# Supplementary Material for “YAMP: a containerised workflow enabling reproducibility in metagenomics research”

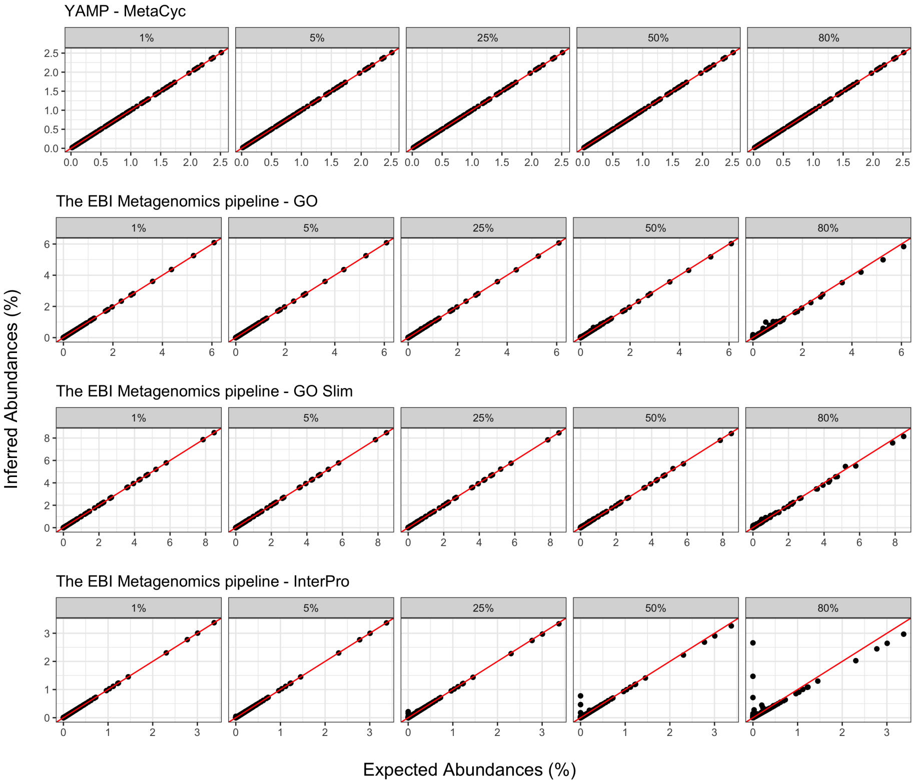
Alessia Visconti[1], Tiphaine C. Martine,[1], and Mario Falchi,[1]
[1]Department of Twin Research and Genetic Epidemiology,
King’s College London, London, UK

Supplementary Table S1: Simulated human oral community. The community includes 13 bacterial species. The genomic coverages, and therefore the relative proportion of the bacterial genomes, follow those suggested in Zhou Q, Su X, Ning K. *Assessment of quality control approaches for metagenomic data analysis.* Scientific reports (2014);4:6957, but for *Prevotella tannerae* which has been substituted with *Alloprevotella tannerae*. * indicates that the GenBank assembly accession number has been reported. We considered as *natural duplicates* the reads which were generated to match the requested coverage.

