

# UC Davis

## UC Davis Previously Published Works

### Title

Recovery of 52 bacterial genomes from the fecal microbiome of the domestic cat (*Felis catus*) using Hi-C proximity ligation and shotgun metagenomics.

### Permalink

<https://escholarship.org/uc/item/14f9m0q5>

### Journal

Microbiology Resource Announcements, 12(10)

### Authors

Rojas, Connie

Gardy, Jennifer

Ganz, Holly

et al.

### Publication Date

2023-10-19

### DOI

10.1128/MRA.00601-23

### Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

# Recovery of 52 bacterial genomes from the fecal microbiome of the domestic cat (*Felis catus*) using Hi-C proximity ligation and shotgun metagenomics

Connie A. Rojas,<sup>1</sup> Jennifer Gardy,<sup>2</sup> Jonathan A. Eisen,<sup>3</sup> Holly H. Ganz<sup>1</sup>

**AUTHOR AFFILIATIONS** See affiliation list on p. 2.

**ABSTRACT** We used Hi-C proximity ligation with shotgun sequencing to retrieve metagenome-assembled genomes (MAGs) from the fecal microbiomes of two domestic cats (*Felis catus*). The genomes were assessed for completeness and contamination, classified taxonomically, and annotated for putative antimicrobial resistance (AMR) genes.

**KEYWORDS** gut microbiome, fecal microbiome, metagenome-assembled genomes (MAGs), domestic cats, gut bacteria, assembly, shotgun metagenomics, Hi-C, antimicrobial-resistance

Domestic cats (*Felis catus*) are obligate carnivores (1) that rely on their gut microbiome for digestion (2–4). Prior studies have mapped the composition of the fecal microbiome in cats (5–8), but no complete bacterial genomes have been reconstructed using metagenomic sequencing. One promising approach is Hi-C technology, which cross-links, digests, and ligates DNA (9) to improve genome assembly. Here we used metagenomic sequencing with Hi-C proximity ligation to reconstruct genomes (MAGs) from two cats and assess the usefulness of this approach for detecting bacteria that may be novel.

We collected fresh fecal material from two domestic shorthairs (Danny and Lil Bub). Danny was a healthy, 9-yr-old neutered male who lived indoors. Lil Bub was an indoor, 4-yr-old spayed female celebrity cat with osteopetrosis. The UC Davis IACUC determined that formal approval was not necessary because fecal material is a waste product.

DNA was isolated from fecal samples using the MoBio PowerFecal DNA Extraction Kit. A Hi-C library was created with the Phase Genomics (Seattle, WA, USA) ProxiMeta Hi-C v4.0 Kit (10). A metagenomic shotgun library was also prepared with ProxiMeta reagents. Sequencing was performed on an Illumina NovaSeq generating PE reads (150 bp) for both Hi-C and shotgun libraries. Sequencing files were uploaded to the Phase Genomics portal for quality filtering with fastp (v0.23.2) (11) and assembly with MEGAHIT (v1.2.9) (12). Assembly quality was assessed with QUAST (v5.0) (13). For these tools and subsequent tools, default parameters were used unless otherwise specified. Hi-C reads were aligned to the assembly using BWA-MEM (v0.7.17) (14). Metagenome deconvolution was performed with ProxiMeta (15, 16), and the resulting MAGs were assessed for quality using CheckM (v1.2.0) (17) and assigned taxonomy with the Genome Taxonomy Database release 202 (gtdbtk v1.5.0) (18). Antimicrobial resistance (AMR) genes were annotated with the NCBI AMRFinderPlus (v3.10.5) (19).

We recovered a total of 52 MAGs with >90% completeness and <5% contamination and nine MAGs with >80% completeness and <5% contamination. The most common bacterial families were *Lachnospiraceae*, *Oscillospiraceae*, *Bacteroidaceae*, and *Erysipelotrichaceae*. Seventeen MAGs were assigned a species-level classification, and among

**Editor** David Rasko, University of Maryland School of Medicine, Baltimore, Maryland, USA

Address correspondence to Holly H. Ganz, [holly@animalbiome.com](mailto:holly@animalbiome.com).

H.H.G. and C.A.R. are employees of AnimalBiome, a company based in Oakland, CA, and have stock options. J.A.E. and J.G. are advisors for AnimalBiome and have stock options.

