Genome sequence of the Listia angolensis microsymbiont

Microvirga lotononidis strain WSM3557T

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Microvirga lotononidis is a recently described species of root-nodule bacteria that is an effective nitrogen-(N) fixing microsymbiont of the symbiotically specific African legume Listia angolensis (Welw. ex Bak.) B.-E. van Wyk & Boatwr. M. lotononidis possesses several properties that are unusual in root-nodule bacteria, including pigmentation and the ability to grow at temperatures of up to 45°C. Strain WSM3557T is an aerobic, motile, Gram-negative, non-spore-forming rod isolated from a L. angolensis root nodule collected in Chipata, Zambia in 1963. This is the first report of a complete genome sequence for the genus Microvirga. Here we describe the features of Microvirga lotononidis strain WSM3557T, together with genome sequence information and annotation. The 7,082,538 high-quality-draft genome is arranged in 18 scaffolds of 104 contigs, contains 6,956 protein-coding genes and 84 RNA-only encoding genes, and is one of 20 rhizobial genomes sequenced as part of the DOE Joint Genome Institute 2010 Community Sequencing Program.

Introduction

Legume-rhizobia symbioses are important components of southern Australian agricultural systems, in which symbiotic N2-fixation provides a significant amount of the nitrogen input that is required to boost food and animal production [1,2]. Traditionally, pasture legumes have been Mediterranean annuals such as medics and subterranean clover [3]. However, recent changes to the rainfall patterns in south-western Western Australia, resulting in a 10-20% decrease in annual rainfall [4], have adversely affected production from these annual legumes. Researchers are therefore seeking to introduce alternative perennial legume species and associated rhizobia that are better adapted to the arid climate and acid, infertile soils found in these systems [2]. Among the perennial, herbaceous forage legumes selected for further study are several species within the papilionoid legume clade Lotononis sensu lato.

Lotononis s. l. is grouped within tribe Crotalarieae, has a centre of origin in South Africa and consists of some 150 species, divided into 15 sections [5]. The taxonomy has recently been revised and the three distinct clades within Lotononis s. l. are now recognized at the generic level as Listia, Leobordea and Lotononis s. s. [6]. Species within the genus Listia are of agronomic interest, as they have potential as perennial pasture legumes that are able to reduce groundwater recharge and assist in preventing dry land salinity in southern Australian agricultural systems [7]. Listia spp. produce stoloniferous roots on their lower branches (a characteristic thought to be associated with the seasonally wet habitats where these species are found) [5] and form lupinoid, rather than indeterminate nodules, in response to infection by rhizobia [7,8]. The symbioses between Listia species and their associated root-nodule bacteria are highly specific. All studied...
host species are nodulated by strains of pigmented methyllobacteria [7,9,10], except for *Listia angolensis*, which is effectively nodulated only by newly described species of *Microvirga* [11]. *Microvirga lotononidis* strain WSM3557 was the type strain for this species. Here we present a set of preliminary classification and general features for *M. lotononidis* strain WSM3557 together with the description of the genome sequence and annotation.

**Classification and general features**

*M. lotononidis* strain WSM3557 is a motile, Gram-negative, non-spore-forming rod with one to several flagella (Figure 1, left and center panel). It is a member of the family *Methylobacteriaceae* in the class *Alphaproteobacteria* (Figure 2). WSM3557 is fast growing, forming 0.5-1.5 mm diameter colonies within 2-3 days. It is moderately thermophilic and has a mean generation time of 1.6 h when grown in broth at the optimum growth temperature of 41°C [15]. WSM3557 is pigmented, an unusual property for rhizobia. Colonies on half Lupin Agar (½LA) [7] are pale pink, opaque, slightly domed, moderately mucoid with smooth margins (Figure 1, right panel). The color develops after several days. WSM3557 is able to tolerate a pH range between 6.0 and 9.5 [11]. Carbon source utilization, cellular fatty acid profiles, polar lipid analysis and respiratory lipoquinone analysis have been described previously [11]. Minimum Information about the Genome Sequence (MIGS) is provided in Table 1.

