Metagenomics

Bioinformatics Applications (PLPTH813)

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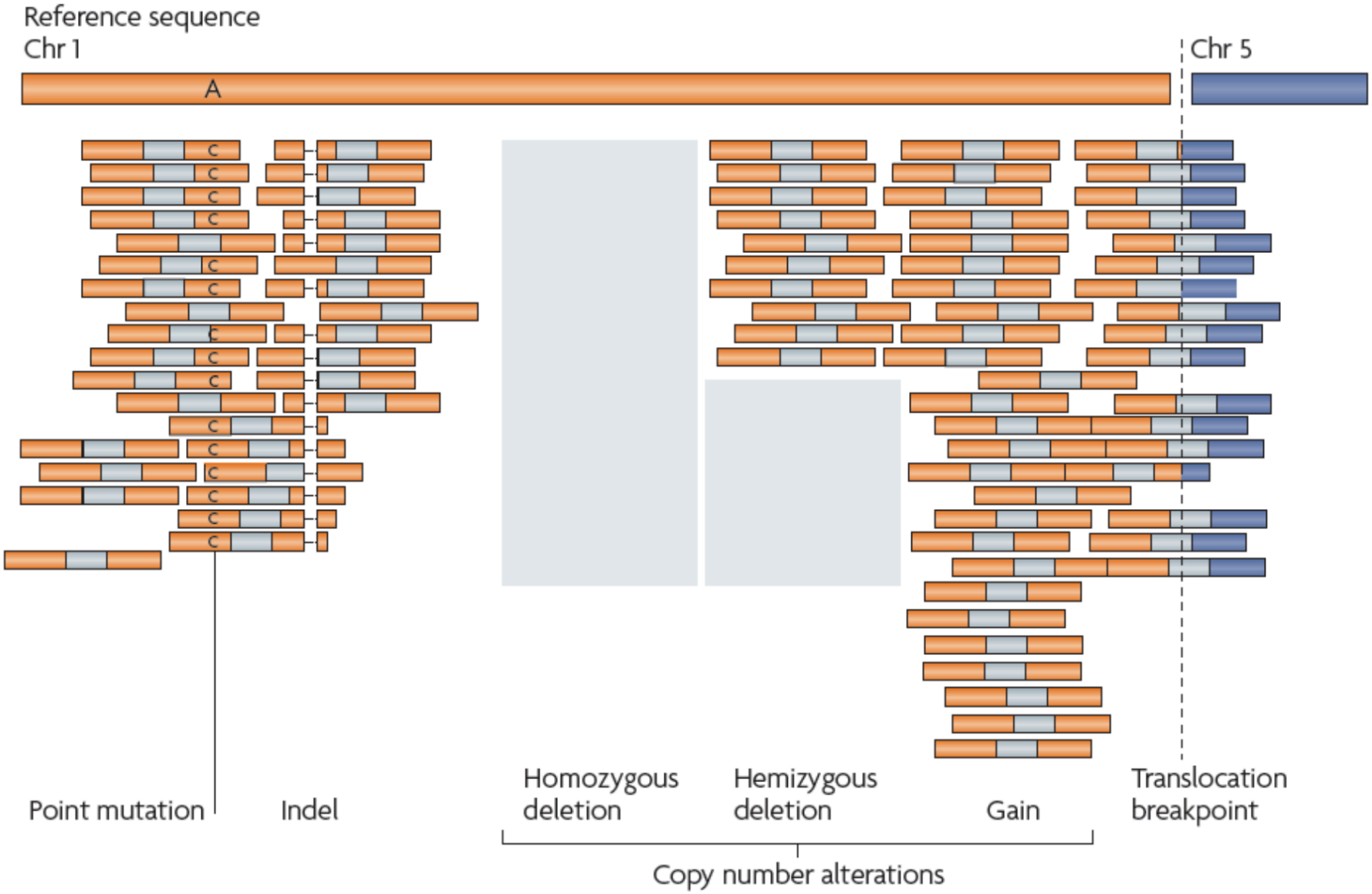
4/16/2019

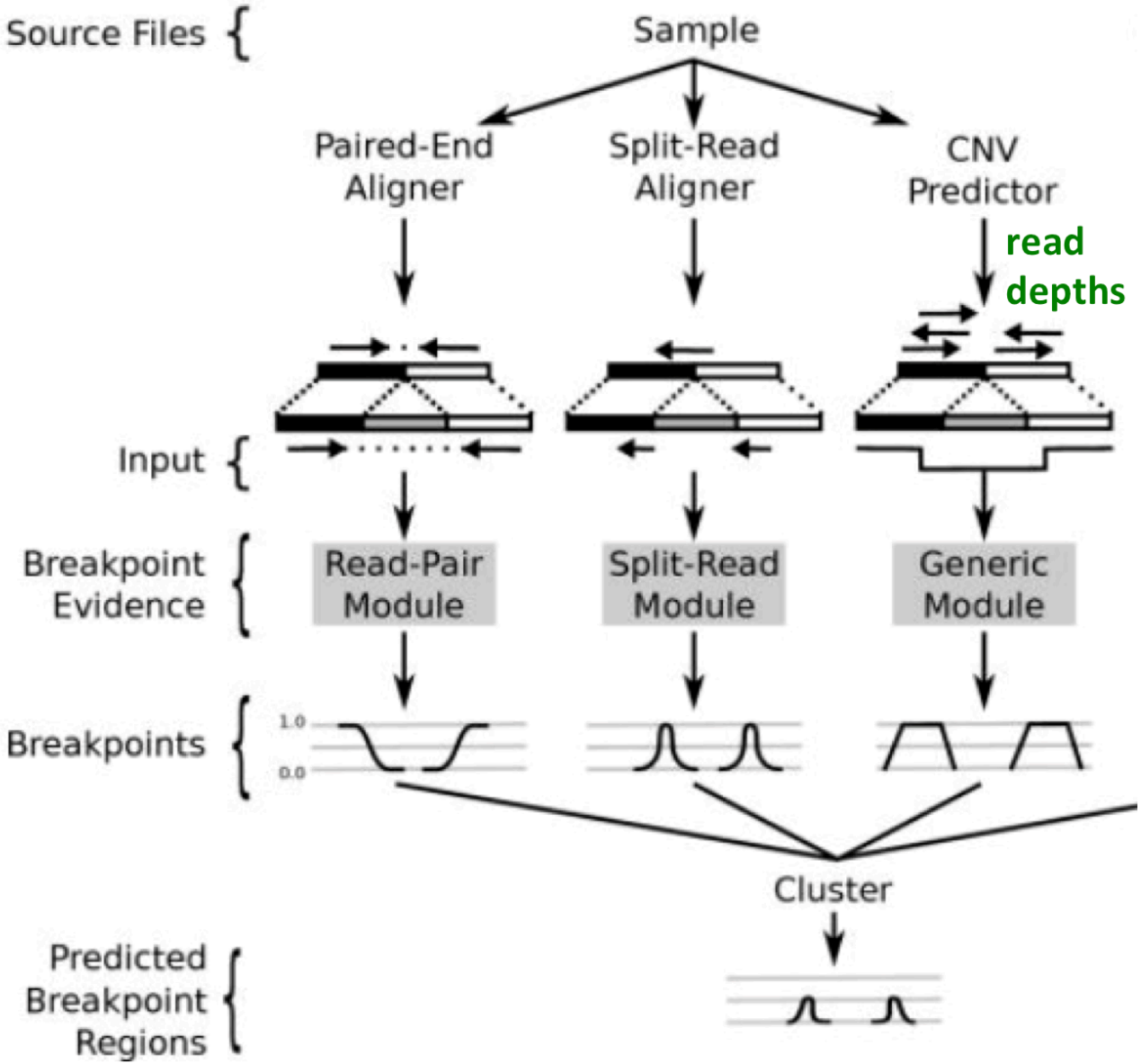
Schedule

16-Apr	microbiome
18-Apr	RNA-Seq_RNA-asm
23-Apr	Oral presentation_microbiome
25-Apr	Oral presentation_comparative-genomics
30-Apr	RNA-Seq_DE
2-May	in-class project: RNA-Seq
7-May	Oral presentation_RNA-Seq
9-May	Review_Q&A