See the funding table on p. 3.

**Received** 6 July 2023

**Accepted** 8 August 2023

**Published** 11 September 2023

Copyright © 2023 Rojas et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

TABLE 1 Overview of data files/data sets

Data description	File types	
	(file extension)	Links
Hi-C Illumina Sequences (PE 150 bp) from Danny (973,507 reads)	Fastq file (.fastq.gz)	NCBI Sequence Read Archive <a href="https://identifiers.org/ncbi/insdc.sra:SRX18058393">https://identifiers.org/ncbi/insdc.sra:SRX18058393</a>
Hi-C Illumina Sequences (PE 150 bp) from Lil Bub (14,782,886 reads)	Fastq file (.fastq.gz)	NCBI Sequence Read Archive <a href="https://identifiers.org/ncbi/insdc.sra:SRX18058392">https://identifiers.org/ncbi/insdc.sra:SRX18058392</a>
Assembled Contigs from Illumina Shotgun Sequences (Danny) (62,536 contigs)	Fasta file (.fa)	NCBI GenBank <a href="https://identifiers.org/nucleotide:JAPFCN000000000.1">https://identifiers.org/nucleotide:JAPFCN000000000.1</a>
	Fasta file (.fa)	NCBI GenBank <a href="https://identifiers.org/nucleotide:JAPFCM000000000.1">https://identifiers.org/nucleotide:JAPFCM000000000.1</a>
Assembled Contigs from Illumina Shotgun Sequences (Lil Bub) (82,672 contigs)		
Statistics on the Assembled Contigs using QUAST	Word document (.docx)	Figshare <a href="https://doi.org/10.6084/m9.figshare.23827035">https://doi.org/10.6084/m9.figshare.23827035</a>
MAG fasta files	Fasta file (.fa)	NCBI BioProject <a href="https://identifiers.org/ncbi/bioproject:PRJNA893230">https://identifiers.org/ncbi/bioproject:PRJNA893230</a>
List of MAGs, their taxonomy, and basic statistics	Excel spreadsheet (.xlsx)	Figshare <a href="https://doi.org/10.6084/m9.figshare.21555165">https://doi.org/10.6084/m9.figshare.21555165</a>
More detailed study methods	Word document (.docx)	Figshare <a href="https://doi.org/10.6084/m9.figshare.21440763.v6">https://doi.org/10.6084/m9.figshare.21440763.v6</a>

them were *Alistipes putredinis*, *Bacteroides fingoldii*, *Campylobacter helveticus*, *Collinsella tanakaei*, *Parabacteroides merdae*, *Prevotella copri*, and *Ruminococcus gnavus*.

A total of 31 unique antimicrobial-resistance genes (AMRs) were detected in contigs and were predicted to encode resistance to tetracycline, erythromycin, kanamycin, and beta-lactam antibiotics. Multidrug efflux transporters, beta-lactamases, and methyltransferases were among the putative proteins.

Our data is limited to two individual cats, but we add to the growing catalog of studies using Hi-C with shotgun sequencing to retrieve quality genomes from mammals, including dogs (20), sheep (21), cows (16), and humans (22).

## ACKNOWLEDGMENTS

We would like to thank Ivan Liachko and Phase Genomics for sequencing our samples and analyzing the data on their ProxiMeta platform. We would also like to thank Lil Bub and Danny for their lasting contributions to our understanding of the cat fecal microbiome.

We extend our appreciation to AnimalBiome and Phase Genomics for funding this work.

## AUTHOR AFFILIATIONS

<sup>1</sup>AnimalBiome, Oakland, California, USA

<sup>2</sup>Bill & Melinda Gates Foundation, Seattle, Washington, USA

<sup>3</sup>Evolution and Ecology, University of California, Davis, California, USA

## AUTHOR ORCID*s*

Connie A. Rojas  <http://orcid.org/0000-0002-6097-8970>

Jennifer Gardy  <http://orcid.org/0000-0001-7255-7631>

Jonathan A. Eisen  <http://orcid.org/0000-0002-0159-2197>

Holly H. Ganz  <http://orcid.org/0000-0003-4938-6226>

## FUNDING

Funder	Grant(s)	Author(s)
AnimalBiome		Holly H. Ganz
Phase Genomics		Holly H. Ganz

