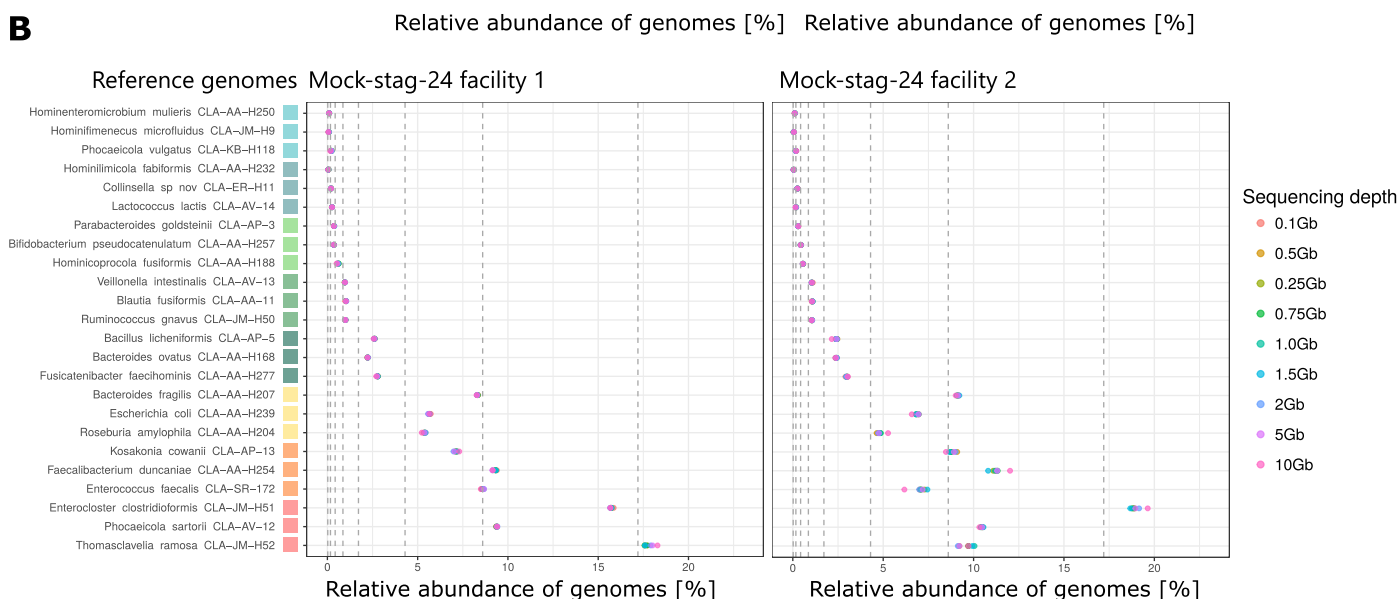
