





Nearly Complete Genome Sequence of *Brugia malayi* Strain FR3

Alan Tracey,^a Jeremy M. Foster,^b Michael Paulini,^c Alexandra Grote,^d John Mattick,^e Yu-Chih Tsai,^f  Matthew Chung,^e James A. Cotton,^a Tyson A. Clark,^f Adam Geber,^d Nancy Holroyd,^a Jonas Korlach,^f Silvia Libro,^b Sara Lustigman,^g Michelle L. Michalski,^h Matthew B. Rogers,ⁱ Alan Twaddle,^d  Julie C. Dunning Hotopp,^{e,j,k} Matthew Berriman,^a Elodie Ghedin^{d,l}

^aWellcome Sanger Institute, Wellcome Genome Campus, Hinxton, Cambridgeshire, United Kingdom

^bDivision of Protein Expression & Modification, New England Biolabs, Ipswich, Massachusetts, USA

^cEuropean Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Wellcome Genome Campus, Hinxton, Cambridgeshire, United Kingdom

^dDepartment of Biology, Center for Genomics and Systems Biology, New York University, New York, New York, USA

^eInstitute for Genome Science, University of Maryland School of Medicine, Baltimore, Maryland, USA

^fPacific Biosciences, Menlo Park, California, USA

^gLaboratory of Molecular Parasitology, Lindsley F. Kimball Research Institute, New York Blood Center, New York, New York, USA

^hDepartment of Biology and Microbiology, University of Wisconsin Oshkosh, Oshkosh, Wisconsin, USA

ⁱDepartment of Surgery, UPMC Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania, USA

^jDepartment of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, Maryland, USA

^kGreenebaum Cancer Center, University of Maryland School of Medicine, Baltimore, Maryland, USA

^lDepartment of Epidemiology, College of Global Public Health, New York University, New York, New York, USA

Alan Tracey, Jeremy M. Foster, Michael Paulini, Alexandra Grote, and John Mattick contributed equally to this work. Author order was determined through a collegial discussion and reflects the sequential order in which their contributions were added to the project.

ABSTRACT Lymphatic filariasis affects ~120 million people and can result in elephantiasis and hydrocele. Here, we report the nearly complete genome sequence of the best-studied causative agent of lymphatic filariasis, *Brugia malayi*. The assembly contains four autosomes, an X chromosome, and only eight gaps but lacks a contiguous sequence for the known Y chromosome.

Brugia malayi is a causative agent of lymphatic filariasis, which affects ~120 million people and can result in elephantiasis and hydrocele. An ~71-Mbp *B. malayi* draft genome sequence was previously produced using Sanger sequencing with 8,180 scaffolds and an N_{50} value of ~93 kbp (1, 2). Here, we used long-read sequencing and manually curated optical maps to complete this *B. malayi* genome.

B. malayi adult worms were obtained directly from the filarial nematode repositories at TRS Labs and the Filariasis Research Reagent Resource Center (FR3) (3)—the two major repositories that both independently maintain the same lineage of *B. malayi* originally from a green leaf monkey (4). High-molecular-weight genomic DNA was prepared by grinding frozen worms in liquid nitrogen and transferring them to 100 mM Tris-HCl (pH 8.5), 50 mM NaCl, 50 mM EDTA, 1% SDS, 1.1% β -mercaptoethanol, and 100 μ g/ml NEB proteinase K at 55°C for 4 h with rocking. DNA was spooled from an ethanol precipitation following a phenol-chloroform extraction. DNA was suspended in Tris-EDTA (TE) (pH 8.0) with 25 μ g/ml Epicentre RNase A at 37°C for 1 h followed by phenol-chloroform extraction, precipitation, and centrifugation at 12,000 $\times g$ at 4°C. Genomic DNA (30 μ g) was sheared to 20 kbp with a Covaris g-TUBE. A 7-kbp Sage Science Blue Pippin size-selected SMRTbell library was prepared, and 11.3 Gbp of sequence data (3,922,808 reads; N_{50} , 17,971 bp) were produced on a Pacific Biosciences RS II instrument (P5-C3; 180 min).

