Genome of *Leptospira borgpetersenii* strain 4E, a highly virulent isolate obtained from *Mus musculus* in southern Brazil

Marcus Redü Eslabão¹, Frederico Schmitt Kremer¹, Rommel Thiago Juca Ramos², Artur Luiz da Costa da Silva³, Vasco Ariston de Carvalho Azevedo³, Luciano da Silva Pinto¹, Éverton Fagone da Silva⁴, Odir Antônio Dellagostin¹/+  

¹Universidade Federal de Pelotas, Núcleo de Biotecnologia, Capão do Leão, RS, Brasil  
²Universidade Federal do Pará, Belém, PA, Brasil  
³Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil  
⁴Universidade Federal de Pelotas, Faculdade de Veterinária, Capão do Leão, RS, Brasil

A previous study by our group reported the isolation and characterisation of *Leptospira borgpetersenii* serogroup Ballum strain 4E. This strain is of particular interest because it is highly virulent in the hamster model. In this study, we performed whole-genome shotgun genome sequencing of the strain using the SOLiD sequencing platform. By assembling and analysing the new genome, we were able to identify novel features that have been previously overlooked in genome annotations of other strains belonging to the same species.

Key words: bioinformatics - genomics - leptospirosis - neglected disease - whole genome shotgun sequencing

The *Leptospira* genus consists of 23 species of bacteria (Boonsilp et al. 2013, Bourhy et al. 2014), of which at least nine are naturally pathogenic, five are opportunists (“intermediary pathogenic”), and the remaining are saprophytes (non-pathogenic). *L. interrogans* is the most commonly reported cause of leptospirosis, which is an infection caused by pathogenic leptospires; however, other species, such as *L. borgpetersenii*, *L. kirschneri*, and *L. santarosai*, are also associated with leptospirosis and are responsible for many infections and deaths both in humans and animals (Guerra 2009). Leptospirosis is a worldwide distributed zoonotic disease that has re-emerged as a public health problem in many countries in recent years, especially in countries located in the tropics (Guerra 2013).

*L. borgpetersenii* serovar Ballum strain 4E was isolated from the suburban area of Pelotas, a city located in southern Brazil, from mice (*Mus musculus*) (da Silva et al. 2010). Previous studies have demonstrated that this strain has a LD50 (lethal dose for 50% of the population) of ~5.18 leptospires in a hamster model. As such, it is more lethal and virulent than are other standard models such as *L. interrogans* serovar Copenhageni strain Fiocruz L1-130 (LD50 = ~80 leptospires) (Diniz et al. 2011). The characterisation of highly virulent strains may provide useful data that can potentially extend our knowledge and understanding of the pathogenesis of these bacteria and lead to the development of new vaccines. Further, it may generate insights that are useful for epidemiological surveillance. In the present study, we performed a whole-genome shotgun analysis of the *L. borgpetersenii* serovar Ballum strain 4E to develop a more comprehensive characterisation of this isolate.

Bacterial culture and DNA extraction were performed in accordance with previously described methods (Kremer et al. 2016b). Whole-genome shotgun sequencing was performed using the ABI SOLiD v. 4 sequencing platform with a 50 base-pair (bp) single-end library.

Raw reads in colour-space FASTA format (csFASTA) were pre-processed using SAET (https://www.thermofisher.com/) and converted into FASTQ format using our in-house Python script cs2q (http://labbioinfo.ufpel.edu.br/cs2q).

Two assembly approaches were evaluated for the *L. borgpetersenii* strain 4E genome: de novo assembly and reference-guided assembly. De novo assembly was performed using Velvet, with different parameters of k-mer length, expected coverage and coverage cutoff, and the assembly metrics were accessed using QUAST (Gurevich et al. 2013). Reference-guided assembly was performed by mapping the reads to the genome of *L. borgpetersenii* serovar Ballum strain 56604 (GenBank: CP012029.1, CP012030.1) using SMART (www.sanger.ac.uk/science/tools/smalt-0). The resulting SAM file was then converted to BAM format and sorted using Samtools before a consensus sequence was extracted using Samtools, BCFTools, VCFutils.pl (Li et al. 2009) and GATk (McKenna et al. 2010). Genome annotation was performed using Genix (Kremer et al. 2016a) and manually reviewed and curated using Artemis (Rutherford et al. 2000).

