

## Supplemental Material for

### Quantification of *Azospirillum brasilense* FP2 in wheat roots by strain-specific real-time qPCR

Maria Isabel Stets<sup>a,b</sup>, Sylvia Alqueres<sup>b</sup>, Emanuel Maltempi de Souza<sup>a</sup>, Fábio de Oliveira Pedrosa<sup>a</sup>, Michael Schmid<sup>b</sup>, Anton Hartmann<sup>b</sup>, and Leonardo Magalhães Cruz<sup>a#</sup>

Department of Biochemistry and Molecular Biology, Federal University of Parana (UFPR), Curitiba, PR, Brazil<sup>a</sup>; Helmholtz Zentrum, German Research Center for Environmental Health (GmbH), Department for Environmental Sciences, Research Unit Microbe-Plant Interactions, Munich, Germany<sup>b</sup>

Running title: qPCR *Azospirillum brasilense* quantification in roots

#Adress correspondence to Leonardo M. Cruz, Department of Biochemistry and Molecular Biology, Federal University of Paraná (UFPR), P. O. BOX 19.046, 81.531-990, Curitiba, PR, Brazil; phone +55 041 3361 1657; e-mail leonardo@ufpr.br

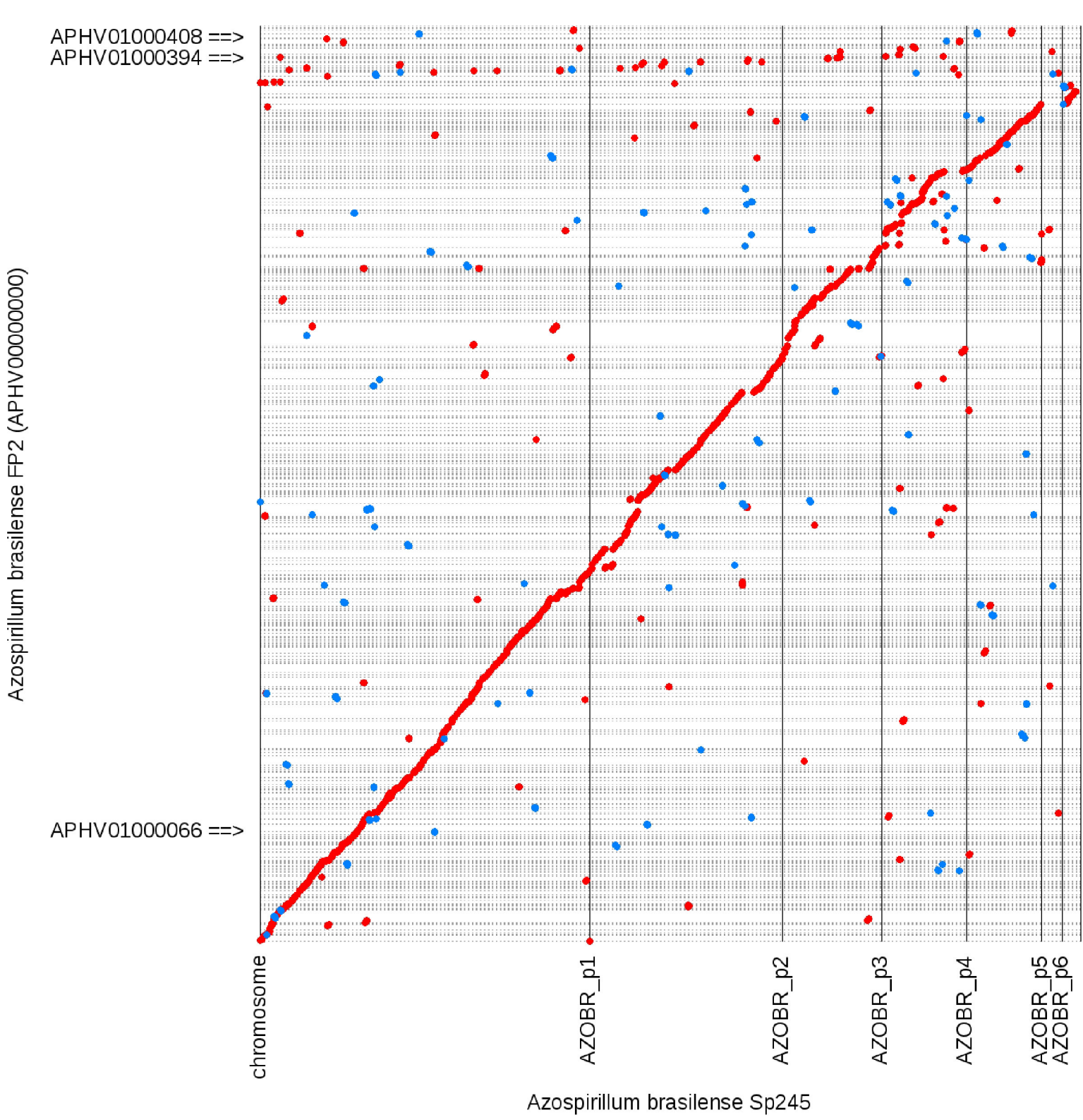
**Figure S1.** Synteny plot between *Azospirillum brasilense*, strains FP2 and Sp245. All 413 contigs from FP2 were aligned against the chromosome and six plasmids from Sp245 using NUCmer and alignment was plotted using mummerplot, both included in MUMmer 3 software package (Kurtz et al., 2004); **(A)** synteny plot shows the comparison of concatenated sequences (FP2 contigs were ordered to optimize the synteny); FP2 contigs, from which primers were designed, are indicated in the "Y" axis; Sp245 sequences are shown in the "X" axis as follows: chromosome (HE577327), AZOBR\_p1 (HE577328), AZOBR\_p2 (HE577329), AZOBR\_p3 (HE577330), AZOBR\_p4 (HE577331), AZOBR\_p5 (HE577332), AZOBR\_p6 (HE477333); **(B)** synteny between contig APHV01000066 of FP2 against Sp245 chromosome region from 880,000 to 940,000 bp.

