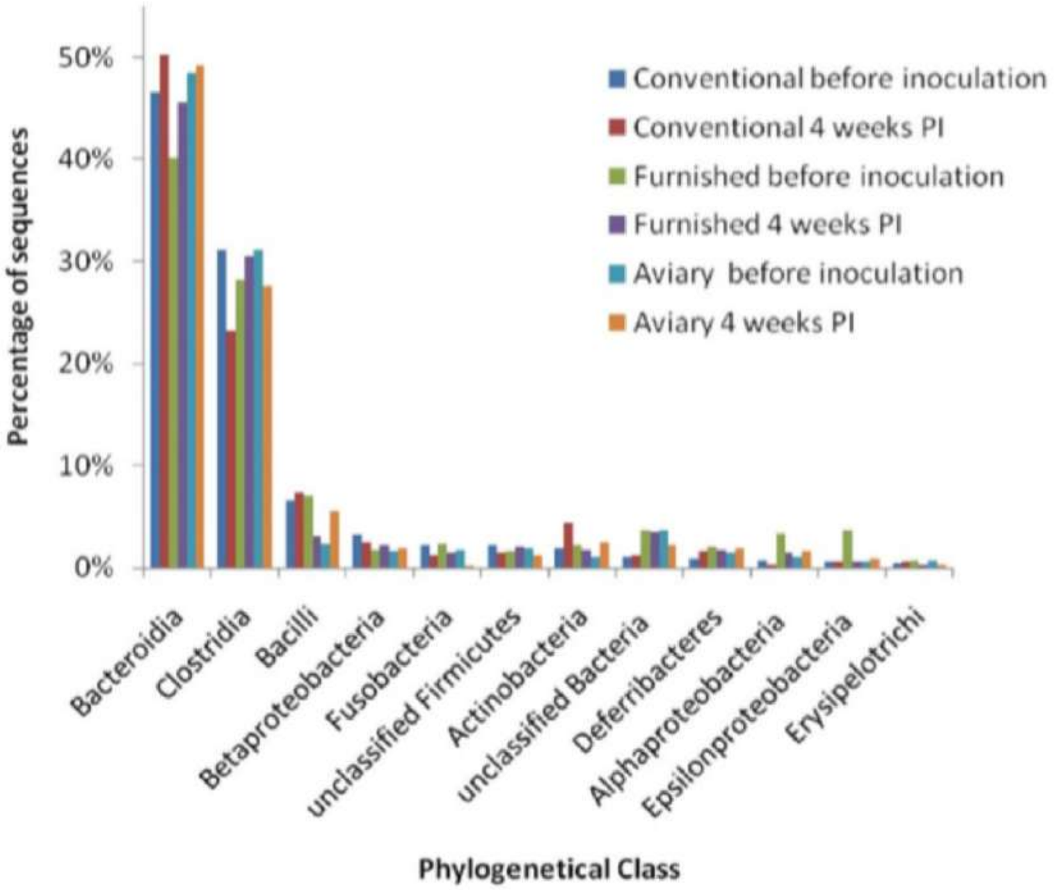
RESEARCH HIGHLIGHT

Tracking down the sources of experimental contamination in microbiome studies

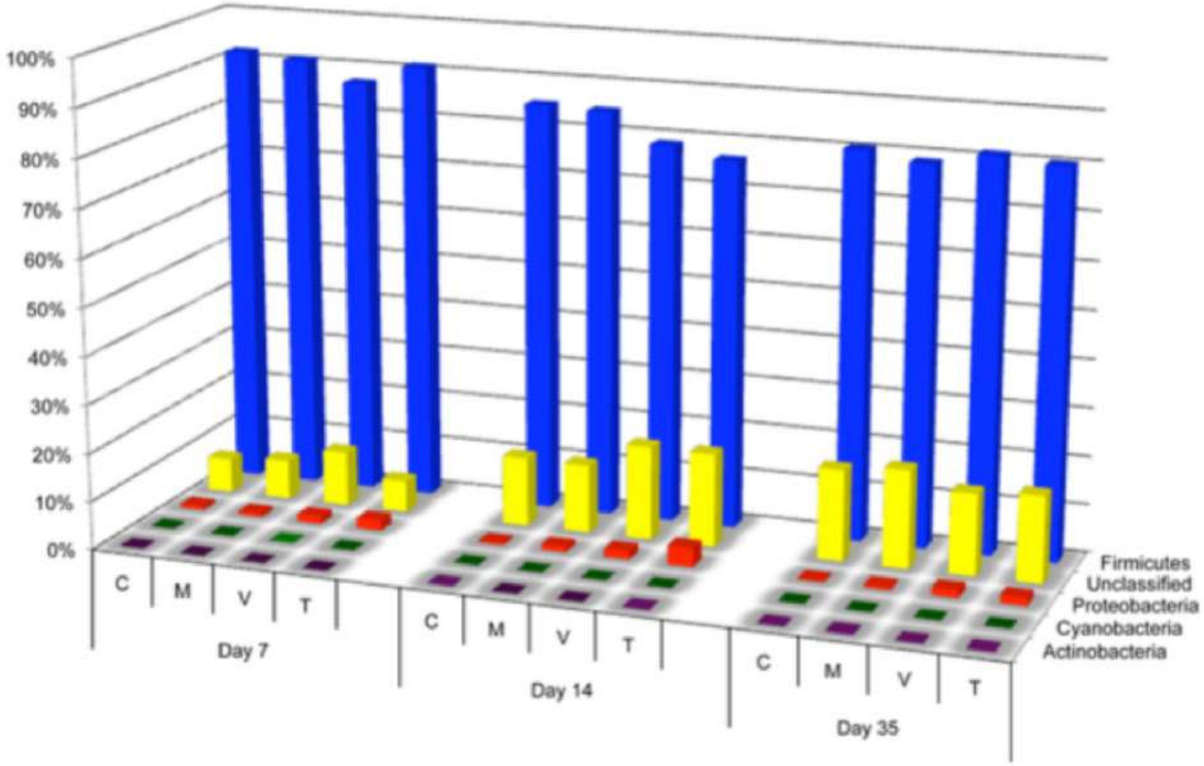
Sophie Weiss[1], Amnon Amir[2], Embriette R Hyde[2], Jessica L Metcalf[2], Se Jin Song[2] and Rob Knight[2,3,4*]

2 papers with different results at the same year

Bacteroidetes >>> rest



firmicutes >>> rest > bacteroidetes

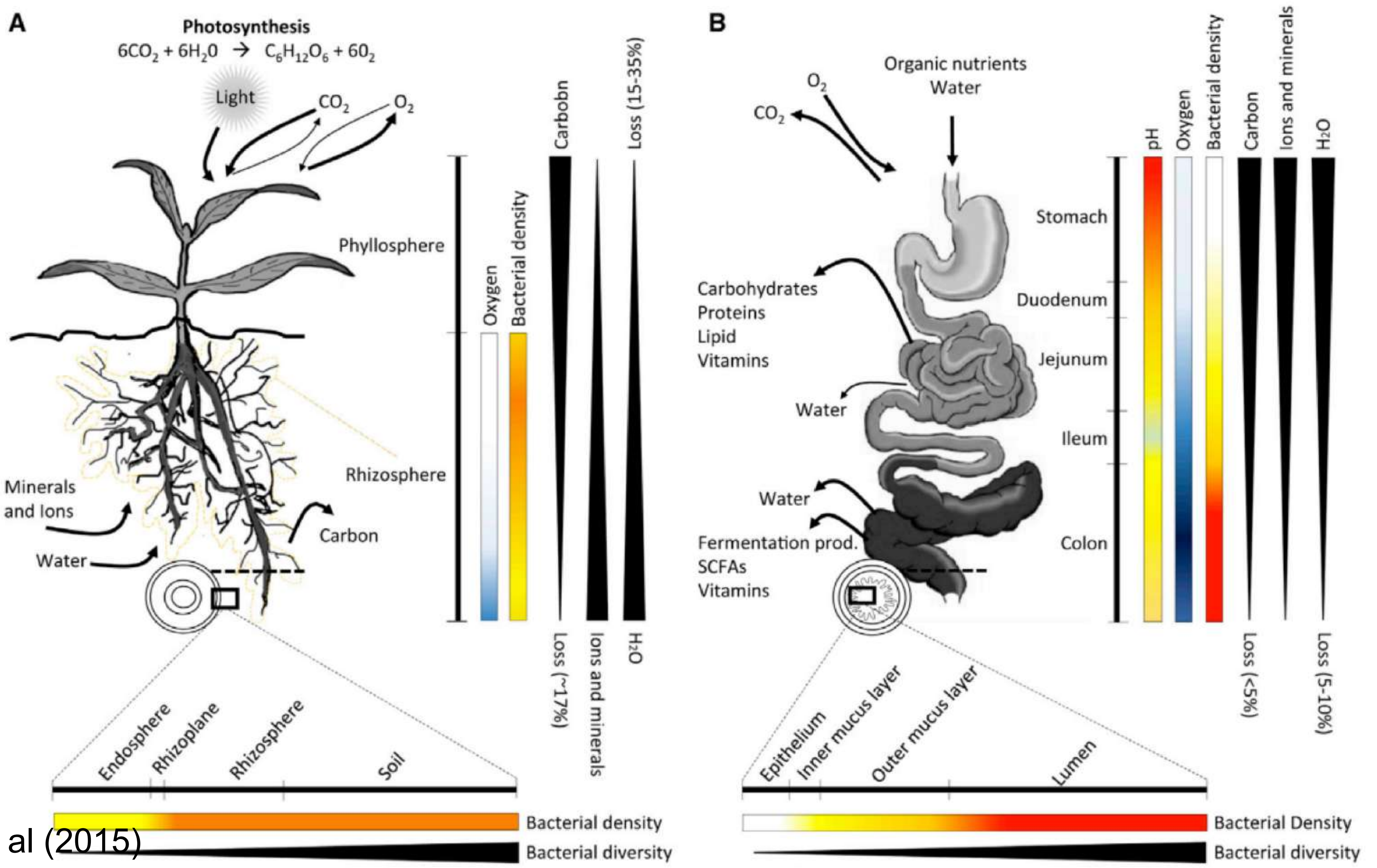


Nordentoft S *et al* (2011) BMC Microbiology

Danzeisen JL *et al* (2011) PLOS one

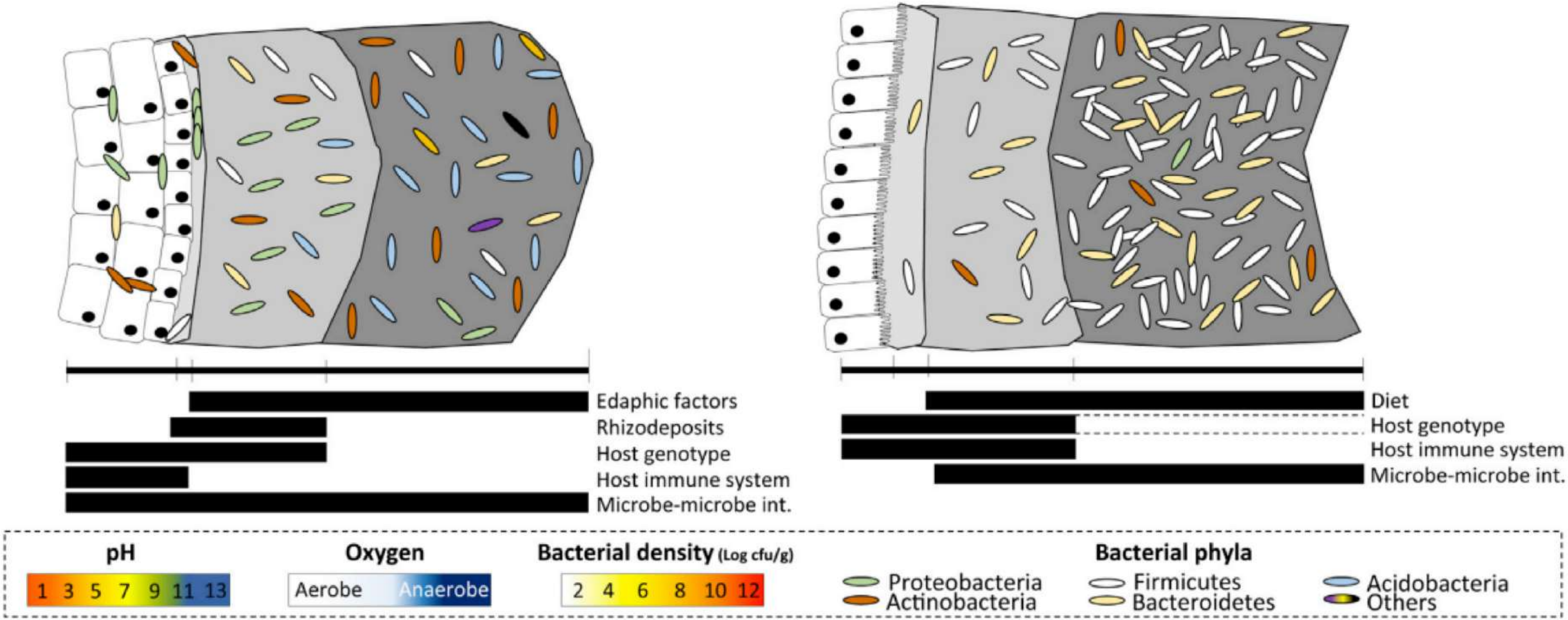
Case studies

Two most common systems



Hacquard et al (2015)

Two most common systems



Hacquard et al (2015)