Variants in sequencing reads



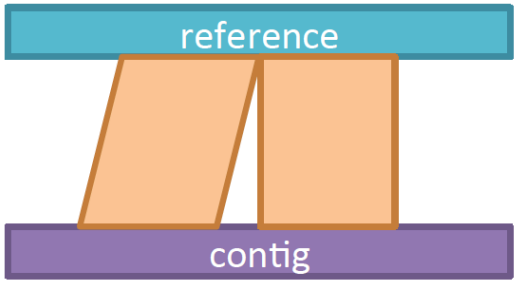
Integrative approaches for SV discovery



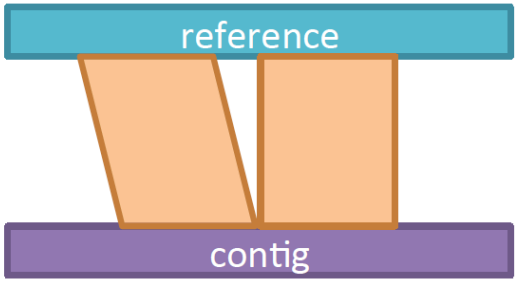
Genome assembly and genome-wide SV discovery

Defined point

Insertion

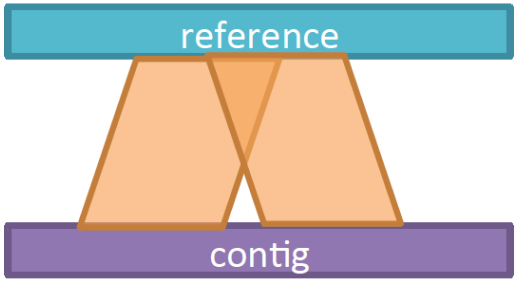


Deletion

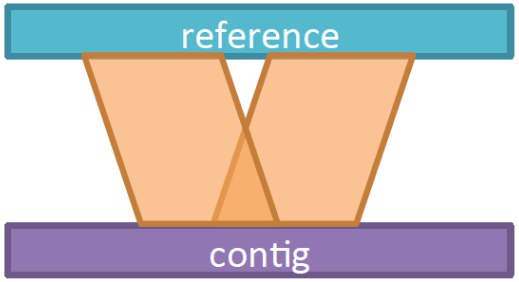


Overlapping alignments suggest tandem repeat

Tandem Expansions



Tandem Contractions



Outline

- Introduction of metagenomics
- Marker gene analysis (rRNA)
- Whole genome analysis
- Metatranscriptome analysis

What is metagenomics?

Terms

- **metagenomics**: The application of high-throughput DNA sequencing to profile the genomic composition of a microbial community in a culture-independent manner.
- **microbiomes**: The community composition, biomolecular repertoire and ecology of microorganisms inhabiting particular environments.
- **microbiota**: The collection of microorganisms (of all types: bacteria, archaea, viruses and eukaryotes) inhabiting a particular environment.

metagenomics

Advances in DNA sequencing have enabled culture-independent profiling of microbial community membership and function - the field of metagenomics. (e.g., a study indicated that cultivation methods found <1% of the bacterial & archaeal species in samples.)

Firmicutes and Bacteroidetes phyla[2]. Culture-independent, genomic approaches have transformed our understanding of the role of the human microbiome in health and many diseases[1]. However, owing to the prevailing perception that our indigenous bacteria are largely recalcitrant to culture, many of their functions and phenotypes remain unknown[3]. Here we describe a novel workflow based on targeted phenotypic culturing linked to large-scale whole-genome sequencing, phylogenetic analysis and computational modelling that demonstrates that a substantial proportion of the intestinal bacteria are culturable. Applying this approach to healthy individuals,

HP Browne, et al. Nature 533:543–546 (2016)

Approaches

- **Marker gene analysis**
- **Whole genome analysis**
- Metatranscriptome analysis

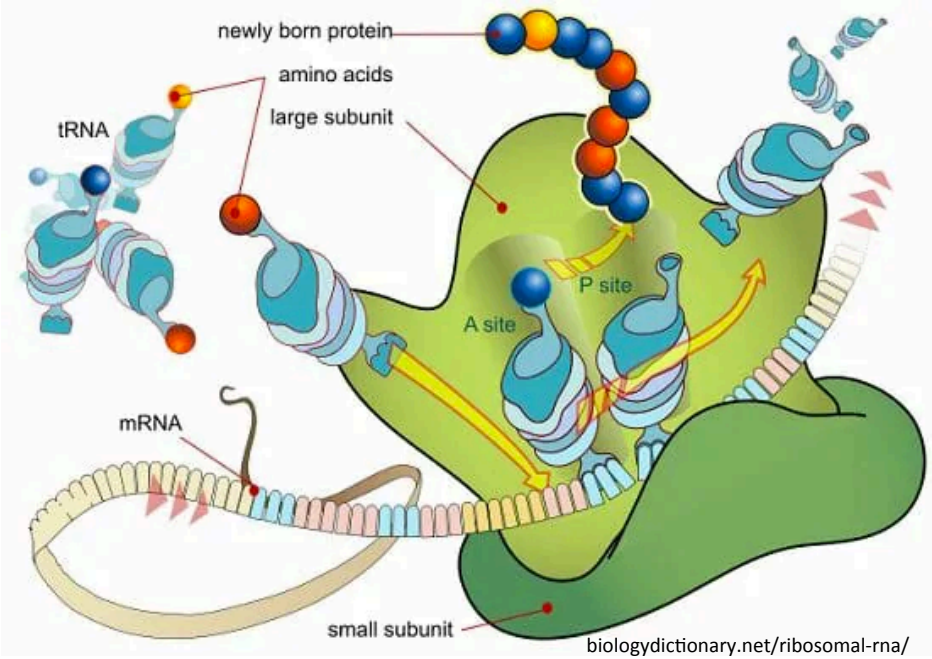
Marker gene analysis

Marker gene sequencing uses ***primers*** that target **a specific region of interest** to determine microbial community of a sample.

- fast
- low-cost
- low-resolution
- Limited information

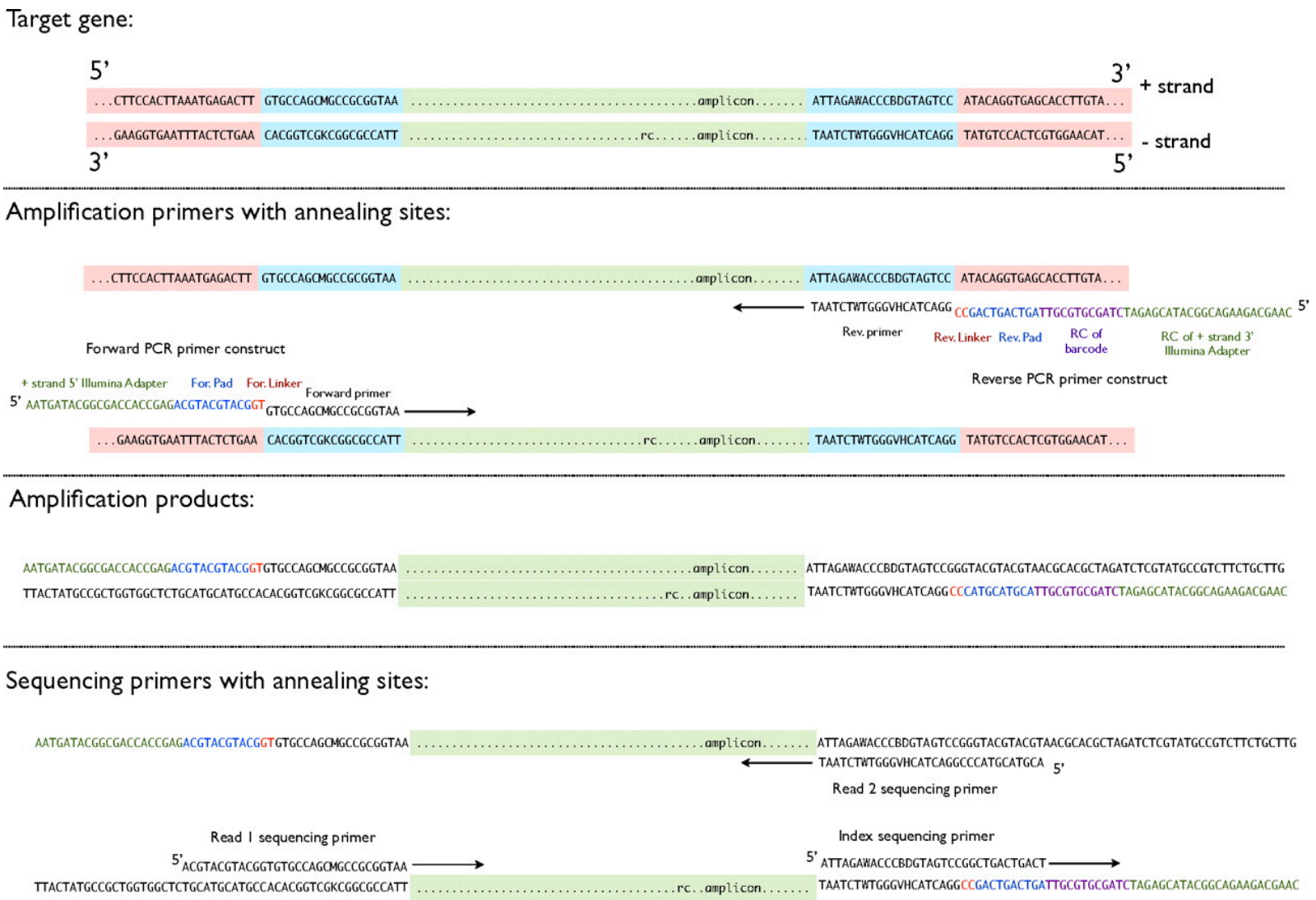
ribosomal RNA

- Conserved sequences within a species
- Generally divergent between species
- Relative short (e.g., 1.5kb 16S rRNA gene)



