# Guardians of the gut: harnessing bioinformatics to study the gut microbiome and faecal microbiota transplantation in intestinal disorders

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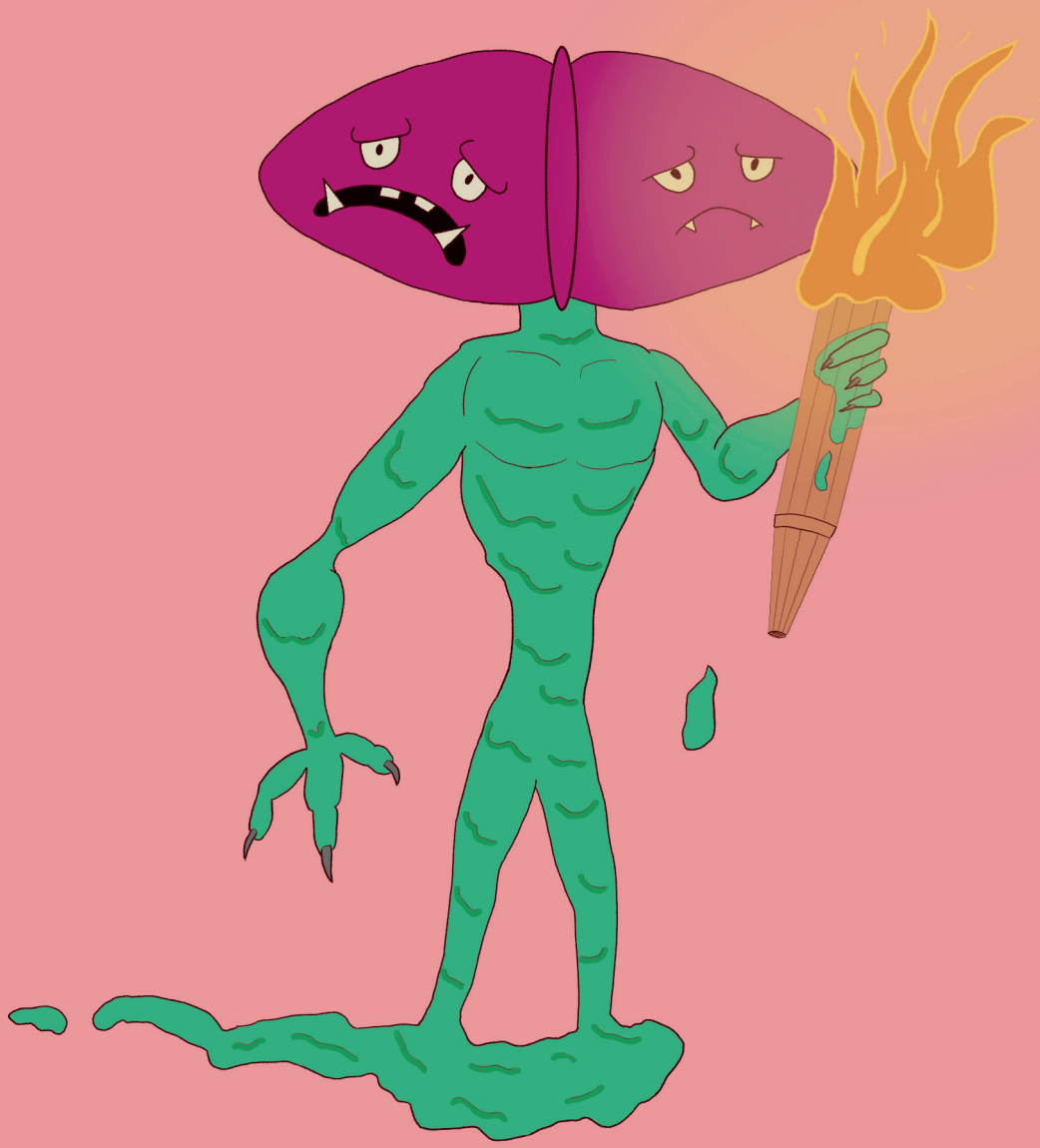

# Chapter 7.2

## Draft and complete genome sequences of 17 *Streptococcus* species

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## Abstract

We present seventeen near-complete and complete genomes of *Streptococcus* species obtained from eight mixed cultures of presumed *Ruminococcus gnavus* isolates. The genomes are classified as eight different *Streptococcus* species and three are unclassified (currently have no species representative available in databases). We provide these high-quality genomes to the scientific community for further scrutinization.