Two most common systems

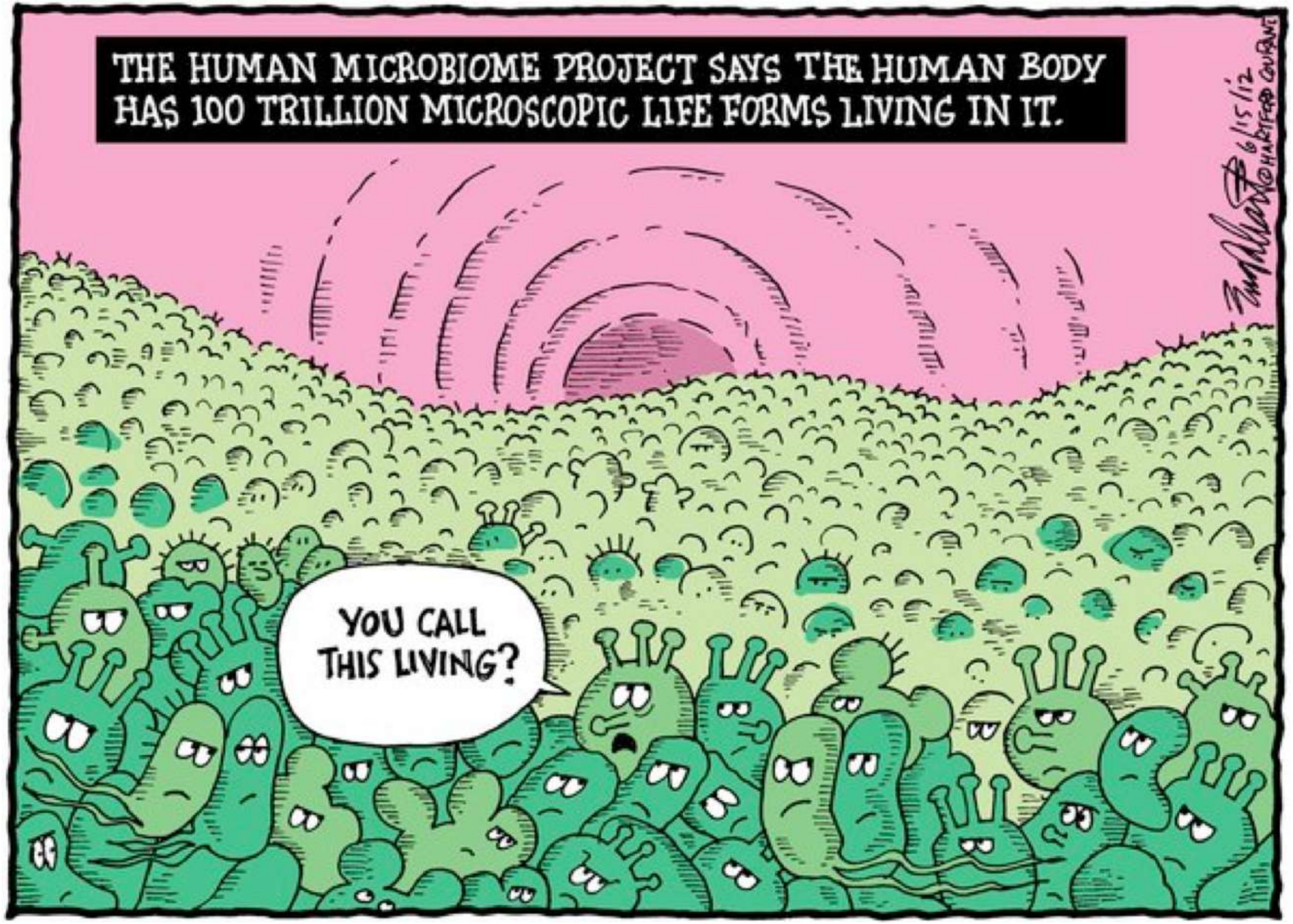
Table 1. Percentage of Shotgun Metagenome Reads Assigned to Each Kingdom of Life across Metagenome Studies

	Cucumber[a]	Wheat[a]	Soybean[b]	Wheat[c]	Oat[c]	Pea[c]	Barley[d]	Gut[e]
Bacteria	99.36	99.45	96	88.5	77.3	73.7	94.04	99.1
Archaea	0.02	0.02	<1	<0.5	<0.5	<0.5	0.054	
Eukaryotes	0.54	0.48	3	3.3	16.6	20.7	5.90	<0.1

[a] Ofek-Lalzar et al. (2014) (metagenomics of rhizoplane samples).
[b] Mendes et al. (2014) (metagenomics of rhizosphere samples).
[c] Turner et al. (2013) (metatranscriptomics of rhizosphere samples).
[d] Bulgarelli et al. (2015) (metagenomics of rhizosphere samples).
[e] Qin et al. (2010) (metagenomics of gut samples).

Hacquard et al (2015)

Human gut microbiome



Human gut microbiome

Vol 464 | 4 March 2010 | doi:10.1038/nature08821 nature

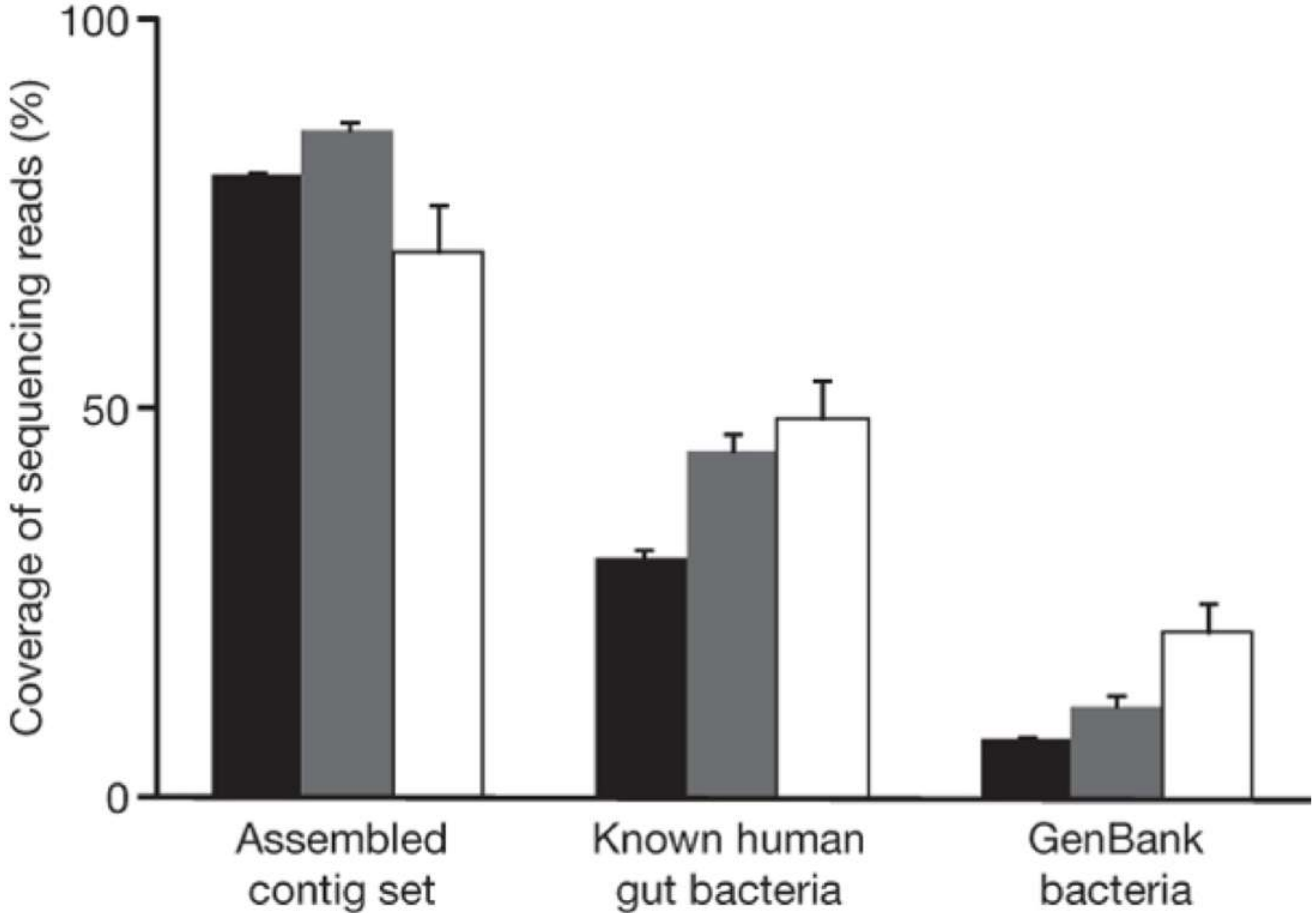
ARTICLES

A human gut microbial gene catalogue established by metagenomic sequencing

Junjie Qin[1]*, Ruiqiang Li[1]*, Jeroen Raes[2,3], Manimozhiyan Arumugam[2], Kristoffer Solvsten Burgdorf[4], Chaysavanh Manichanh[5], Trine Nielsen[4], Nicolas Pons[6], Florence Levenez[6], Takuji Yamada[2], Daniel R. Mende[2], Junhua Li[1,7], Junming Xu[1], Shaochuan Li[1], Dongfang Li[1,8], Jianjun Cao[1], Bo Wang[1], Huiqing Liang[1], Huisong Zheng[1], Yinlong Xie[1,7], Julien Tap[6], Patricia Lepage[6], Marcelo Bertalan[9], Jean-Michel Batto[6], Torben Hansen[4], Denis Le Paslier[10], Allan Linneberg[11], H. Bjørn Nielsen[9], Eric Pelletier[10], Pierre Renault[6], Thomas Sicheritz-Ponten[9], Keith Turner[12], Hongmei Zhu[1], Chang Yu[1], Shengting Li[1], Min Jian[1], Yan Zhou[1], Yingrui Li[1], Xiuqing Zhang[1], Songgang Li[1], Nan Qin[1], Huanming Yang[1], Jian Wang[1], Søren Brunak[9], Joel Doré[6], Francisco Guarner[5], Karsten Kristiansen[13], Oluf Pedersen[4,14], Julian Parkhill[12], Jean Weissenbach[10], MetaHIT Consortium†, Peer Bork[2], S. Dusko Ehrlich[6] & Jun Wang[1,13]