**Symbiotaxonomy**

*M. lotononidis* strain WSM3557 nodulates (Nod+) and fixes N2 effectively (Fix+) with *Listia angolensis*; nodulates and is partially effective on *Leobordea platycarpa*, *Leobordea bolusii* and *Lotononis crumanina* and nodulates but is unable to fix N2 (Nod+, Fix−) with *Leobordea longiflora*, *Leobordea stipulosa* and *Lotononis falcata* [8]. It forms occasional ineffective nodules with *Phaseolus vulgaris*, but is unable to nodulate *Crotalaria juncea*, *Indigofera patens*, *Lotus corniculatus*, *Lupinus angustifolius*, or *Macroptilium atropurpureum* [11].

**Genome sequencing and annotation**

**Genome project history**

This organism was selected for sequencing on the basis of its environmental and agricultural relevance to issues in global carbon cycling, alternative energy production, and biogeochemical importance, and is part of the Community Sequencing Program at the U.S. Department of Energy, Joint Genome Institute (JGI) for projects of relevance to agency missions. The genome project is deposited in the Genomes OnLine Database [14] and an improved high-quality-draft genome sequence in IMG. Sequencing, finishing and annotation were performed by the JGI. A summary of the project information is shown in Table 2.

**Growth conditions and DNA isolation**

*Microvirga lotononidis* WSM3557 was grown to mid-logarithmic phase in TY rich medium [23] on a gyratory shaker at 28°C. DNA was isolated from 60 mL of cells using a CTAB (Cetyl trimethyl ammonium bromide) bacterial genomic DNA isolation method [24].

**Figure 1.** Images of *Microvirga lotononidis* strain WSM3557 using scanning (Left) and transmission (Center) electron microscopy as well as light microscopy to visualize colony morphology on a solid medium (Right).
Table 1. Classification and general features of *Microvirga lotononidis*. strain WSM3557T in accordance to the MIGS recommendations [16,17].

| MIGS ID | Property                     | Term                                              | Evidence code |
|---------|------------------------------|---------------------------------------------------|---------------|
|         | Domain                       | Bacteria                                          | TAS [17]      |
|         | Phylum                       | Proteobacteria                                    | TAS [18]      |
|         | Class                        | Alphaproteobacteria                               | TAS [18]      |
| Current classification | Order                        | Rhizobiales                                       | TAS [19]      |
|         | Family                       | Methylbacteriaceae                                | TAS [20]      |
|         | Genus                        | Microvirga                                        | TAS [21]      |
|         | Species                      | Microvirga lotononidis                            | TAS [15]      |
| Gram stain |                              | Negative                                          | TAS [15]      |
| Cell shape |                              | Rod                                               | TAS [15]      |
| Motility |                              | Motile                                            | TAS [15]      |
| Sporulation |                              | Non-sporulating                                   | TAS [15]      |
| Temperature range |                        | Mesophile                                         | TAS [15]      |
| Optimum temperature |                        | 41°C                                              | TAS [15]      |
| Salinity |                              | Non-halophile                                     | TAS [15]      |
| MIGS-22 | Oxygen requirement           | Aerobic                                           | TAS [15]      |
| Carbon source |                        | L-arabinose, D-cellobiose, D-fructose, D-glucose, glycerol, D-mannitol, acetate, succinate & glutamate | TAS [15]      |
| Energy source |                              | Chemoorganotroph                                 | TAS [15]      |
| MIGS-6  | Habitat                      | Soil, root nodule on host                         | TAS [15]      |
| MIGS-15 | Biotic relationship          | Free living, symbiotic                            | TAS [15]      |
| MIGS-14 | Pathogenicity                | Non-pathogenic                                    | NAS           |
| Biosafety level |                        | 1                                                 | NAS           |
| Isolation |                              | Root nodule of *Listia angolensis*                | TAS [15]      |
| MIGS-4  | Geographic location          | Chipata, Zambia                                   | TAS [15]      |
| MIGS-5  | Nodule collection date       | April 1963                                        | TAS [15]      |
| MIGS-4.1 | Longitude                   | 32.63                                             | TAS [15]      |
| MIGS-4.2 | Latitude                    | -13.65                                            | TAS [15]      |
| MIGS-4.3 | Depth                       | Not recorded                                      |               |
| MIGS-4.4 | Altitude                    | 1000                                              | IDA           |

