Metagenomika - Metatranskriptomika

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Metatranskriptomika

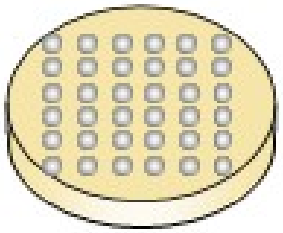
- Zkoumá odpověď mikroorganismů na změny prostředí
- Sleduje se genová exprese = aktivita mikroorganismů

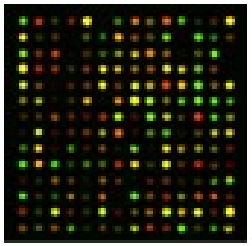
Metatranskriptomika

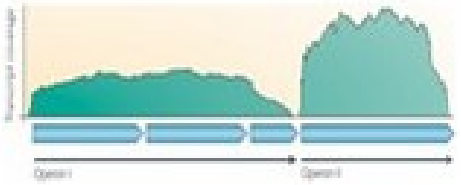
- Diverzita exprimovaných genů
- Abundance exprimovaných genů – nejvíce exprimované geny – nejpotřebnější metabolické dráhy v daném prostředí (genová exprese je velice rozdílná (foldy), nutná velká prosekvenovanost)
- Rozdíl v expresi při různých podmínkách (hledání biomarkerů)

Jak šel čas

- Příprava cDNA knihoven pomocí klonování, Sangerovo sekvenování
- Microarraye
- RNA-seq



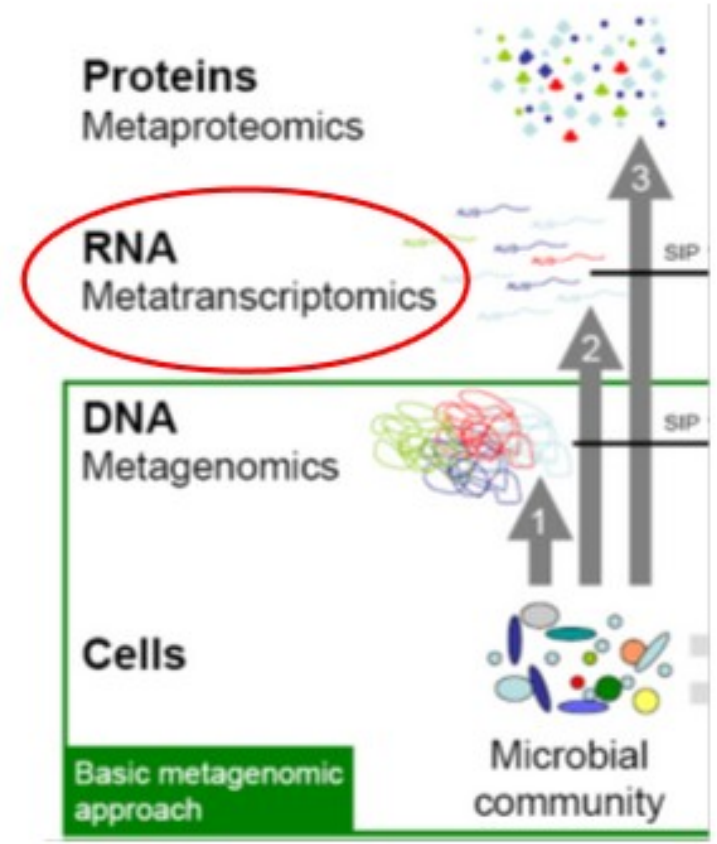




Sorek & Cossart, *NRG* (2010) 11, 9-16

Metatranskriptomika

- Kódující - mRNA
- Nekódující - rRNA, tRNA, regulační RNA…



Warnecke & Hugenholtz (2007) Genome Biology

Metatranskritpomika - problémy

- Málo RNA ve vzorcích
- Nestabilita RNA – rozkládá se v minutách
- mRNA tvoří pouze 1-5%, zbytek většinou rRNA

Postup

- Nejčastěji se používají Ribo-Zero magnetické kuličky
- Odstraní se bakteriální rRNA a následně eukaryontní rRNA (záleží na vzorku)