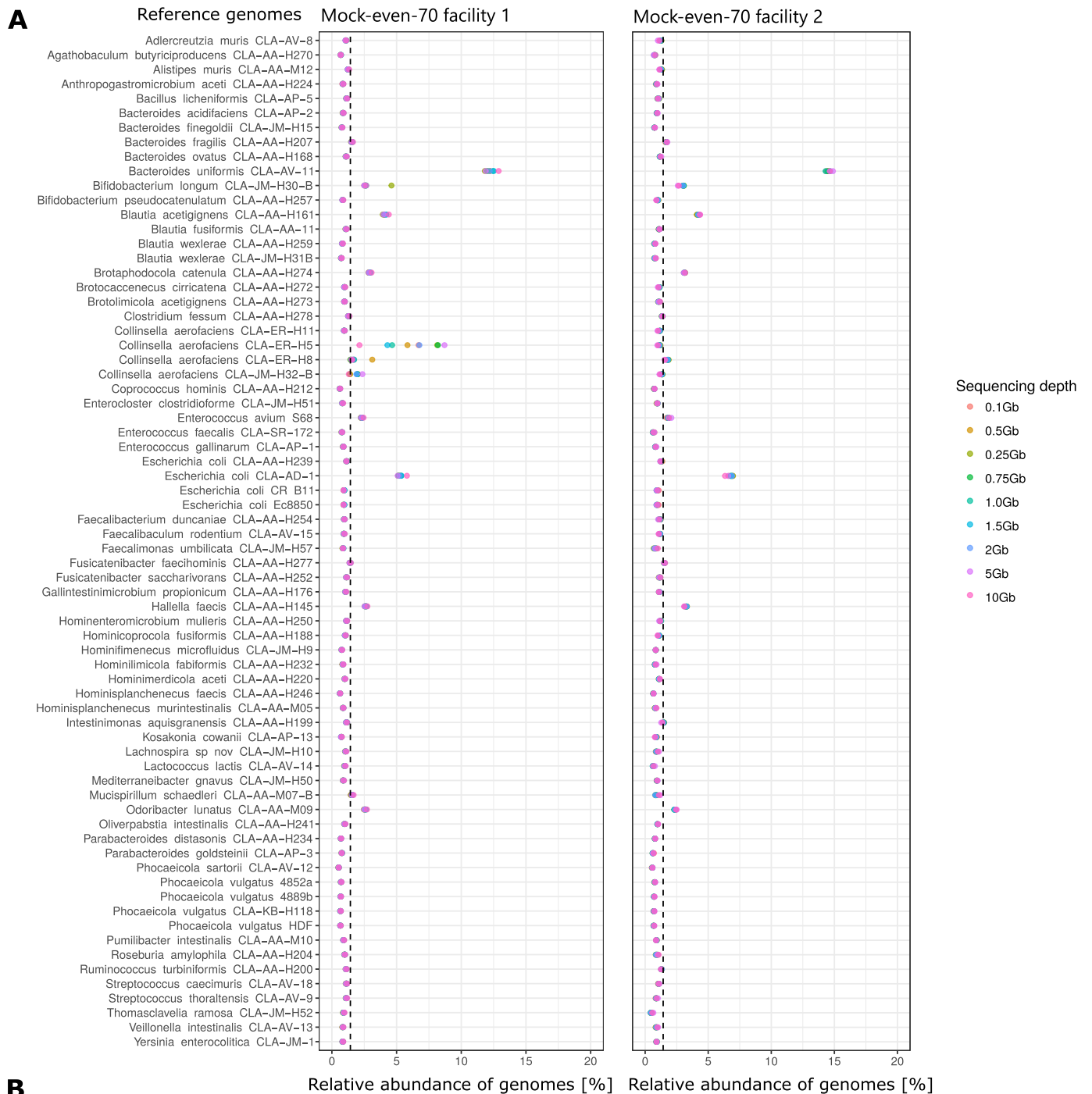
