



Complete genome sequence of the well-studied nicotine-degrading microorganism *Paenarthrobacter nicotinovorans* strain ATCC 49919

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1. Nicotine: toxic to more than humans



The tobacco industry produces 300 000 tonnes of non-recyclable nicotine-containing waste (18 g/per kg waste).

DOI: 10.1006/wmre.1997.0091, DOI: 10.1155/2013/125385

The waste is classified as:



Toxic Release Inventory

“toxic and hazardous”
(if nicotine content > 0.05% w/w)



GHS06



GHS09

This waste is simply discarded in the environment!

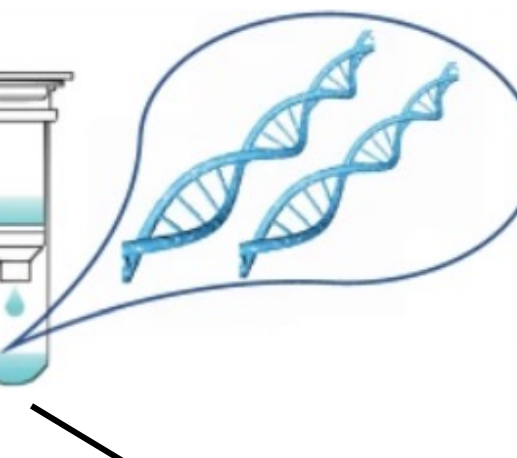
CAN NICOTINE-CONTAINING WASTE, SOIL AND WATER BE DECONTAMINATED?

WE CAN USE NICOTINE-DEGRADING MICROORGANISMS!