Efektivita odstranění rRNA

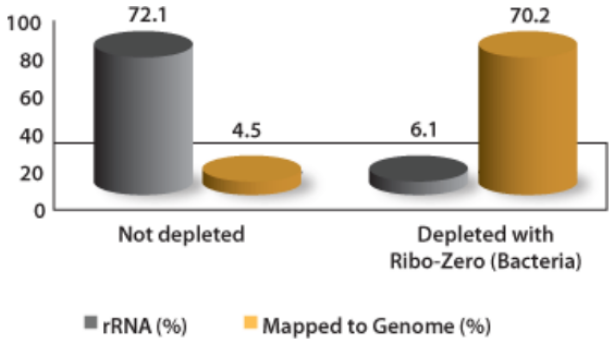
Sample	% rRNA	% Map (rumen metagenome)	% Other
No depletion control	82.4	3.4	10.5
Ribo-Zero Metabacteria	15.9	27.7	55.2
Ribo-Zero Metabacteria + Human/Mouse /Rat	4.9	26.7	56.3

Effective even on complex metatranscriptome samples.

- Cindi Hoover, JGI

Ribo-Zero kuličky - bakterie

Ribo-Zero (Bacteria) rRNA depletion increases metatranscriptome mapping efficiency



Organism	Gram +/–	% of Pool
Prochlorococcus marinus pastoris CMP 1986	–	1
Pediococcus pentosaceus	+	49
Acinetobacter sp. ADP1	–	21
Cyanobacterium synechocystis PCC 6803	–	25
Synechococcus elongates PCC 7942	–	4

A mixture of known bacteria containing gram-positive and gram-negative strains was created by Joint Genome Institute (JGI). The mixture was sequenced either without depletion (6.08m reads) or after depletion by Ribo-Zero (Bacteria) (6.82m reads). The percent of total reads that mapped to one of the bacterial strains increased 11.5 fold following depletion by Ribo-Zero (Bacteria) for an improved sequencing efficiency of 11.5x.

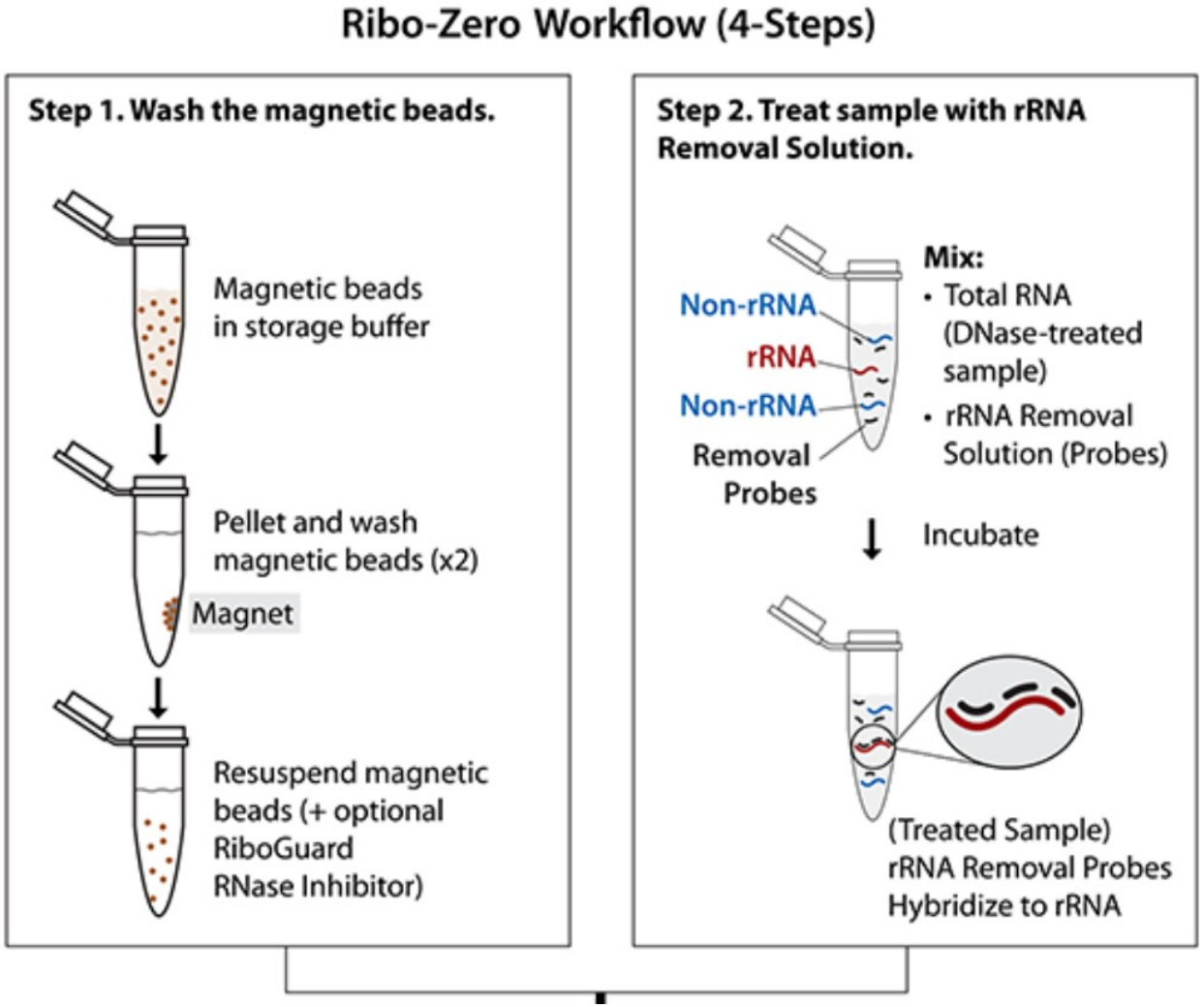
Ribo-Zero (Bacteria) removes rRNA from gram-positive and gram-negative bacteria