Ribosomal RNA	Eukaryotic	Bacterial
Large subunit	28S rRNA (3354 nt) 5S rRNA (120 nt) 5.8S rRNA (154 nt) 47 proteins	23S rRNA (2839 nt) 5S rRNA (122 nt) 33 proteins
Small subunit	18S rRNA (1753 nt) 32 proteins	16S rRNA (1504 nt) 20 proteins

16S rRNA sequencing



PNAS, 108:4516–4522 (2011)

QIIME

- Quality filtering of reads
- OTU (operational taxonomic unit) picking:

1. *de novo*: : all-to-all alignments and clustering
2. closed-reference: OTU aligned to pre-defined cluster centroids in a reference database. OTU *excluded* if no matches
3. **open-reference**: aligned to the reference & *de novo*

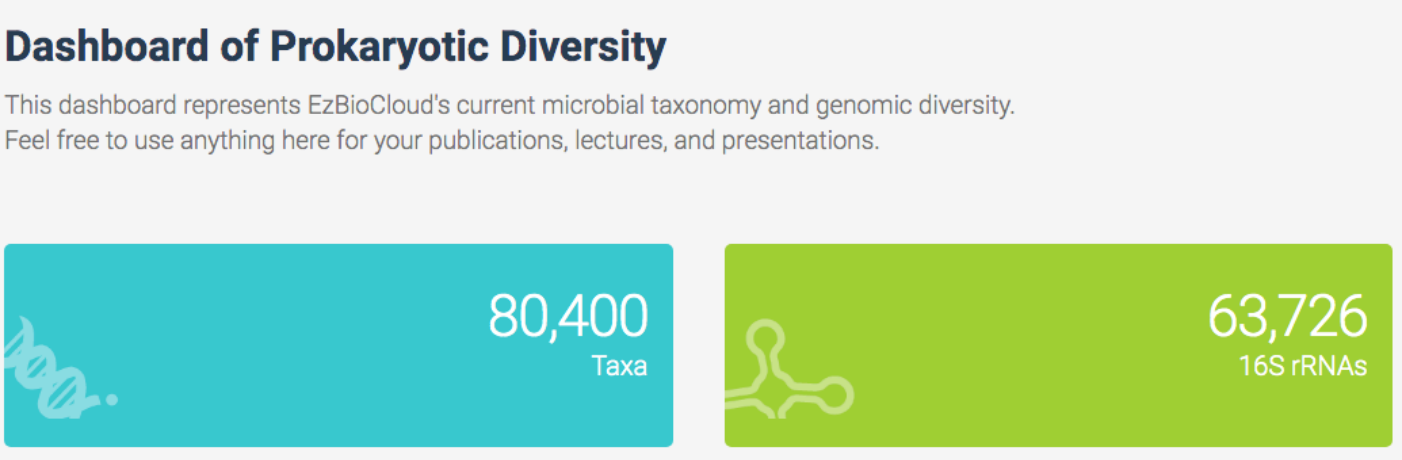
- Output:

OTU table or the number of times each OTU is observed in each sample.

16s rRNA (bacteria)

16S rRNA database: the Ribosomal Database Project, Greengenes, SILVA, EzTaxon

ezbiocloud database (www.ezbiocloud.net)

