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# Identification of genomic regions for high-resolution taxonomic profiling using long-read sequencing technology

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## Taxonomic profiling: who is in the community?



### The widespread MiSeq illumina amplicon sequencing approach:

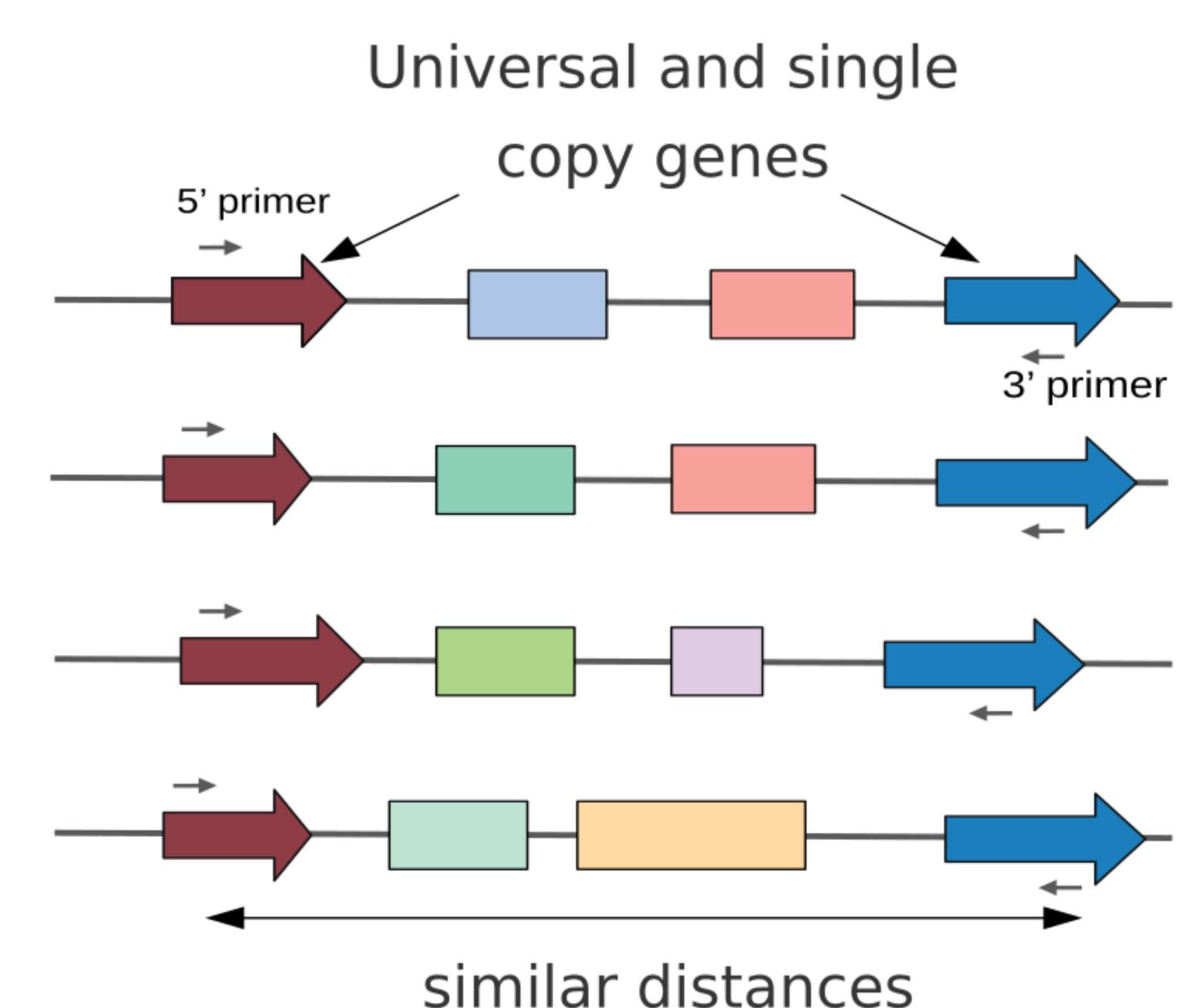
Target a small part of the 16S RNA gene or alternative markers (*rpoB*, *recA* and *gyrB*) [1].