rRNA Removed					
Gram-Negative			Gram-Positive		
23S	16S	5S	23S	16S	5S

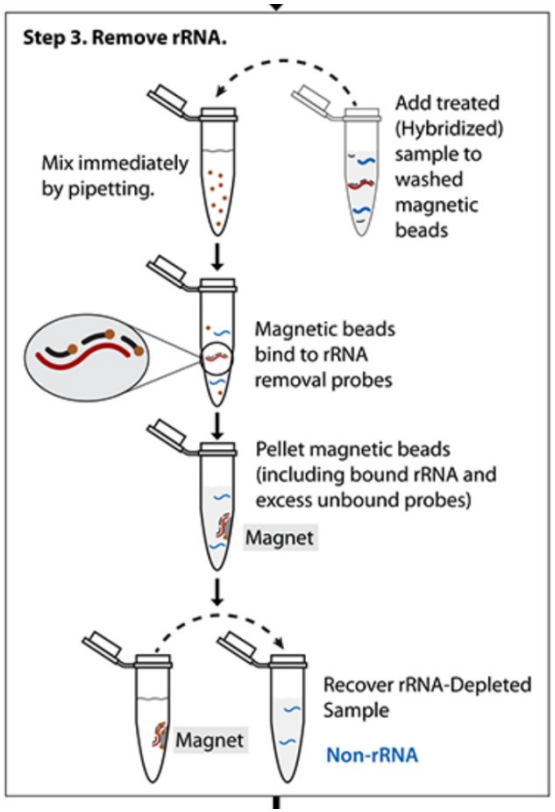
Ribo-Zero (Bacteria) removes ribosomal RNA from gram-positive, gram-negative and mixed samples.

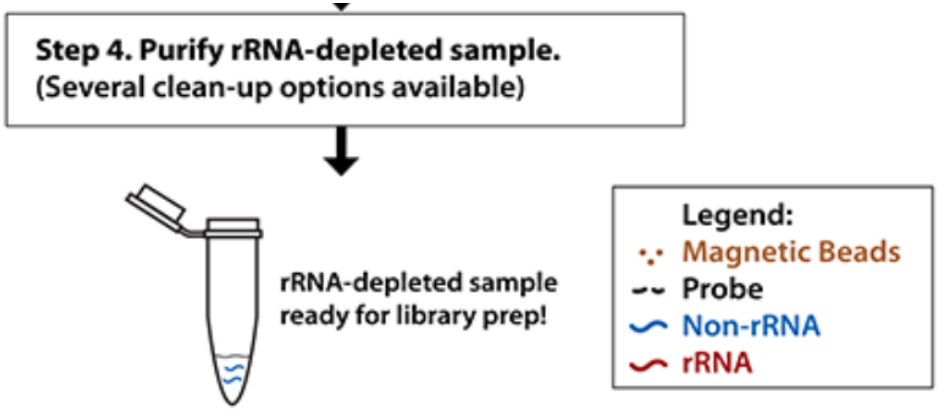
Postup odstranění rRNA pomocí Ribo-Zero kuliček - 4 kroky

Virtually all rRNA is depleted from intact, degraded and badly degraded (FFPE) samples in 2 hours.