Evidence codes – IDA: Inferred from Direct Assay; TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the Gene Ontology project [22].
Figure 2. Phylogenetic tree showing the relationships of *Microvirga lotononidis* WSM3557 (shown in blue print) with some of the root nodule bacteria in the order *Rhizobiales* based on aligned sequences of the 16S rRNA gene (1,255 bp internal region). All sites were informative and there were no gap-containing sites. Phylogenetic analyses were performed using MEGA, version 5.05 [12]. The tree was built using the maximum likelihood method with the General Time Reversible model. Bootstrap analysis [13] with 500 replicates was performed to assess the support of the clusters. Type strains are indicated with a superscript T. Strains with a genome sequencing project registered in GOLD [14] are in bold print and the GOLD ID is mentioned after the accession number. Published genomes are designated with an asterisk.

**Genome sequencing and assembly**

The improved high quality draft genome of *Microvirga lotononidis* WSM3557\(^T\) was generated at the DOE Joint Genome Institute (JGI) using a combination of Illumina [25] and 454 technologies [26]. An Illumina GAii shotgun library comprising 71,475,016 reads totaling 5,432.1 Mb reads and 1 paired end 454 library with an average insert size of 10 Kb which produced 582,107 reads totaling 113.9 Mb of 454 data were generated for this genome. All general aspects of library construction and sequencing performed at the JGI can be found at [24]. The initial draft assembly contained 444 contigs in 1 scaffold. The 454 paired end data was assembled together with Newbler, version 2.3 PreRelease-6/30/2009. The Newbler consensus sequences were computationally shredded into 2 Kb overlapping fake reads (shreds). Illumina sequencing data was assembled with Velvet, version 1.0.13 [27], and the consensus sequences were computationally shredded into 1.5 Kb overlapping fake reads (shreds). The 454 Newbler consensus shreds, the Illumina Velvet consensus shreds and the read pairs in the 454 paired end library using parallel phrap, version SPS - 4.24 (High Performance Software, LLC) were integrated. The software Consed [28-30] was used in the following finishing process. Illumina data was used to correct potential base errors and increase consensus quality, using the software Polisher developed at JGI (Alla Lapidus, unpublished). Possible mis-assemblies were corrected using gapResolution (Cliff Han, unpublished), Dupfinisher [31], or sequencing cloned bridging

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PCR fragments with subcloning. Gaps between contigs were closed by editing in Consed, by PCR and by Bubble PCR (J-F Cheng, unpublished) primer walks. A total of 303 additional reactions were necessary to close gaps and to raise the quality of the finished sequence. The estimated genome size is 7.2 Mb and the final assembly is based on 59.7 Mb of 454 draft data which provides an average 8.3× coverage of the genome and 2,160 Mb of Illumina draft data which provides an average 300× coverage of the genome.

**Genome annotation**
Genes were identified using Prodigal [32] as part of the DOE-JGI Annotation pipeline [33], followed by a round of manual curation using the JGI GenePRIMP pipeline [34]. The predicted CDSs were translated and used to search the National Center for Biotechnology Information (NCBI) non-redundant database, UniProt, TIGRFam, Pfam, PRIAM, KEGG, COG, and InterPro databases. These data sources were combined to assert a product description for each predicted protein. Non-coding genes and miscellaneous features were predicted using tRNAscan-SE [35], RNAMer [36], Rfam [37], TMHMM [38], and SignalP [39]. Additional gene prediction analyses and functional annotation were performed within the Integrated Microbial Genomes (IMG-ER) platform [40].