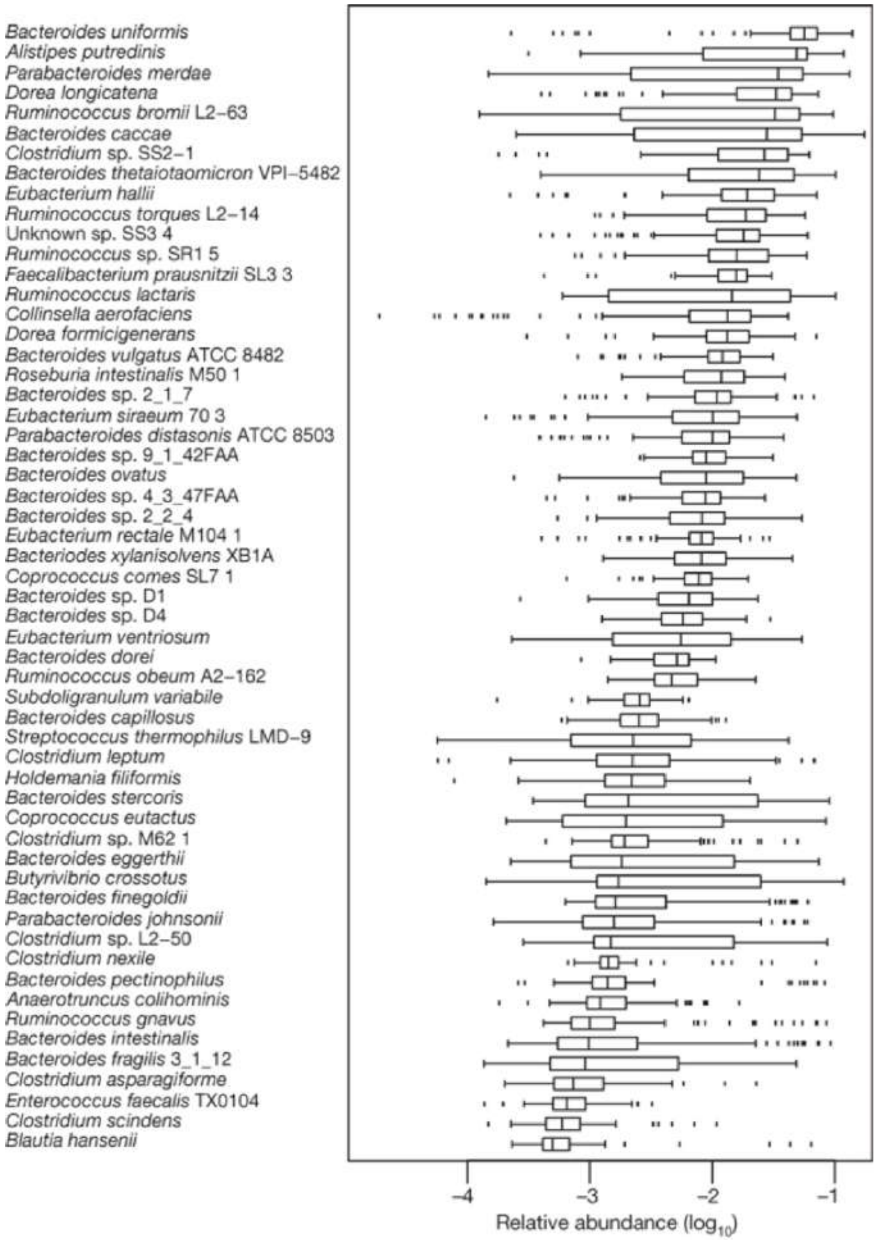
doi:10.1038/nature08821

Human gut microbiome



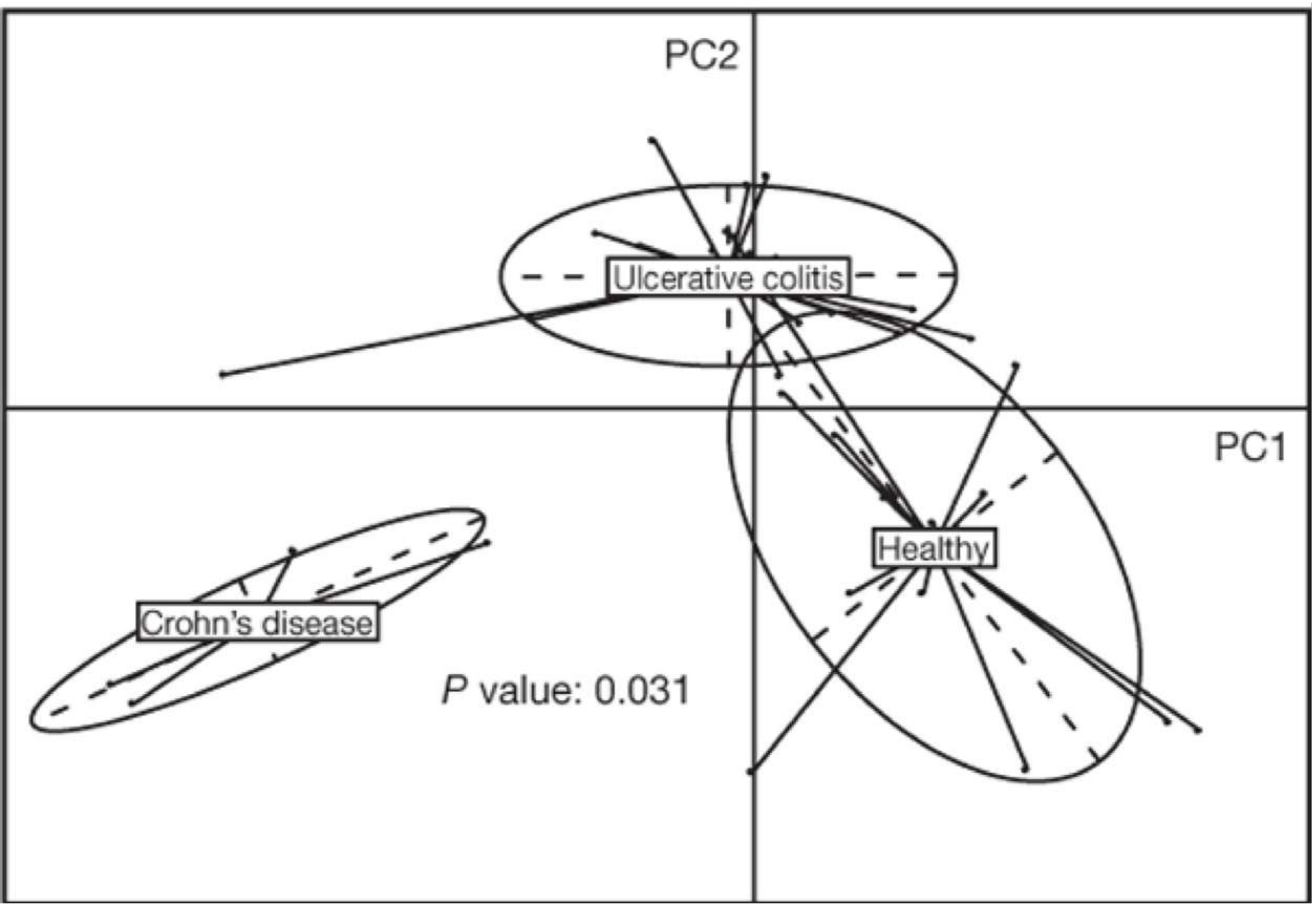
doi:10.1038/nature08821

Human gut microbiome



doi:10.1038/nature08821

Human gut microbiome

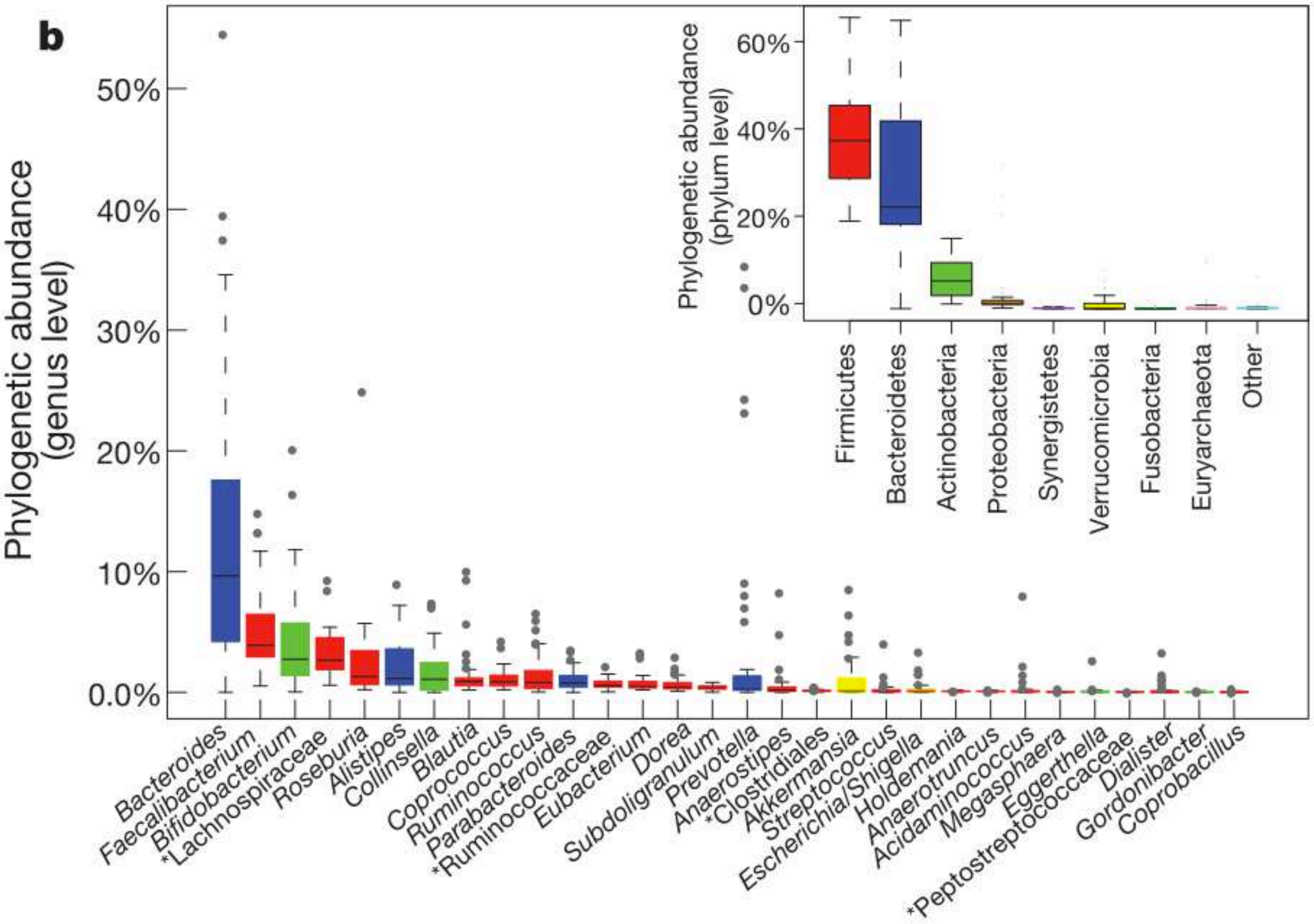


We can check which OTUs constitute the clustering (and separation) patterns

-> Biology
-> Biomarkers

doi:10.1038/nature08821

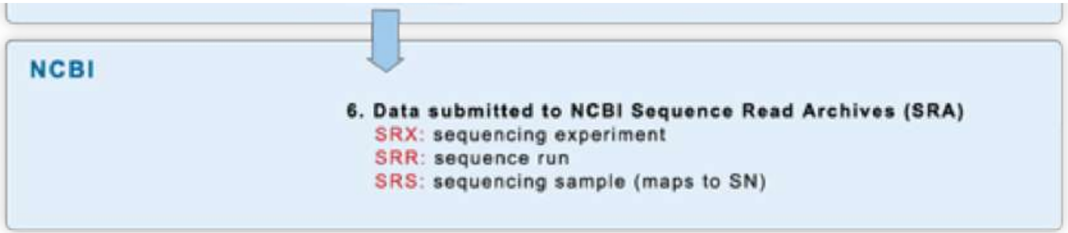
Human gut microbiome



doi:10.1038/nature09944

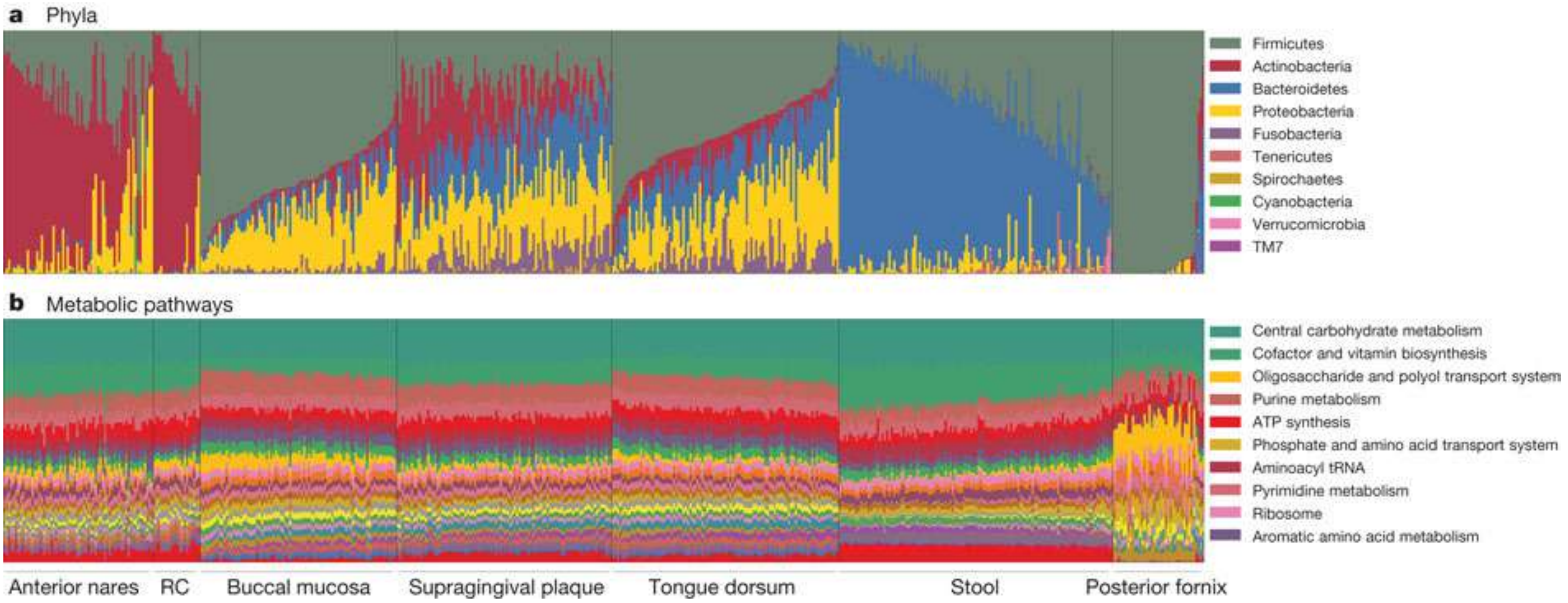
Table 1 | HMP donor samples examined by 16S and WGS

Body region	Body site	Total samples	Total 16S samples	V13 samples	V13 read depth (M)*	V35 samples	V35 read depth (M)*	Samples V13 and V35	Total WGS samples	Total read depth (G)†	Filtered reads (%)‡	Human reads (%)§	Remaining read depth (G)†	Samples 16S and WGS
Gut	Stool	352	337	193	1.4	328	2.4	184	136	1,720.7	15	1	1,450.6	124
Oral cavity	Buccal mucosa	346	330	184	1.3	314	1.7	168	107	1,438.0	9	82	136.7	91
	Hard palate	325	325	179	1.2	310	1.7	164	1	10.9	20	25	5.9	1
	Keratinized gingiva	335	329	183	1.3	319	1.7	173	6	72.3	5	47	34.4	0
	Palatine tonsils	337	332	189	1.2	315	1.9	172	6	74.8	2	80	13.5	1
	Saliva	315	310	166	0.9	292	1.5	148	5	55.7	1	91	4.2	0
	Subgingival plaque	334	328	186	1.2	314	1.8	172	7	92.1	5	79	15.3	1
	Supragingival plaque	345	331	192	1.3	316	1.9	177	115	1,500.7	15	40	674.8	101
	Throat	331	325	176	1.0	312	1.7	163	7	78.8	4	79	13.6	1
	Tongue dorsum	348	332	193	1.3	320	2.0	181	122	1,620.1	15	19	1,084.3	106
Airway	Anterior nares	316	302	169	1.0	283	1.2	150	84	1,129.9	3	96	14.3	70
Skin	Left antecubital fossa	269	269	158	0.7	221	0.5	110	0	NA	NA	NA	0	NA
	Left retroauricular crease	313	312	188	1.6	295	1.5	171	9	126.3	9	73	22.1	8
	Right antecubital fossa	274	274	158	0.7	229	0.5	113	0	NA	NA	NA	0	NA
	Right retroauricular crease	319	316	190	1.4	304	1.6	178	15	181.9	18	59	42.4	12
Vagina	Mid-vagina	145	143	91	0.6	140	1.0	88	2	22.6	0	99	0.2	0
	Posterior fornix	152	142	89	0.6	136	1.0	83	53	702.1	6	90	25.2	43
	Vaginal introitus	142	140	87	0.6	131	0.9	78	3	36.5	1	98	0.6	1
	Total	5,298	5,177	2,971	19	4,879	26.3	2,673	681	8,863.3	11	49	3,538.1	560