## AUTHOR CONTRIBUTIONS

Connie A. Rojas, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing | Jennifer Gardy, Conceptualization, Funding acquisition, Investigation, Resources, Writing – review and editing | Jonathan A. Eisen, Conceptualization, Funding acquisition, Investigation, Supervision, Validation, Writing – review and editing | Holly H. Ganz, Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review and editing

## DATA AVAILABILITY

All genomic sequence data is under NCBI BioProject [PRJNA893230](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA893230). Raw Hi-C Illumina sequences are in the Sequence Read Archive (accessions [SRR22078165](https://www.ncbi.nlm.nih.gov/sra/SRR22078165) and [SRR22078166](https://www.ncbi.nlm.nih.gov/sra/SRR22078166)), unannotated assemblies are in NCBI GenBank (accessions [JAPFCN010000000](https://www.ncbi.nlm.nih.gov/genbank/JAPFCN010000000) and [JAPFCM010000000](https://www.ncbi.nlm.nih.gov/genbank/JAPFCM010000000)), and MAGs are also in NCBI GenBank. Please refer to Table 1 for links to the data.

## REFERENCES

- Bradshaw JWS, Goodwin D, Legrand-Defréтин V, Nott HMR. 1996. Food selection by the domestic cat, an obligate carnivore. *Comp Biochem Physiol A Physiol* 114:205–209. [https://doi.org/10.1016/0300-9629\(95\)02133-7](https://doi.org/10.1016/0300-9629(95)02133-7)
- Cabral L, Persinoti GF, Paixão DAA, Martins MP, Morais MAB, Chinaglia M, Domingues MN, Sforca ML, Pirolla RAS, Generoso WC, Santos CA, Maciel LF, Terrapon N, Lombard V, Henrissat B, Murakami MT. 2022. Gut microbiome of the largest living rodent harbors unprecedented enzymatic systems to degrade plant polysaccharides. *Nat Commun* 13:629. <https://doi.org/10.1038/s41467-022-28310-y>
- Xie F, Jin W, Si H, Yuan Y, Tao Y, Liu J, Wang X, Yang C, Li Q, Yan X, Lin L, Jiang Q, Zhang L, Guo C, Greening C, Heller R, Guan LL, Pope PB, Tan Z, Zhu W, Wang M, Qiu Q, Li Z, Mao S. 2021. An integrated gene catalog and over 10,000 metagenome-assembled genomes from the gastrointestinal microbiome of ruminants. *Microbiome* 9:137. <https://doi.org/10.1186/s40168-021-01078-x>
- Milani C, Alessandri G, Mancabelli L, Mangifesta M, Lugli GA, Viappiani A, Longhi G, Anzalone R, Duranti S, Turroni F, Ossiprandi MC, van Sinderen D, Ventura M, Björkroth J. 2020. Multi-omics approaches to decipher the impact of diet and host physiology on the mammalian gut microbiome. *Appl Environ Microbiol* 86. <https://doi.org/10.1128/AEM.01864-20>
- Johnston KL, Swift NC, Forster-van Hijfte M, Rutgers HC, Lampion A, Ballèvre O, Batt RM. 2001. Comparison of the bacterial Flora of the duodenum in healthy cats and cats with signs of gastrointestinal tract disease. *J Am Vet Med Assoc* 218:48–51. <https://doi.org/10.2460/javma.2001.218.48>
- Badri DV, Jackson MI, Jewell DE. 2021. Dietary protein and carbohydrate levels affect the gut microbiota and clinical assessment in healthy adult cats. *J Nutr* 151:3637–3650. <https://doi.org/10.1093/jn/nxab308>
- Young W, Moon CD, Thomas DG, Cave NJ, Bermingham EN. 2016. Pre- and post-weaning diet alters the faecal metagenome in the cat with differences in vitamin and carbohydrate metabolism gene abundances. *Sci Rep* 6:34668. <https://doi.org/10.1038/srep34668>
- Barry KA, Middelbos IS, Vester Boler BM, Dowd SE, Suchodolski JS, Henrissat B, Coutinho PM, White BA, Fahey GC, Swanson KS. 2012. Effects of dietary fiber on the feline gastrointestinal metagenome. *J Proteome Res* 11:5924–5933. <https://doi.org/10.1021/pr3006809>
- van Berkum NL, Lieberman-Aiden E, Williams L, Imakaev M, Gnirke A, Mirny LA, Dekker J, Lander ES. 2010. Hi-C: a method to study the three-dimensional architecture of genomes. *J Vis Exp*, no. 39:1869. <https://doi.org/10.3791/1869>
- Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragozcy T, Telling A, Amit I, Lajoie BR, Sabo PJ, Dorschner MO, Sandstrom R, Bernstein B, Bender MA, Groudine M, Gnirke A, Stamatoyannopoulos J, Mirny LA, Lander ES, Dekker J. 2009. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science* 326:289–293. <https://doi.org/10.1126/science.1181369>
- Chen S, Zhou Y, Chen Y, Gu J. 2018. Fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* 34:i884–i890. <https://doi.org/10.1093/bioinformatics/bty560>
- Li D, Liu C-M, Luo R, Sadakane K, Lam T-W. 2015. MEGAHIT: An ultra-fast single-node solution for large and complex metagenomics assembly via succinct de bruijn graph. *Bioinformatics* 31:1674–1676. <https://doi.org/10.1093/bioinformatics/btv033>
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>
- Li H, Durbin R. 2010. Fast and accurate long-read alignment with burrows-wheeler transform. *Bioinformatics* 26:589–595. <https://doi.org/10.1093/bioinformatics/btp698>
- Maximilian O. Press, Wiser AH, Kronenberg ZN, Langford KW, Shakya M, Lo C-C, Mueller KA, Sullivan ST, Chain PSG, Liachko I. 2017. Hi-C deconvolution of a human gut microbiome yields high-quality draft genomes and reveals plasmid-genome interactions. [bioRxiv](https://doi.org/10.1101/166072).
- Stewart RD, Auffret MD, Warr A, Wiser AH, Press MO, Langford KW, Liachko I, Snelling TJ, Dewhurst RJ, Walker AW, Roehe R, Watson M. 2018. Assembly of 913 microbial genomes from metagenomic sequencing of the cow rumen. *Nat Commun* 9:1–11. <https://doi.org/10.1038/s41467-018-03317-6>
- Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. Checkm: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 25:1043–1055. <https://doi.org/10.1101/gr.186072.114>
- Chaumeil P-A, Mussig AJ, Hugenholtz P, Parks DH. 2019. GTDB-TK: a toolkit to classify genomes with the genome taxonomy database. *Bioinformatics* 36:1925–1927. <https://doi.org/10.1093/bioinformatics/btz848>