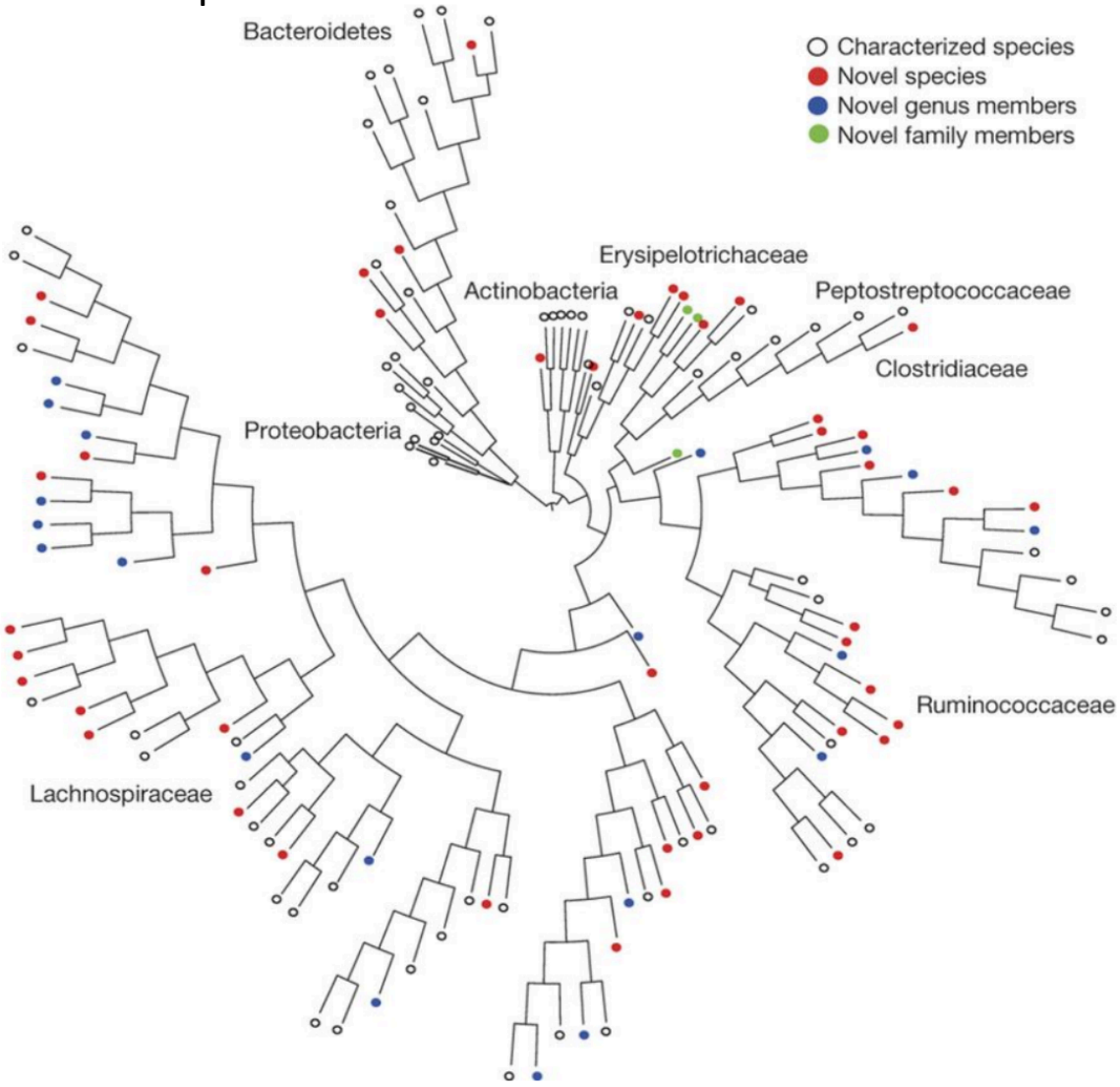


- bacteria: 60,883
- archaea: 2,843

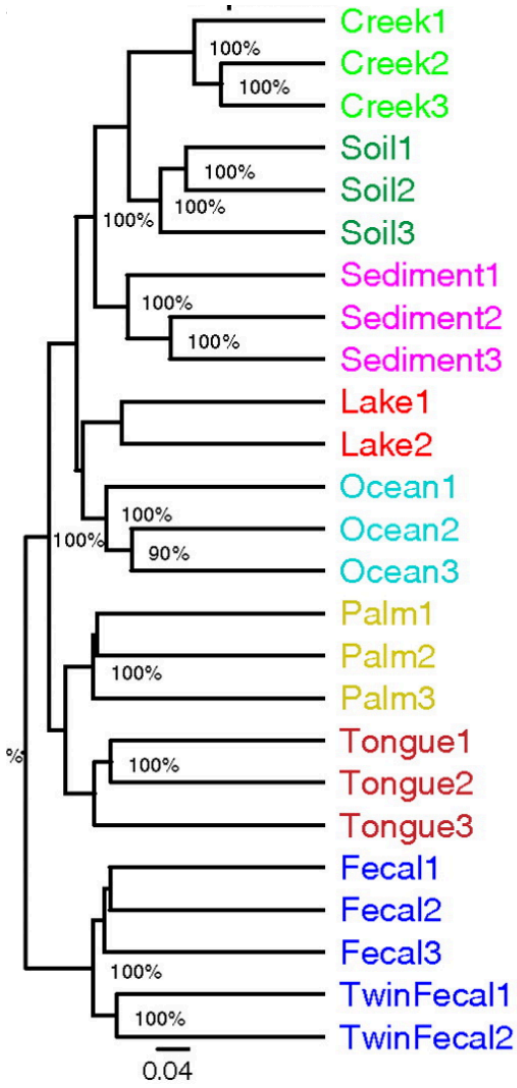
taxonomic classification: to assign taxonomic names to microbial sequences in the data

Microbial communities

From one sample

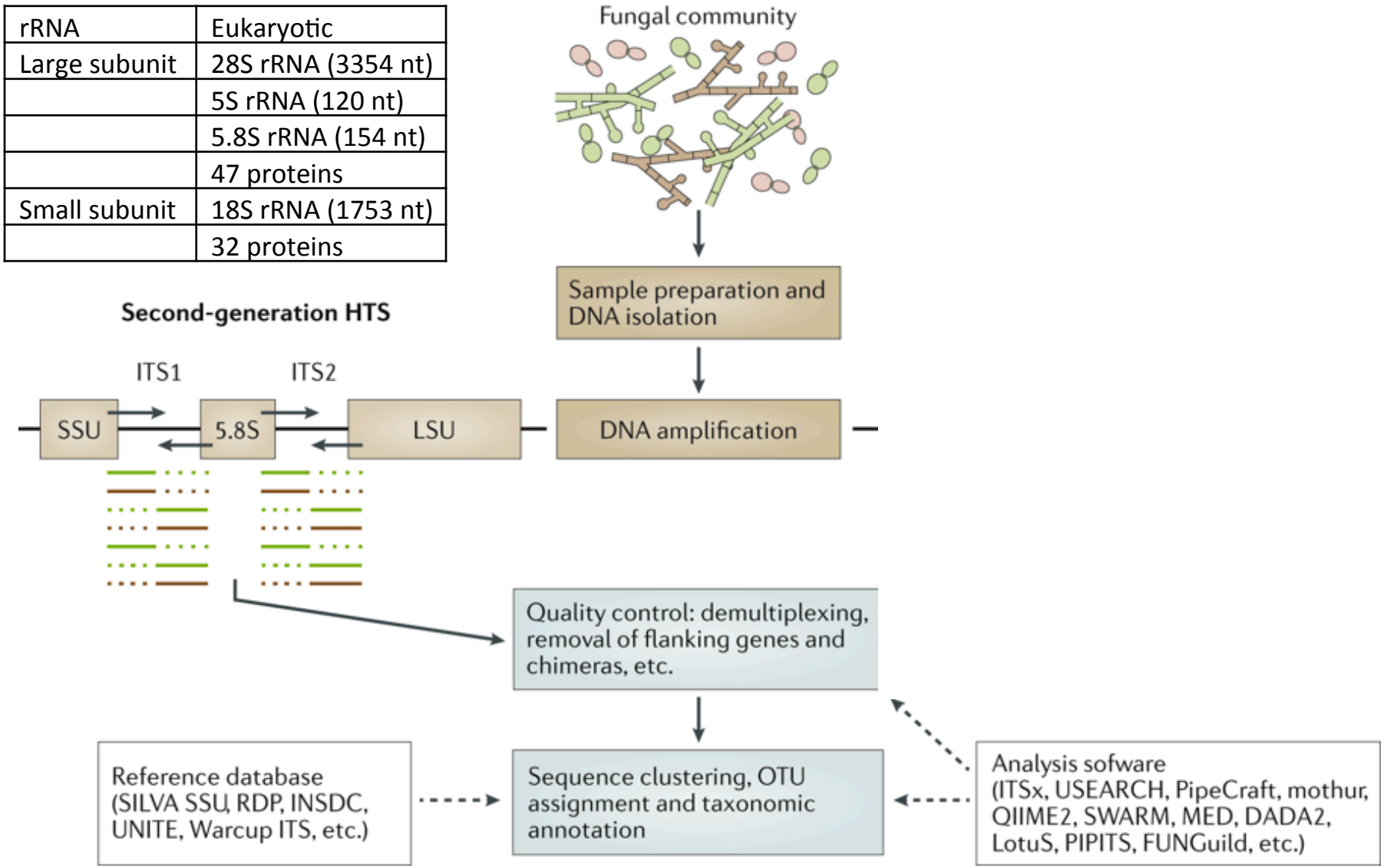


From diverse samples



ribosome DNA (fungal example)

rRNA	Eukaryotic
Large subunit	28S rRNA (3354 nt)
	5S rRNA (120 nt)
	5.8S rRNA (154 nt)
	47 proteins
Small subunit	18S rRNA (1753 nt)
	32 proteins



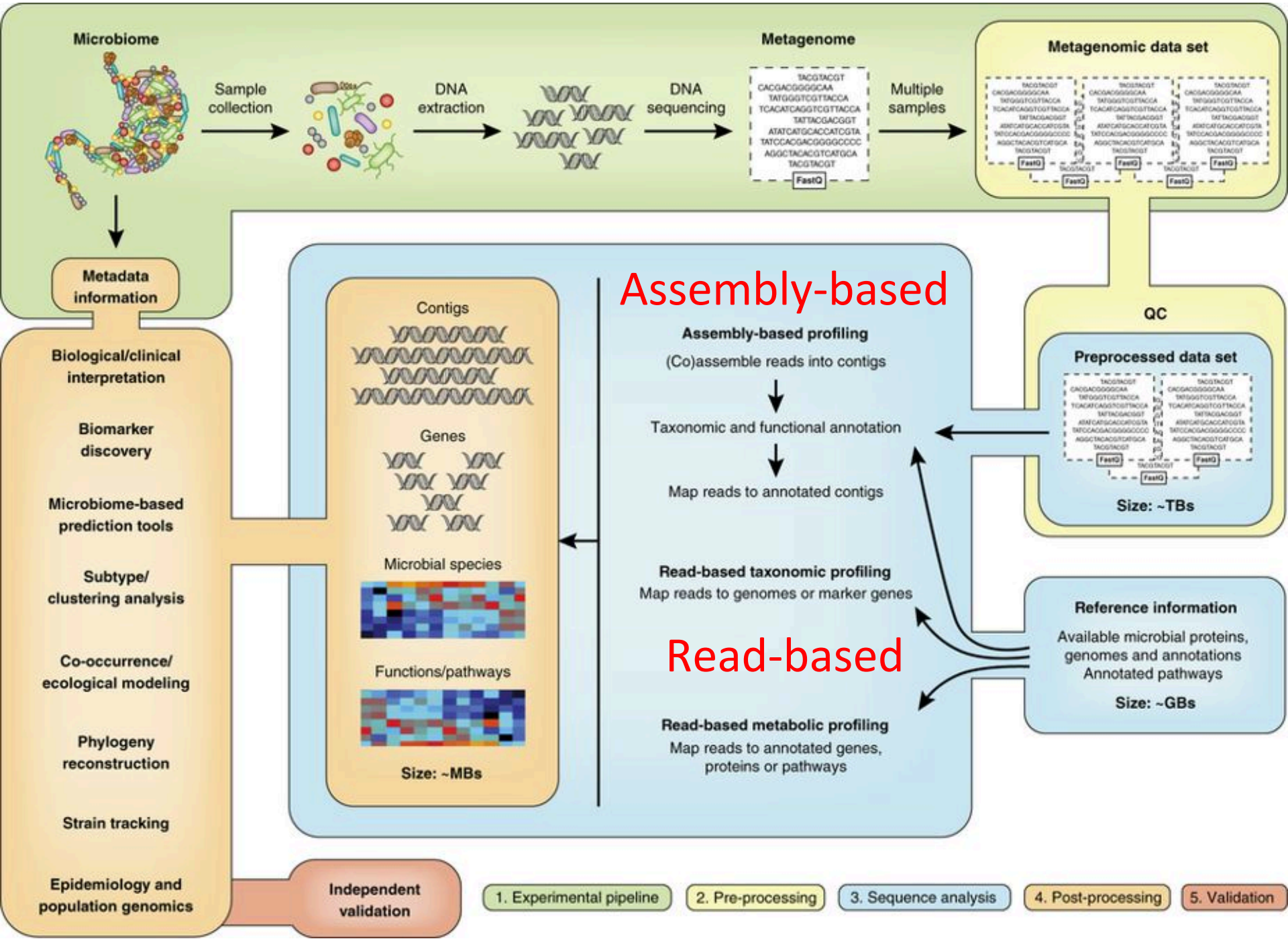
RH Nilsson et al. Nat Rev Microbiol 17:95–109 (2019)