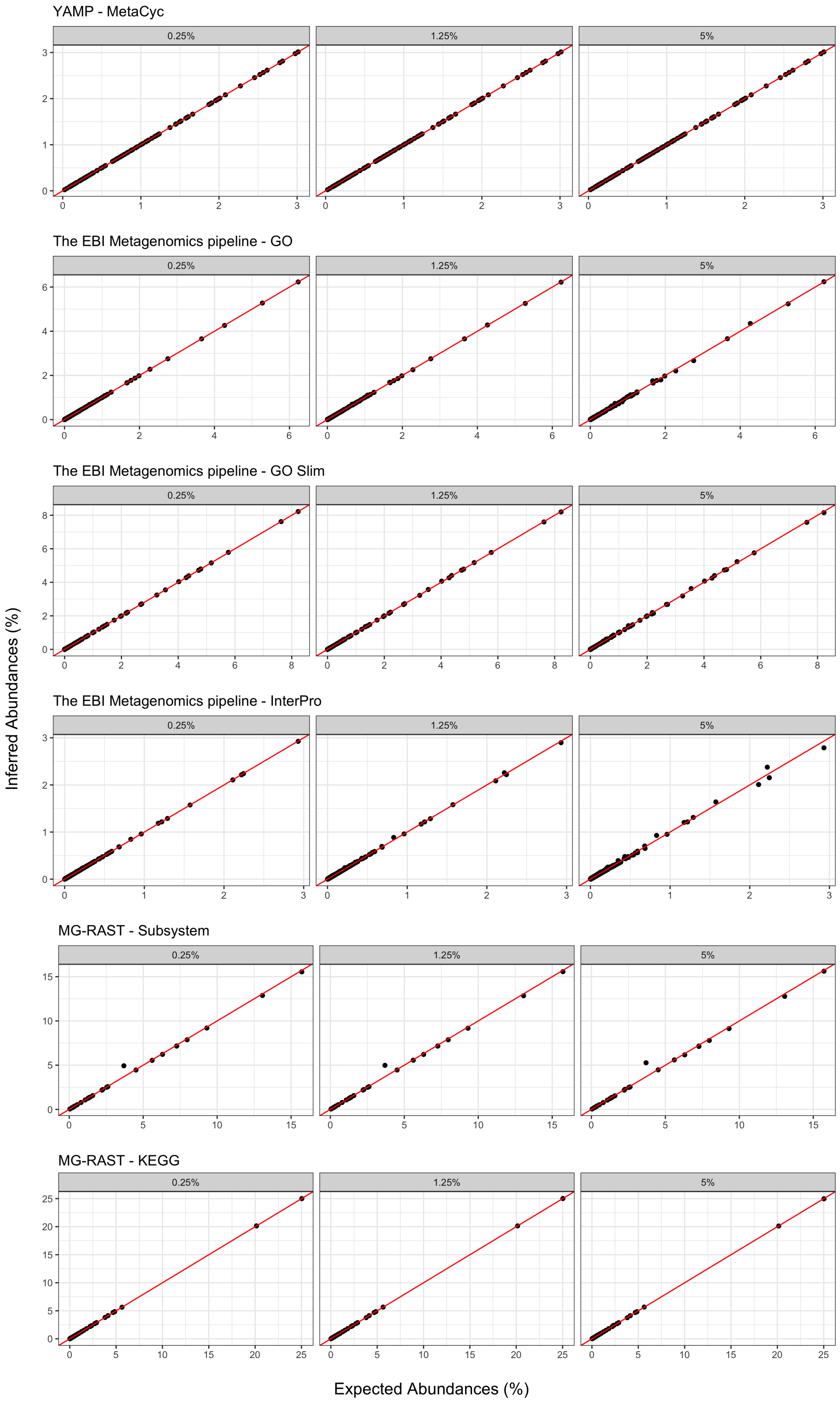
| **Species** | **RefSeq Accession number** | **%GC content** | **Genomic Coverage** | **Reads number** | **Reads percentage** | **Natural duplicates Reads (%)** |
|---:|:---:|:---:|:---:|---:|---:|---:|
| *Rothia dentocariosa* | GCF_002861025.1 | 53.75 | 6 | 149,659 | 22.80 | 8435 (5.64) |
| *Porphyromonas endodontalis* | GCF_000174815.1 | 47.52 | 5 | 103,225 | 15.73 | 2531 (2.45) |
| *Streptococcus peroris* | GCF_000187585.1 | 39.15 | 5 | 81,996 | 12.49 | 786 (0.96) |
| *Alloprevotella tannerae* | GCF_000159995.1 | 46.56 | 2 | 51,714 | 7.88 | 1501 (2.90) |
| *Porphyromonas gingivalis* | GCF_002892575.1 | 48.36 | 2 | 47,597 | 7.25 | 18 (0.04) |
| *Veillonella atypica* | GCF_001546845.1 | 38.88 | 2 | 41,977 | 6.40 | 521 (1.24) |
| *Megasphaera micronuciformis* | GCF_000165735.1 | 45.44 | 2 | 35,310 | 5.38 | 1101 (3.12) |
| *Prevotella veroralis* | GCF_000613325.1 | 41.80 | 1 | 28,344 | 4.32 | 294 (1.04) |
| *Prevotella denticola* | GCA_001062325.1* | 50.05 | 1 | 25,990 | 3.96 | 20 (0.08) |
| *Campylobacter rectus* | GCF_000174175.1 | 44.85 | 1 | 25,131 | 3.83 | 133 (0.53) |
| *Actinomyces odontolyticus* | GCF_002847525.1 | 65.46 | 1 | 23,415 | 3.57 | 38 (0.16) |
| *Veillonella parvula* | GCF_002847925.1 | 38.57 | 1 | 21,505 | 3.28 | 248 (1.15) |
| *Veillonella dispar* | GCF_001553315.1 | 38.57 | 1 | 20,454 | 3.12 | 195 (0.95) |

Supplementary Table S2: Simulated dataset. We report here the European Nucleotide Archive (ENA) project and sample accession numbers for the datasets used in the two simulation studies.