3. Sequencing and assembly

A. gDNA extraction

Qiagen DNeasy UltraClean Microbial Kit

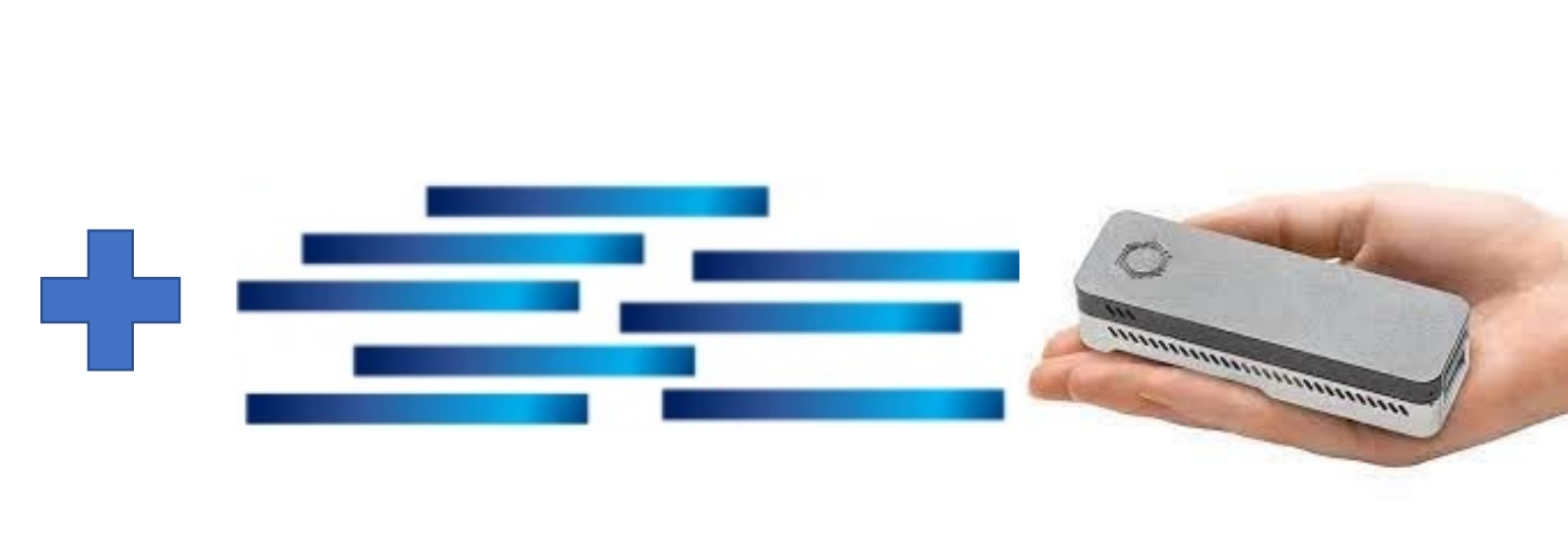


Qiagen MagAttract HMW DNA + size-selection

B. Library preparation and sequencing

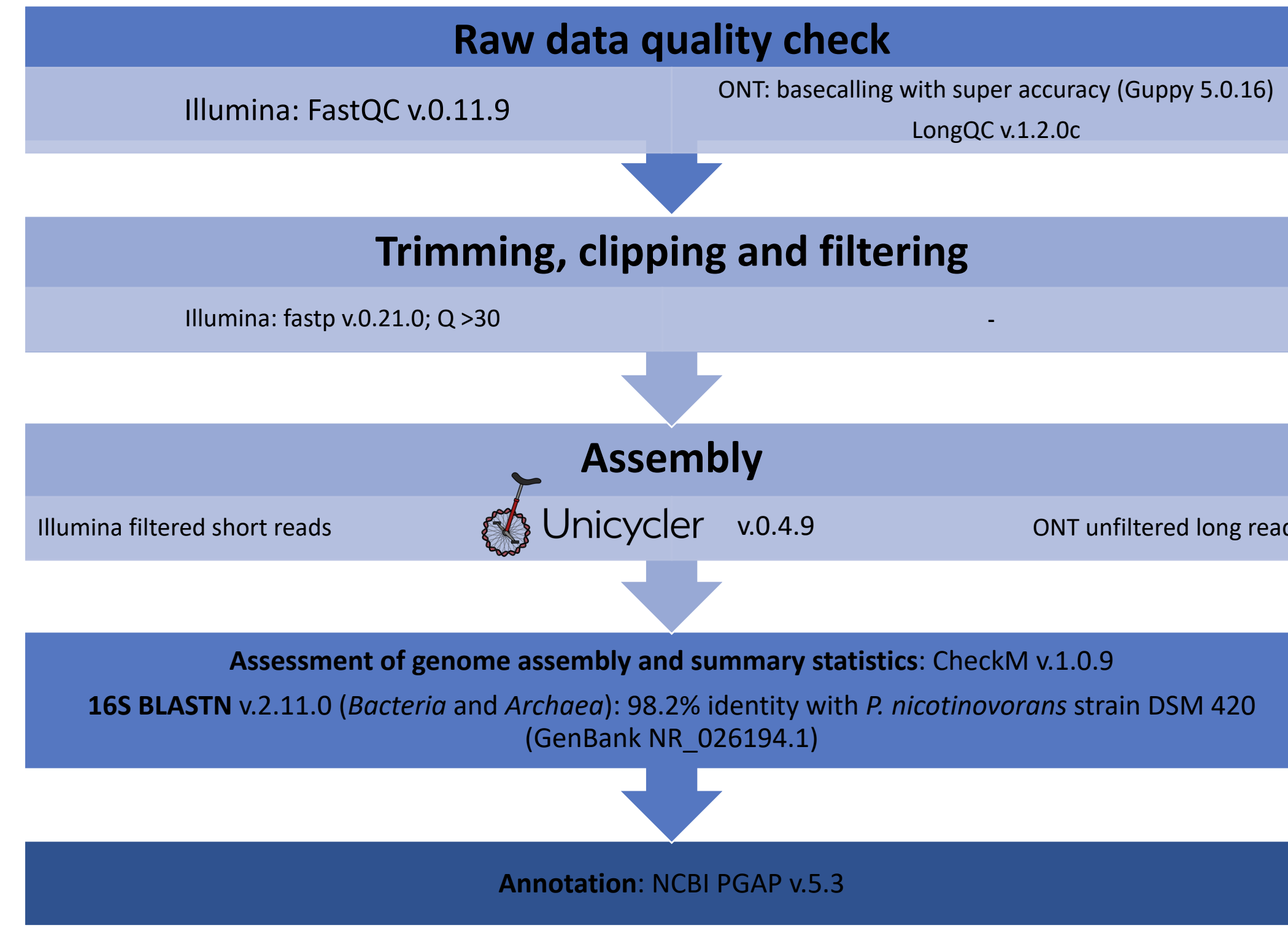


Illumina NovaSeq 6000 PCR-Free
paired-end short-reads (470 bp)