### Limits of the approach:

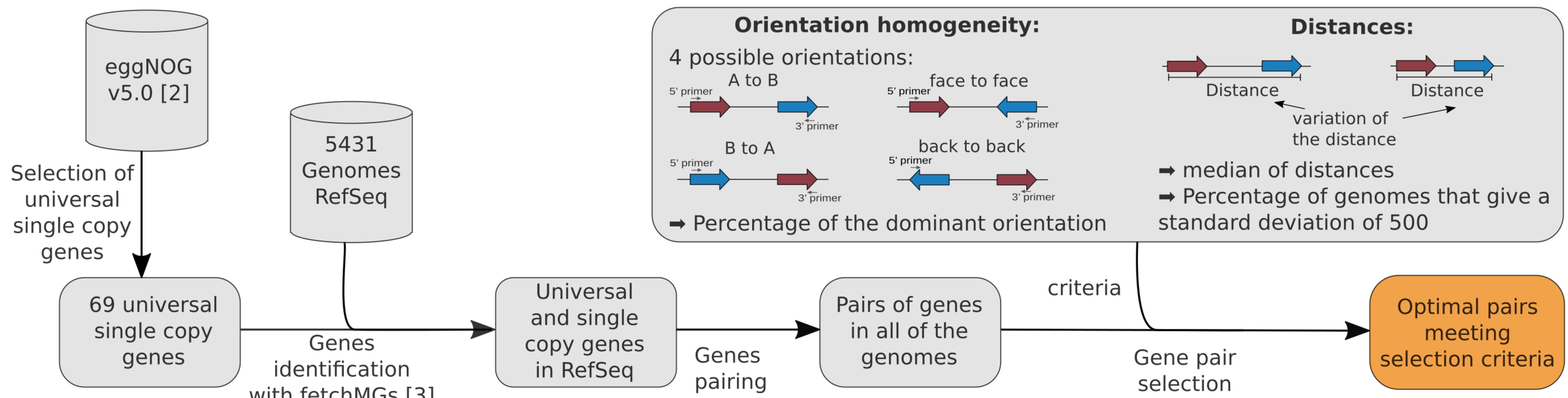
- Amplicon size of around 450pb
- Poor resolution for closely related organisms
- Multicopy affects the abundance estimation