**Genome properties**
The genome is 7,082,538 nucleotides with 63.00% GC content (Table 3) and comprised of 18 scaffolds (Figures 3a, 3b and Figure 3c) of 104 contigs. From a total of 7,040 genes, 6,956 were protein encoding and 84 RNA only encoding genes. The majority of genes (67.64%) were assigned a putative function while the remaining genes were annotated as hypothetical. The distribution of genes into COGs functional categories is presented in Table 4.

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**Table 2. Genome sequencing project information for Microvirga lotononidis WSM3557T**

| MIGS ID | Property | Term |
|---------|----------|------|
| MIGS-31 | Finishing quality | Improved high quality draft |
| MIGS-28 | Libraries used | Illumina GAii shotgun and paired end 454 libraries |
| MIGS-29 | Sequencing platforms | Illumina GAii and 454 GS FLX Titanium technologies |
| MIGS-31.2 | Sequencing coverage | 8.3× 454 paired end, 300× Illumina |
| MIGS-30 | Assemblers | Velvet, version 1.0.13; Newbler, version 2.3- PreRelease-6/30/2009; phrap, version SPS - 4.24 |
| MIGS-32 | Gene calling method | Prodigal |
| GOLD ID | | Gi06493 |
| NCBI project ID | | 65303 |
| Database: IMG | | 2508501114 |
| Project relevance | | Symbiotic N fixation, agriculture |

**Table 3. Genome Statistics for Microvirga lotononidis WSM3557T**

| Attribute | Value | % of Total |
|-----------|-------|------------|
| Genome size (bp) | 7,082,538 | 100.00 |
| DNA coding region (bp) | 5,991,598 | 84.60 |
| DNA G+C content (bp) | 4,462,203 | 63.00 |
| Number of scaffolds | 18 | |
| Number of contigs | 104 | |
| Total genes | 7,040 | 100.00 |
| RNA genes | 84 | 1.19 |
| rRNA operons* | 1 | |
| Protein-coding genes | 6,956 | 98.81 |
| Genes with function prediction | 4,762 | 67.64 |
| Genes assigned to COGs | 5,117 | 72.68 |
| Genes assigned Pfam domains | 5,358 | 76.11 |
| Genes with signal peptides | 656 | 9.32 |
| Genes with transmembrane helices | 1,480 | 21.02 |
| CRISPR repeats | 0 | |

*1 full-length and 1 partial 23s rRNA gene, 3 partial 5s rRNA genes
**Figure 3a.** Graphical map of the genome of *M. lotononidis* WSM3557T (scaffolds MLG.1-MLG.9). From the bottom to the top of each scaffold: Genes on forward strand (color by COG categories as denoted by the IMG platform), Genes on reverse strand (color by COG categories), RNA genes (tRNAs green, sRNAs red, other RNAs black), GC content, GC skew.
Figure 3c. Color code for Figure 3a and 3b.

Figure 3b. Graphical map of the genome of *Microvirga lotononidis* strain WSM3557T (scaffolds MLG.10-MLG.18). From the bottom to the top of each scaffold: Genes on forward strand (color by COG categories as denoted by the IMG platform), Genes on reverse strand (color by COG categories), RNA genes (tRNAs green, sRNAs red, other RNAs black), GC content, GC skew.
### Table 4. Number of protein coding genes of *Microvirga* sp. WSM3557T associated with the general COG functional categories.