Oxford Nanopore Technologies
MinION long-reads

C. Data analysis and *de-novo* hybrid genome assembly



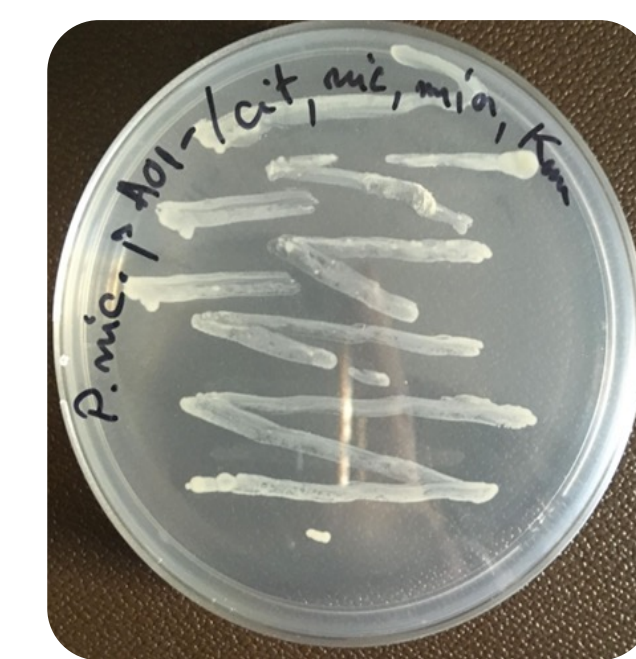
5. Assembled genomes

Strain (BioSample accession no.)	Sequencing technology*	Raw data				Filtered data			
		SRA accession no.	Size (kbp)	No. of reads	GC (%)	Size (kbp)	No. of reads	Read length data (bp)	GC (%)
<i>P. nicotinovorans</i> ATCC 49919 (SAMN17383832)	SRS/Illumina	SRR13483967	2,091,873	13,853,462	63.05	1,934,725	12,812,750	150 (read length)	63.10
		SRR13483966	2,137,039	14,152,578	63.00	1,966,789	13,025,092		63.82
		SRR13483965	2,139,192	14,166,832	63.03	1,944,165	12,875,262		63.12
		SRR17074807	2,080,033	13,775,050	63.14	2,021,849	13,389,726		63.12
		SRR17074806	2,078,919	13,767,678	63.23	2,010,658	13,315,616		63.17
SRR17074805	2,116,302	14,015,248	63.14	2,054,871	13,608,418	63.13	63.13		
LRS/MinION	SRR17083591	3,373,804	288,121	62.70			11,709.6 (mean), 21,146 (N50)		
Total		16,017,162	84,018,969		15,306,861	79,314,985			
Mean				63.04				63.17	
<i>P. nicotinovorans</i> nic- (SAMN23721560)	SRS/Illumina	SRR17187325	2,083,609	13,798,734	63.36	2,020,264	13,379,230	150 (read length)	63.33
		SRR17187324	2,128,302	14,094,714	63.35	2,056,827	13,621,370		63.34
		SRR17187323	2,087,609	13,825,226	63.33	2,017,195	13,358,906		63.29
	LRS/MinION	SRR17187322	524,233	58,746	62.83			8,923.7 (mean), 16,496 (N50)	
Total		6,823,753	41,777,420		6,618,519	40,418,252			
Mean				63.22				63.20	