Postup odstranění rRNA pomocí Ribo-Zero kuliček - 4 kroky





Celkový postup

- Vzorkování
  - Správný odběr vzorku
  - Stabilizace vzorku (např. RNA later)
  - Uskladnění (-80°C)
- Příprava knihovny
  - Izolace RNA, např. MoBio (speciální na RNA, popř. RNA +DNA)
  - Odstranění DNA (DNáza)
  - Odstranění rRNA (bakteriální, eukaryotické – Ribo-Zero)
  - Syntéza cDNA, odstranění RNA
  - Připojení adaptorů, kontrola knihovny

Kombinace meta technik

Computational and Structural Biotechnology Journal 13 (2015) 390–401

COMPUTATIONAL AND STRUCTURAL BIOTECHNOLOGY JOURNAL

ELSEVIER

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

Mini Review

Combining metagenomics, metatranscriptomics and viromics to explore novel microbial interactions: towards a systems-level understanding of human microbiome

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ARTICLE INFO

Article history:
Received 11 March 2015
Received in revised form 1 June 2015
Accepted 4 June 2015
Available online 9 June 2015

Keywords:
Metagenomics
Metatranscriptomics
Viromics
Human microbiome
Systems-level
Bioinformatics

ABSTRACT

The advances in experimental methods and the development of high performance bioinformatic tools have substantially improved our understanding of microbial communities associated with human niches. Many studies have documented that changes in microbial abundance and composition of the human microbiome is associated with human health and diseased state. The majority of research on human microbiome is typically focused in the analysis of one level of biological information, i.e., metagenomics or metatranscriptomics. In this review, we describe some of the different experimental and bioinformatic strategies applied to analyze the 16S rRNA gene profiling and shotgun sequencing data of the human microbiome. We also discuss how some of the recent insights in the combination of metagenomics, metatranscriptomics and viromics can provide more detailed description on the interactions between microorganisms and viruses in oral and gut microbiomes. Recent studies on viromics have begun to gain importance due to the potential involvement of viruses in microbial dysbiosis. In addition, metatranscriptomic combined with metagenomic analysis have shown that a substantial fraction of microbial transcripts can be differentially regulated relative to their microbial genomic abundances. Thus, understanding the molecular interactions in the microbiome using the combination of metagenomics, metatranscriptomics and viromics is one of the main challenges towards a system level understanding of human microbiome.