| Code | Value | Percentage | Description                                              |
|------|-------|------------|----------------------------------------------------------|
| J    | 200   | 3.52       | Translation, ribosomal structure and biogenesis          |
| A    | 1     | 0.02       | RNA processing and modification                          |
| K    | 397   | 6.98       | Transcription                                            |
| L    | 431   | 7.58       | Replication, recombination and repair                     |
| B    | 7     | 0.12       | Chromatin structure and dynamics                          |
| D    | 38    | 0.67       | Cell cycle control, mitosis and meiosis                  |
| Y    | 0     | 0.00       | Nuclear structure                                         |
| V    | 72    | 1.27       | Defense mechanisms                                       |
| T    | 374   | 6.58       | Signal transduction mechanisms                           |
| M    | 254   | 4.47       | Cell wall/membrane biogenesis                            |
| N    | 76    | 1.34       | Cell motility                                            |
| Z    | 0     | 0.00       | Cytoskeleton                                             |
| W    | 1     | 0.02       | Extracellular structures                                 |
| U    | 73    | 1.28       | Intracellular trafficking and secretion                  |
| O    | 178   | 3.13       | Posttranslational modification, protein turnover, chaperones|
| C    | 307   | 5.40       | Energy production conversion                             |
| G    | 435   | 7.65       | Carbohydrate transport and metabolism                    |
| E    | 612   | 10.76      | Amino acid transport metabolism                          |
| F    | 105   | 1.85       | Nucleotide transport and metabolism                      |
| H    | 193   | 3.39       | Coenzyme transport and metabolism                        |
| I    | 179   | 3.15       | Lipid transport and metabolism                           |
| P    | 319   | 5.61       | Inorganic ion transport and metabolism                   |
| Q    | 171   | 3.01       | Secondary metabolite biosynthesis, transport and catabolism|
| R    | 677   | 11.91      | General function prediction only                         |
| S    | 586   | 10.31      | Function unknown                                         |
| -    | 1,923 | 27.32      | Not in COGS                                             |

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Microvirga lotononidis strain WSM3557T

References

1. Herridge DF, Peoples MB, Boddey RM. Global inputs of biological nitrogen fixation in agricultural systems. *Plant Soil* 2008; 311:1-18.  
   http://dx.doi.org/10.1007/s11104-008-9668-3

2. Howieson JG, Yates RJ, Foster K, Real D, Besier B. Prospects for the future use of legumes. In: Dilworth MJ, James EK, Sprent JI, Newton WE, editors. Leguminous Nitrogen-Fixing Symbioses. London, UK: Elsevier; 2008. p 363-394.

3. Loi A, Howieson JG, Nutt BJ, Carr SJ. A second generation of annual pasture legumes and their potential for inclusion in Mediterranean-type farming systems. *Aust J Exp Agric* 2005; 45:289-299.  
   http://dx.doi.org/10.1071/EA03134

4. George RJ, Speed RJ, Simons JA, Smith RH, Ferdowsian R, Raper GP, Bennett DL. Long-term groundwater trends and their impact on the future extent of dryland salinity in Western Australia in a variable climate. Salinity Forum 2008. 2008.

5. van Wyk BE. A Synopsis of the Genus Lotononis (Fabaceae: Crotalarieae). Cape Town, South Africa: Rustica Press; 1991.

6. Boatwright JS, Wink M, van Wyk BE. The generic concept of Lotononis (Crotalarieae, Fabaceae): Reinstatement of the genera *Euchlora*, *Leobordea* and *Listia* and the new genus *Ezoaloba*. *Taxon* 2011; 60:161-177.

7. Yates RJ, Howieson JG, Reeve WG, Nandasena KG, Law IJ, Bräu L, Ardley JK, Nistelberger HM, Real D, O’Hara GW. *Lotononis angolensis* forms nitrogen fixing, lupinoid nodules with phylogenetically unique, fast-growing, pink-pigmented bacteria, which do not nodulate L. *baïnesii* or L. *listii*. *Soil Biol Biochem* 2007; 39:1680-1688.  
   http://dx.doi.org/10.1016/j.soilbio.2007.01.025

8. Ardley JK. Symbiotic specificity and noduleation in the southern African legume clade *Lotononis s. l.* and description of novel rhizobial species within the Alphaproteobacterial genus *Microvirga*: Murdoch University, Murdoch, WA, Australia; 2012.

