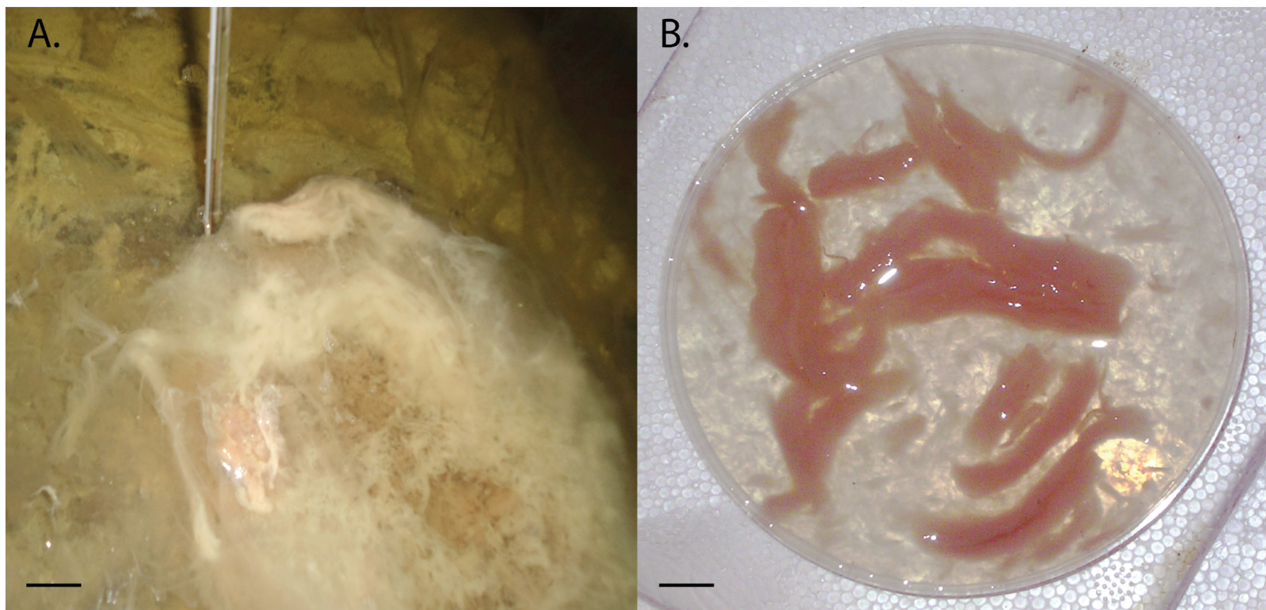
SUPPLEMENTARY MATERIALS

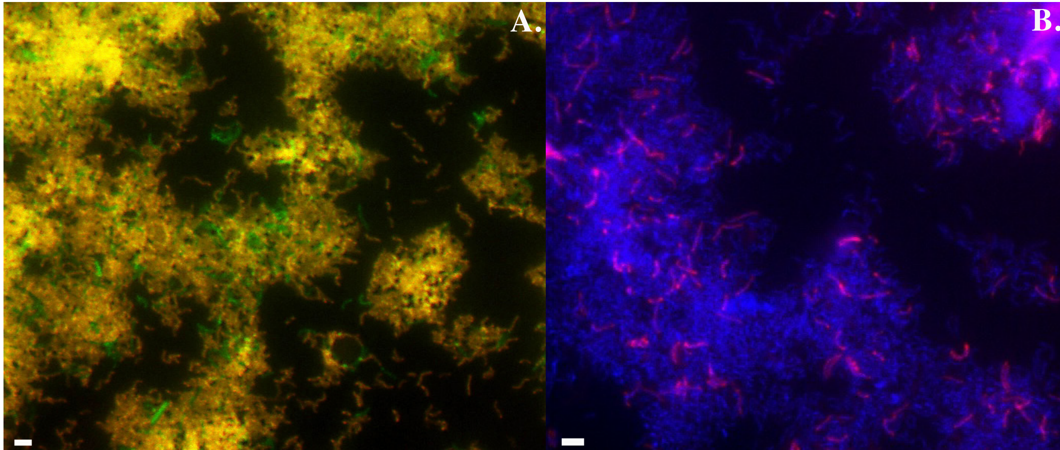
Supplementary Table S1. Statistics of reads mapped to the assembled *Leptospirillum* Group IV UBA BS genome. Inferred read error (IRE) = % total number of mapped errors such as INDELS and SNPs divided by the number of mapped bases (gsMapper - Roche/454).