A variant calling analysis using Samtools, BCFTools, and VCFutils.pl that was based on the BAM file generated from the aligned reads was performed to identify single nucleotide polymorphisms (SNPs) and insertions and deletions (INDELs). The effect of each variant was inferred based on the annotation of *L. borgpetersenii* serovar Ballum strain 56604 using Snpeff (Reumers 2004).

The reference-guided assembly covered > 99.99% of the reference sequence, with a mean coverage of ~
A lack of coverage was identified in five assembly gaps, which were associated with mobile elements, such as transposons, that can change their positions in the genome and usually result in gaps in reference-guided assemblies or collapses in a single contig in de novo assembly from short reads, even when they are present in multiple copies. The de novo assemblies generated by Velvet were highly fragmented, with more than 5,000 contigs and a very low N50 (53), thus making it inappropriate for any downstream analysis.

An overview of the features identified in the genome of *L. borgpetersenii* serovar Ballum strain 4E is shown in Table I. We identified a total of 3469 coding DNA sequences (CDSs), 37 transfer-RNAs (tRNAs), 4 ribosomal RNAs (rRNAs), one transfer-messenger RNA (tmRNA) and five riboswitch loci. Although the protein-coding genes found were almost the same as those identified in the genome of the 56604 strain, by using our annotation pipeline, we were able to identify new non-coding features that were overlooked in the reference annotation: a tmRNA gene and riboswitches. TmRNAs act as tRNAs and contain a small open reading frame (ORF) in their structure that encodes a peptide responsible for many regulation processes, including targeting proteins for degradation (Hayes & Keiler 2010). Riboswitches are non-coding motifs that are present in the untranslated regions (UTRs) of some messenger RNAs (mRNAs) that act as cis-regulatory elements and bind specific metabolites to inhibit the gene expression. Riboswitches are typically found in genes associated with vitamin metabolism, e.g., cobalamin (Garst et al. 2011, Serganov & Nudler 2013). Previous studies have demonstrated that riboswitch-regulated cobalamin (B12) autotrophy is a virulence factor in the *Leptospira* genus (Fouts et al. 2016). Therefore, a deeper annotation of the non-coding features may provide a better description of the resulting transcriptome.

The genes that presented missense mutations in the variant calling analysis are displayed in Table II, and their locations in the genome of *L. borgpetersenii* strain 4E are illustrated in Figure. A total 41 genes were predicted as being affected by missense mutations in the variant calling analysis, although 33 of them had only one mutation. One of the genes, LB4E_3373, which encodes a protein from the PF07598 family, presented 27 missense SNPs compared with the genome of the strain 56604. The orthologous genes from the PF07598 family have already been associated with adaptation to the host in *L. interrogans* and regulation of gene expression during the life cycle and infection (Lehmann et al. 2013).

Another highly mutated gene, LB4E_1801, contains 10 single-nucleotide polymorphisms, but its function remains unclear, and no BLAST hit in Uniprot (Apweiler et al. 2004) could allow a deeper annotation or provide any clue regarding its molecular function. We also identified five mutations in a gene that encodes an M23 peptidase (LB4E_1800), which has already been associated with fibronectin binding in *Leptospira* and other closely related genera, such as *Treponema*, and may contribute to the pathogenesis process.

Although de novo assembly is usually preferred for microbial organisms, it is associated with many drawbacks in obtaining a finished genome (Miller et al. 2010). Therefore, reference-guided assembly, based on an already-finished genome, may be a more reasonable approach to assembly when a closely related reference is available. In our case, both the 4E and 56604 strains belonged to the same species and serovar, so there was no requirement for a de novo assembly in this case. In fact, the SOLiD sequencing platform offers a high-throughput platform, short read length (50 bp) and high accuracy (Liu et al. 2012); as such, it is more suitable for re-sequencing/reference-guided assembly than de novo assembly.