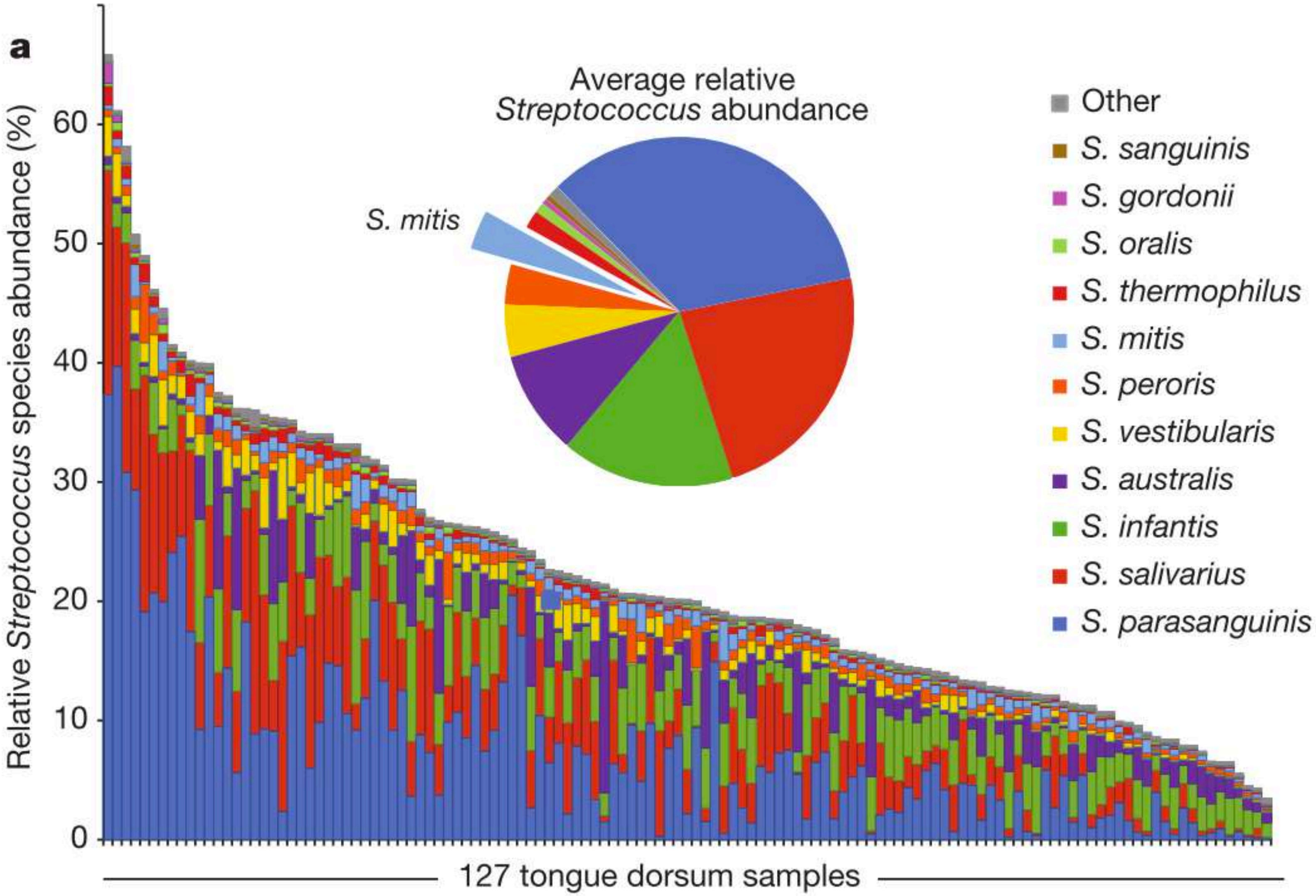
doi:10.1038/nature11209

Human microbiome



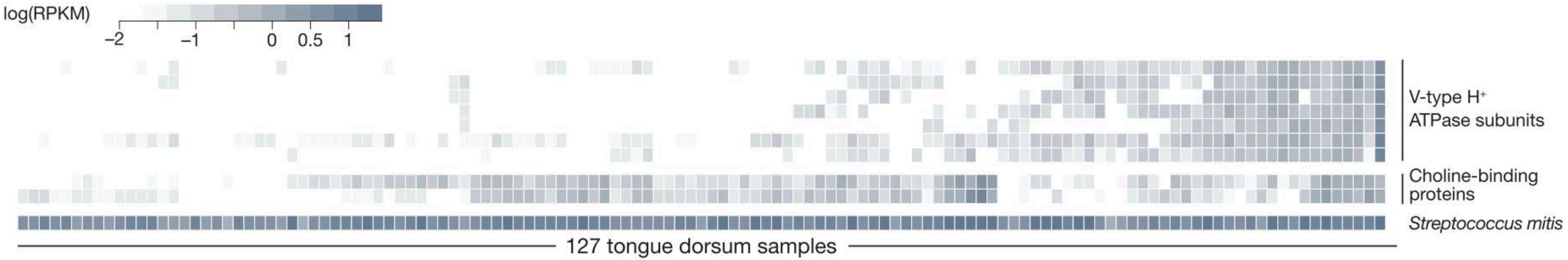
doi:10.1038/nature11234

Inter-individual variation in the microbiome proved to be specific, functionally relevant and personalized