Sample	Seq.	% Reads mapped	Inferred read error	% total dataset	Reference
UBABS	Sanger	36.06	0.57	2.57	This study
UBA	Sanger	14.56	1.75	1.07	(Lo et al., 2007)
5way CG	Sanger	22.70	4.96	1.32	(Tyson et al., 2004)

Supplementary Figure S1. Images of UBA BS-like biofilms. Scale bars = 1 cm. A) Photograph of biofilms growing in the A-drift of the Richmond Mine; B) Photograph of a UBABS biofilm on a petri dish.



Supplementary Figure S2. Sample UBA BS Dec10 (collected on 12/28/10, at pH 0.98 and temperature 38 °C). A) general bacteria (green) and *L. ferrodiazotrophum* (yellow). B) DAPI-stain (blue) and *Leptospirillum* group II 5wayCG (red/pink). Scale bar = 1 μm.



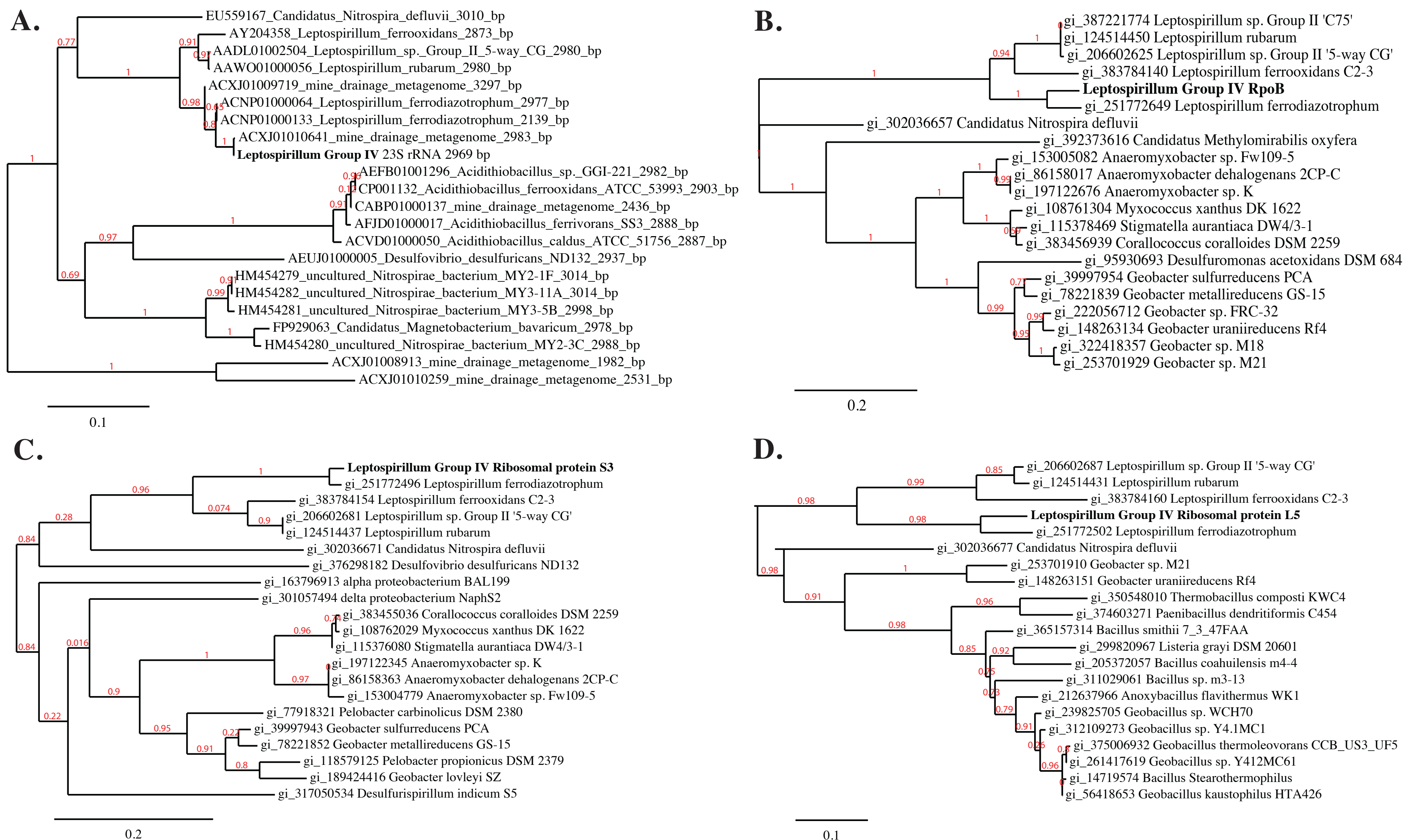
Supplementary Figure S3. Estimation of genome completeness in *Leptospirillum* group IV UBA BS based on 30 single copy genes. Some genes in *Leptospirillum* group IV UBA BS are split due to gaps within genes.