**Figure S2.** *Azospirillum brasilense* FP2 annotated genomic regions used for strain-specific primer pairs design. Contigs are identified by NCBI accession numbers; the gray box in upper ruler shows the contig region spanning the CDSs in the figure (shown in light blue and numbered sequentially); primer binding positions are shown in green as follows: Azo-2 in contig APHV01000066, AzoR5.x in contig APHV01000394, and AzoR2.x in contig APHV01000408 (with "x" meaning the primer version).

**Figure S3.** Quantitative real-time PCR (qPCR) standard curve for *Azospirillum brasilense* FP2. The standard curve was generated by plotting Ct values against log CFU equivalent/ g of wheat root; DNA was extracted from 100  $\mu$ L of each serial dilution of *A. brasilense* FP2 culture mixed with 100 mg of crushed plant-root tissues.

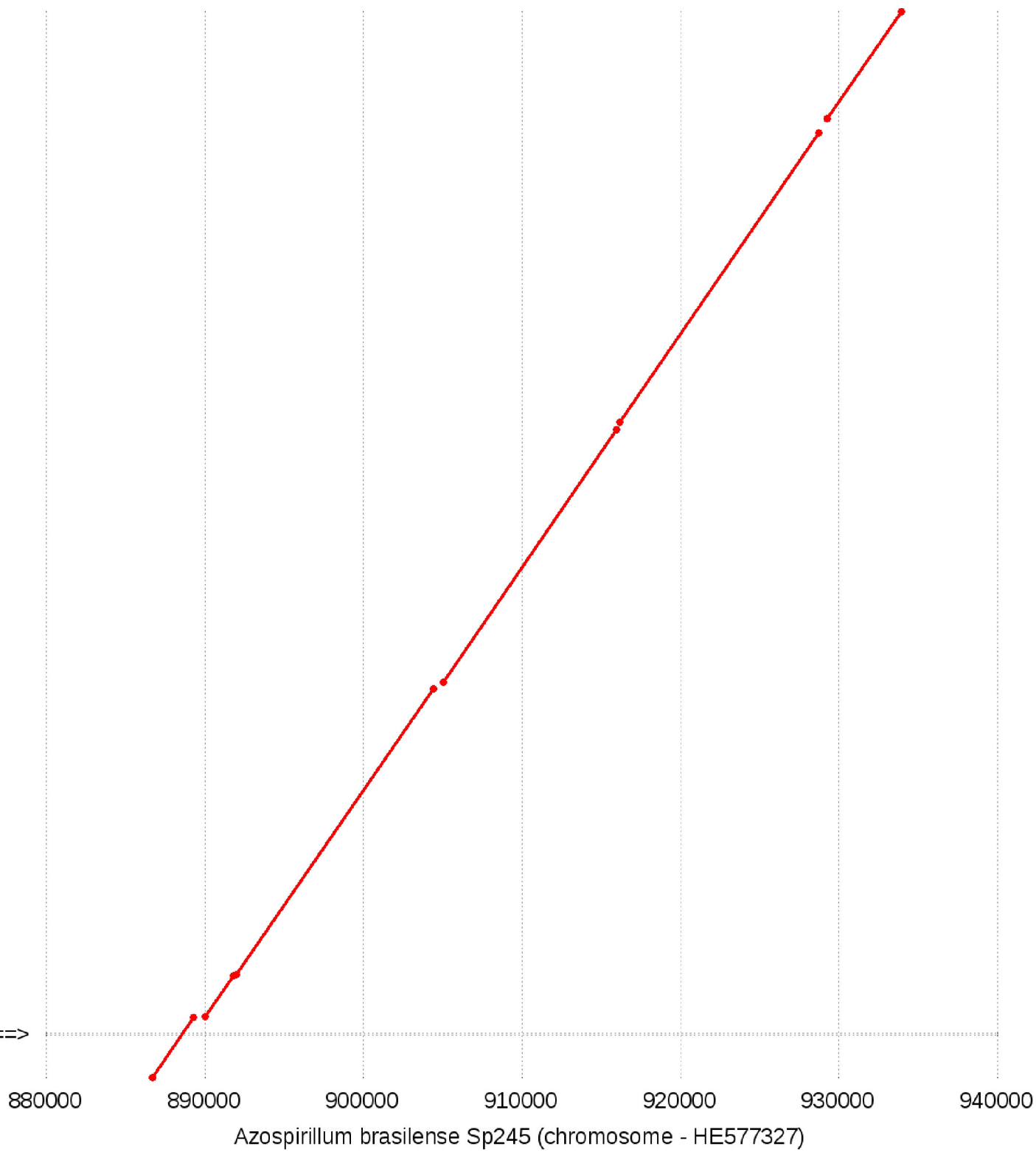
**Figure S4.** Comparison for the quantification of *Azospirillum brasilense* FP2 associated with wheat roots in all three inoculation experiments performed under sterile and non-sterile conditions and inoculated with *Azospirillum brasilense* FP2

alone or co-inoculated with *Azospirillum brasilense* NH, *Herbaspirillum seropedicae* Z67, *Gluconacetobacter diazotrophicus* DSM 5601, and *Azospirillum lipoferum* DSM 1691 by qPCR method. Values for qPCR are mean of three strain-specific primer pairs. For each day, different letters indicate statistical difference at  $p < 0.01$ .