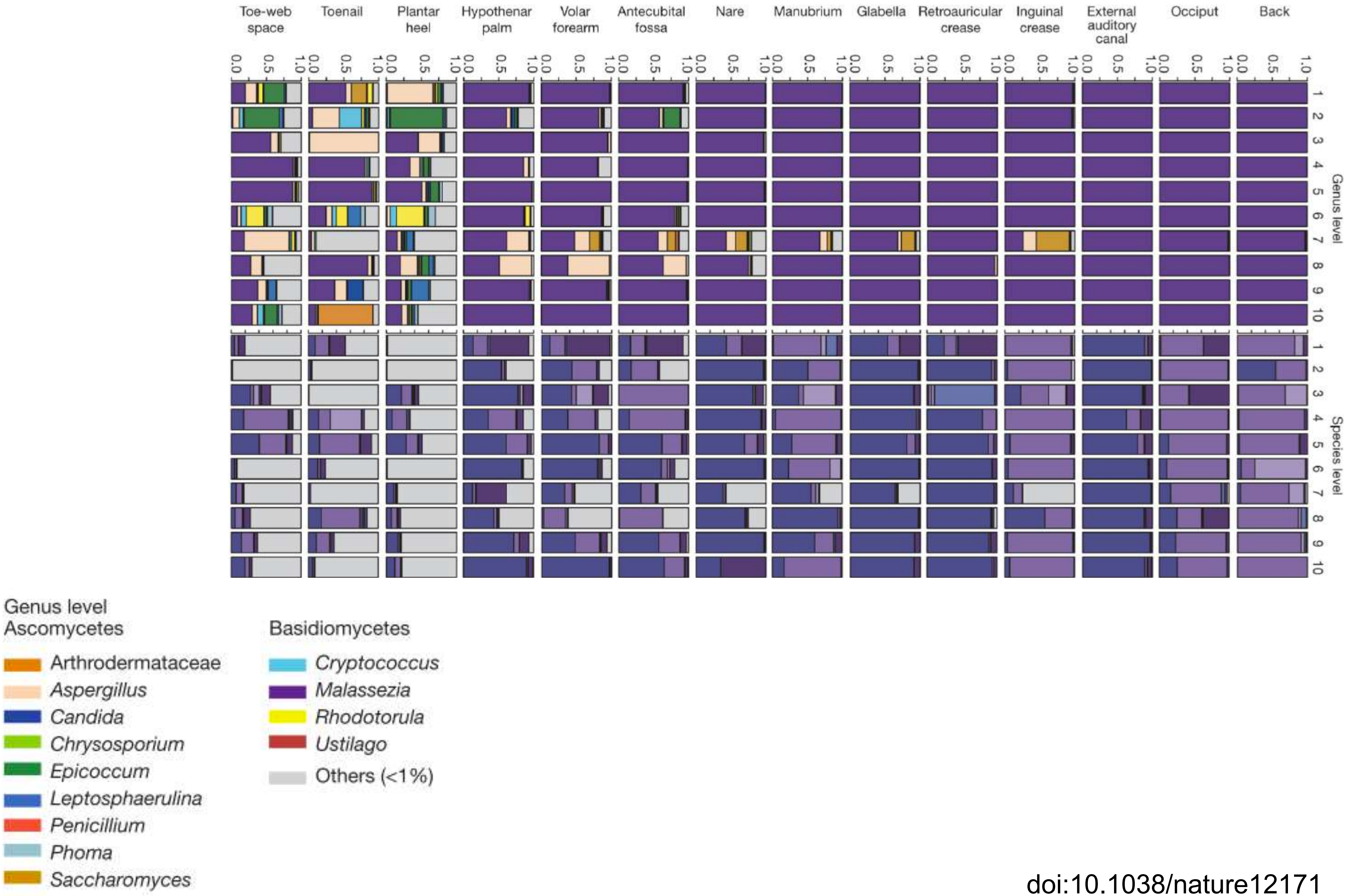
doi:10.1038/nature11234

Gene loss & Structural variants are common



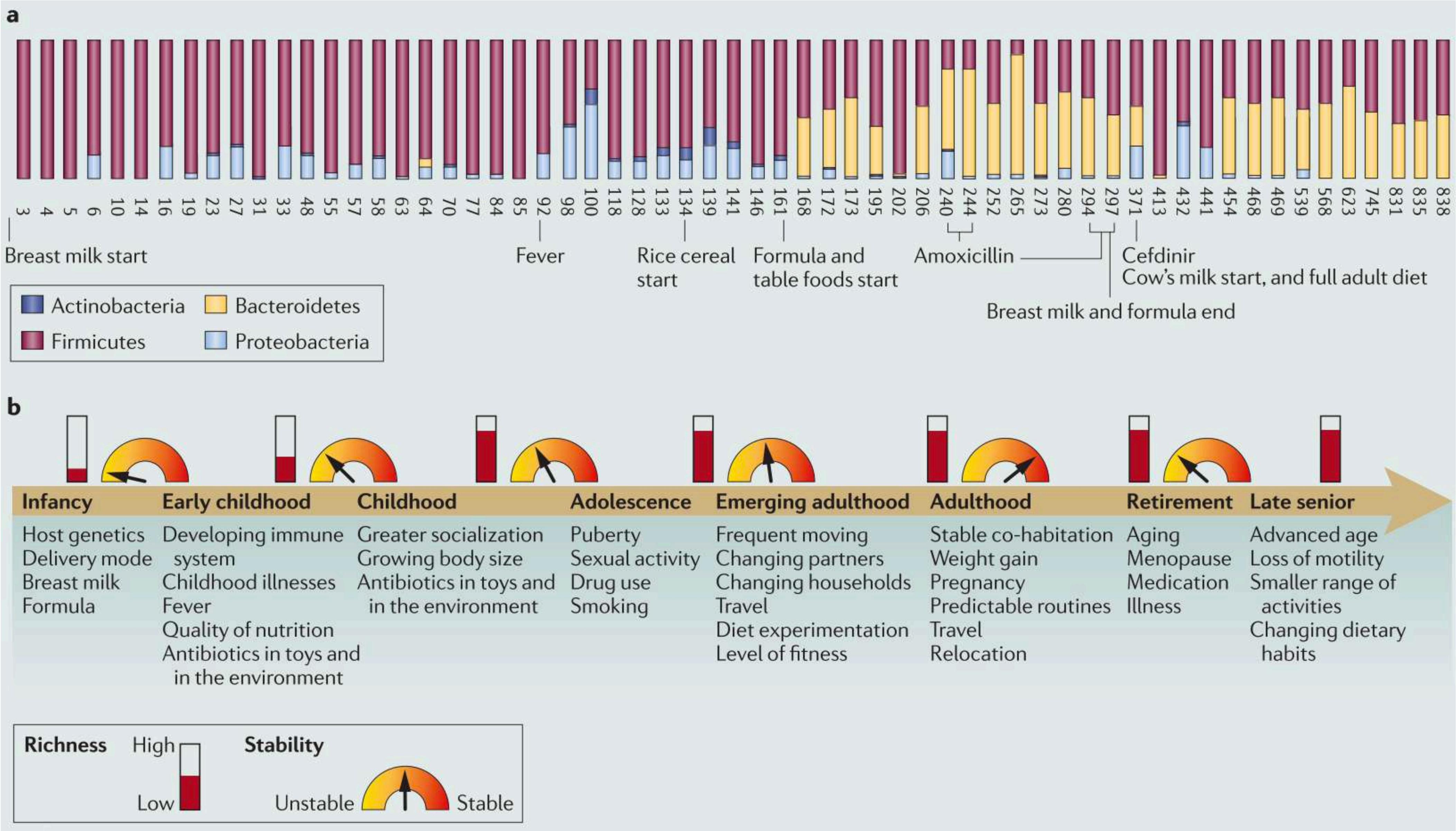
doi:10.1038/nature11234

Skins



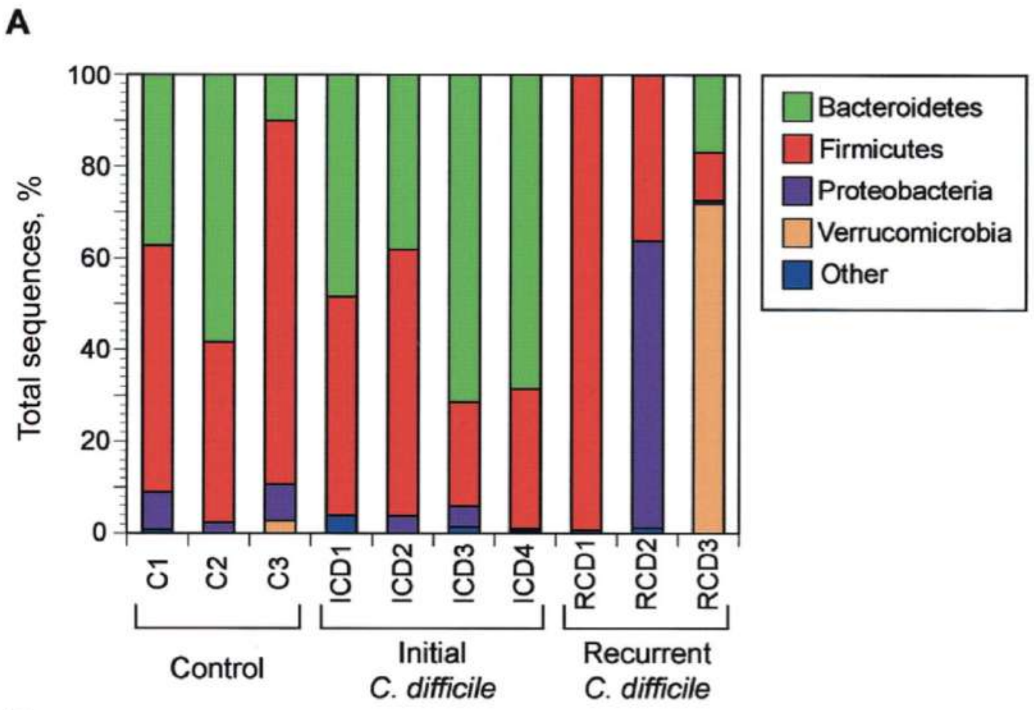
doi:10.1038/nature12171

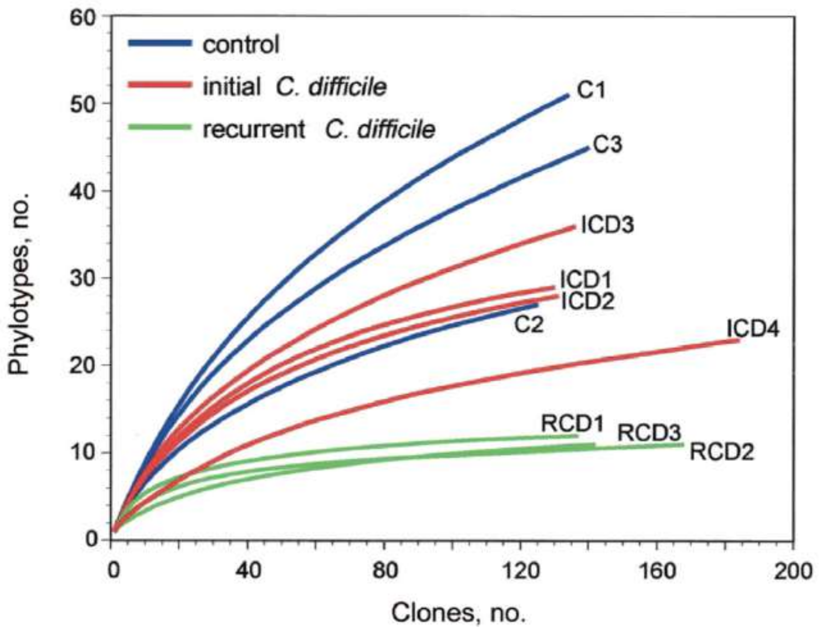
The gut microbiome during life



doi:10.1038/nrmicro2540

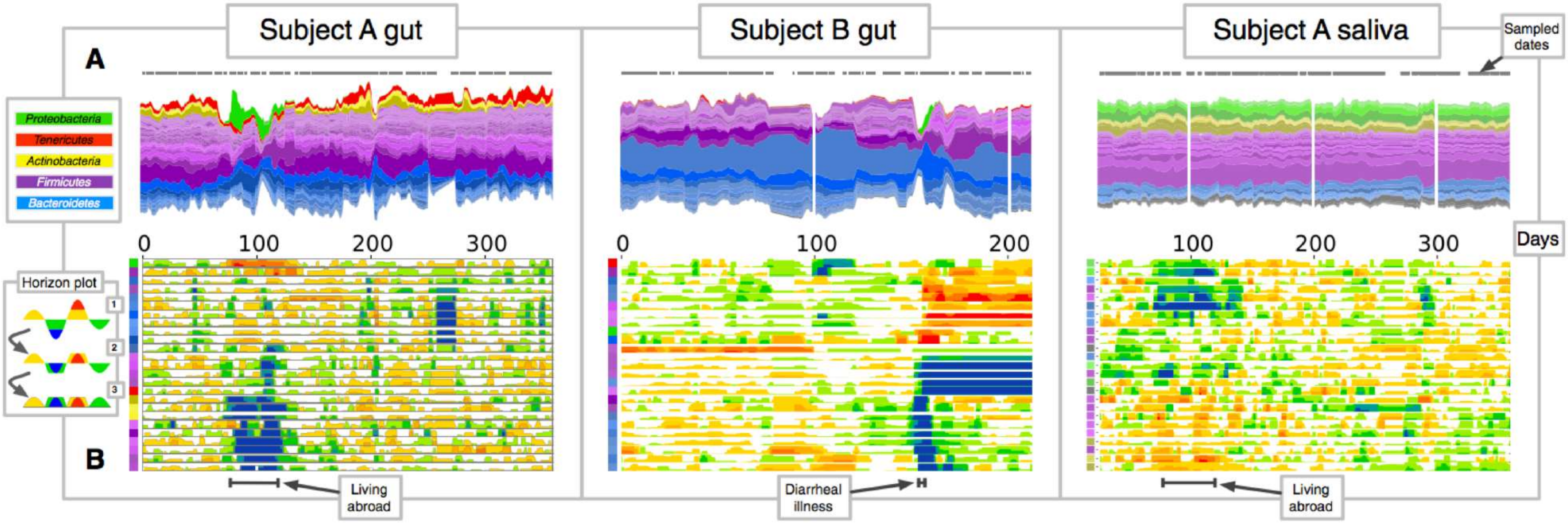
Decreased diversity with *Clostridium difficile* – assciated diarrhea





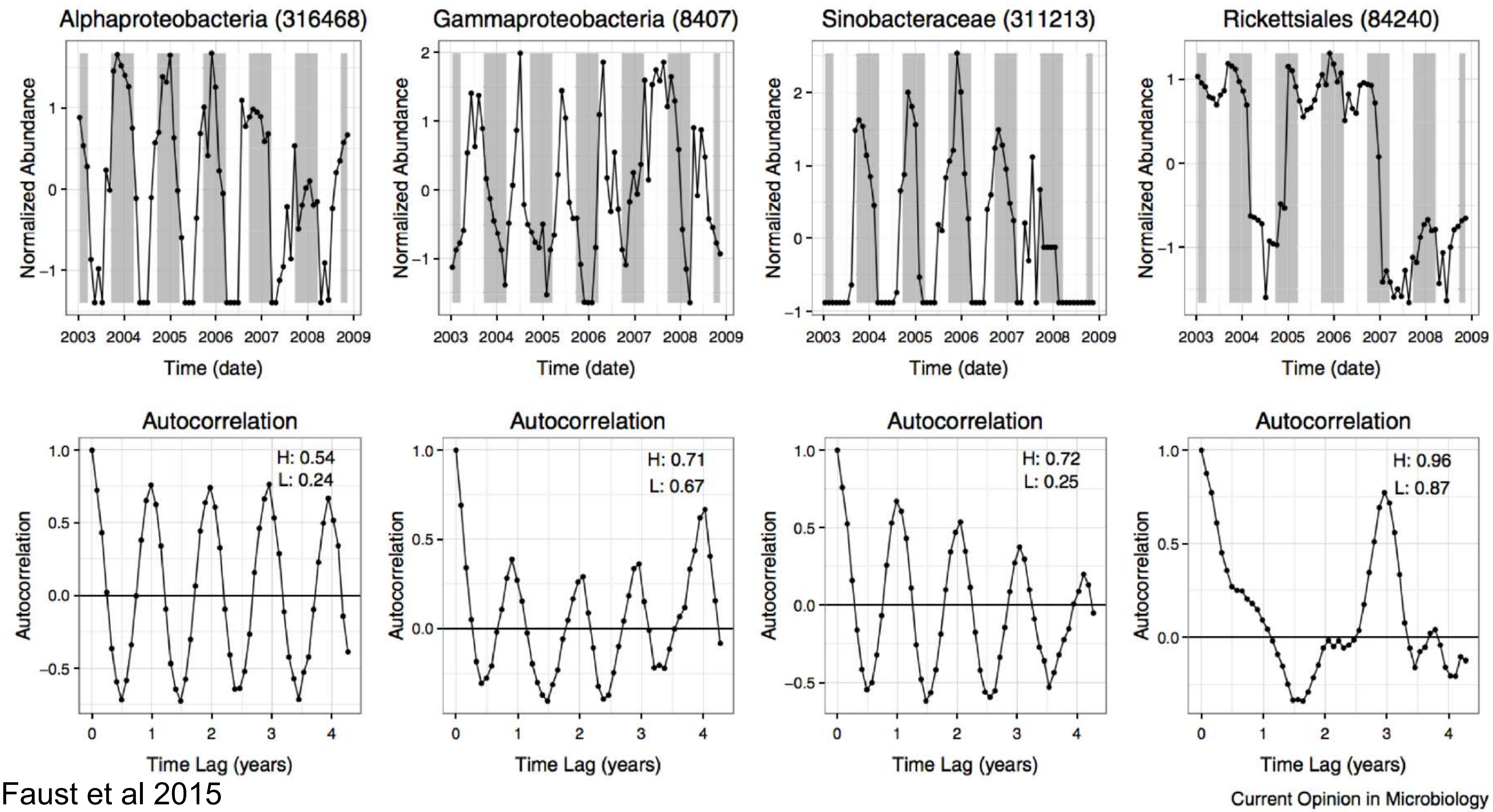
doi:10.1086/525047

Tracking microbiome on a daily scale



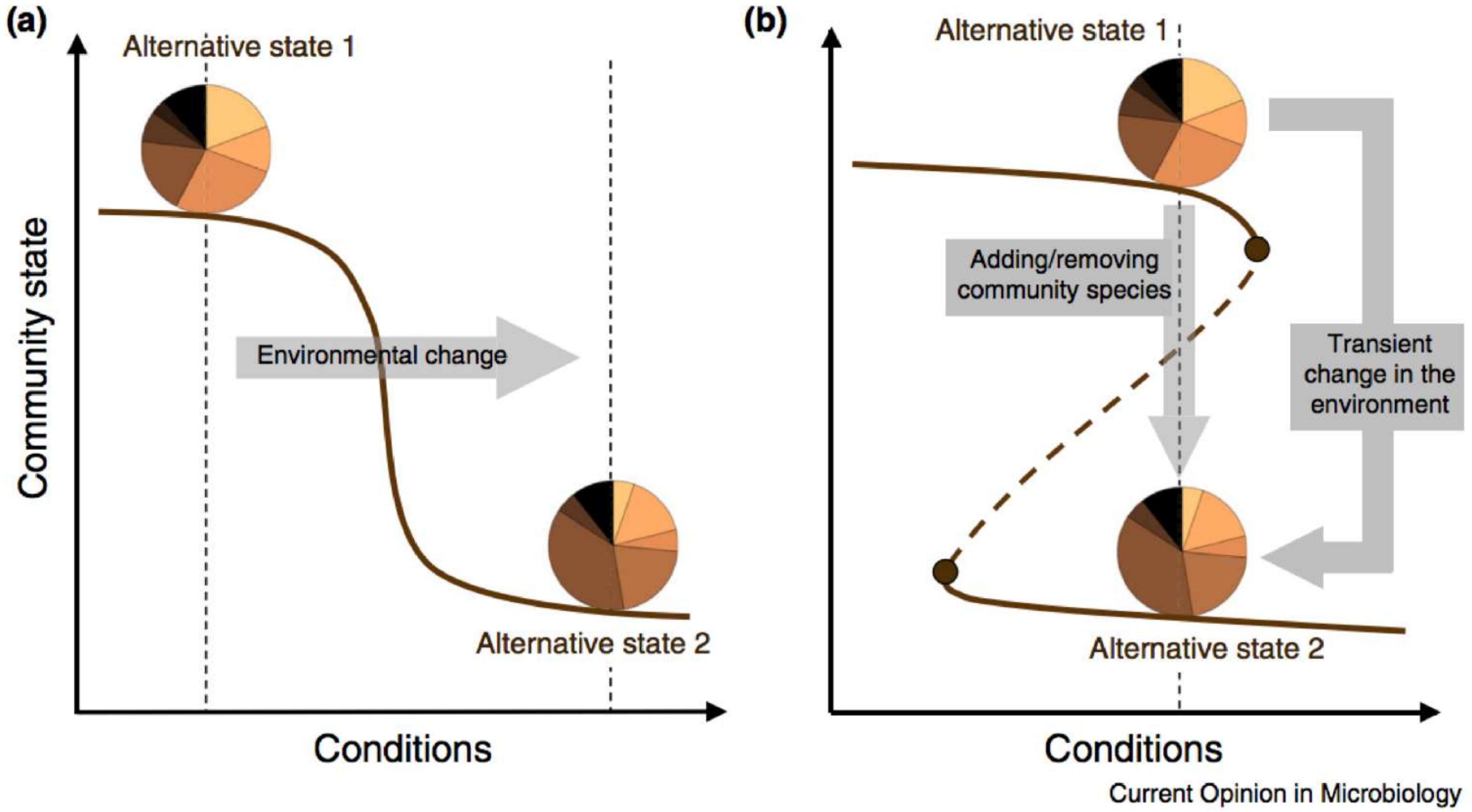
David et al. Genome Biology 2014, 15:R89

Tracking microbiome spanning 6 years



Faust et al 2015

Tracking microbiome on a daily scale



Faust et al 2015

Question: What community gets reset and what don't?

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A. Shade, J.S. Read, N.D. Youngblut, N. Fierer, R. Knight, T.K. Kratz, N.R. Lottig, E.E. Roden, E.H. Stanley, J. Stombaugh, et al.
Lake microbial communities are resilient after a whole-ecosystem disturbance
ISME J, 6 (2012), pp. 2153–2167

Yes

L. Dethlefsen, D.A. Relman
Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation
Proc Natl Acad Sci U S A, 108 (2011), pp. 4554–4561

No

L.A. David, A.C. Materna, J. Friedman, M.I. Campos-Baptista, M.C. Blackburn, A. Perrotta, S.E. Erdman, E.J. Alm
Host lifestyle affects human microbiota on daily timescales
Genome Biol, 15 (2014), p. R89

Yes and No

Faust et al 2015

ARTICLE

OPEN

doi:10.1038/nature23889

Strains, functions and dynamics in the expanded Human Microbiome Project

Jason Lloyd-Price[1,2]*, Anup Mahurkar[3]*, Gholamali Rahnavard[1,2], Jonathan Crabtree[3], Joshua Orvis[3], A. Brantley Hall[2], Arthur Brady[3], Heather H. Creasy[3], Carrie McCracken[3], Michelle G. Giglio[3], Daniel McDonald[4], Eric A. Franzosa[1,2], Rob Knight[4,5], Owen White[3] & Curtis Huttenhower[1,2]

The characterization of baseline microbial and functional diversity in the human microbiome has enabled studies of microbiome-related disease, diversity, biogeography, and molecular function. The National Institutes of Health Human Microbiome Project has provided one of the broadest such characterizations so far. Here we introduce a second wave of data from the study, comprising 1,631 new metagenomes (2,355 total) targeting diverse body sites with multiple time points in 265 individuals. We applied updated profiling and assembly methods to provide new characterizations of microbiome personalization. Strain identification revealed subspecies clades specific to body sites; it also quantified species with phylogenetic diversity under-represented in isolate genomes. Body-wide functional profiling classified pathways into universal, human-enriched, and body site-enriched subsets. Finally, temporal analysis decomposed microbial variation into rapidly variable, moderately variable, and stable subsets. This study furthers our knowledge of baseline human microbial diversity and enables an understanding of personalized microbiome function and dynamics.