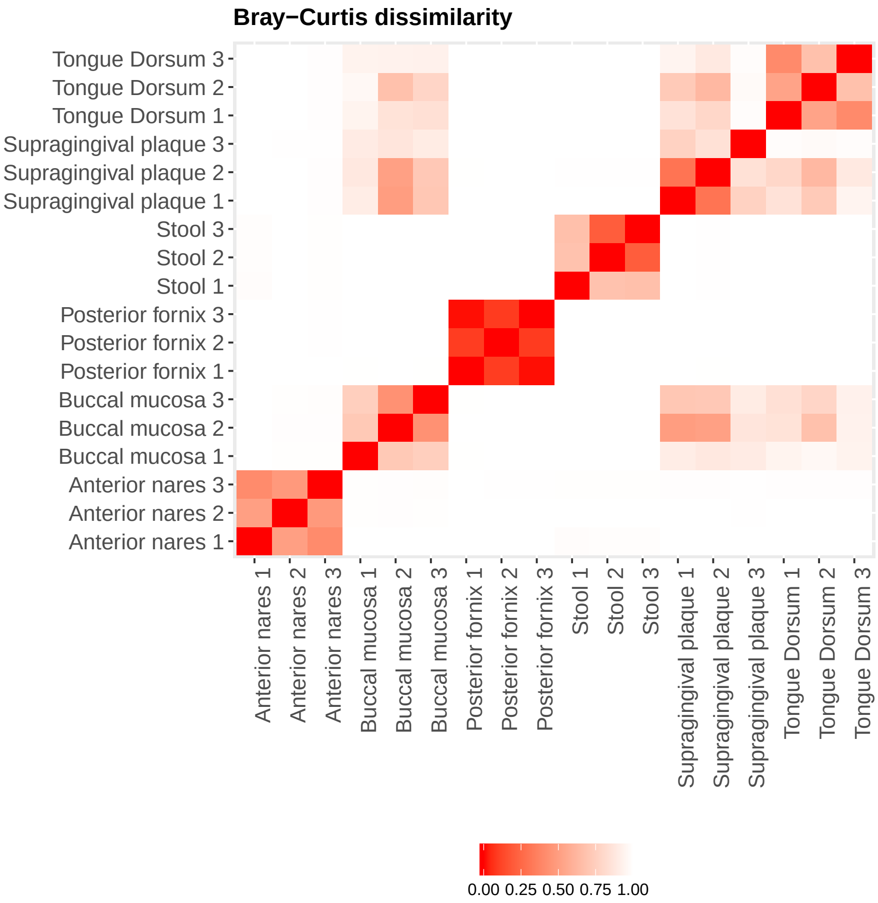
| Simulated Study | Project Accession Number | Sample Accession Number | Setting |
|---:|---:|---:|---:|
| Human Contamination | PRJEB25791 | ERS2327609 | Baseline |
| | | ERS2327610 | 1% contamination |
| | | ERS2327611 | 5% contamination |
| | | ERS2327612 | 25% contamination |
| | | ERS2327613 | 50% contamination |
| | | ERS2327614 | 80% contamination |
| Artificial Duplication | PRJEB26333 | ERS2418541 | Baseline |
| | | ERS2418542 | 0.25% duplicates |
| | | ERS2418543 | 1.25% duplicates |
| | | ERS2418544 | 5.00% duplicates |



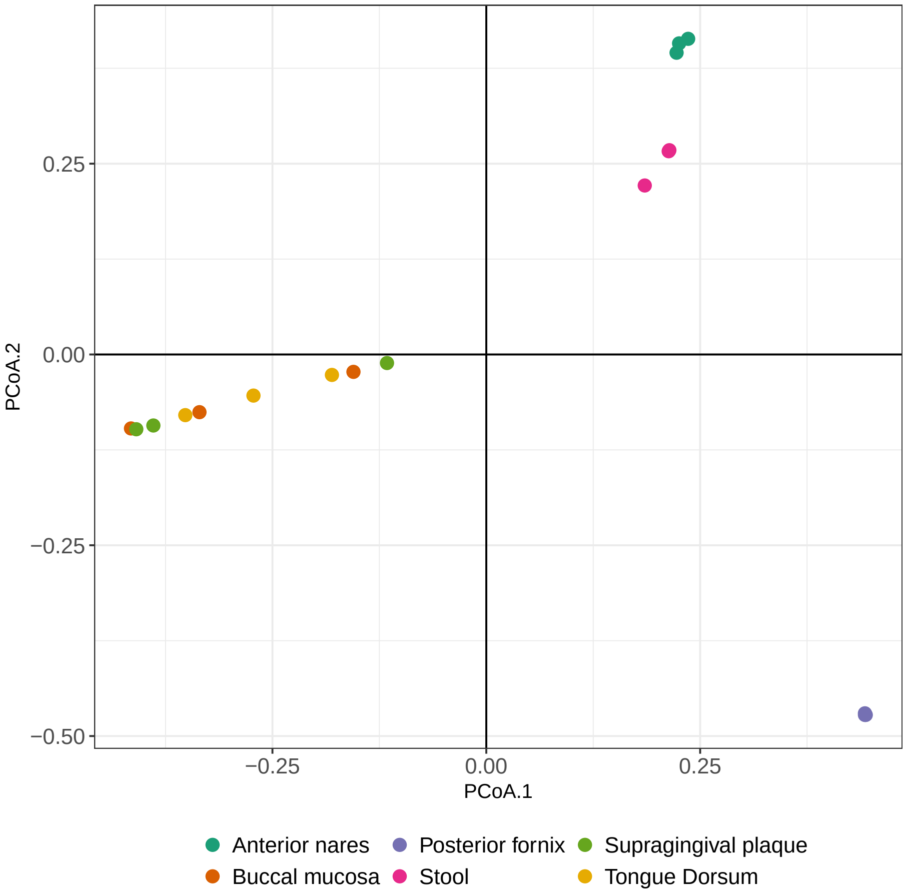
Supplementary Figure S1: Results of the first simulation study (human contamination). Each scatterplot represents the relationship between the expected functional annotations (*i.e.*, inferred without human contamination) and those inferred at different human contamination levels. For YAMP, we report results obtained with the MetaCyc database; for the EBI metagenomics pipeline (v4.1), we report results on the Gene Ontology (GO), GO Slim, and InterPro databases.