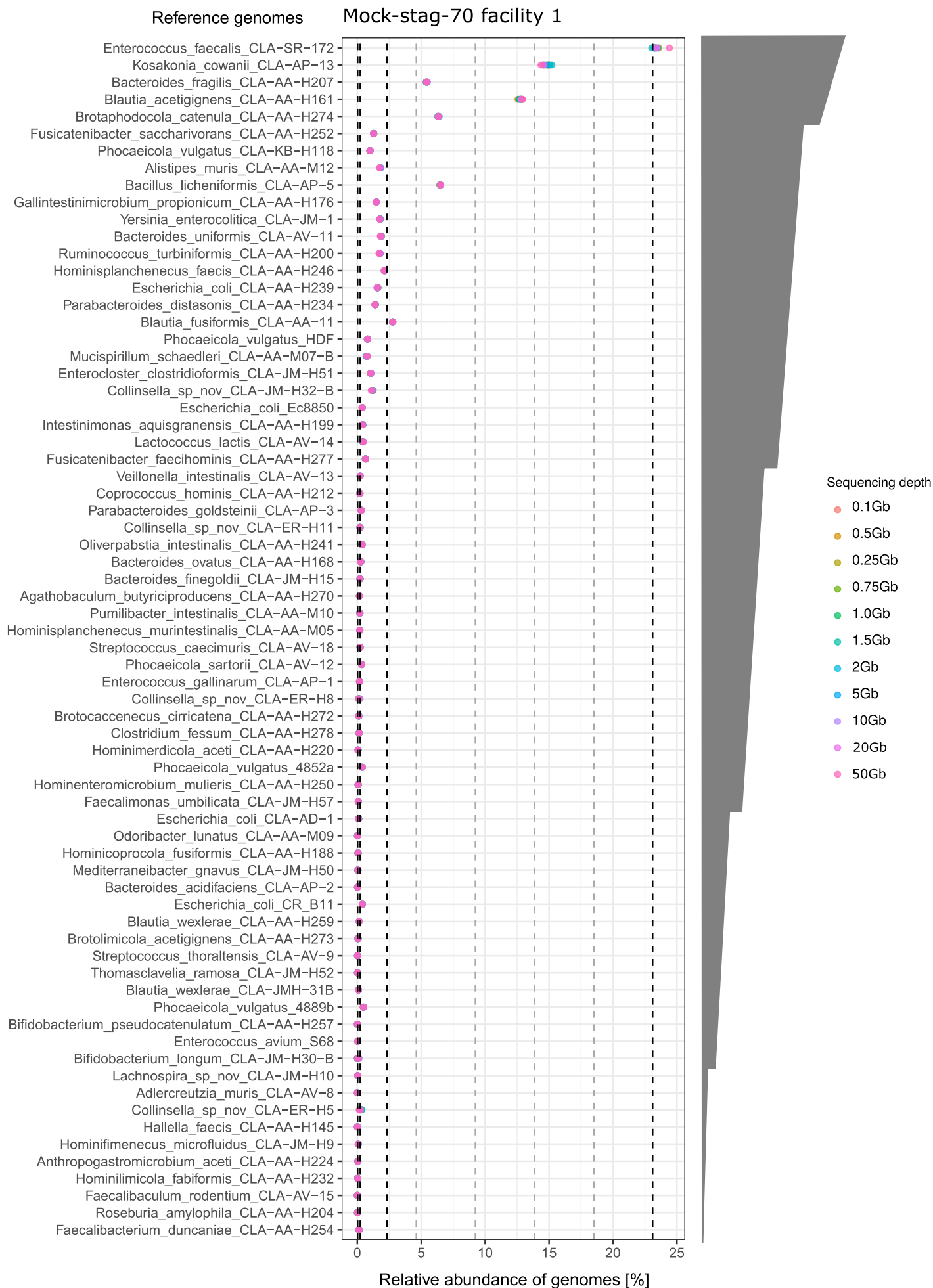