The SOLiD sequencing process requires two hybridisation reactions to identify each base, so the probability of an erroneous identification or an artificial insertion/deletion tends to be much smaller compared with other platforms, such as Illumina and IonTorrent. In fact, in cases of sequencing artefacts, the decoding process of the colour-space data (csFASTA) to nucleotide-space format (FASTA) (based on nucleotide transitions) would generate an apparently random sequence after the erroneous base position, which probability would not align to the reference genome in the read mapping process (during a variant calling study) or be used in the assembly of a contig (in a de novo assembly). The reliability of this platform has already been demonstrated by previous studies, such as the benchmarking study performed by Ratan et al. (2013), which compared the accuracy of three different NGS platforms (ABI SOLiD, Illumina HiSeq and Roche 454 FLX) in the identification of SNPs in a human sample. In this case, the number of SNPs identified by SOLiD that were validated by mass-spectrometry was higher that what was observed in the other platforms. Therefore, although SOLiD is not a first option for microbial genomics, for which benchtop platforms are usually preferred, it may still be a valuable tool when aiming for a more accurate identification of mutations.

Finally, a de novo assembly using SOLiD data resulted in a more fragmented draft genome than other sequencing technologies because the short read length implies that there are many difficulties for the assem-

### Table I

Features identified in the draft genome of the *Leptospira borgpetersenii* serovar Ballum strain 4E during the annotation

| Chromosome | CDSs | tRNAs | rRNAs | mRNA | Riboswitches | Gaps |
|------------|------|-------|-------|------|--------------|------|
| I          | 3120 | 4     | 37    | 2    | 0            | 21   |
| II         | 331  | 0     | 0     | 0    | 5            | 5    |

*a: includes other families of non-coding RNAs predicted by GeniX which are neither tRNAs nor rRNAs; b: considered as assembly gaps runs of “Ns” with length equal or longer than five nucleotides, and those shorter than this threshold were considered INDELs or base-calling errors.*
bly algorithms due to the occurrence of repeated regions along the genome that may be collapsed by the de Bruijn graphs (Alkan et al. 2010); as such, this method would not be appropriate in this case.

In the context of *Leptospira* research, genomic data from highly virulent strains might provide useful information for the development of new vaccines and diagnostic methods and improve the understanding of bacterial pathogenesis and pathogen-host interactions. The presence of a high number of mutations in a gene that encodes a protein from the PF07598 family, which has already been suggested to be related to its pathogenesis in previous studies, may be one of the reasons for the greater virulence observed in this strain, although further studies are necessary to validate this relationship. Additionally, the availability of genomic characterisation from this strain might be useful for future epidemiological surveillance studies in southern Brazil.

### TABLE II

Genes containing missense mutations identified in the genome of *Leptospira borgpetersenii* strain 4E based on the variant calling analysis using the genome of *L. borgpetersenii* strain 56604 as reference