Kombinace meta technik

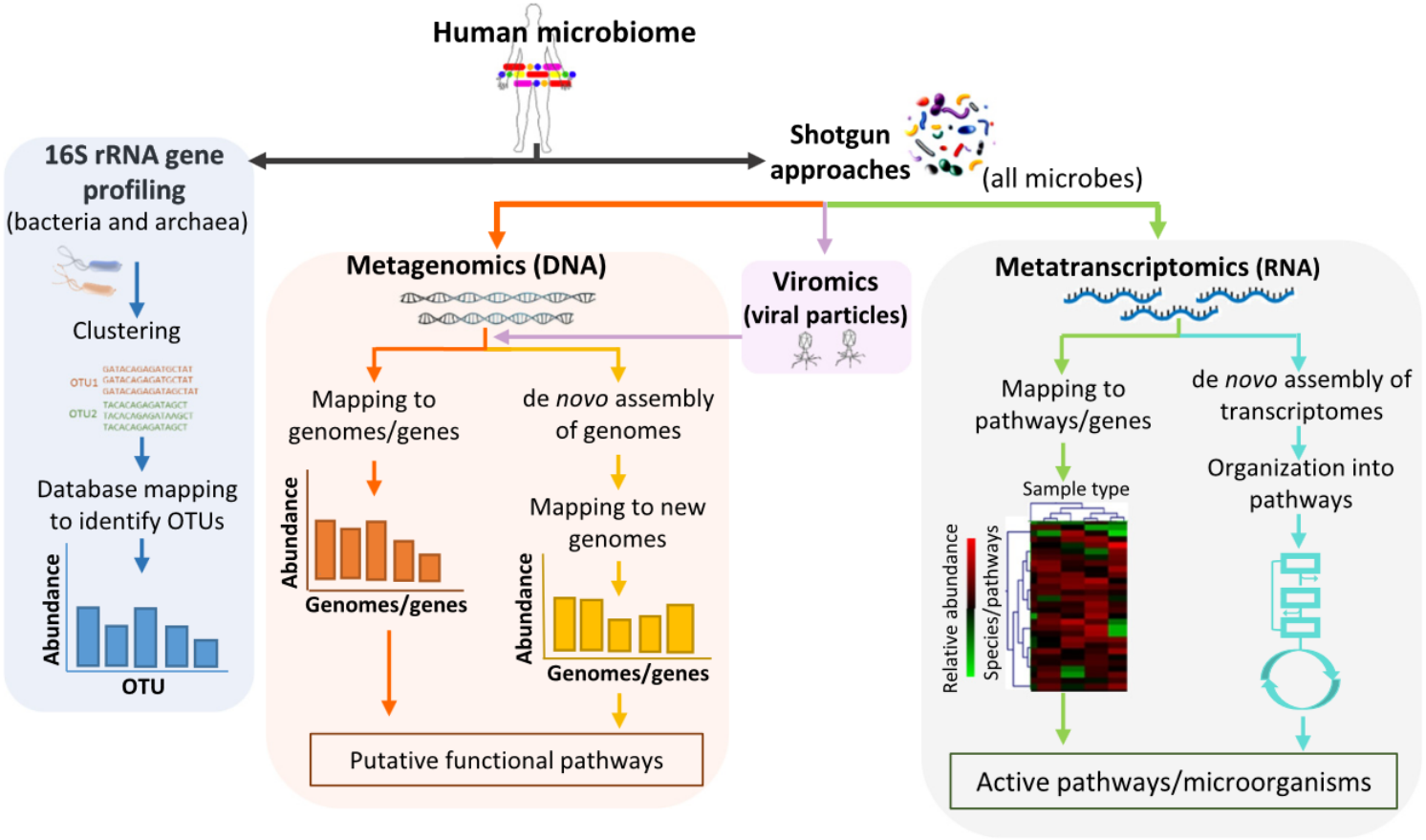

Fig. 1. Different sequencing and bioinformatic strategies for human microbiome analysis. In the 16S rRNA gene profiling the raw sequences obtained are passed through quality filters to minimize the presence of sequencing artifacts. The resulting filtered sequence reads are clustered into operational taxonomic units (OTUs), which represent similar organisms. After that, taxonomic identity is assigned for each OTU based in sequence homology against known 16S rRNA gene databases and the relative abundance of each OTU is calculated for each sample. The resulting OTUs table is also used for quantifying population diversity within and between the samples, as the alpha and beta diversity measurements, respectively. In the shotgun approaches, metagenomic, metatranscriptomic and viromic analyses are performed. In the metagenomic analysis, the DNA sequences obtained can either be mapped to reference genomes/genes or used for *de novo* assembly of genomes. Then the relative abundance of the present genomes/genes and the functional potential of the sequences can be assessed using functional annotated databases. In viromics analysis, first the viral particles (VPs) must be enriched and posteriorly sequenced to obtain the virus genomes. Furthermore, to analyze the active genes and species of the microbiome, the metatranscriptomic analysis is applied and the obtained RNA sequences are mapped to reference pathways and genes. The results are used to identify the active pathways, genes and microorganisms. Thus, the relative abundance of each active pathway/gene/microorganism in the human microbiome is determined. The *de novo* assembly of genomes and transcriptomes can be also performed to identify novel genomes or pathways.

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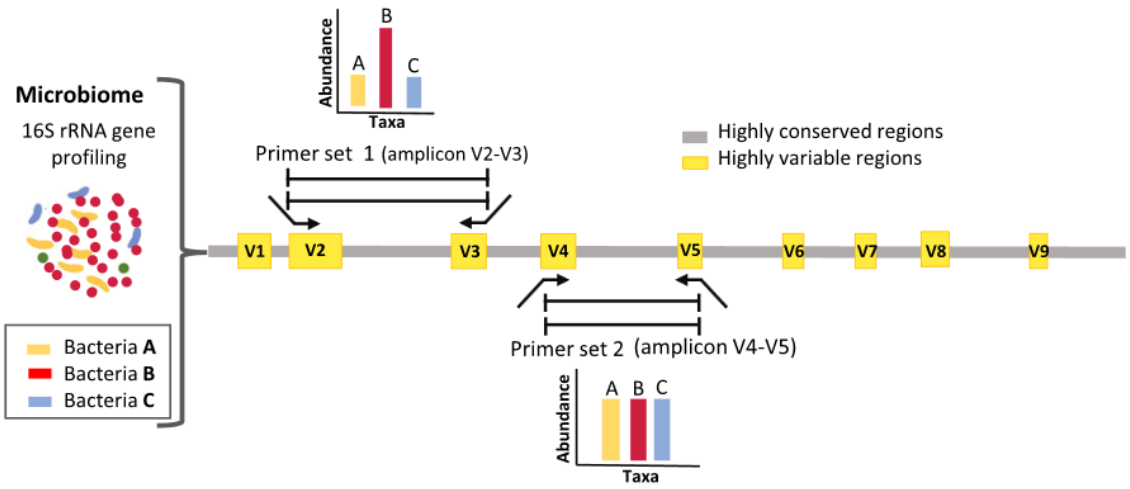