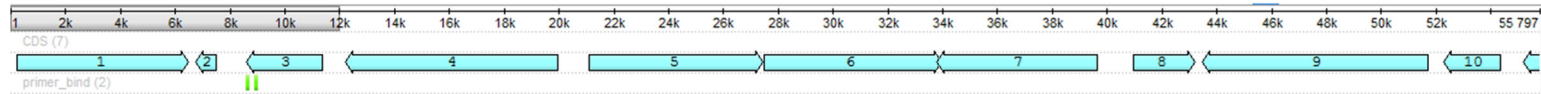


Azospirillum brasilense FP2 (contig APHV01000066)

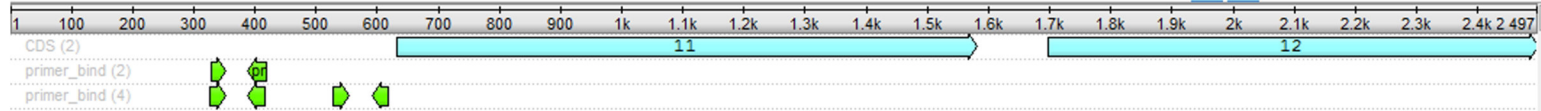
Azo-2 ==>



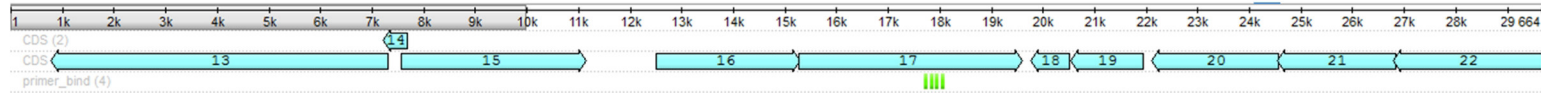
## Contig APHV01000066



## Contig APHV01000394

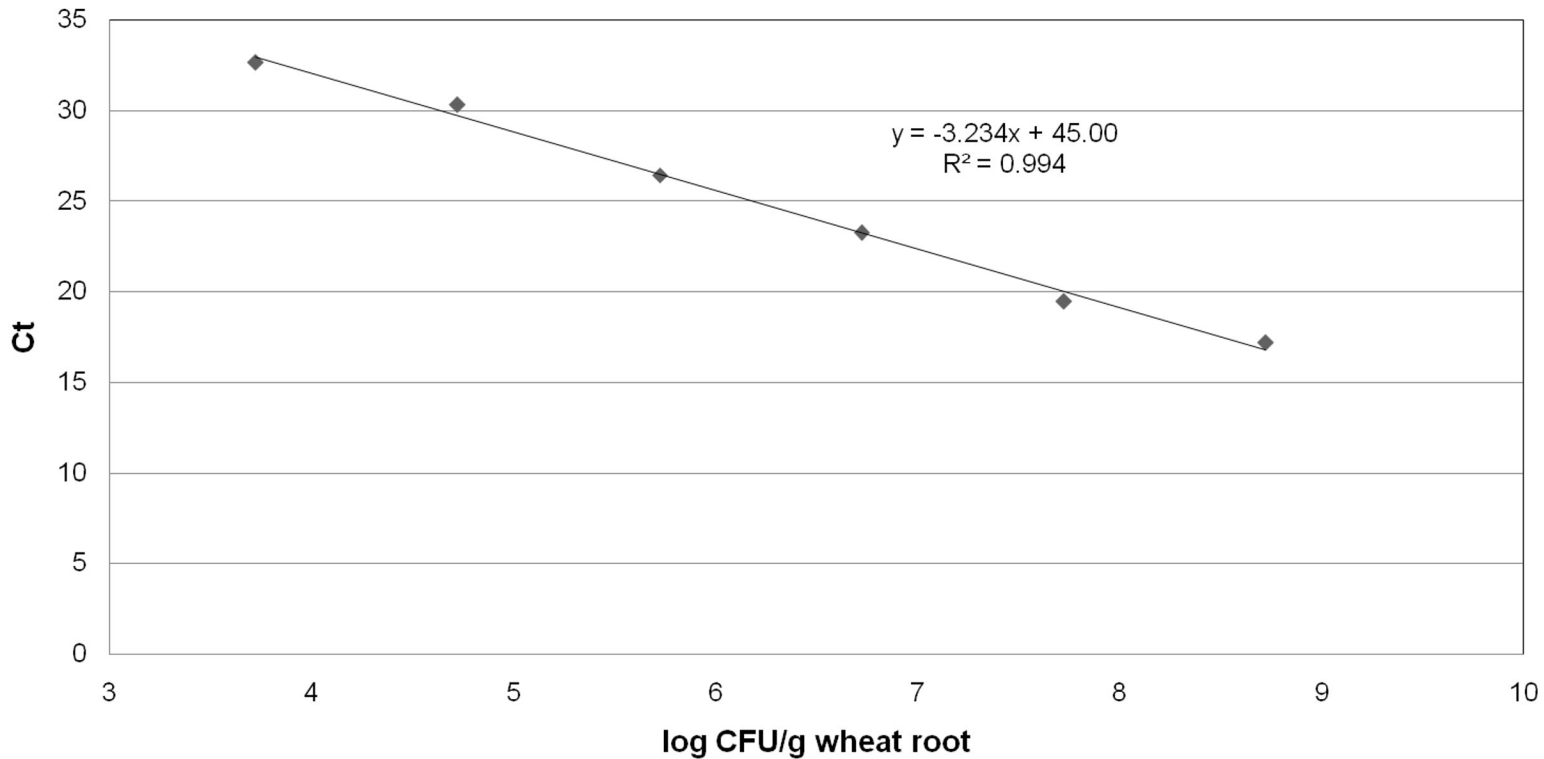


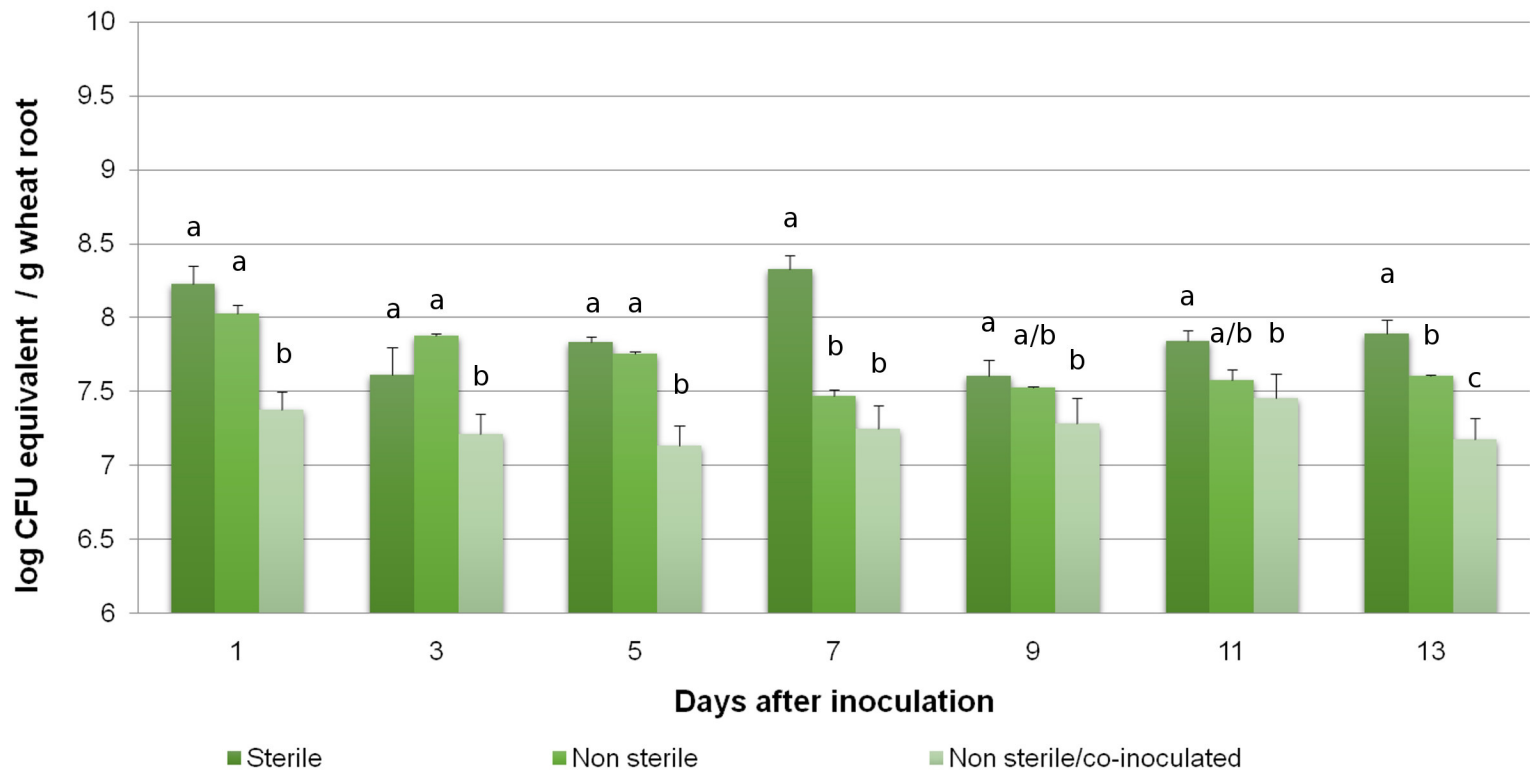
## Contig APHV01000408



ID <sup>1</sup>	Start	End	Length	Strand <sup>2</sup>	Annotation
<b>Contig APHV01000066 (55,797bp; GC 68.1%; 56 ORF)</b>					
1	44	1366	1323		NAD synthetase (EC 6.3.1.5)/Glutamine amidotransferase chain of NAD synthetase
2	1477	1608	132	c	Hypothetical protein
3	1874	2443	570	c	Hypothetical protein
4	2649	4286	1638	c	Indole-3-pyruvate decarboxylase (EC 4.1.1.74)