	CTP synthetase	DNA gyrase A	DNA gyrase B	EF-Ts	frr gene	histidyl tRNA synthetase	initiation factor IF3	Leucyl tRNA synthetase	NusA	phenylalanyl tRNA synth.	Phosphoglycerate kinase	RecA	Ribosomal L1
L. rubarum	1	1	1	1	1	1	1	1	1	1	1	1	1
L. group II 5wayCG	1	1	1	1	1	1	1	1	1	1	1	1	1
L. ferrodiazotrophum	1	1	1	1	1	1	1	1	1	1	1	1	1
L. group IV	·	·	·	·	1	1	1	2	1	1	2	1	1

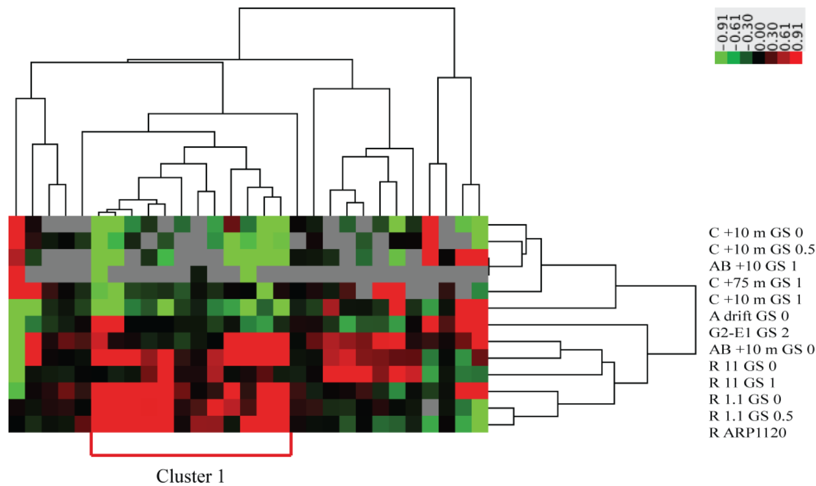
	Ribosomal L2	Ribosomal L3	Ribosomal L4	Ribosomal L5	Ribosomal L6	Ribosomal L10	Ribosomal L11	Ribosomal L13	Ribosomal L14	Ribosomal L15	Ribosomal L16	Ribosomal L18	Ribosomal L19
L. rubarum	1	1	1	1	1	1	1	1	1	1	1	1	1
L. group II 5wayCG	1	1	1	1	1	1	1	1	1	1	1	1	1
L. ferrodiazotrophum	1	1	1	1	1	1	1	1	1	1	1	1	1
L. group IV	1	1	1	1	1	1	1	·	1	·	1	·	1