Fig. 2. Importance of primer selection for the amplification of the hypervariable regions of the 16S rRNA gene. The figure illustrates how choosing different sets of primers for the amplification of different hypervariable regions of the 16S rRNA gene has an influence in the resulting abundance of hypothetical bacteria A, B and C. For example, in this figure, the species abundance distribution obtained using primer set 1 shows a more similar distribution to that observed in the microbiome than the abundance obtained from primer set 2. In a similar manner, Kuczynski et al [2], demonstrated that using the universal primer set F515–R806 (which is typically used to amplify a great coverage of bacteria and archea) in skin samples showed poor results for the identification of *Propionibacterium*, however the use of primer set F27–R338 was better to identify this bacteria [2].

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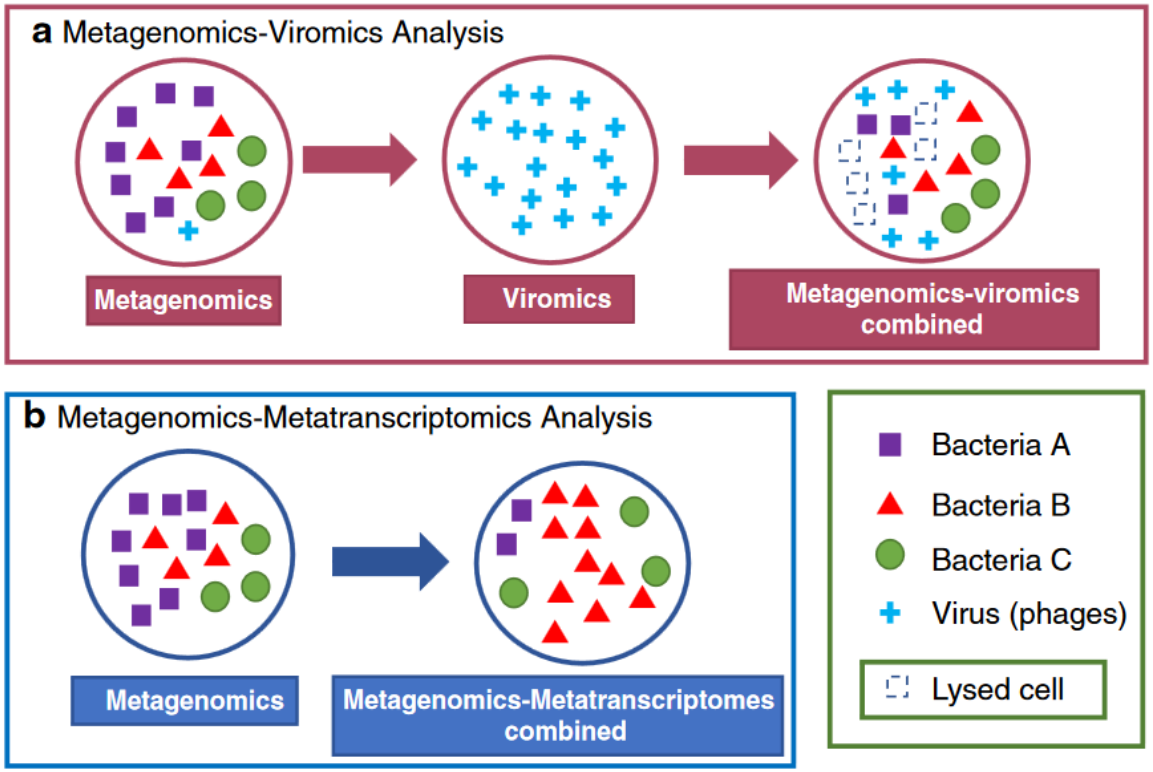