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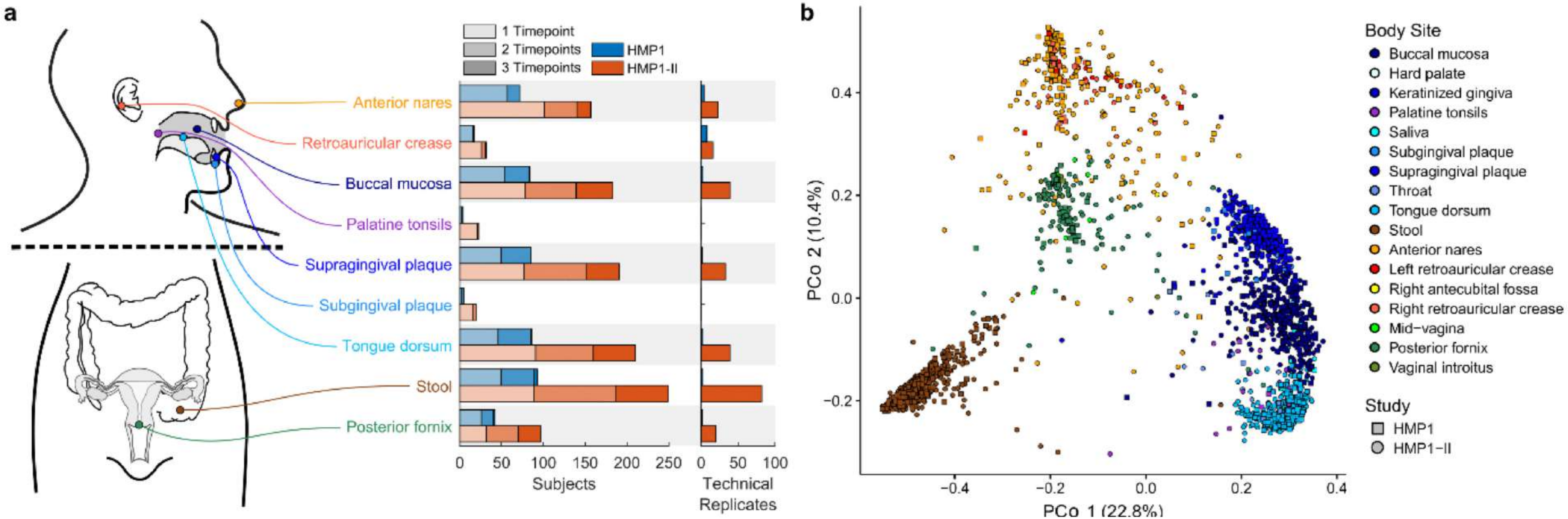
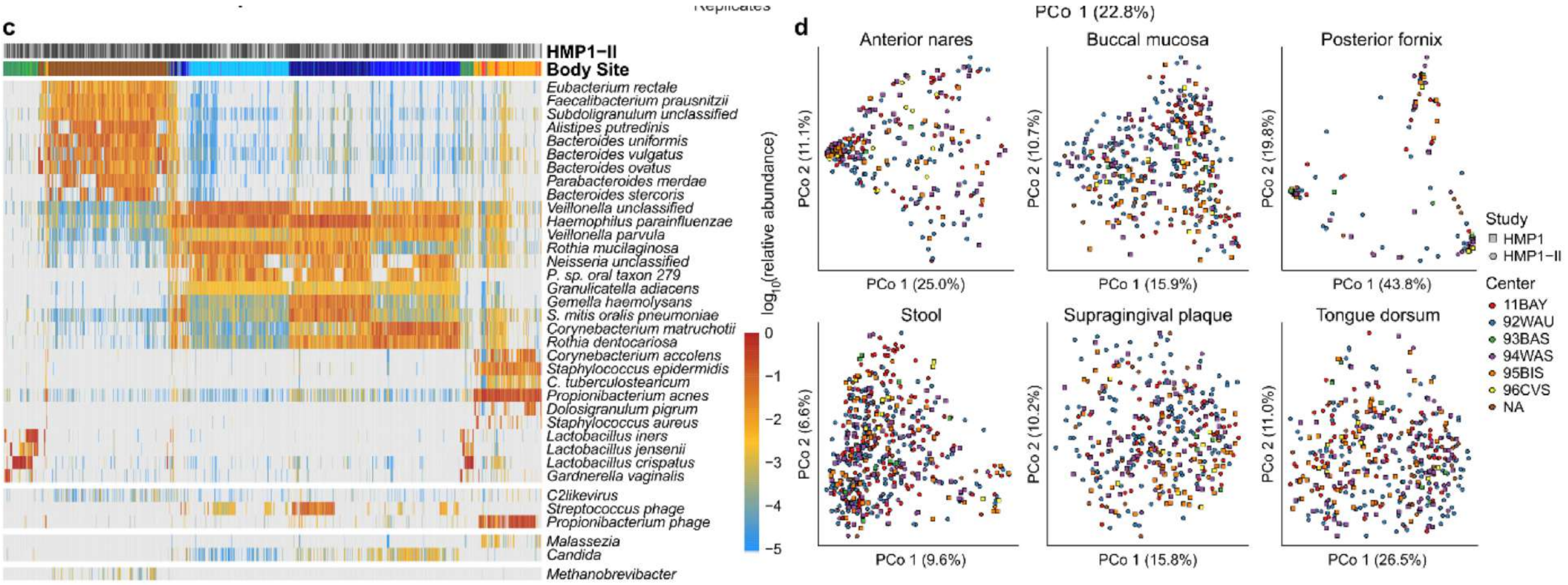
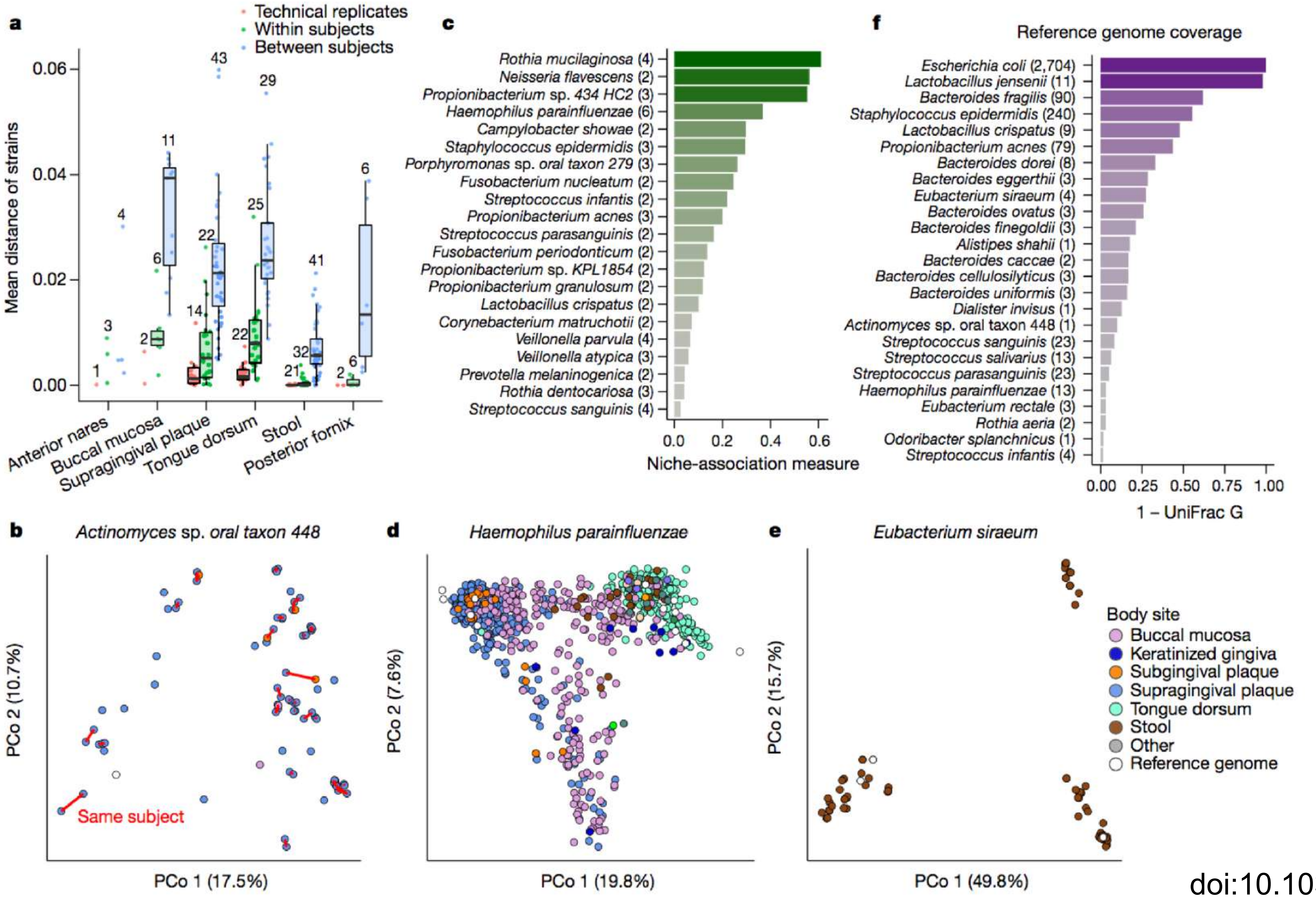
Published online 20 September 2017.



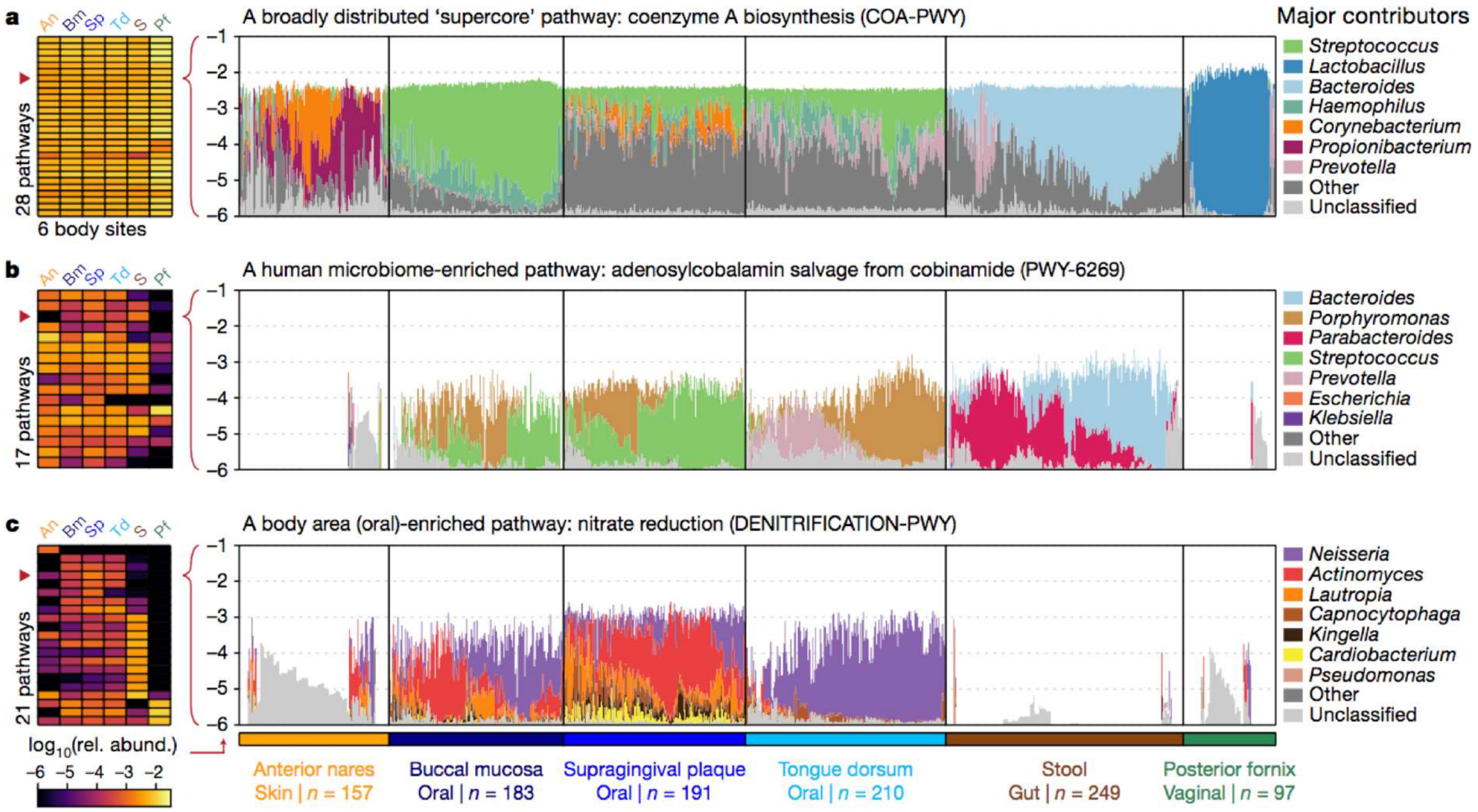
Extended Data Figure 1 | Extended body-wide metagenomic taxonomic profiles in HMP1-II. a, The combined HMP1-II datasets include a total of 2,355 metagenomes (724 previously published and 1,631 new, including 252 technical replicates). These span the project's six targeted body sites (anterior nares, buccal mucosa, supragingival plaque, tongue dorsum, stool, and posterior fornix) in addition to at least 20 samples each from 3 additional sites, of the 18 total sampled sites: retroauricular crease, palatine tonsils, and subgingival plaque. Metagenomes are now available for at least one body site for a total of 265 individuals. **b**, PCoA using Bray–Curtis distances among all microbes at the species level. **c**, Relative abundances of the most prevalent and abundant microbes (bacterial, viral, eukaryotic, and archaeal) among all body sites, as profiled by MetaPhlAn2[20]. Prevalent eukaryotic microbes are shown at the genus level. **d**, Taxonomic profiles do not vary more between sequencing centres, batches, or clinical centres than they do among individuals within body sites. Ordinations show Bray–Curtis principal coordinates of species-level abundances at each body site. Within-site ecological structure is as expected[1], with no divergence associated with technical variables along the first two ordination axes.