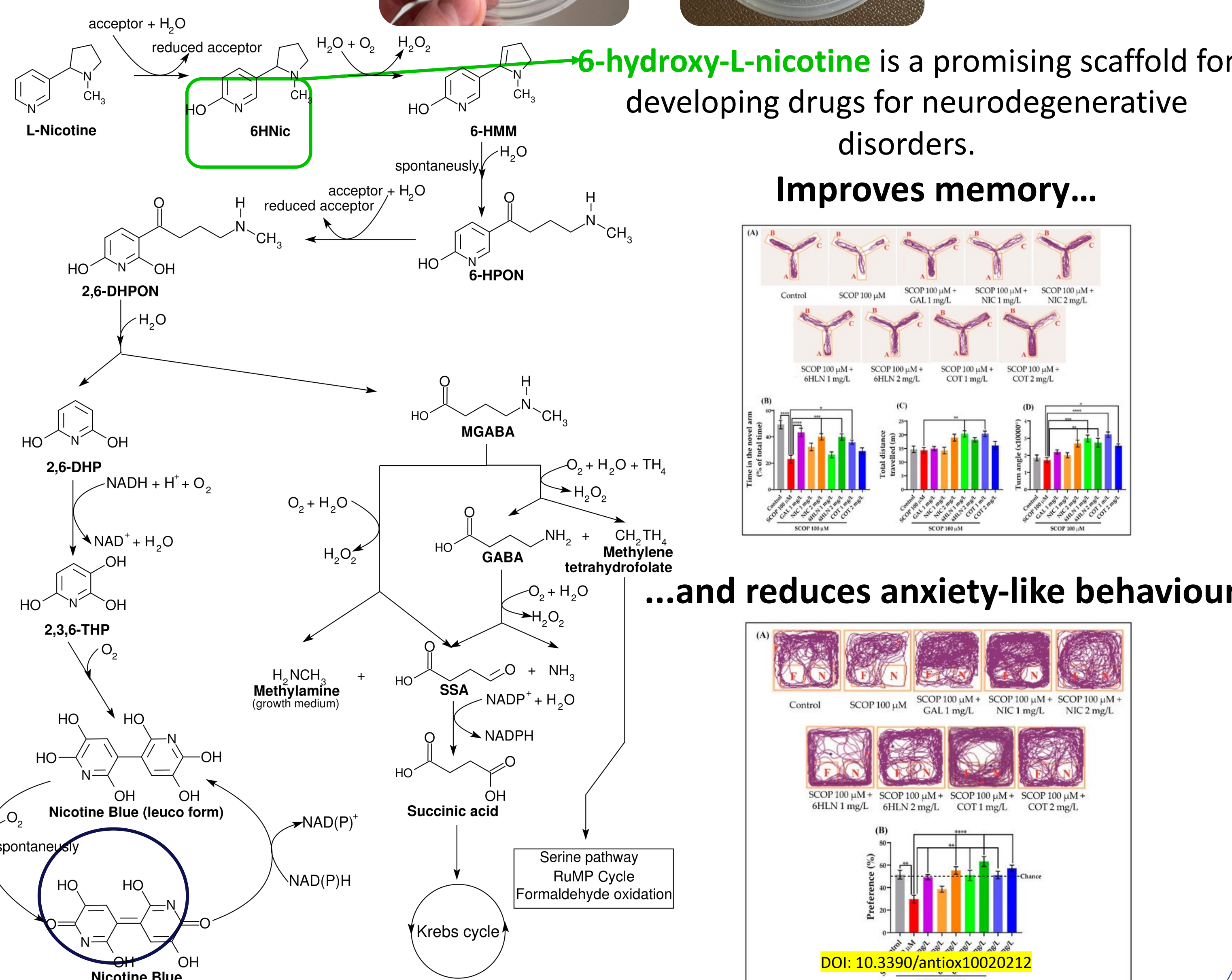
*SRS, short-read sequencing; LRS, long-read sequencing. In the case of SRS, multiple sequencing runs were performed for each sample using the same sequencing library. Each sequencing run was deposited as an SRA entry.

2. Nicotine degradation in *Paenarthrobacter nicotinovorans*

Nicotine-Blue (NB) metabolite accumulating in the growth medium of *P. nicotinovorans* strain ATCC 49919



P. nicotinovorans nic- cured derivative lacking the pAO1 megaplasmid



6-hydroxy-L-nicotine is a promising scaffold for developing drugs for neurodegenerative disorders.