Fig. 3. Molecular interactions explored using metagenomics–viromics and metagenomics–metatranscriptomics analyses. The interactions between microorganisms in the human microbiome can be better studied combining omics analysis. (a) In this panel is illustrated how phages can interact and affect the microbial diversity by infecting their host bacteria and thus promoting homeostasis or disbyosis [179,24]. This type of interaction can be explored using viromics combined with metagenomics. (b) The species abundance of the three hypothetical bacteria can be different depending on the used analysis (metagenomics or metagenomics combined with metatranscriptomics) [171]. The data integration and normalization when metagenomics is combined with metatranscriptomics is important because the metatranscriptomics data can correspond to different species abundance and/or to differentially expressed transcriptomes.

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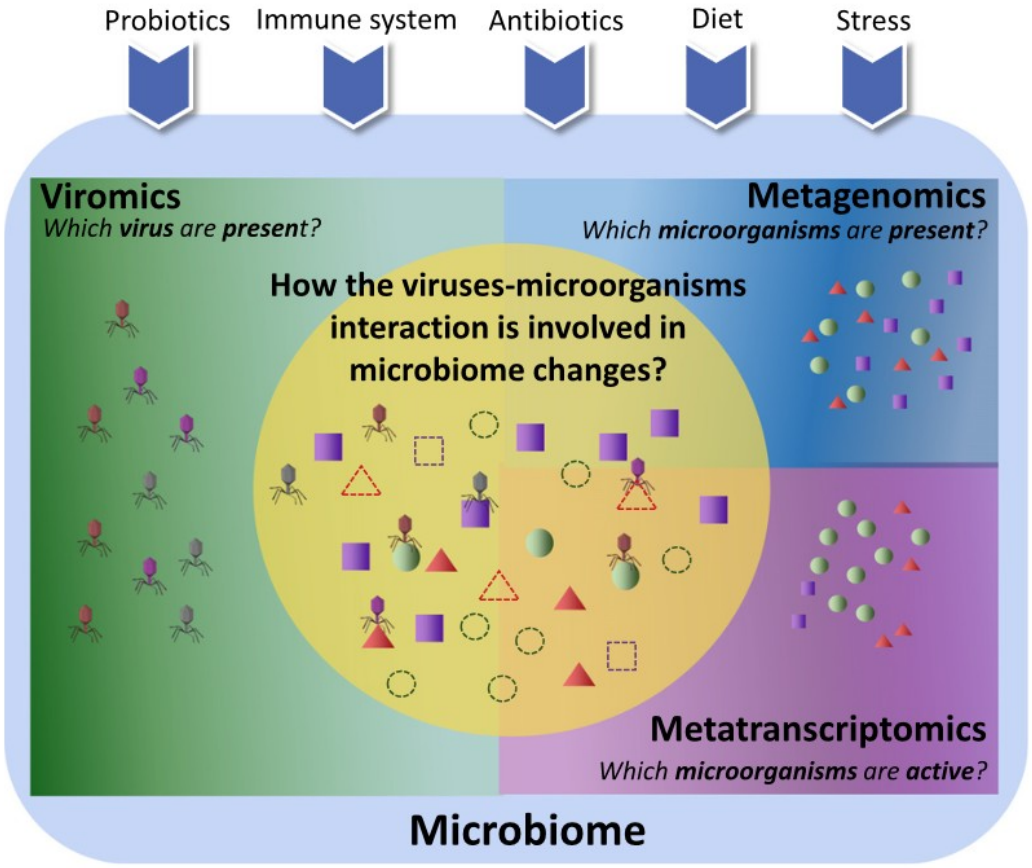

Fig. 4. Towards a systems level understanding of human microbiome. The use of only one analysis to study the human microbiome (viromics, metagenomics or metatranscriptomics) provides a partial view of the complete ecological system. In a combined approach, the metagenomic analysis can give us a view of the microorganism's abundance and functions available in the microbiome, while the metatranscriptomic analyses combined with metagenomics can show us which of these microorganisms and functions are actually active. Finally, the integration of viromics analysis with the other omics data can provide information about the role that viruses play within the microbiome. The combined analyses can offer a better understanding of the role that external factors like diet, immune system and probiotics are playing in shaping the human microbiome abundance and composition. Thus, an integrated systems analysis (orange circle) seems necessary to have a better understanding of molecular mechanisms and their interactions in human microbiome.