For optical mapping, individual phosphate-buffered saline (PBS)-washed *B. malayi* male worms were placed into ~50- μ l plugs of 1% InCert agarose (Lonza, Rockland, ME)

Citation Tracey A, Foster JM, Paulini M, Grote A, Mattick J, Tsai Y-C, Chung M, Cotton JA, Clark TA, Geber A, Holroyd N, Korlach J, Libro S, Lustigman S, Michalski ML, Rogers MB, Twaddle A, Dunning Hotopp JC, Berriman M, Ghedin E. 2020. Nearly complete genome sequence of *Brugia malayi* strain FR3. *Microbiol Resour Announc* 9:e00154-20. <https://doi.org/10.1128/MRA.00154-20>.

Editor Christina A. Cuomo, Broad Institute

Copyright © 2020 Tracey 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/).

Address correspondence to Julie C. Dunning Hotopp, jdhotopp@som.umaryland.edu, or Matthew Berriman, mb4@sanger.ac.uk.

Received 19 February 2020

Accepted 14 May 2020

Published 11 June 2020

in PBS that were extruded into 1 ml of 50°C 1% (wt/vol) *N*-lauroylsarcosine, 2 mg/ml proteinase K, and 0.5 M EDTA (pH 9.5) and incubated overnight with rocking at 50°C. Plugs were washed 5 times for 1 h each in TE (pH 8.0), with rocking at 4°C, and then stored at 4°C in 0.5 M EDTA (pH 8.0). Stretched and immobilized DNA was digested with NEB SpeI and AflII separately and fluorescently stained, generating ~80× optical data depth. An OpGen Argus optical mapping system (2015 version), with proprietary MapManager (2015 version) and MapSolver version 3.1 software, resolved a 96.58-Mbp *B. malayi* SpeI optical map of 17 contigs and a 77.57-Mbp AflII optical map of 12 contigs.

The 1,895,591 PacBio subreads that passed a 0.75 quality filter (N_{50} , 8,771 bp; mean, 5,930 bp) were assembled into 1,371 contigs with HGAP version 2 *de novo* assembly and compared to the *de novo* SpeI and AflII optical maps using MapSolver version 3.1. The genome was manually edited with publicly available capillary (2), Roche 454 (SRA accession number [PRJNA10729](https://www.ncbi.nlm.nih.gov/SRA/acc.cgi?acc=PRJNA10729)), and Illumina (5) reads mapped to the PacBio contigs with Gap5 (6). Errors were corrected with three iterations of iCORN2 (7) with Bowtie mapping (8) using a tile path of 40× sequencing depth using pseudoreads created with the script to_perfect_reads (<https://github.com/sanger-pathogens/Fastaq>) using the prior publicly available WormBase assembly release 242 (WS242), followed by 3 further iterations using Illumina reads. Automated gap filling (24 iterations) was performed using IMAGE version 2.4.1 (9) and the Illumina reads. PBSuite_14.6.24 and smrtanalysis-2.2.0.133377, PBJelly (10), and Quiver were used to close gaps, add additional scaffolding, error correct, and trim. Introduced errors were corrected with three further iterations of iCORN using Illumina reads. Aligned sprai version 0.9.9.1-corrected (<https://bioconda.github.io/recipes/sprai/README.html>) PacBio reads were used for manual extension of sequence contigs, reducing the total gap count to 8. Default software parameters were used unless otherwise noted.

The resulting 87-Mbp assembly of 196 scaffolds has a GC content of 28% and an N_{50} value of 14.2 Mbp with 4 autosomes and an X chromosome but is lacking a contiguous Y chromosome despite numerous efforts to assemble it.

Data availability. This *B. malayi* v4 assembly with the WS270 annotation can be accessed at NCBI (accession number [GCA_000002995.5](https://www.ncbi.nlm.nih.gov/GenBank/ accession/GCA_000002995.5)), WormBase (http://www.wormbase.org/species/b_malayi), and WormBase-Para-Site (http://parasite.wormbase.org/Brugia_malayi_prjna10729/Info/Index/). The PacBio data are available at the SRA under accession number [SRX3461807](https://www.ncbi.nlm.nih.gov/SRA/acc.cgi?acc=SRX3461807).