19. Feldgarden M, Brover V, Gonzalez-Escalona N, Frye JG, Haendiges J, Haft DH, Hoffmann M, Pettengill JB, Prasad AB, Tillman GE, Tyson GH, Klimke W. 2021. Amrfinderplus and the reference gene catalog facilitate examination of the genomic links among antimicrobial resistance, stress response, and virulence. *Sci Rep* 11:12728. <https://doi.org/10.1038/s41598-021-91456-0>
20. Cuscó A, Pérez D, Viñes J, Fàbregas N, Francino O. 2022. Novel canine high-quality metagenome-assembled genomes, prophages and host-associated plasmids provided by long-read metagenomics together with hi-c proximity ligation. *Microb Genom* 8:000802. <https://doi.org/10.1099/mgen.0.000802>
21. Bickhart DM, Kolmogorov M, Tseng E, Portik DM, Korobeynikov A, Tolstoganov I, Uritskiy G, Liachko I, Sullivan ST, Shin SB, Zorea A, Andreu VP, Panke-Buisse K, Medema MH, Mizrahi I, Pevzner PA, Smith TPL. 2022. Generating lineage-resolved, complete metagenome-assembled genomes from complex microbial communities. *Nat Biotechnol* 40:711–719. <https://doi.org/10.1038/s41587-021-01130-z>
22. Ivanova V, Chernevskaia E, Vasiluev P, Ivanov A, Tolstoganov I, Shafranskaya D, Ulyantsev V, Korobeynikov A, Razin SV, Beloborodova N, Ulianov SV, Tyakht A. 2021. Hi-C metagenomics in the icu: exploring clinically relevant features of gut microbiome in chronically critically ill patients. *Front. Microbiol* 12. <https://doi.org/10.3389/fmicb.2021.770323>