





Draft Genome Sequence of French Guiana Leishmania (Viannia) guyanensis Strain 204-365, Assembled Using Long Reads

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ABSTRACT We present here the draft genome sequence for Leishmania (Viannia) quyanensis. The isolate was obtained from a clinical case of cutaneous leishmaniasis in French Guiana. Genomic DNA was sequenced using PacBio and MiSeg platforms.

uman leishmaniasis is a spectrum of diseases of public health concern which affects millions of people, causing thousands of deaths worldwide (1, 2). Cutaneous leishmaniasis (CL) is the most prevalent clinical manifestation of the disease caused by several Leishmania parasites in the Leishmania and Viannia subgenera, which may put patients at risk of developing other forms of the disease (3). Mucocutaneous leishmaniasis (MCL) is a sequela that affects mucosal tissues of the respiratory and alimentary tracts, resulting in severe facial deformities. MCL may occur via the recurrence of misdiagnosed and/or suboptimal/not treated cases of CL caused by some species in the Viannia subgenus (4-7). Therefore, species-specific diagnosis is often critical for clinical management, i.e., prognosis of the disease's progression, treatment options, and how to monitor for relapse and sequelae (8, 9). However, as the development of species-level diagnostic tests for clinical application depends on a robust genomic database, we are working on identifying species-specific targets by sequencing and comparing the genomes of several Leishmania species. Here, we describe the draft genome sequence of L. (V.) guyanensis, which was isolated from a clinical case of cutaneous leishmaniasis in French Guiana in 2004. The strain was identified using our traditional diagnostic approach (10, 11) and by comparing the sequence of Leishmania sp. genes P6GD, ICD, MDH, and MPI generated on an ABI 3130xl DNA analyzer (Applied Biosystems, CA), with the NCBI nucleotide database using BLAST.

Genomic DNA was extracted from parasites cultured in RPMI 1640 axenic medium (Life Technologies, CA) using the MagAttract high-molecular-weight (HMW) DNA kit (Qiagen, MD), following the manufacturer's instructions, and DNA quality was evaluated using the 2200 TapeStation system (Agilent Technologies, DE). Dual-indexed sequencing libraries were prepared using NEBNext Ultra library prep reagents (New England BioLabs, MA) and barcoding indices synthesized by the CDC's Biotechnology Core Facility. Sequencing was performed on a MiSeq platform (Illumina, CA) using the MiSeq 2×250 -cycle sequencing kit. Upon completion, sequence reads were filtered for read quality, basecalled, and demultiplexed using default settings for bcl2fastq (version 2.19), resulting in 13,902,528 reads. In addition, a 20-kb DNA library was prepared following the standard PacBio 20-kb procedure (Pacific Biosciences, CA) and size selected with BluePippin (Sage Science, MA). The final size-selected library was bound to polymerase using the DNA/polymerase binding kit P6 version 2, loaded onto 5

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TABLE 1 Assembly metrics for L. guyanensis 204-365 and LgCL085^a

Characteristic	204-365 contigs	LgCL085 scaffolds	LgCL085 contigs
No.	123	270	1,734
Total size (bp)	33,816,023	31,014,322	30,875,308
Longest (bp)	2,583,876	2,586,957	307,213
Shortest (bp)	12,939	1,009	48
Mean size (bp)	274,927	114,868	17,806
Medium size (bp)	47,844	4,383	5,985
N_{50} length (bp)	683,170	967,189	47,920
L ₅₀	16	11	182

^a See reference 14.

single-molecule real-time (SMRT) cells, and sequenced with C4 version 2 chemistry for 360-min movies on the RS II instrument (Pacific Biosciences) to obtain 251,394 reads, with a mean read length of 12,942 bp after using default filter settings.

The PacBio reads were *de novo* assembled ($80 \times$ coverage; GC content, 57%) using Canu version 1.6 (12) (minReadLength = 5,000, minOverlapLength = 1,000), and very low-coverage ($<10 \times$) contigs were removed from the assembly. Illumina reads were used to polish and correct contigs using unicycler_polish (Unicycler package version 4.4) (12, 13).

As shown in Table 1, the proposed assembly is significantly contiguous in comparison to the previously published short-read assembly of the *L. (V.) guyanensis* LgCL085 genome (14).

Data availability. The genome contigs of *L. (V.) guyanensis* 204-365 have been deposited in GenBank under accession number QVNO00000000, SRA accession numbers SRR7867261, SRR7867262, SRR7867269, SRR7867270, SRR7867271, and SRR8179913, and BioProject number PRJNA484340.

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