Tools for marker gene analysis

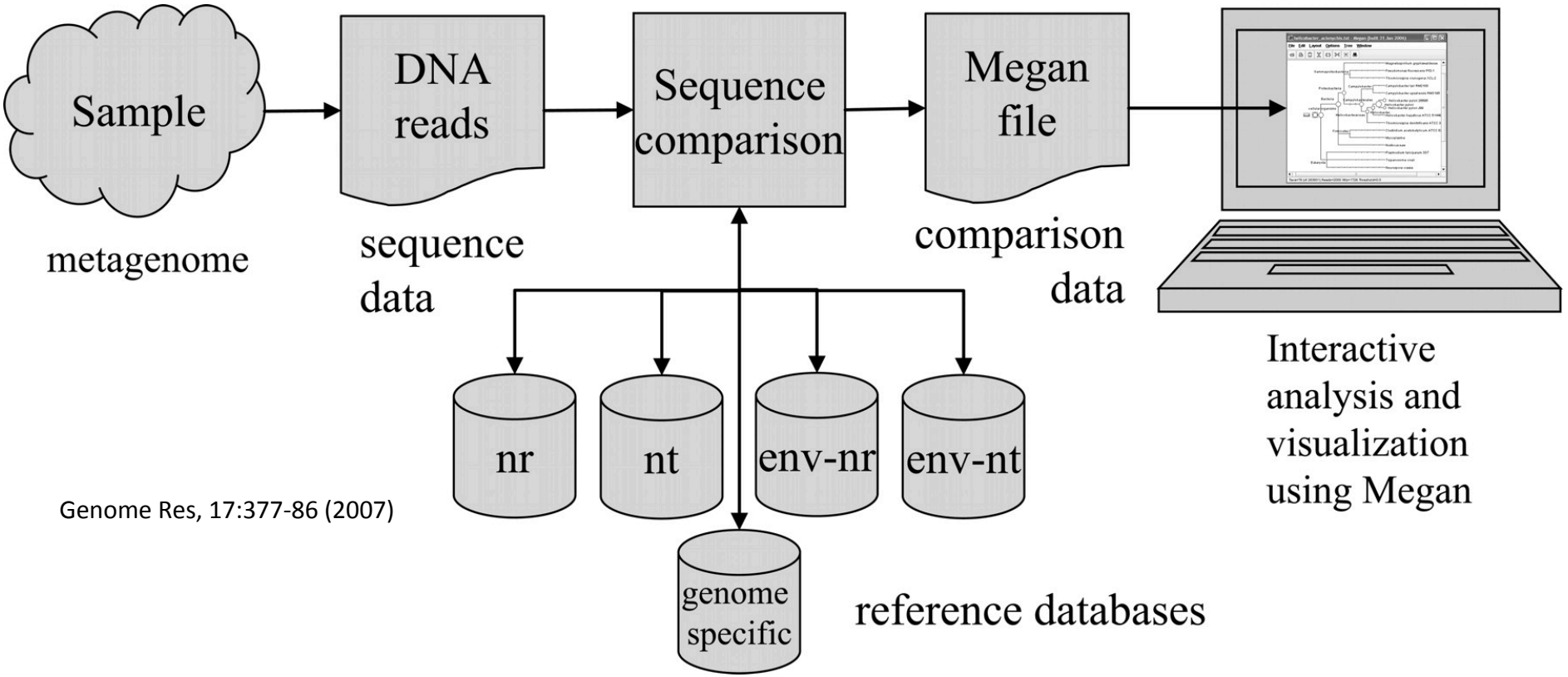
Name	**Description and link**
DADA2	Amplicon sequence variant analysis pipeline
LotuS	Full pipeline for amplicon data
mothur	**Versatile software suite (designed mostly for 16S rRNA)**
AMPtk	Full pipeline for amplicon data
OBITools	Versatile software package
PipeCraft	Full pipeline for amplicon data (with graphical user interface)
PIPITS	Full pipeline for fungal ITS amplicon data (only for Illumina data)
QIIME	**Full pipeline for amplicon data (designed mostly for 16S rRNA)**
SEED2	Full pipeline for amplicon data (with graphical user interface; on Windows)
Microbiology.se	Tools, including ITSx and Metaxa2, for processing ITS, SSU and LSU data
USEARCH	Versatile software package
VSEARCH	Versatile software package

Whole-metagenome analysis

- Sequencing of "all" DNA, including viral and eukaryotic DNA.
- High taxonomic resolution: to species or strain levels
- Assembly of whole microbial genomes
- Relatively expensive


Microbiome
Sample collection
DNA extraction
DNA sequencing
Metagenome
Multiple samples
Metagenomic data set
Metadata information
Biological/clinical interpretation
Biomarker discovery
Microbiome-based prediction tools
Subtype/ clustering analysis
Co-occurrence/ ecological modeling
Phylogeny reconstruction
Strain tracking
Epidemiology and population genomics
Independent validation
Contigs
Genes
Microbial species
Functions/pathways
Size: ~MBs
Assembly-based
Assembly-based profiling
(Co)assemble reads into contigs
Taxonomic and functional annotation
Map reads to annotated contigs
Read-based taxonomic profiling
Map reads to genomes or marker genes
Read-based
Read-based metabolic profiling
Map reads to annotated genes, proteins or pathways
QC
Preprocessed data set
Size: ~TBs
Reference information
Available microbial proteins, genomes and annotations
Annotated pathways
Size: ~GBs
1. Experimental pipeline
2. Pre-processing
3. Sequence analysis
4. Post-processing
5. Validation