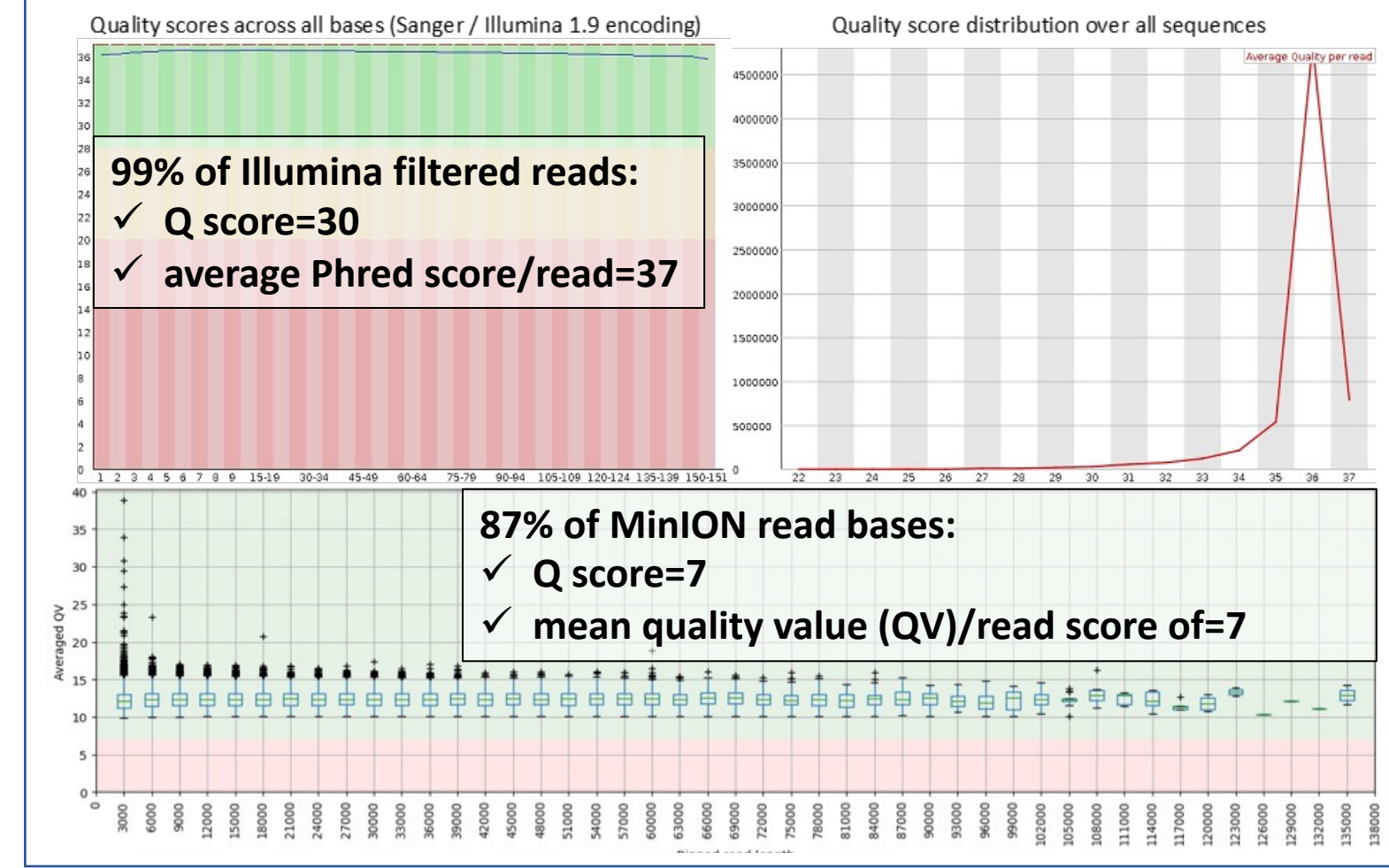
Improves memory...