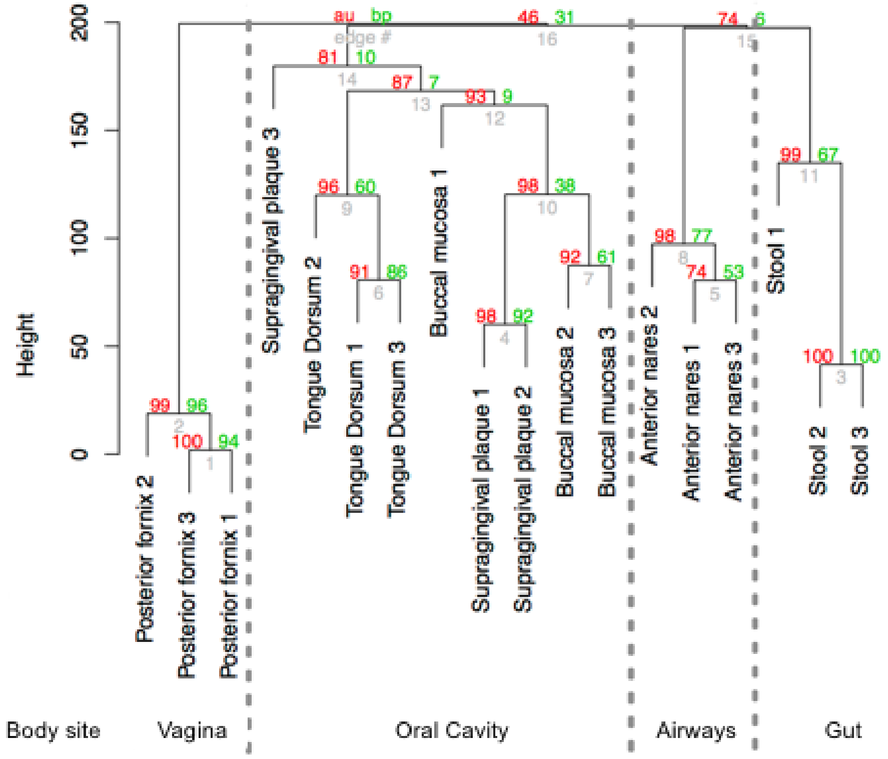
Supplementary Figure S2: Results of the second simulation study (artificial duplicates). Each scatterplot represents the relationship between the expected functional annotations (*i.e.*, inferred without duplicated reads) and those inferred at different levels of artificial duplication. For YAMP, we report results obtained with the MetaCyc database; for the EBI metagenomics pipeline (v4.1), we report results on the Gene Ontology (GO), GO Slim, and InterPro databases; for MG-RAST (v.4.0.3) we report results on the SEED Subsystem and the KEGG databases.



Supplementary Figure S3: Bray-Curtis dissimilarity for the 18 analysed samples. The Bray-Curtis dissimilarity values were evaluated using the species relative abundances as estimated by YAMP using MetaPhlAn2 and the *vegdist* function in the `vegan` R package.



Supplementary Figure S4: Principal coordinate analysis (PCoA) for the 18 analysed samples. PCoA was evaluated on the Bray-Curtis dissimilarity values using the *pcoa* function in the `ape` R package. PCoA shows that species composition is sufficient to discriminate among body sites, even though it has limited ability in distinguishing among different loci in the oral cavity.



Supplementary Figure S5: Hierarchical clustering for the 18 analysed samples. Hierarchical clustering was computed using the Manhattan distance among species relative abundances. Values at branches are, in red, the approximately unbiased (AU) P values, in green, the bootstrap probability (BP) values (percentages), and, in grey, the edge number.