9. Norris DO. A red strain of *Rhizobium* from *Lotononis bainesii* Baker. *Aust J Agric Res* 1958; 9:629-632.  
   http://dx.doi.org/10.1071/AR9580629

10. Jaftha JB, Strijdom BW, Steyn PL. Characterization of pigmented methylotrophic bacteria which nodule *Lotononis bainesii*. *Syst Appl Microbiol* 2002; 25:440-449.  
   PubMed  
   http://dx.doi.org/10.1078/0723-2020-00124

11. Ardley JK, Parker MA, De Meyer SE, Trengove RD, O’Hara GW, Reeve WG, Yates RJ, Dilworth MJ, Willems A, Howieson JG. *Microvirga lupini* sp. nov., *Microvirga lotononidis* sp. nov. and *Microvirga zambiensis* sp. nov. are alphaproteobacterial root-nodule bacteria that specifically nodulate and fix nitrogen with geographically and taxonomically separate legume hosts. *Int J Syst Evol Microbiol* 2011; 62:2579-2588. PubMed  
   http://dx.doi.org/10.1099/ijs.0.035097-0

12. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular evolutionary genetics analysis using Maximum Likelihood, evolutionary distance, and Maximum Parsimony methods. *Mol Biol Evol* 2011; 28:2731-2739. PubMed  
   http://dx.doi.org/10.1093/molbev/msr121

13. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 1985; 39:783-791.  
   http://dx.doi.org/10.2307/2408678

14. Liolios K, Mavromatis K, Tavemarakis N, Kyrpides NC. The Genomes On Line Database (GOLD) in 2007: status of genomic and metagenomic projects and their associated metadata. *Nucleic Acids Res* 2008; 36(Database issue):D475-D479. PubMed  
   http://dx.doi.org/10.1093/nar/gkm884

15. Ardley JK, Parker MA, De Meyer SE, Trengove RD, O’Hara GW, Reeve WG, Yates RJ, Dilworth MJ, Willems A, Howieson JG. *Microvirga lupini* sp. nov., *Microvirga lotononidis* sp. nov., and *Microvirga zambiensis* sp. nov. are Alphaproteobacterial root nodule bacteria that specifically nodulate and fix nitrogen with geographically and taxonomically separate legume hosts. *Int J Syst Evol Microbiol* 2011; 62:2579-2588. PubMed  
   http://dx.doi.org/10.1099/ijs.0.035097-0

16. Field D, Garrity G, Gray T, Morrison N, Selengut J, Sterk P, Tatusova T, Thomson N, Allen M, Angiuoli SV, et al. Towards a richer description of our complete collection of genomes and metagenomes "Minimum Information about a Genome Sequence " (MIGS) specification. *Nat Biotechnol* 2008; 26:541-547. PubMed  
   http://dx.doi.org/10.1038/nbt1360

17. Woese CR, Kandler O, Wheelis ML. Towards a natural system of organisms: proposal for the domains *Archaea*, *Bacteria*, and *Eucarya*. *Proc Natl Acad Sci USA* 1990; 87:4576-4579. PubMed  
   http://dx.doi.org/10.1073/pnas.87.12.4576
18. Garrity GM, Bell JA, Libum T. Class I. 
Alpha proteobacteria class. In: Garrity GM, Brenner DJ, Krieg NR, Staley JT, editors. Bergey’s Manual of Systematic Bacteriology. Second ed: New York: Springer - Verlag; 2005. p 195.

19. Kuykendall LD. Order VI. Rhizobiales ord. nov. In: Garrity GM, Brenner DJ, Krieg NR, Staley JT, editors. Bergey’s Manual of Systematic Bacteriology. Second ed. New York: Springer - Verlag; 2005. p 324.

20. Garrity GM, Bell JA, Libum TG. Family IX. Methylobacteriaceae. In: Garrity GM, Brenner DJ, Krieg NR, Staley JT, editors. Bergey’s Manual of Systematic Bacteriology. Second ed. Volume 2. New York: Springer - Verlag; 2005. p 567.