| Locus tag         | Strain 56604 | Strain 4E | SNPs | Product                                      |
|-------------------|--------------|-----------|------|----------------------------------------------|
| LBBP_04290        | LB4E_3373    | 27        |      | PF07598 family protein<sup>a</sup>          |
| LBBP_02267        | LB4E_1801    | 10        |      | Hypothetical protein                         |
| LBBP_04295        | LB4E_3378    | 5         |      | Integrase core domain protein               |
| LBBP_02266        | LB4E_1800    | 5         |      | M23 family peptidase<sup>a</sup>            |
| LBBP_03954        | -            | 3         |      | Hypothetical protein                         |
| LBBP_02437        | LB4E_1928    | 3         |      | Hypothetical protein                         |
| LBBP_01389        | LB4E_1117    | 3         |      | PPE protein<sup>a</sup>                     |
| LBBP_04424        | -            | 2         |      | Hypothetical protein                         |
| LBBP_04423        | LB4E_3488    | 1         |      | Transposase                                  |
| LBBP_04394        | LB4E_3464    | 1         |      | Putative EF-P lysine aminoaacylase GenX      |
| LBBP_04178        | LB4E_3280    | 1         |      | Transposase                                  |
| LBBP_04013        | LB4E_3222    | 1         |      | AraC family transcriptional regulator        |
| LBBP_03775        | -            | 1         |      | Hypothetical protein                         |
| LBBP_03455        | LB4E_2709    | 1         |      | PF07600 family protein                       |
| LBBP_03226        | LB4E_2530    | 1         |      | Flagellin domain protein<sup>a</sup>        |
| LBBP_02875        | LB4E_2269    | 1         |      | Hypothetical protein                         |
| LBBP_02823        | LB4E_2227    | 1         |      | Transposase                                  |
| LBBP_02742        | LB4E_2163    | 1         |      | Dolichyl-phosphate-mannose-protein
mnnosyltransferase                           |
| LBBP_02514        | LB4E_1991    | 1         |      | Stage II sporulation protein E               |
| LBBP_02460        | LB4E_1947    | 1         |      | Hypothetical protein                         |
| LBBP_02259        | LB4E_1792    | 1         |      | DNA-directed RNA polymerase subunit beta     |
| LBBP_01965        | LB4E_1576    | 1         |      | Ribosomal RNA small subunit methyltransferase H |
| LBBP_01593        | LB4E_1288    | 1         |      | 1-aminocyclopropane-1-carboxylate deaminase  |
| LBBP_01564        | LB4E_1267    | 1         |      | Tyrosine recombinate XerD                    |
| LBBP_01436        | LB4E_1154    | 1         |      | Oma87-like Outer membrane protein            |
| LBBP_01392        | LB4E_1120    | 1         |      | Hypothetical protein                         |
| LBBP_01368        | LB4E_1098    | 1         |      | Hypothetical protein                         |
| LBBP_01318        | -            | 1         |      | Hypothetical protein                         |
| LBBP_01157        | LB4E_1157    | 1         |      | DNA repair protein RecN                      |
| LBBP_01063        | LB4E_0848    | 1         |      | tRNA nucleotidyltransferase/poly(A) polymerase family protein |
| LBBP_00977        | LB4E_0716    | 1         |      | Uncharacterized protein                      |
| LBBP_00916        | LB4E_1376    | 1         |      | Flagellar motor switch protein FliN          |
| LBBP_00894        | LB4E_0702    | 1         |      | Transketolase, pyridine binding domain protein |
| LBBP_00821        | LB4E_0643    | 1         |      | Putative coroporphyrinogen dehydrogenase     |
| LBBP_00739        | LB4E_0580    | 1         |      | Transposase                                  |
| LBBP_00738        | -            | 1         |      | Hypothetical protein                         |
| LBBP_00468        | -            | 1         |      | Hypothetical protein                         |
| LBBP_00376        | LB4E_0356    | 1         |      | Hypothetical protein                         |
| LBBP_00318        | LB4E_0266    | 1         |      | RND transporter, Hydrophobe/Amphiphile
Efflux-1 (HAE1)/Heavy Metal Efflux (HME) family, permease protein |
| LBBP_00116        | LB4E_0102    | 1         |      | NUDIX hydrolase                              |

<sup>a</sup>: potentially related to pathogenesis.
Map of the two chromosomes of *Leptospira borgpetersenii* strain 4E. Genes identified as mutated (non-synonymous mutations) based on comparison with *L. borgptersenii* strain 56604 are indicated in blue, and non-mutated genes are indicated in red.
Nucleotide sequence accession number - The complete genome of *L. borgpetersenii* strain 4E is available at GenBank under the accession codes CP015814.2 (chromosome I) and CP015815.2 (chromosome II). The raw reads from this sequencing project are available at the NCBI Short Read Archive under accession code SRR5266483.

AUTHORS’ CONTRIBUTION

MRE, FSK and RTJR - Performed the bioinformatics analysis; VACA and ALCS - supervised the whole-genome sequencing procedure using the SOLiD platform; LSP - supervised the bioinformatics analysis; EFS and OAD - isolated the strain; MRE, FSK and OAD - Wrote the manuscript; OAD - supervised the main project.

REFERENCES

Alkan C, Sajjadian S, Eichler EE. Limitations of next-generation genome sequence assembly. Nat Methods. 2010; 8(1): 61-5.

Apweiler R, Bairoch A, Wu CH, Barker WC, Boeckmann B, O’Donovan C, et al. UniProt: the universal protein knowledgebase. Nucleic Acids Res. 2004; 32(Database issue): 115-9.