ACKNOWLEDGMENTS

This project was funded in part by the Burroughs-Wellcome Fund to E.G., federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under grant number U19AI110820 to J.C.D.H., J.M.F., and M.L.M., and core support from Wellcome (grants WT098051 and WT206194) to the Wellcome Sanger Institute and Medical Research Council (UK) funding to M.B. and M.P. (grant MR/L001020/1).

We thank Karen Brooks for help with manual finishing.

REFERENCES

- Ghedini E, Wang S, Foster JM, Slatko BE. 2004. First sequenced genome of a parasitic nematode. *Trends Parasitol* 20:151–153. <https://doi.org/10.1016/j.pt.2004.01.011>.
- Ghedini E, Wang S, Spiro D, Caler E, Zhao Q, Crabtree J, Allen JE, Delcher AL, Guiliano DB, Miranda-Saavedra D, Angiuoli SV, Creasy T, Amedeo P, Haas B, El-Sayed NM, Wortman JR, Feldblyum T, Tallon L, Schatz M, Shumway M, Koo H, Salzberg SL, Schobel S, Perteau M, Pop M, White O, Barton GJ, Carlow CKS, Crawford MJ, Daub J, Dimmic MW, Estes CF, Foster JM, Ganatra M, Gregory WF, Johnson NM, Jin J, Komuniecki R, Korf I, Kumar S, Laney S, Li B-W, Li W, Lindblom TH, Lustigman S, Ma D, Maina CV, Martin DMA, McCarter JP, McReynolds L, et al. 2007. Draft genome of the filarial nematode parasite *Brugia malayi*. *Science* 317:1756–1760. <https://doi.org/10.1126/science.1145406>.
- Michalski ML, Griffiths KG, Williams SA, Kaplan RM, Moorhead AR. 2011. The NIH-NIAID Filariasis Research Reagent Resource Center. *PLoS Negl Trop Dis* 5:e1261. <https://doi.org/10.1371/journal.pntd.0001261>.
- Buckley JJ, Edeson JF. 1956. On the adult morphology of *Wuchereria* sp. (*malayi*?) from a monkey (*Macaca irus*) and from cats in Malaya, and on *Wuchereria pahangi* n.sp. from a dog and a cat. *J Helminthol* 30:1–20. <https://doi.org/10.1017/S0022149X00032922>.
- Ioannidis P, Johnston KL, Riley DR, Kumar N, White JR, Olarte KT, Ott S, Tallon LJ, Foster JM, Taylor MJ, Dunning Hotopp JC. 2013. Extensively duplicated and transcriptionally active recent lateral gene transfer from a bacterial *Wolbachia* endosymbiont to its host filarial nematode *Brugia malayi*. *BMC Genomics* 14:639. <https://doi.org/10.1186/1471-2164-14-639>.
- Bonfield JK, Whitwham A. 2010. Gap5—editing the billion fragment

- sequence assembly. *Bioinformatics* 26:1699–1703. <https://doi.org/10.1093/bioinformatics/btq268>.
7. Otto TD, Sanders M, Berriman M, Newbold C. 2010. Iterative Correction of Reference Nucleotides (iCORN) using second generation sequencing technology. *Bioinformatics* 26:1704–1707. <https://doi.org/10.1093/bioinformatics/btq269>.
 8. Langmead B. 2010. Aligning short sequencing reads with Bowtie. *Curr Protoc Bioinformatics* Chapter 11:Unit 11.7. <https://doi.org/10.1002/0471250953.bi1107s32>.
 9. Tsai IJ, Otto TD, Berriman M. 2010. Improving draft assemblies by iterative mapping and assembly of short reads to eliminate gaps. *Genome Biol* 11:R41. <https://doi.org/10.1186/gb-2010-11-4-r41>.
 10. English AC, Richards S, Han Y, Wang M, Vee V, Qu J, Qin X, Muzny DM, Reid JG, Worley KC, Gibbs RA. 2012. Mind the gap: upgrading genomes with Pacific Biosciences RS long-read sequencing technology. *PLoS One* 7:e47768. <https://doi.org/10.1371/journal.pone.0047768>.