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Closed and High-Quality Bacterial Genome Sequences of the Oligo-Mouse-Microbiota Community

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ABSTRACT The Oligo-Mouse-Microbiota (OMM¹²) gnotobiotic murine model is an increasingly popular model in microbiota studies. However, following Illumina and PacBio sequencing, the genomes of the 12 strains could not be closed. Here, we used genomic chromosome conformation capture (Hi-C) data to reorganize, close, and improve the quality of these 12 genomes.

The Oligo-Mouse-Microbiota (OMM¹²) is a murine bacterial synthetic community (12 strains) introduced in axenic mice. The resulting gnotobiotic murine model has been increasingly used for diverse gut microbiota studies across several animal facilities (1–4). This bacterial consortium comprises members from the 5 major phyla naturally present in mice microbiota and reconstitutes its main functionalities, such as metabolism and colonization resistance against pathogens (5).

Despite two rounds of sequencing using PacBio and Illumina technologies (6, 7), one-half of the genomes currently accessible remain made of 2 to 20 contigs (Table 1). In particular, the genome of *Bacteroides caecimuris*, the most abundant bacterium of the OMM¹² consortium, consists of 19 contigs (5). Improving scaffolding would facilitate a number of downstream analyses, such as prophage prediction.

We used genomic chromosome conformation capture (Hi-C), a technique that uses chemical fixation (formaldehyde) to *in vivo* cross-link nearby DNA sequences, digestion with restriction enzymes, DNA extremities refilling with biotinylated nucleotides, and finally, proximity ligation, DNA extraction, and deep sequencing. The relative ligation frequencies between nonadjacent DNA segments reflect the average three-dimensional (3D) organization of the genome of interest (8) and also, because of the polymer nature of DNA, the relative distance separating them along the chromosome. The latter property has therefore been exploited to bridge scaffolding gaps resulting, for instance, from the presence of repeated elements (9). Hi-C scaffolding is now commonly used in sequencing projects of large eukaryotic genomes but applies to bacteria as well (10, 11).

A Hi-C metagenomic protocol for mammalian gut samples developed in our laboratory (12) was applied to fecal pellets from the OMM¹² mice bred at Institut Pasteur (protocol 20.173 approved by the veterinary staff and under authorization APAFIS 26874 by the national ethics committee). Libraries were prepared using streptavidin beads to capture biotinylated ligation junction as described previously (13) and sequenced with an Illumina NextSeq 550 system to generate a total of 101,182,905 reads of 35 bp. The quality of the reads was first verified with FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc>). Then, for each bacterium, the python library hicstuff (<https://github.com/koszulab/hicstuff>) was used to generate Hi-C contact maps using the most recent OMM¹² genomes (6). Contigs were reorganized (i.e., scaffolded) based on their relative contact frequencies (14). When necessary, preexisting contigs were split and rearranged to better fit the expected 3D structure, based on the contact data. In order for users to detect junctions

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TABLE 1 Description and accession numbers of the OMM¹² genomes^a

Strain	Genome size (bp)	Previous no. of contigs ^b	No. of genes	DSM no.	Accession no.	Reads (%) ^c
<i>Akkermansia muciniphila</i> YL44	2,737,357	1	2,717	26127	CP065322	2.8
<i>Acutalibacter muris</i> KB18	3,802,913	1	4,271	26090	CP065321	0.002
<i>Bifidobacterium animalis</i> YL2	2,021,936	2	1,740	26074	CP065311	0.0007
<i>Bacteroides caecimuris</i> I48	4,800,606	19	4,322	26085	CP065319	55.8
<i>Blautia coccoides</i> YL58	5,128,582	1	5,351	26115	CP065312	20.5
<i>Enterocloster clostridioformis</i> YL32	7,157,610	16	7,955	26114	CP065314	4.9
<i>Clostridium innocuum</i> I46	4,452,146	1	4,966	26113	CP065320	5.6
<i>Enterococcus faecalis</i> KB1	3,025,655	1	2,919	32036	CP065317	0.1
<i>Flavonifractor plautii</i> YL31	3,813,715	5	4,049	26117	CP065315	0.38
<i>Limosilactobacillus reuteri</i> I49	2,063,624	3	1,958	32035	CP065318	0.99
<i>Muribaculum intestinale</i> YL27	3,307,069	1	2,957	28989	CP065316	4.5
<i>Turicimonas muris</i> YL45	2,887,949	20	2,753	26109	CP065313	0.089

^a Raw data can be accessed at [SRX9907181](https://www.ncbi.nlm.nih.gov/sra/SRX9907181).^b Garzetti et al. (6).^c Unmapped reads = 4.34 %.

between contigs, contigs were separated by $10 \times N$ in the final fasta file. We successfully closed the genomes of the 12 strains except for *Flavonifractor plautii*, where the position of a 70,760-bp region could not be assigned into the main scaffold. The genome of *F. plautii* therefore remains split in 2 scaffolds. An automatic annotation was then performed using RAST (15). The access to a single scaffold of high quality will greatly improve the accuracy of genomic analyses performed by the community of users of the OMM¹² gnotobiotic mice.

Data availability. The 12 reassembled and closed genome sequences of the OMM¹² bacteria as well as the FastQ reads have been deposited under the accession numbers provided in Table 1 under the BioProject number [PRJNA680355](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA680355).

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