Boonsilp S, Thaipadungpanit J, Amornchai P, Wuthiekanun V, Bailey MS, Holden MTG. A single multilocus sequence typing (MLST) scheme for seven pathogenic *Leptospira* species. PLoS Negl Trop Dis. 2013; 7(1): e1954.

Bourhy P, Collet L, Brisse S, Picardeau M. *Leptospira mayottensis* sp. nov., a pathogenic species of the genus *Leptospira* isolated from humans. Int J Syst Evol Microbiol. 2014; 64(12): 4061-7.

da Silva EF, Félix SR, Cerqueira GM, Fagundes MQ, Neto ACP, Grassmann AA, et al. Preliminary characterization of *Mus musculus*-derived pathogenic strains of *Leptospira borgpetersenii* serogroup Ballum in a hamster model. Am J Trop Med Hyg. 2010; 83(2): 336-7.

Diniz JA, Félix SR, Bonel-Raposo J, Seixas Neto ACP, Vasconcellos FA, Grassmann AA, et al. Highly virulent *Leptospira borgpetersenii* strain characterized in the hamster model. Am J Trop Med Hyg. 2011; 85(2): 271-4.

Fouts DE, Matthias MA, Adhikarla H, Adler B, Amorim-Santos L, Berg DE, et al. What makes a bacterial species pathogenic?: comparative genomic analysis of the genus *Leptospira*. PLoS Negl Trop Dis. 2016; 10(2): e0004403.

Garst AD, Edwards AL, Batey RT. Riboswitches: structures and mechanisms. Cold Spring Harb Perspect Biol. 2011; 3(6): a003533.

Guerra MA. Leptospirosis. J Am Vet Med Assoc. 2009; 234(4): 472-8.

Guerra MA. Leptospirosis: public health perspectives. Biologicals. 2013; 41(5): 295-7.

Gurevich A, Saveliev V, Vyahhi N, Tesler G. QUAST: quality assessment tool for genome assemblies. Bioinformatics. 2013; 29(8): 1072-5.

Hayes CS, Keiler KC. Beyond ribosome rescue: tmRNA and co-translational processes. FEBS Lett. 2010; 584(2): 413-9.

Kremer FS, Eslabão MR, Dellagostin OA, Pinto LS. Genix: a new online automated pipeline for bacterial genome annotation. FEMS Microbiol Lett. 2016a; 363(23): fnw263.

Kremer FS, Eslabão MR, Jorge S, Oliveira NR, Labonde J, Santos MNP, et al. Draft genome of the *Leptospira interrogans* strains, Aceguá, RCA, Prea, and Capivara, obtained from wildlife maintenance hosts and infected domestic animals. Mem Inst Oswaldo Cruz. 2016b; 111(4): 280-3.

Lohmann JS, Fouts DE, Haft DH, Cannella AP, Ricaldi JN, Brinkac L, et al. Pathogenomic inference of virulence-associated genes in *Leptospira interrogans*. PLoS Negl Trop Dis. 2013; 7(10): e2468.

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The sequence alignment/map format and SAMtools. Bioinformatics. 2009; 25(12): 2078-9.

Liu L, Li Y, Li S, Hu N, He Y, Pong R, et al. Comparison of next-generation sequencing systems. J Biomed Biotechnol. 2012; 2012: 251364.

McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010; 20(9): 1297-303.

Miller JR, Koren S, Sutton G. Assembly algorithms for next-generation sequencing data. Genomics. 2010; 95(6): 315-27.

Ratan A, Miller W, Guillory J, Stinson J, Seshagiri S, Schuster S. Comparison of sequencing platforms for single nucleotide variants in a human sample. PLoS One. 2013; 8(2): e55089.

Reumers J. SNPeffect: a database mapping molecular phenotypic effects of human non-synonymous coding SNPs. Nucleic Acids Res. 2004; 33(Database issue): 527-32.

Rutherford K, Parkhill J, Crook J, Horsnell T, Rice P, Rajandream M-A, et al. Artemis: sequence visualization and annotation. Bioinformatics. 2000; 16(10): 944-5.

Serganov A, Nudler E. A decade of riboswitches. Cell. 2013; 152: 17-24.