## Announcement

In a project focusing on isolating and sequencing genomes of *Ruminococcus gnavus* (reference *R. gnavus* manuscript), we sent presumed pure *R. gnavus* DNA for long-read PacBio circular consensus sequencing (CCS) to the Leiden Genome Technology Center (Leiden University Medical Center, Leiden, Netherlands). The DNA libraries were prepared using the SMRTbell prep kit 3.0 kit and sequenced on the Sequel II platform (Pacific Biosciences of California, Inc., CA, USA). Raw reads were assembled with Flye[1] (version 2.9.2) and resulting contigs were classified using the Contig Annotation Tool[2] (CAT; version 5.2.3). Contigs were reoriented to start at the *dnaA* for putative chromosomes using dnaapler[3] (version 0.3.0). To our surprise, eight of the samples returned complete *Streptococcus* genomes and short (7-55kbp), predicted *R. gnavus* contigs with an estimated depth of coverage of 4X. As we suppose these Streptococci are lab contaminants, we cannot be certain of their exact origin. Nonetheless, we aimed to generate complete genomes and improved assemblies of *Streptococcus* genomes by using a metagenome assembly approach with metaFlye[4] (version 2.9.2). This yielded up to 194 contigs per sample with a median length of 23kbp. We separated the contigs classified as *Streptococcus* using seqkit[5] (version 0.16.0) and did a quality control consisting of: 1) a length and GC-content check by QUAST[6] (version 5.0.2), 2) completeness and contamination estimation by CheckM[7] (version 1.0.13), CheckM2[8] (version 1.0.1) and BUSCO[9] (version 5.4.3; using flag '--auto-lineage-prok'), 3) gene annotation with Bakta[10] (version 1.6.1), and 4) taxonomic classification with GTDB-Tk[11] (version 2.3.2) using the Genome Taxonomy database (GTDB) version r207_v2. All tools were run with default parameters unless stated otherwise. Relevant statistics of each genome are listed in table 1. Furthermore, to determine the relatedness between the *Streptococcus* genomes and infer if they might originate from a common source, we used fastANI[12] (version 1.33) to calculate pairwise average nucleotide identity (ANI; figure 1). We found five *Streptococcus equinus* (NCBI taxonomy; GTDB: *Streptococcus sp001556435*) highly similar genomes (ANI ≥ 99.9%) from five different samples, four *S. oralis* (GTDB: 2 × identical *S. oralis_S* and 2 × *S. oralis_Y*) from three different samples, and six genomes with slightly different classifications that are all close to *S. parasanguinis* (ANI ≥ 93.8%) from three different samples (1×3, 1×2, 1×1 genome/sample). We found one genome classified as *S. sp902363395* (GTDB), which is unknown

to NCBI, that remotely resembles (ANI ~86.4%) the *parasanguinis*-like genomes. The final genome was classified as *S. sanguinis* SK49 (GTDB: *S. sanguinis_C*) and is only remotely similar to the other genomes (ANI ≤ 80%).

All raw reads and assembled genomes are available through the European Nucleotide Archive under project number PRJEB76410.