Benchmarking of shotgun sequencing depth reveals the potential and limitations of shallow metagenomics and strain-level analysis

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Supplementary Fig. S1: Relative abundance per reference genome
 Relative abundances based on reference genomes in (A) Mock-even-70 in facility 1 and 2, (B) Mock-stag-24 in facility 1 and 2. Sequencing depths are indicated by the coloured dots. The theoretical relative abundances are indicated by vertical dashed lines. For Mock-stag-24 in panel B, the reference genomes are ranked from top to bottom by increasing DNA amount in the mixture indicated by coloured boxes (from blue to red; 0.04, 0.4, 1, 2, 4, 10, 20, 40 ng)(concentrations are provided in **Supplementary Data S1**).



Supplementary Fig. S2: Relative abundance per reference genome

Relative abundances based on reference genomes in Mock-stag-70 in facility 1.

Sequencing depths are indicated by the coloured dots. The theoretical relative abundances are indicated by vertical dashed lines. The reference genomes are ranked from top to bottom by decreasing DNA amount in the mixture indicated by the grey gradient (concentrations are provided in **Supplementary Data S1**).

Mock-even-70 facility 1

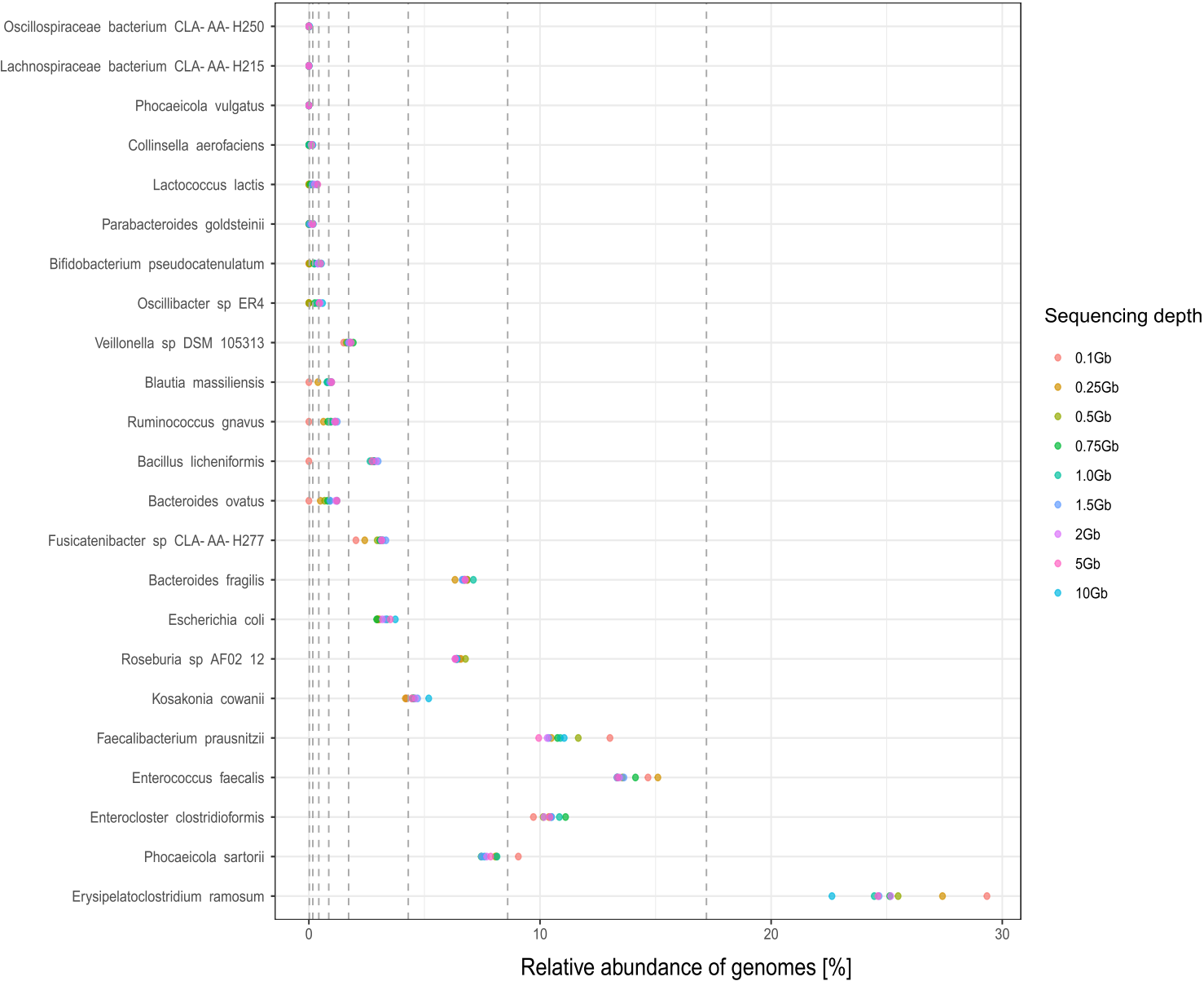
Species assigned by Metaphlan



Supplementary Fig. S3: Relative abundance after “non-supervised” taxonomic assignment using MetaPhlAn4 in Mock-even-70. Sequencing depths are indicated by the coloured dots. The theoretical relative abundance is indicated by a vertical dashed line.