21. Kanso S, Patel BKC. Microvirga subterranea gen. nov., sp. nov., a moderate thermophile from a deep subsurface Australian thermal aquifer. Int J Syst Evol Microbiol 2003; 53:401-406. PubMed [http://dx.doi.org/10.1099/ijis.0.02348-0]

22. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet 2000; 25:25-29. PubMed [http://dx.doi.org/10.1038/75556]

23. Reeve WG, Tiwari RP, Worsley PS, Dilworth MJ, Glenn AR, Howieson JG. Constructs for insertional mutagenesis, transcriptional signal localization and gene regulation studies in root nodule and other bacteria. Microbiology 1999; 145:1307-1316. PubMed [http://dx.doi.org/10.1099/13500872-145-6-1307]

24. http://my.jgi.doe.gov/general/index.html

25. Bennett S. Solexa Ltd. Pharmacogenomics 2004; 5:433-438. PubMed [http://dx.doi.org/10.1517/14622416.5.4.433]

26. Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen YJ, Chen Z, et al. Genome sequencing in microfabricated high-density picolitre reactors. Nature 2005; 437:376-380. PubMed

27. Zerbino DR. Using the Velvet de novo assembler for short-read sequencing technologies. Current Protocols in Bioinformatics 2010;Chapter 11:Unit 11 5.

28. Ewing B, Green P. Base-calling of automated sequencer traces using phred. II. Error probabilities. Genome Res 1998; 8:175-185. PubMed [http://dx.doi.org/10.1101/gr.8.3.175]

29. Ewing B, Hillier L, Wendl MC, Green P. Base-calling of automated sequencer traces using phred. I. Accuracy assessment. Genome Res 1998; 8:175-185. PubMed [http://dx.doi.org/10.1101/gr.8.3.175]

30. Gordon D, Abajian C, Green P. Consed: a graphical tool for sequence finishing. Genome Res 1998; 8:195-202. PubMed [http://dx.doi.org/10.1101/gr.8.3.195]

31. Han C, Chain P. Finishing repeat regions automatically with Dupfinisher. In: Valafar HRAH, editor. Proceeding of the 2006 international conference on bioinformatics & computational biology: CSREA Press; 2006. p 141-146.

32. Hyatt D, Chen GL, Locascio PF, Land ML, Larmier FW, Hauser LJ. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics 2010; 11:119. PubMed [http://dx.doi.org/10.1186/1471-2105-11-119]

33. Mavromatis K, Ivanova NN, Chen IM, Szeto E, Markowitz VM, Kyrpides NC. The DOE-JGI Standard operating procedure for the annotations of microbial genomes. Stand Genomic Sci 2009; 1:63-67. PubMed [http://dx.doi.org/10.4056/sigs.632]

34. Pati A, Ivanova NN, Mikhailova N, Ovchinnikova G, Hooper SD, Lykidis A, Kyrpides NC. GenePRIMP: a gene prediction improvement pipeline for prokaryotic genomes. Nat Methods 2010; 7:455-457. PubMed [http://dx.doi.org/10.1038/nmeth.1457]

35. Lowe TM, Eddy SR. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 1997; 25:955-964. PubMed

36. Lagesen K, Hallin P, Rodland EA, Staerfeldt HH, Rognes T, Ussery DW. RNAmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res 2007; 35:3100-3108. PubMed [http://dx.doi.org/10.1093/nar/gkm160]

37. Griffiths-Jones S, Bateman A, Marshall M, Khanna A, Eddy SR. Rfam: an RNA family database. Nucleic Acids Res 2003; 31:439-441. PubMed [http://dx.doi.org/10.1093/nar/gkg006]

38. Krogh A, Larsson B, von Heijne G, Sonnhammer ELL. Predicting transmembrane protein topology with a hidden Markov model: Application to complete genomes. J Mol Biol 2001; 305:567-580. PubMed [http://dx.doi.org/10.1006/jmbi.2000.4315]

http://standardsingenomics.org
39. Bendtsen JD, Nielsen H, von Heijne G, Brunak S. Improved prediction of signal peptides: SignalP 3.0. *J Mol Biol* 2004; **340**:783-795. PubMed
   http://dx.doi.org/10.1016/j.jmb.2004.05.028

40. Markowitz VM, Mavromatis K, Ivanova NN, Chen IM, Chu K, Kyrpides NC. IMG ER: a system for microbial genome annotation expert review and curation. *Bioinformatics* 2009; **25**:2271-2278. PubMed
   http://dx.doi.org/10.1093/bioinformatics/btp393