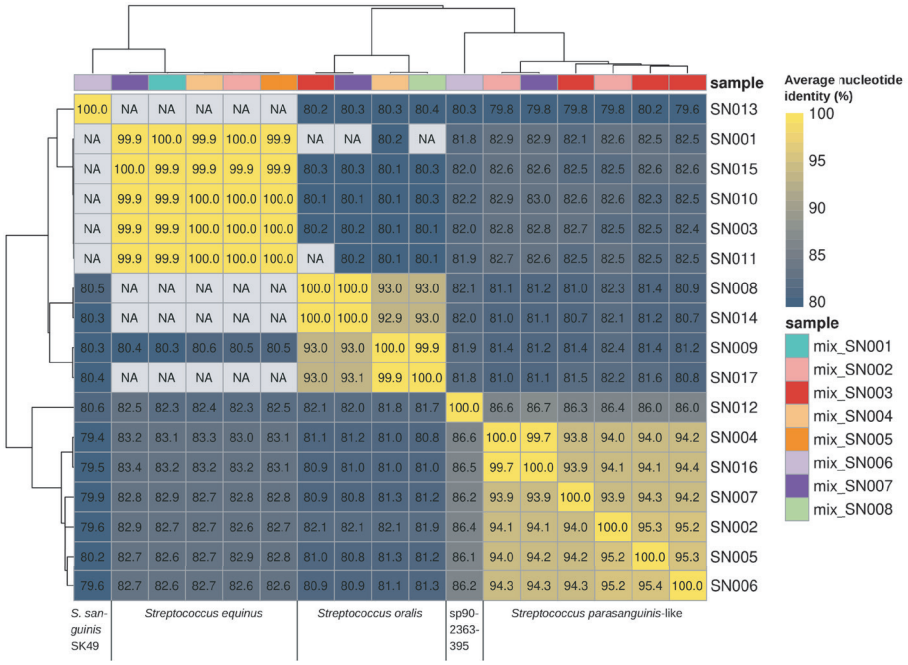


**Figure 1. Whole-genome comparison of 17 *Streptococcus* genomes.** Whole-genome sequences of 17 *Streptococcus* bacteria were compared using Average Nucleotide Identity (ANI). Values in cells indicate percentage ANI. Genomes are annotated with the sample they derive from.

7.2

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**Table 1. Streptococcus genome assembly characteristics and quality measures.** Initial assemblies were made with Flye. In case of multiple linear contigs, a secondary assembly was made using metaFlye. The most complete assembly was selected from either approach.

| Genome | Length (bp) | Predicted genes | GC% | Circular? | Species | CheckM completeness | CheckM contamination | Assembly method | Depth of coverage |
|---|---|---|---|---|---|---|---|---|---|
| *SN001* | 2140162 | 1914 | 40.12 | Y | *Streptococcus equinus* | 99.69 | 0.15 | metaFlye | 17 |
| *SN002* | 2223664 | 2093 | 41.52 | N | *Streptococcus* sp. HMSC072G04 | 100 | 0.09 | Flye | 13 |
| *SN003* | 2141365 | 1913 | 40.11 | Y | *Streptococcus equinus* | 99.69 | 0.15 | Flye | 21 |
| *SN004* | 2160498 | 2021 | 42.06 | N | *Streptococcus* sp. | 100 | 0.17 | metaFlye | 18 |
| *SN005* | 2125957 | 2016 | 41.72 | Y | *Streptococcus parasanguinis* | 99.66 | 0.11 | Flye | 120 |
| *SN006* | 2081198 | 1934 | 42.11 | Y | *Streptococcus parasanguinis* | 100 | 0.07 | Flye | 120 |
| *SN007* | 2118644 | 2017 | 41.95 | Y | *Streptococcus* sp. | 100 | 0.23 | Flye | 158 |
| *SN008* | 2068017 | 1940 | 41.06 | Y | *Streptococcus oralis* | 99.87 | 0.04 | Flye | 25 |
| *SN009* | 2117116 | 2018 | 40.83 | Y | *Streptococcus oralis* | 99.87 | 0.06 | metaFlye | 24 |
| *SN010* | 2147719 | 1922 | 40.12 | Y | *Streptococcus equinus* | 99.69 | 0.15 | metaFlye | 25 |
| *SN011* | 2141236 | 1914 | 40.11 | N | *Streptococcus equinus* | 99.69 | 0.15 | metaFlye | 14 |
| *SN012* | 2025938 | 1881 | 42.21 | Y | *Streptococcus* sp. | 100 | 0.17 | metaFlye | 21 |
| *SN013* | 2316732 | 2233 | 43 | N | *Streptococcus sanguinis* | 100 | 0 | metaFlye | 20 |
| *SN014* | 2068026 | 1939 | 41.06 | Y | *Streptococcus oralis* | 99.87 | 0.04 | metaFlye | 117 |
| *SN015* | 2141124 | 1914 | 40.12 | Y | *Streptococcus equinus* | 99.69 | 0.15 | metaFlye | 87 |
| *SN016* | 2166044 | 2028 | 42.02 | Y | *Streptococcus* sp. | 100 | 0.17 | Flye | 153 |
| *SN017* | 2119531 | 2017 | 40.83 | Y | *Streptococcus oralis* | 99.87 | 0.6 | metaFlye | 48 |