Mock-stag-24 facility 1

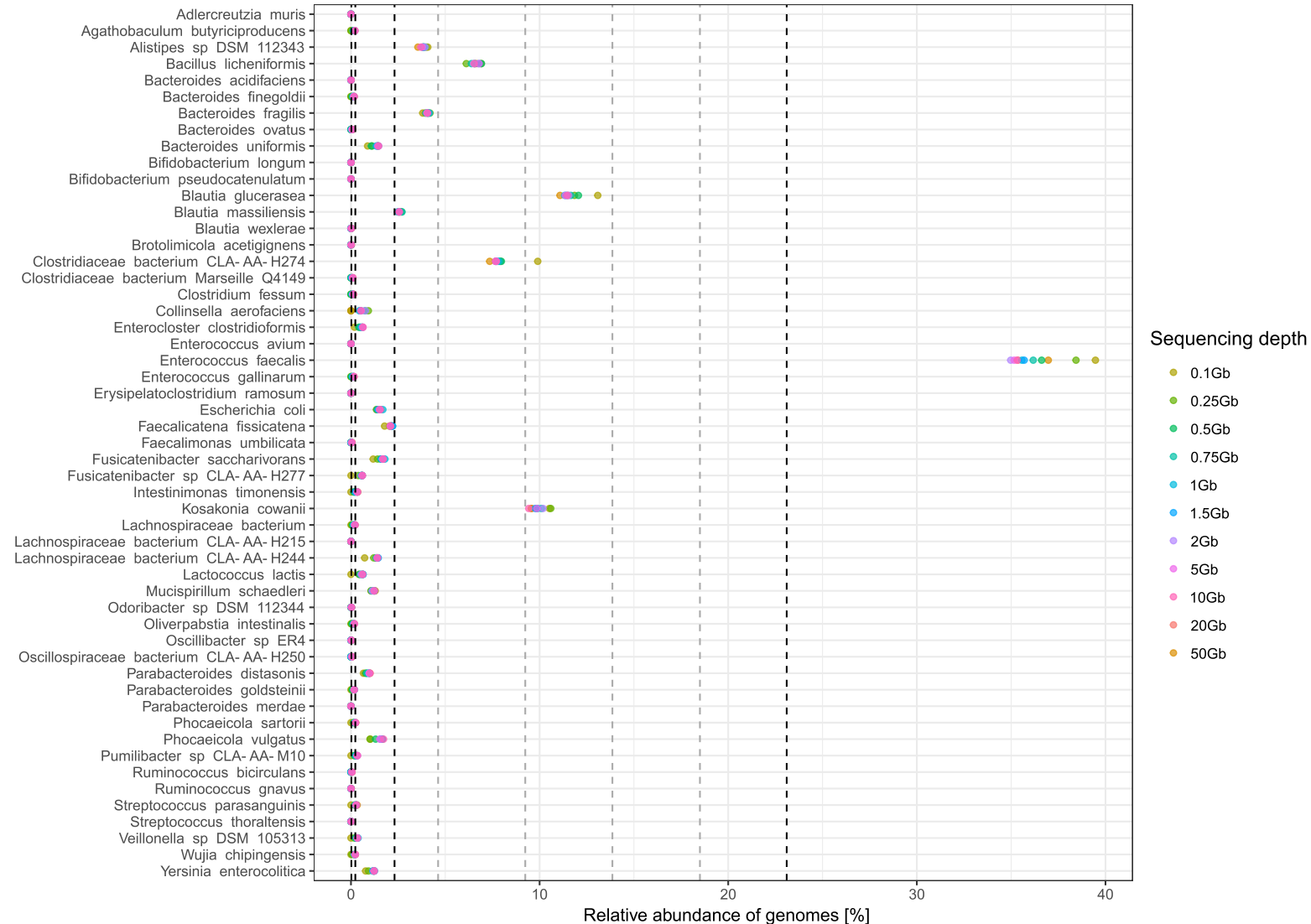
Species assigned by Metaphlan



Supplementary Fig. S4: Relative abundance after “non-supervised” taxonomic assignment using MetaPhlAn4 in Mock-stag-24. Sequencing depths are indicated by the coloured dots. The theoretical relative abundances are indicated by vertical dashed lines.

Species assigned by Metaphlan

Mock-stag-70 facility 1



Supplementary Fig. S5: Relative abundance after “non-supervised” taxonomic assignment using MetaPhlAn4 in Mock-stag-70. Sequencing depths are indicated by the coloured dots. The theoretical relative abundances are indicated by vertical dashed lines.