...and reduces anxiety-like behaviour

A complete genome is not available

4. Technical Validation

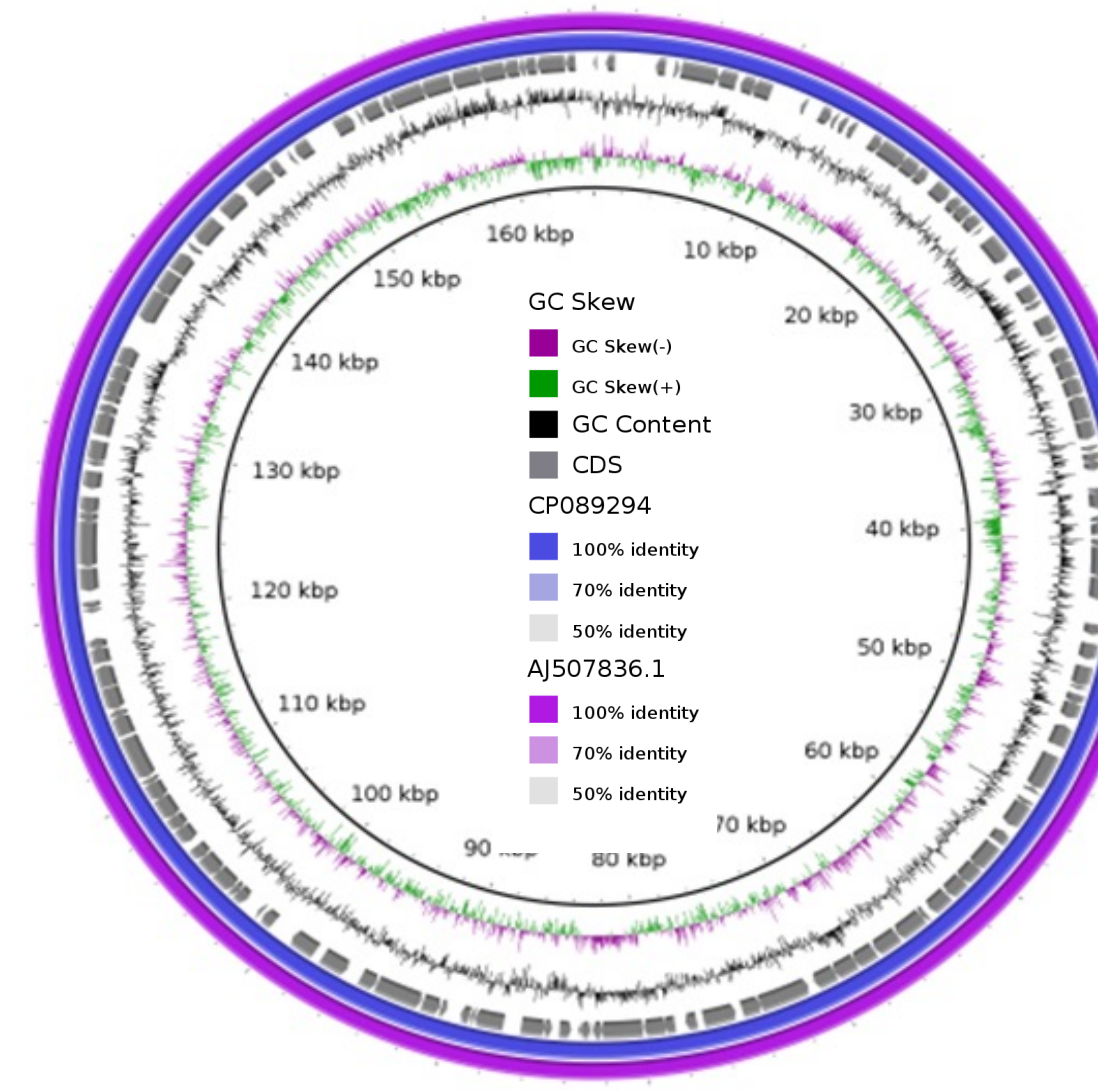
A. Data quality check



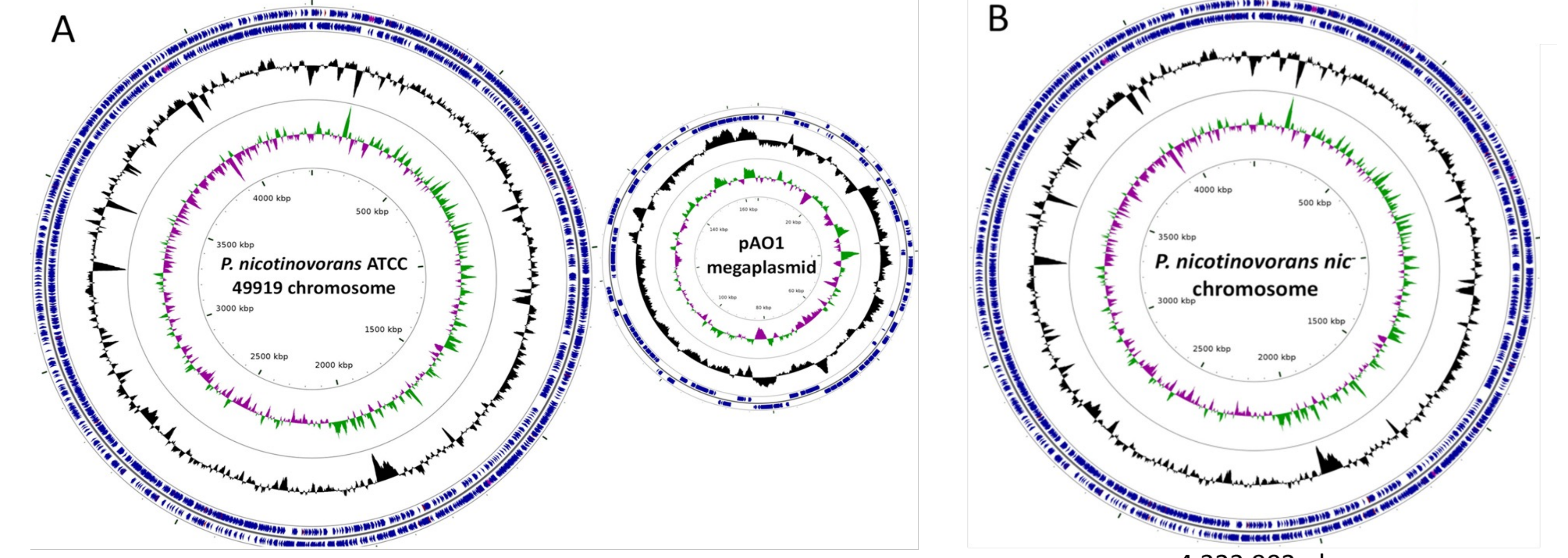
B. Positive controls

1st: the previously described sequence of the pAO1 megaplasmid (GenBank AJ507836.1)

2nd: the sequence of *P. nicotinovorans* nic- (genome should contain only one replisome corresponding to the bacterial chromosome)



The assembled pAO1 megaplasmid has 99.99% identity with the previously described sequence, which is 4 bp shorter. This is in good agreement with the observation from the original submitters of the pAO1 sequence that an irresolvable compression may increase the reported size of the plasmid by up to 5 bp.



4,316,184 pb GenBank: CP089293.1
4,323,902 pb GenBank: CP089515.1
Circular maps of the genomes sequenced: (A) The *P. nicotinovorans* ATCC 49919 chromosome (left) and its megaplasmid, pAO1 (right); (B) the *P. nicotinovorans* nic- chromosome. The maps were generated using Circular Genome Viewer (CGView) v.1.14 DOI:10.1093/bioinformatics/bti054.

	<i>Paenarthrobacter nicotinovorans</i> ATCC 49919	<i>Paenarthrobacter nicotinovorans</i> nic-
# contigs	2	1
Size	Chromosome: 4,316,184 bp pAO1 megaplasmid: 165,141 bp	4,323,902 bp
GC %	Chromosome: 63.2 pAO1 megaplasmid: 59.7	63.2
# CDS	Chromosome: 3953 pAO1 megaplasmid: 145	3957
# rRNA	6	6
# tRNA	54	52