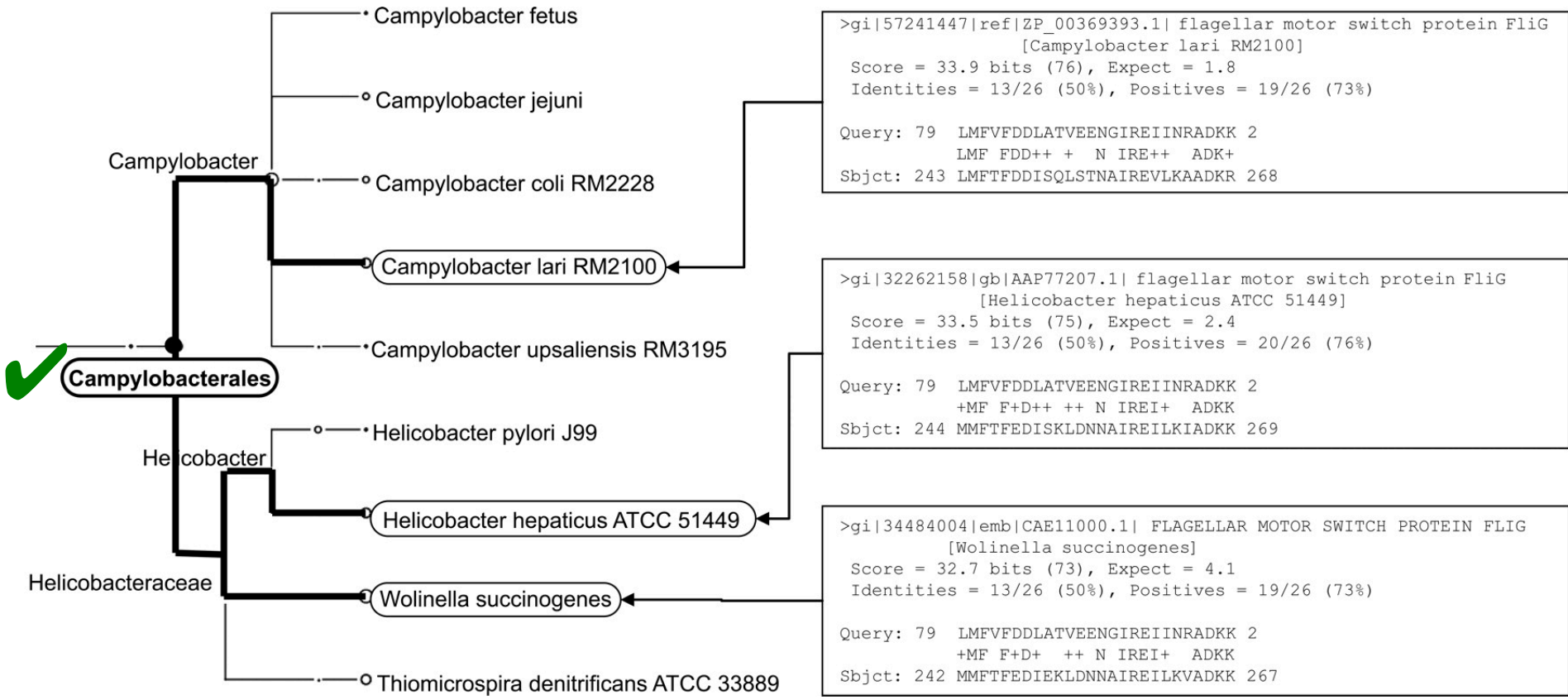
Read-based analysis (Megan)



Genome Res, 17:377-86 (2007)

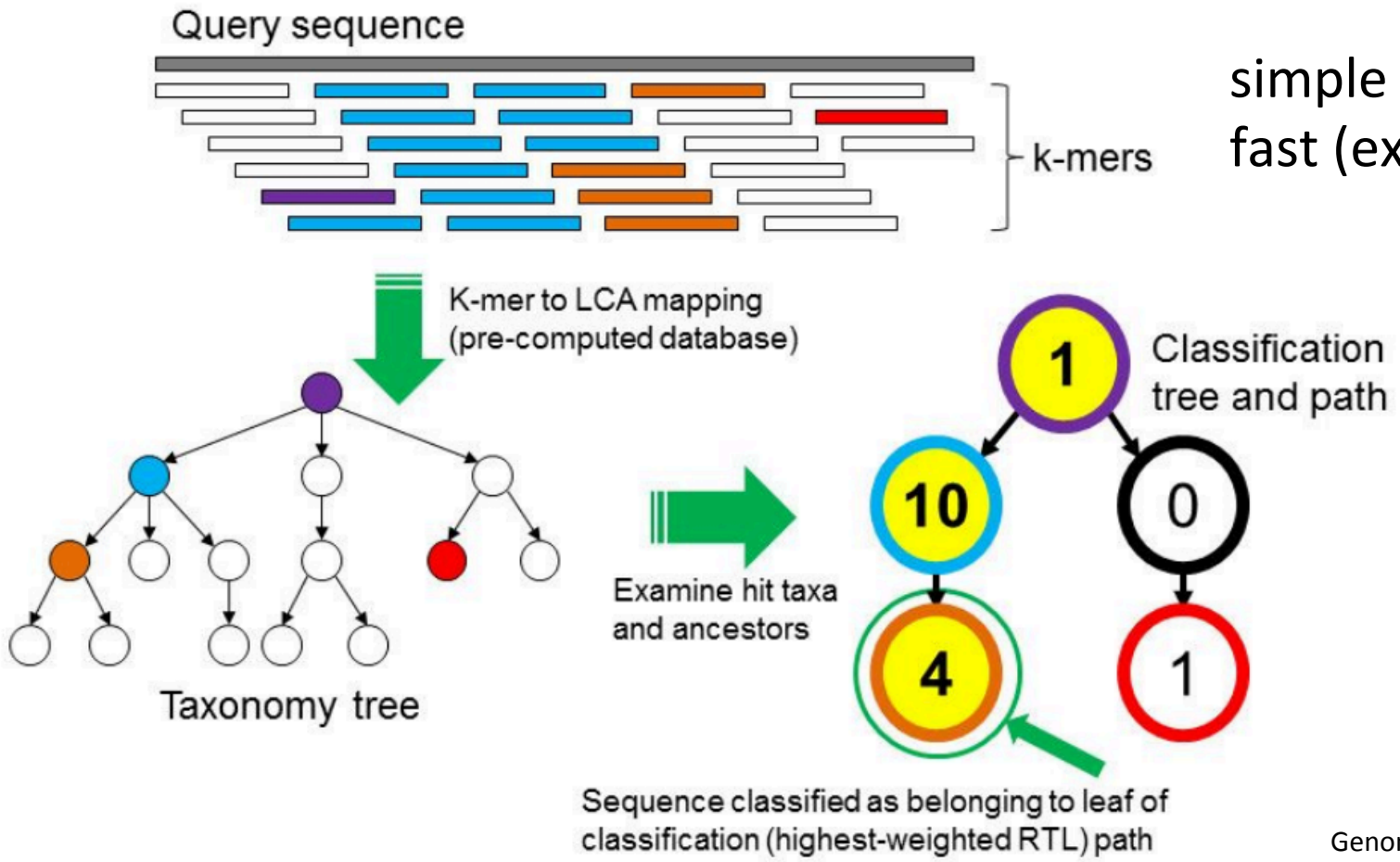
DNA sequences were aligned against databases of known sequences (e.g., BLAST). MEGAN then assigns taxonomy and summaries the results.

LCA: lowest common ancestor



A simple LCA algorithm assigns reads to taxa
(e.g., the read was assigned to *Campylobacterales*)

Read-based analysis (Kraken: k-mer analysis)



simple
fast (exact match)

Genome Biology, 15:R46 (2014)

Kraken has a ***database*** that contains records consisting of a k-mer and the LCA of all organisms whose genomes contain that k-mer.

Assembly-based analysis

Metagenome *de novo* assembly is conceptually similar to whole-genome assembly (de Bruijn graph).

Challenges:
1. Typically a number of species
2. Uneven sequencing coverage of each genome
3. Multiple strains from a species

Resulting in fragmented assemblies (e.g., million of contigs)

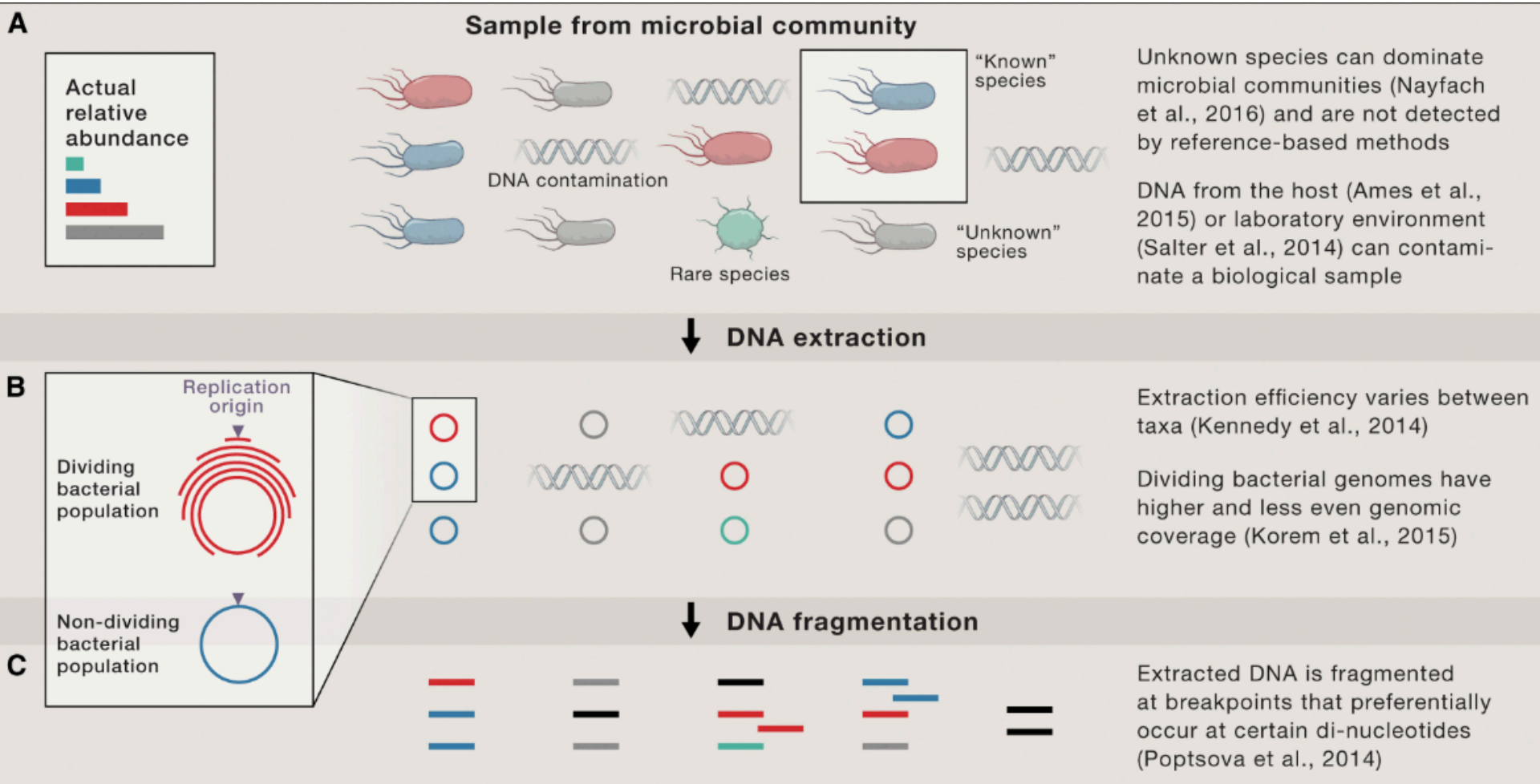
metagenomic assemblers: MEGAHIT and metaSPAdes