	Ribosomal L20	Ribosomal L22	Ribosomal L27	Ribosomal S2	Ribosomal S3	Ribosomal S5	Ribosomal S9	Ribosomal S10	Ribosomal S11	Ribosomal S13	Ribosomal S19	RpoB	valyl tRNA synth.
L. rubarum	1	1	1	1	1	1	1	1	1	1	1	1	1
L. group II 5wayCG	1	1	1	1	1	1	1	1	1	1	1	1	1
L. ferrodiazotrophum	1	1	1	1	1	1	1	1	1	1	1	1	1
L. group IV	·	1	·	·	1	·	·	1	1	1	1	2	2

Supplementary Figure S4. Phylogenetic analysis of: A) 23S rRNA gene tree, B) Protein tree for RpoB, C) ribosomal protein S3, and D) ribosomal protein L5. Sequences were aligned and trees were constructed in the MABL website (Dereeper et al., 2008).



Supplementary Figure S5. Clustering of transcriptomic reads mapping to *Leptospirillum* Group IV UBA BS genes. Cluster 1: (2) IstB domain-containing proteins, (3) hypothetical proteins, (1) ATP synthase, (1) PFOR, (1) type-III restriction enzyme, (1) glycogen operon protein GlgX, (2) transporters, and (2) transposases. Red: overrepresented, green: underrepresented, grey: not identified by transcriptomics.



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Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F. et al. (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Res* **36**: W465-469.

Lo, I., Denef, V.J., Verberkmoes, N.C., Shah, M.B., Goltsman, D., DiBartolo, G. et al. (2007) Strain-resolved community proteomics reveals recombining genomes of acidophilic bacteria. *Nature* **446**: 537-541.

Tyson, G.W., Chapman, J., Hugenholtz, P., Allen, E.E., Ram, R.J., Richardson, P.M. et al. (2004) Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature* **428**: 37-43.

Fluorescence *in situ* hybridization protocol

Fixation:
1. Wash samples with PBS pH 1, then with PBS pH 7. Centrifuge and discard supernatant.
2. Add about 500ul of fixative (PFA 4%)
3. Incubate for about 2 hours at 4 degrees C.
4. Centrifuge and discard supernatant
5. Add 1 volume of PBS pH 7.0 + 1 volume of ethanol 100%

Hybridization:
1. Put 5ul of fixed sample on a well on the slide.
2. Let air dry
3. Prepare hybridization buffer in a 2ml eppendorf tube:
    798ul of miliQ water
    800ul of formamide
    360ul of NaCl 5M
    40ul of buffer Tris-HCl pH 8.0
    2ul of SDS 10%
4. Dehydrate slide in 50%, 80% and 90% ethanol, for 3 minutes each
5. Let air dry
6. Prepare hybridization chamber by placing a kimwipe in a 50ml falcon tube, and add about 1.8ml of hybridization buffer.
7. Add 8ul of hybridization buffer to each well
8. Add 1ul of each probe to each well
9. Place slide in hybridization chamber and incubate at 46 degrees C for about 2 hours
10. Prepare washing buffer in a 50ml falcon tube, warm to 48 degrees in water bath:
    48 ml miliQ water
    460 ul of NaCl 5M
    1 ml of buffer Tris-HCl pH 8.0
    50 ul of SDS 10%
11. Rinse the slide with a little bit of warm (48 degrees) washing buffer and place slide in the washing buffer tube. Let wash for 15 minutes at 48 degrees in the water bath
12. Rinse slide with miliQ water
13. Let air dry
14. Mount media for visualization (Vectashield) and analyze results.