Extended Data Figure 1 | Extended body-wide metagenomic taxonomic profiles in HMP1-II. a, The combined HMP1-II datasets include a total of 2,355 metagenomes (724 previously published and 1,631 new, including 252 technical replicates). These span the project's six targeted body sites (anterior nares, buccal mucosa, supragingival plaque, tongue dorsum, stool, and posterior fornix) in addition to at least 20 samples each from 3 additional sites, of the 18 total sampled sites: retroauricular crease, palatine tonsils, and subgingival plaque. Metagenomes are now available for at least one body site for a total of 265 individuals. **b**, PCoA using Bray–Curtis distances among all microbes at the species level. **c**, Relative abundances of the most prevalent and abundant microbes (bacterial, viral, eukaryotic, and archaeal) among all body sites, as profiled by MetaPhlAn2[20]. Prevalent eukaryotic microbes are shown at the genus level. **d**, Taxonomic profiles do not vary more between sequencing centres, batches, or clinical centres than they do among individuals within body sites. Ordinations show Bray–Curtis principal coordinates of species-level abundances at each body site. Within-site ecological structure is as expected[1], with no divergence associated with technical variables along the first two ordination axes.



doi:10.1038/nature23889



a
An Bm Sp Td S Pf
28 pathways
6 body sites
A broadly distributed 'supercore' pathway: coenzyme A biosynthesis (COA-PWY)
Major contributors
Streptococcus
Lactobacillus
Bacteroides
Haemophilus
Corynebacterium
Propionibacterium
Prevotella
Other
Unclassified
b
An Bm Sp Td S Pf
17 pathways
A human microbiome-enriched pathway: adenosylcobalamin salvage from cobinamide (PWY-6269)
Bacteroides
Porphyromonas
Parabacteroides
Streptococcus
Prevotella
Escherichia
Klebsiella
Other
Unclassified
c
An Bm Sp Td S Pf
21 pathways
A body area (oral)-enriched pathway: nitrate reduction (DENITRIFICATION-PWY)
Neisseria
Actinomyces
Lautropia
Capnocytophaga
Kingella
Cardiobacterium
Pseudomonas
Other
Unclassified
−1
−2
−3
−4
−5
−6
log10(rel. abund.)
−6 −5 −4 −3 −2
Anterior nares
Skin | n = 157
Buccal mucosa
Oral | n = 183
Supragingival plaque
Oral | n = 191
Tongue dorsum
Oral | n = 210
Stool
Gut | n = 249
Posterior fornix
Vaginal | n = 97

doi:10.1038/nature25973

Environment dominates over host genetics in shaping human gut microbiota

Daphna Rothschild[1,2]*, Omer Weissbrod[1,2]*, Elad Barkan[1,2]*, Alexander Kurilshikov[3], Tal Korem[1,2], David Zeevi[1,2], Paul I. Costea[1,2], Anastasia Godneva[1,2], Iris N. Kalka[1,2], Noam Bar[1,2], Smadar Shilo[1,2], Dar Lador[1,2], Arnau Vich Vila[3,4], Niv Zmora[5,6,7], Meirav Pevsner-Fischer[5], David Israeli[8], Noa Kosower[1,2], Gal Malka[1,2], Bat Chen Wolf[1,2], Tali Avnit-Sagi[1,2], Maya Lotan-Pompan[1,2], Adina Weinberger[1,2], Zamir Halpern[7,9], Shai Carmi[10], Jingyuan Fu[3,11], Cisca Wijmenga[3,12], Alexandra Zhernakova[3], Eran Elinav[5]§ & Eran Segal[1,2]§

Human gut microbiome composition is shaped by multiple factors but the relative contribution of host genetics remains elusive. Here we examine genotype and microbiome data from 1,046 healthy individuals with several distinct ancestral origins who share a relatively common environment, and demonstrate that the gut microbiome is not significantly associated with genetic ancestry, and that host genetics have a minor role in determining microbiome composition. We show that, by contrast, there are significant similarities in the compositions of the microbiomes of genetically unrelated individuals who share a household, and that over 20% of the inter-person microbiome variability is associated with factors related to diet, drugs and anthropometric measurements. We further demonstrate that microbiome data significantly improve the prediction accuracy for many human traits, such as glucose and obesity measures, compared to models that use only host genetic and environmental data. These results suggest that microbiome alterations aimed at improving clinical outcomes may be carried out across diverse genetic backgrounds.

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Published online 28 February 2018.

doi:10.1038/nature25973

- 1,046 healthy Israeli adults
- 16S rRNA + metagenomics
- Genotyping 712,540 SNPs
- Questionnaires

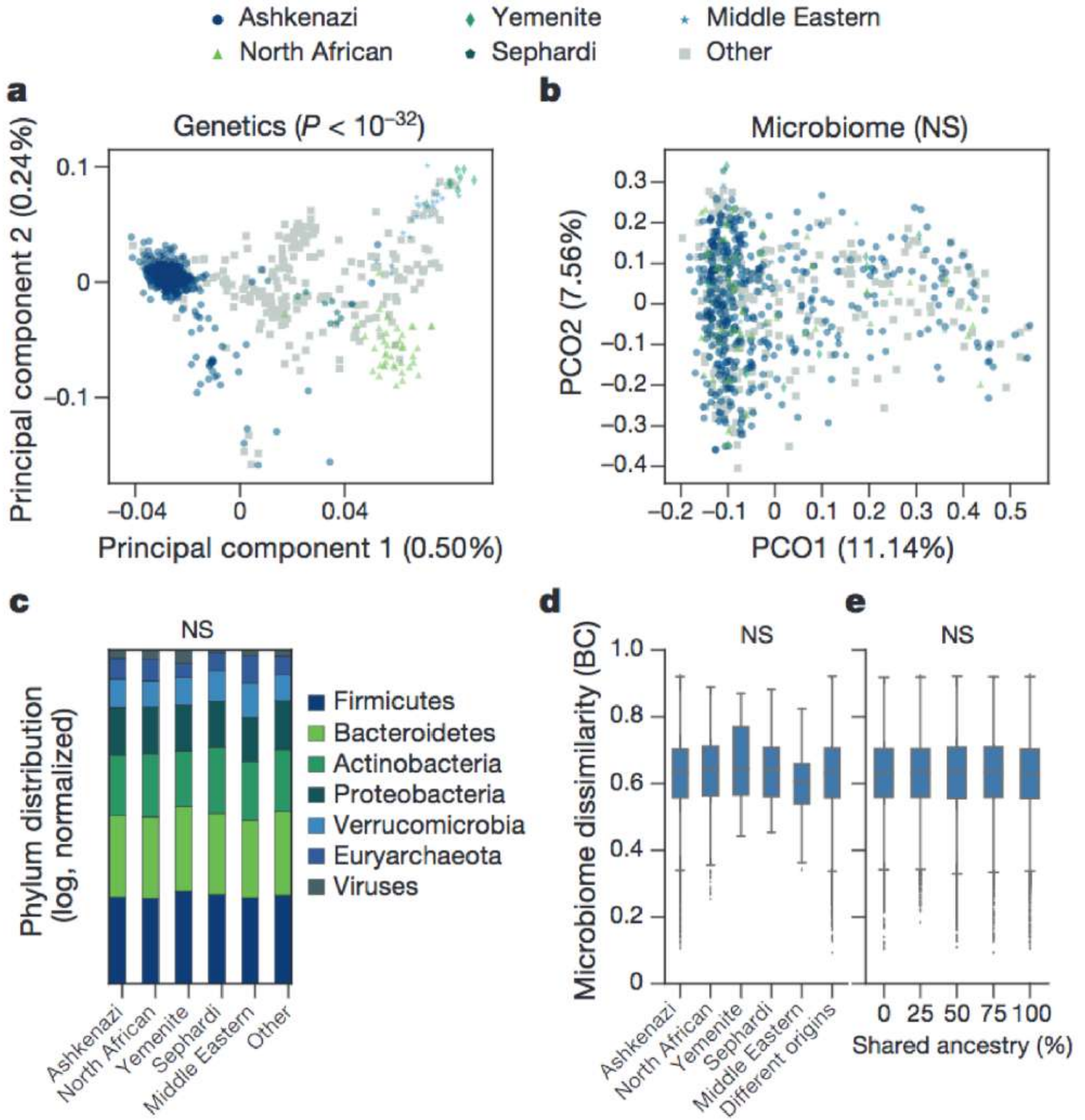

Figure 1 | Genetic ancestry is not significantly associated with microbiome composition. **a**, Genetic principal components are strongly associated with self-reported ancestry, with Ashkenazi ($n = 345$), North African ($n = 42$), Middle Eastern ($n = 24$), Sephardi ($n = 10$), Yemenite ($n = 8$) and admixed/other (other) ($n = 286$) ancestries ($P < 10^{-32}$; Kruskal–Wallis). **b**, As in **a**, but for microbiome principal coordinate analysis ($P > 0.08$; Kruskal–Wallis). **c**, The distribution of average phylum abundance among 582 non-admixed individuals (in log scale, normalized to sum to 1.0) is not associated with ancestry ($P > 0.05$; Kruskal–Wallis). NS, not significant. **d**, Box plots of Bray–Curtis (BC) dissimilarities across all pairs of 737 individuals for whom the ancestries of all grandparents are known, demonstrating that microbiome composition is not associated with ancestry ($P > 0.06$; Kruskal–Wallis test for the top five Bray–Curtis PCOs). $n = 105{,}570$ (Ashkenazi), 1,711 (North African), 528 (Middle Eastern), 136 (Sephardi) and 78 (Yemenite) same ancestry pairs; $n = 61{,}048$ different ancestry pairs. The lower and upper limits of the boxes represent the 25% and 75% percentiles, respectively, and the top and bottom whiskers represent the 5% and 95% percentiles, respectively. **e**, Box plots of Bray–Curtis dissimilarities across pairs of 946 individuals (including admixed individuals), organized according to shared ancestry fraction (the fraction of grandparents of the same ancestry), for pairs with 0% ($n = 167{,}618$), 25% ($n = 33{,}119$), 50% ($n = 100{,}163$), 75% ($n = 34{,}187$) and 100% ($n = 111{,}898$) shared ancestry fractions. The lower and upper limits of the boxes represent the 25% and 75% percentiles, respectively, and the top and bottom whiskers represent the 5% and 95% percentiles, respectively. The figure demonstrates that microbiome similarity is not associated with ancestral similarity ($P = 0.73$; Mantel test).

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b

Phenotype	Microbiome association index Israeli cohort	Microbiome association index LLD cohort	Genetic heritability (literature)
HDL	35.9%***	27.9%***	23.9%–48%
Lactose cons.	35.5%***	N/A	N/A
Waist circ.	28.8%***	26%***	15%–24%
Hip circ.	27.1%***	28%***	10.6%–27%
Glycaemic status	24.5%***	N/A	N/A
BMI	24.5%***	27.8%***	14%–32%
WHR	23.9%***	6.9%*	12%–14%
Fasting glucose	21.9%***	8%**	9%–33%
HbA1c%	16.1%*	8.4%	21%–32%
Creatinine	12.3%*	6.7%	19%–25%
Height	3.2%	25.9%***	33%–68%
Total cholesterol	0%	13.5%	14%–53%

indicate a greater confidence in the estimation. **b**, b^2 estimates from the analysis of 715 individuals with measured genotyped and gut microbiomes from the Israeli cohort (left column) and of 836 individuals from the LLD cohort (middle column) are comparable to previous genetic heritability estimates[27–34] (right column). *FDR < 0.05, **FDR < 0.01 and ***FDR < 0.001. Cons., consumption, circ., circumference. **c**, b^2 estimates

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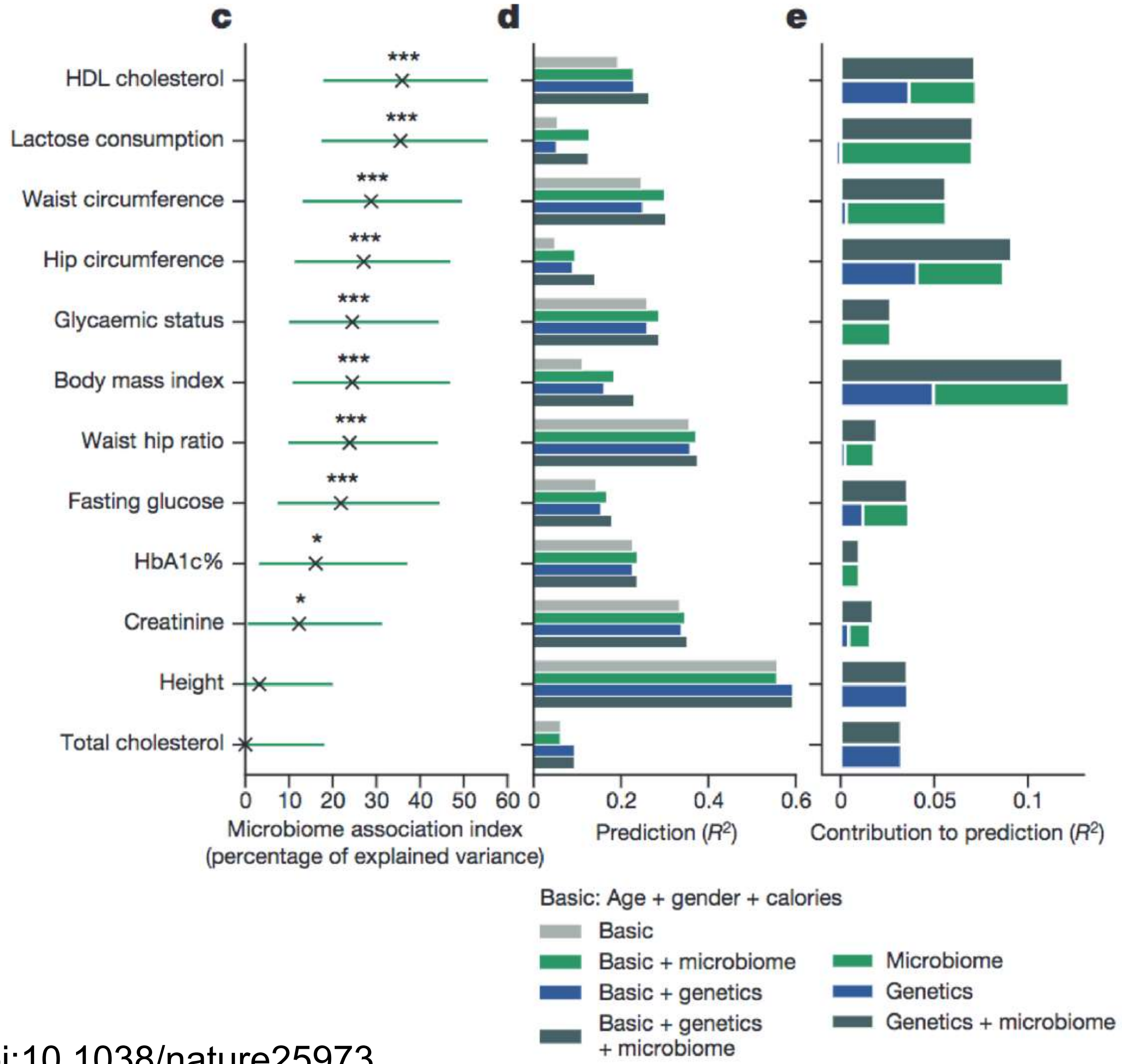

***FDR < 0.001. Cons., consumption, circ., circumference. **c**, b^2 estimates of several human phenotypes and their 95% confidence intervals, evaluated using 715 individuals. *FDR < 0.05, **FDR < 0.01 and ***FDR < 0.001. **d**, Phenotype prediction accuracy for 715 individuals, evaluated using a LMM under different sets of predictive features (measured using coefficient of determination (R^2)), using four different models for each phenotype: (i) 'Basic', age, gender and diet features; (ii) 'Basic + microbiome', basic features and relative abundances of bacterial genes; (iii) 'Basic + genetics', basic features and host genotypes; and (iv) 'Basic + genetics + microbiome': basic features, relative abundances of bacterial genes and host genotypes. **e**, The additive contribution of microbiome and genetics to prediction performance evaluated using a LMM across 715 individuals, over a model that includes only basic features. The joint contribution of microbiome and genetics is similar to the sum of the individual contributions, suggesting these are independent contributions.