Contig binning: to group contigs into species

Number of genomes and what genomes (species)

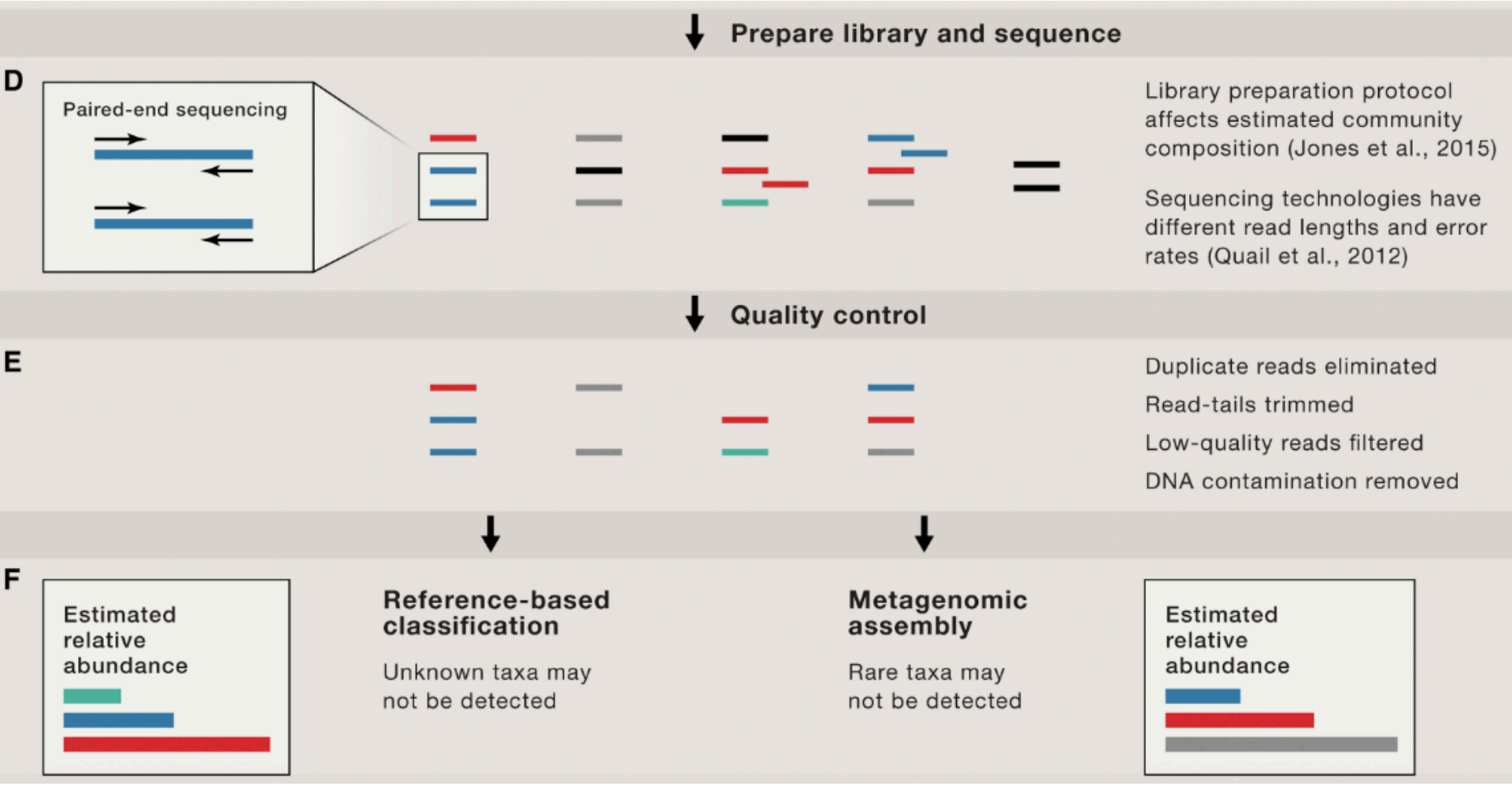
- Taxonomic classification: contig aligned against known genomes
- Sequence composition is used for binning contigs from unknown genomes
  1. Genomes have particular combinations of bases (tetramers)
  2. **Sequencing coverage**: sequences from the same genome have similar sequencing depth
- Contigs are reconstructed into metagenomic assembled genomes (MAGs)

Example of bacterial whole-genome metagenomics (I)



S Nayfach et al., Cell, 166:1103-1116 (2016)

Example of bacterial whole-genome metagenomics (II)



S Nayfach et al., Cell, 166:1103-1116 (2016)

Metatranscriptome

Metatranscriptomics uses RNA sequencing to profile transcription in microbiomes, providing information on active functional outputs of the microbiome.