### Our strategy using long read approach with Oxford Nanopore Technologies:

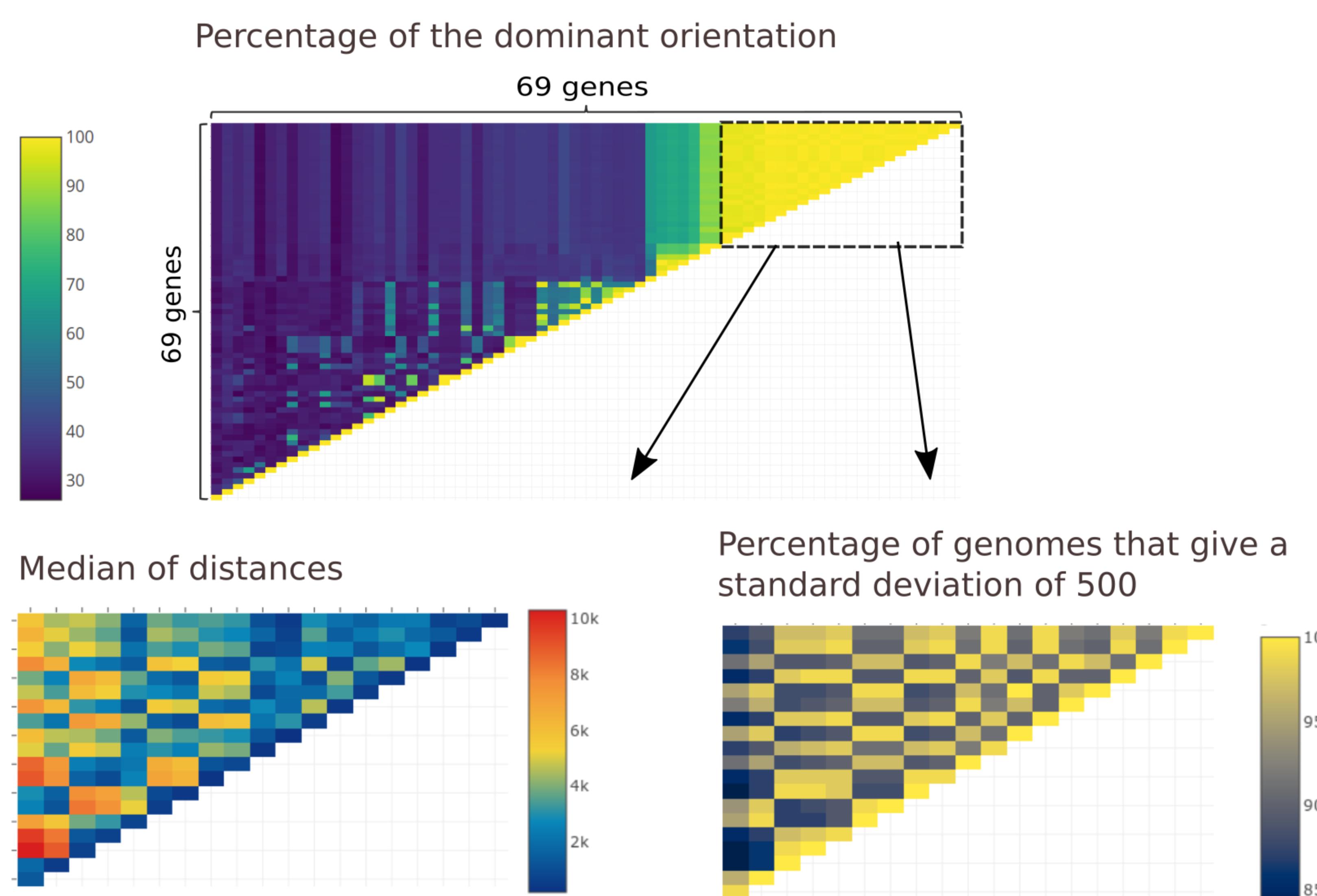
Identify genomic regions bounded by two universal and single copy genes.



## Genomic regions identification workflow



## Optimal pair selection



### Pair filtering:

Percentage of dominant orientation > 95%

2kb < median < 8kb

Percentage of genomes that give a standard deviation of 500 > 95%

2346 possible pairs

110 pairs

80 pairs

38 pairs

## Conclusion and perspectives

Our approach enables the identification of several pairs of genes found in **single copy** and displaying **consistent distances and orientations** across bacterial genomes. These pairs are very promising targets to enhance taxonomic profiling with long read sequencing technologies.

### The next steps of the project are:

- Investigate how well these regions can discriminate closely related organisms.
- Design primers in order to amplify the chosen regions.
- Test out the chosen regions in a wet lab experiment on a real microbial community.

[1] Poirier, Simon, et al. "Deciphering intra-species bacterial diversity of meat and seafood spoilage microbiota using *gyrB* amplicon sequencing: A comparative analysis with 16S rDNA V3-V4 sequencing." *PLoS one* 13.9 (2018): e0204629.

[2] Huerta-Cepas, Jaime, et al. "eggNOG 5.0: a hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses." *Nucleic acids research* 47.D1 (2018): D309-D314.

[3] Milanese, Alessio, et al. "Microbial abundance, activity and population genomic profiling with mOTUs2." *Nature communications* 10.1 (2019): 1014.