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Box 1 | **Ten areas of microbiome inquiry that should be pursued**

- Understanding microbiome characteristics in relation to families: which features are inherited and which are not?*
- Understanding secular trends in microbiome composition: which taxonomic groups have been lost or gained?‡
- For diseases that have changed markedly in incidence in recent decades, do changes in the microbiome have a role? Notable examples include childhood-onset asthma, food allergies, type 1 diabetes, obesity, inflammatory bowel disease and autism.*‡
- Do particular signatures of the metagenome predict risks for specific human cancers and other diseases that are associated with ageing? Can these signatures be pursued to better understand oncogenesis? (Work on *Helicobacter pylori* provides a clear example of this.)*
- How do antibiotics perturb the microbiome, both in the short-term and long-term? Does the route of administration matter?*
- How does the microbiome affect the pharmacology of medications? Can we 'micro-type' people to improve pharmacokinetics and/or reduce toxicity? Can we manipulate the microbiome to improve pharmacokinetic stability?*‡
- Can we harness knowledge of microbiomes to improve diagnostics for disease status and susceptibility?*
- Can we harness the close mechanistic interactions between the microbiome and the host to provide hints for the development of new drugs?‡
- Specifically, can we harness the microbiome to develop new narrow-spectrum antibiotics?‡
- Can we use knowledge of the microbiota to develop true probiotics (and prebiotics)?*‡

*Areas currently under investigation. ‡Proposed areas for investigation.

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ARTICLE

OPEN
doi:10.1038/nature24621

A communal catalogue reveals Earth's multiscale microbial diversity

Luke R. Thompson[1,2,3], Jon G. Sanders[1], Daniel McDonald[1], Amnon Amir[1], Joshua Ladau[4], Kenneth J. Locey[5], Robert J. Prill[6], Anupriya Tripathi[1,7,8], Sean M. Gibbons[9,10], Gail Ackermann[1], Jose A. Navas-Molina[1,11], Stefan Janssen[1], Evguenia Kopylova[1], Yoshiki Vázquez-Baeza[1,11], Antonio González[1], James T. Morton[1,11], Siavash Mirarab[12], Zhenjiang Zech Xu[1], Lingjing Jiang[1,13], Mohamed F. Haroon[14], Jad Kanbar[1], Qiyun Zhu[1], Se Jin Song[1], Tomasz Kosciolek[1], Nicholas A. Bokulich[15], Joshua Lefler[1], Colin J. Brislawn[16], Gregory Humphrey[1], Sarah M. Owens[17], Jarrad Hampton-Marcell[17,18], Donna Berg-Lyons[19], Valerie McKenzie[20], Noah Fierer[20,21], Jed A. Fuhrman[22], Aaron Clauset[19,23], Rick L. Stevens[24,25], Ashley Shade[26,27,28], Katherine S. Pollard[4], Kelly D. Goodwin[3], Janet K. Jansson[16], Jack A. Gilbert[17,29], Rob Knight[1,11,30] & The Earth Microbiome Project Consortium*

Our growing awareness of the microbial world's importance and diversity contrasts starkly with our limited understanding of its fundamental structure. Despite recent advances in DNA sequencing, a lack of standardized protocols and common analytical frameworks impedes comparisons among studies, hindering the development of global inferences about microbial life on Earth. Here we present a meta-analysis of microbial community samples collected by hundreds of researchers for the Earth Microbiome Project. Coordinated protocols and new analytical methods, particularly the use of exact sequences instead of clustered operational taxonomic units, enable bacterial and archaeal ribosomal RNA gene sequences to be followed across multiple studies and allow us to explore patterns of diversity at an unprecedented scale. The result is both a reference database giving global context to DNA sequence data and a framework for incorporating data from future studies, fostering increasingly complete characterization of Earth's microbial diversity.

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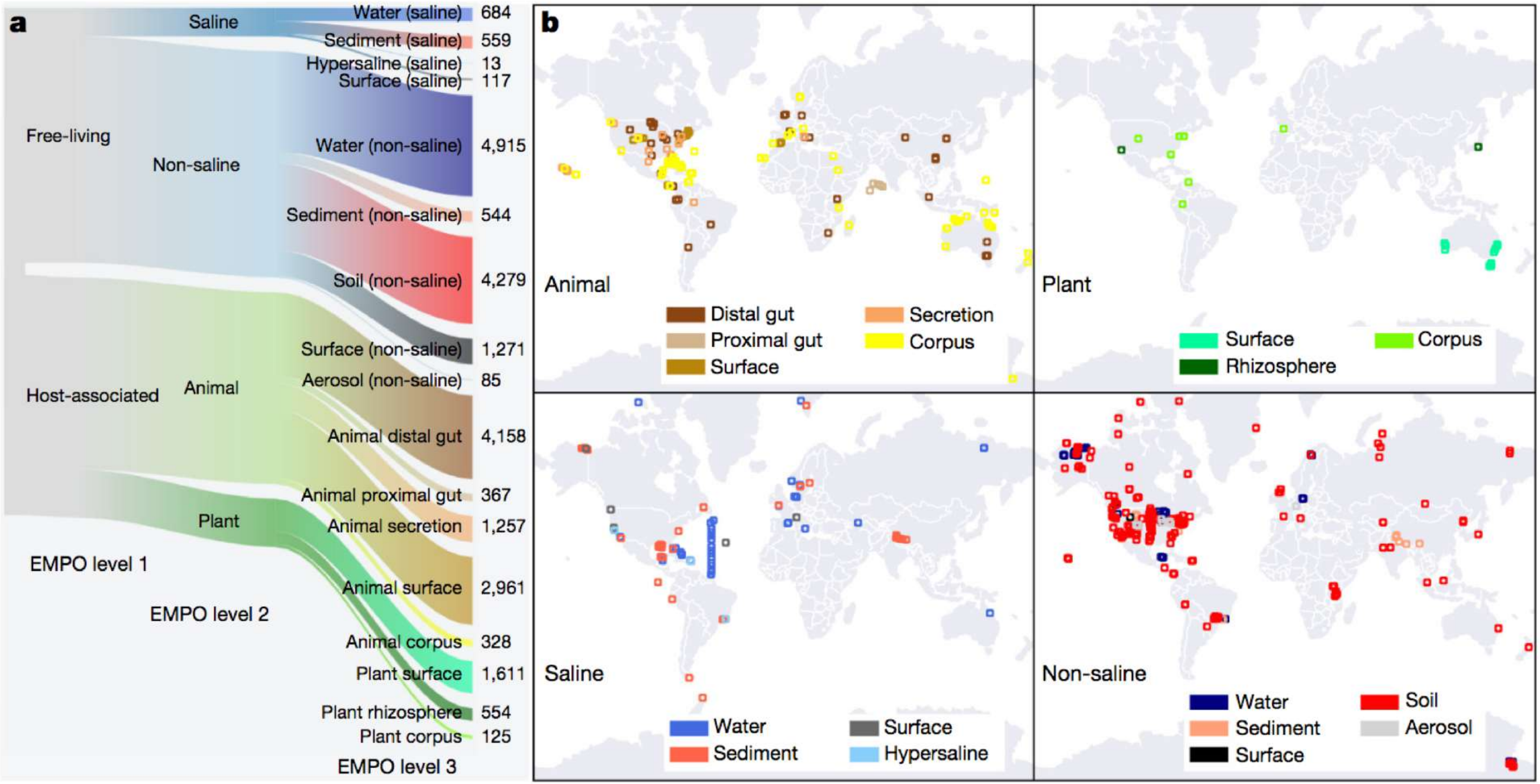
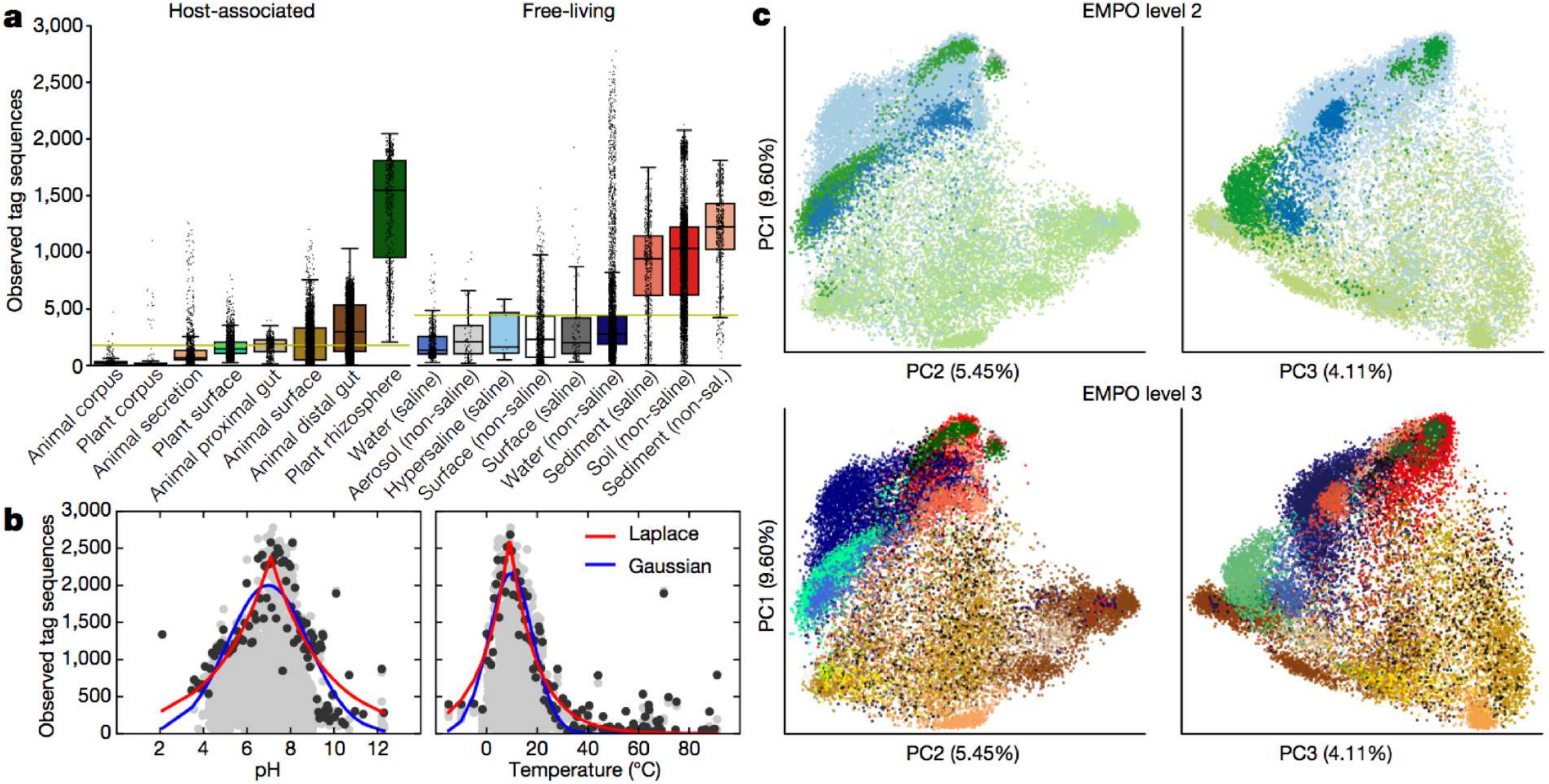

a
Saline
Free-living
Non-saline
Host-associated
Animal
Plant
EMPO level 1
EMPO level 2
EMPO level 3
Water (saline) 684
Sediment (saline) 559
Hypersaline (saline) 13
Surface (saline) 117
Water (non-saline) 4,915
Sediment (non-saline) 544
Soil (non-saline) 4,279
Surface (non-saline) 1,271
Aerosol (non-saline) 85
Animal distal gut 4,158
Animal proximal gut 367
Animal secretion 1,257
Animal surface 2,961
Animal corpus 328
Plant surface 1,611
Plant rhizosphere 554
Plant corpus 125
b
Animal
Distal gut
Proximal gut
Surface
Secretion
Corpus
Plant
Surface
Rhizosphere
Corpus
Saline
Water
Sediment
Surface
Hypersaline
Non-saline
Water
Sediment
Surface
Soil
Aerosol


a
Host-associated
Free-living
Observed tag sequences
3,000
2,500
2,000
1,500
1,000
5,00
5,00
0
Animal corpus
Plant corpus
Animal secretion
Plant surface
Animal proximal gut
Animal surface
Animal distal gut
Plant rhizosphere
Water (saline)
Aerosol (non-saline)
Hypersaline (saline)
Surface (non-saline)
Surface (saline)
Water (non-saline)
Sediment (saline)
Soil (non-saline)
Sediment (non-sal.)
b
Observed tag sequences
pH
Temperature (°C)
Laplace
Gaussian
c
EMPO level 2
EMPO level 3
PC1 (9.60%)
PC2 (5.45%)
PC3 (4.11%)

Additional references

A good introductory popular science video about microbiome
http://www.youtube.com/watch?v=5DTrENdWvvM

A clinician's guide to microbiome analysis
https://www.nature.com/articles/nrgastro.2017.97.pdf

TED talk
https://www.ted.com/talks/rob_knight_how_our_microbes_make_us_who_we_are

#Coursera
https://zh-tw.coursera.org/learn/microbiome