1. To acquire high-quality RNA might be challenging (e.g., feces)
2. rRNA contamination

Metatranscriptome analysis

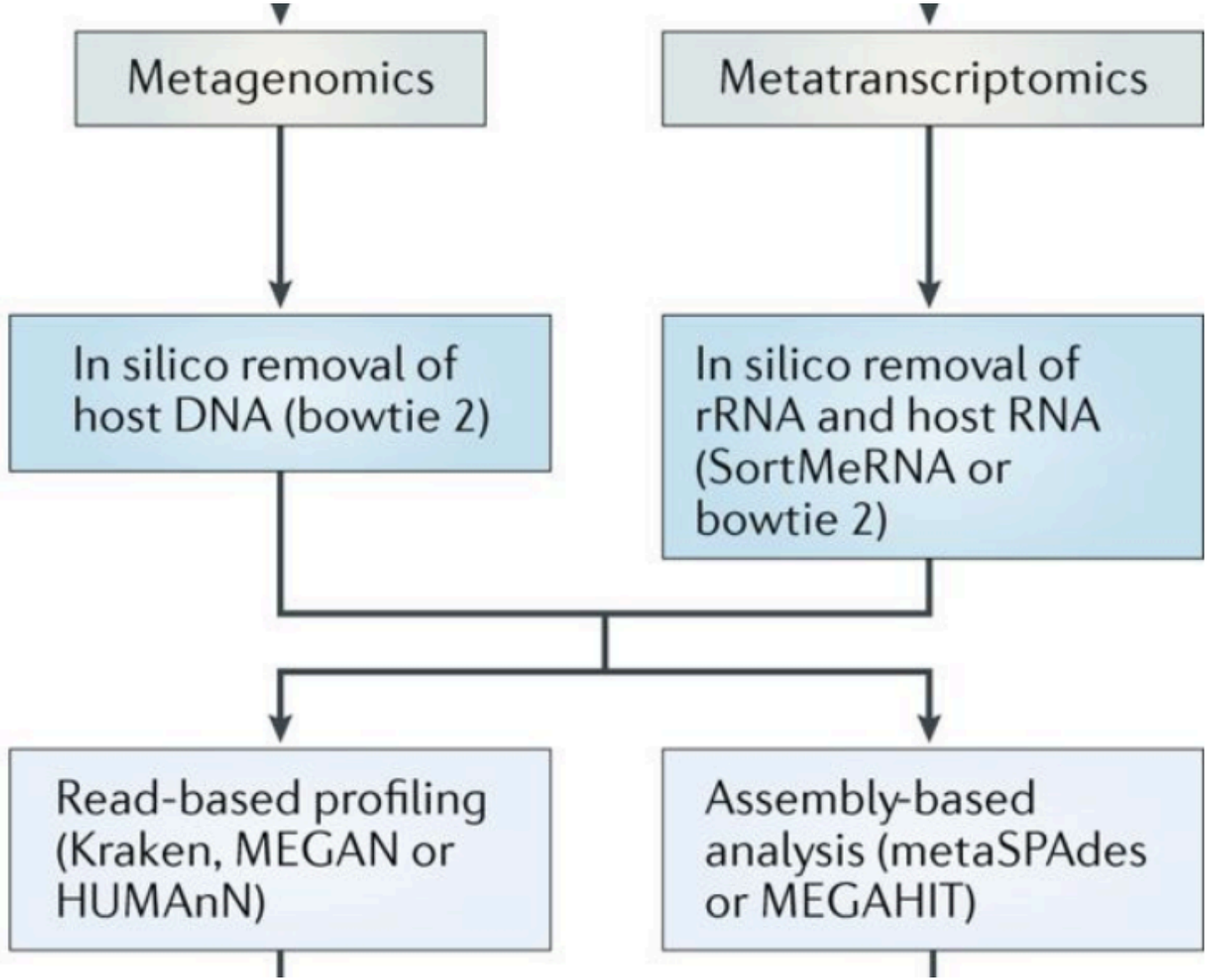
1. mRNA or gene expression is the focus
2. rRNA removal during library prep but not complete

Generally, the following reads are discarded:

- non-mRNA reads
- reads derived from hosts

RNA-Seq analyzing tools for assembly and differential expression are useful for metatranscriptome analysis.

Metagenome and metatranscriptome analysis

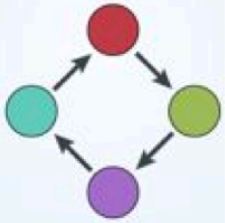


Strengths and weaknesses of metagenomic studies

Strengths

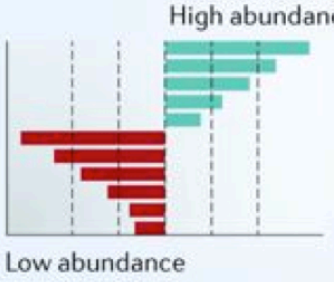
Inclusion of a large number of samples

Integrative workflows

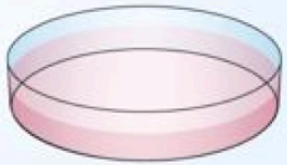


Time to result

Detection of differentially abundant taxa

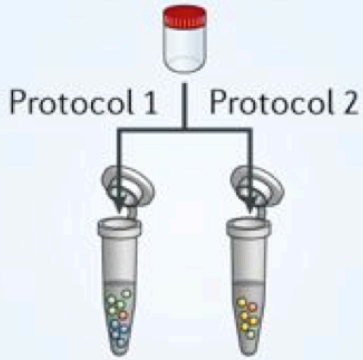


Detection of uncultured bacteria



Weaknesses

Extraction bias



The bacterial diversity that is captured is a function of the extraction protocol

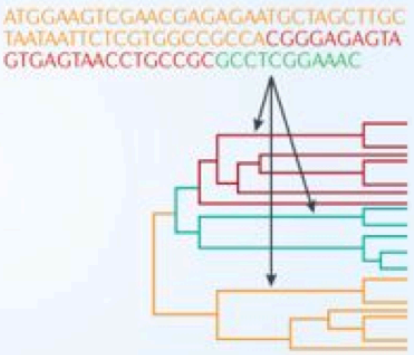
Primer bias

Species identification is dependent on the targeted hypervariable region

Bioinformatics biases

Variations in methodology

Discrimination between species is difficult owing to amplicon length



biases (cont.)

Technical biases from DNA extraction, primers, PCR, and sequencing procedure

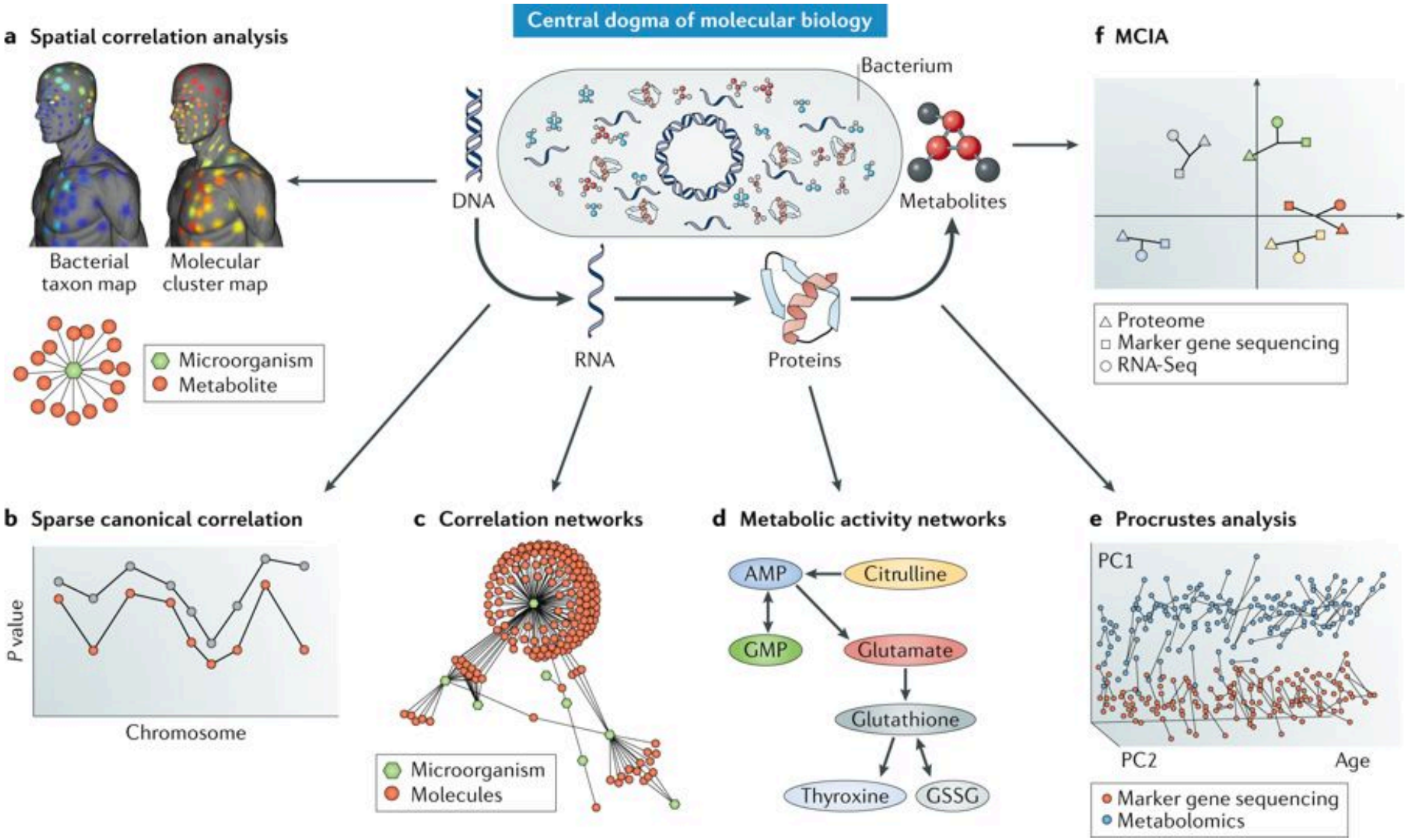
Index (barcode) switching: chimeric products during PCR

solution: less PCR cycles and chimaera filtering?

Clustering Issue: satellite OTUs introduced by sequencing errors could lead to the overestimation of richness of the microbial community.

solution: removal of rare OTUs and multiple rounds of clustering

High-level analyses



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