5	4537	5880	1344		Glutamyl-tRNA(Gln) synthetase (EC 6.1.1.24)
6	5915	7288	1374		Cysteinyl-tRNA synthetase (EC 6.1.1.16)
7	7293	8525	1233	c	Glucans biosynthesis protein C (EC 2.1.-.-)
8	8805	9269	465		Putative preQ0 transporter
9	9382	11121	1740	c	Diguanylate cyclase/phosphodiesterase (GGDEF & EAL domains) with PAS/PAC sensor(s)
10	11268	11681	414	c	Diguanylate cyclase/phosphodiesterase (GGDEF & EAL domains) with PAS/PAC sensor(s)
<b>Contig APHV01000394 (2,497bp; GC 67.5%; 2 ORF)</b>					
11	633	1577	945		Site-specific recombinases, DNA invertase Pin homologs
12	1699	2496	798		Hypothetical protein; Pfam CATH domain related to peptidases in peptidase clan CD that includes the caspases
<b>Contig APHV01000408 (29,664bp; GC 66.1%; 31 ORF)</b>					
13	282	2456	2175	c	Phage T7 exclusion protein
14	2453	2584	132	c	Hypothetical protein
15	2552	3745	1194		TniA putative transposase
16	4218	5144	927		TniB NTP-binding protein
17	5148	6593	1446		TniQ domain containing protein
18	6689	6919	231	c	Hypothetical protein
19	6945	7391	447	c	Hypothetical protein
20	7479	8282	804	c	Enoyl-CoA hydratase (EC 4.2.1.17)
21	8297	9049	753	c	3-oxoacyl-[acyl-carrier protein] reductase (EC 1.1.1.100)
22	9053	10204	1152	c	3-ketoacyl-CoA thiolase (EC 2.3.1.16)

<sup>1</sup>ORFs are numbered according to figure; <sup>2</sup>c, complementary strand;







## References

**Kurtz S, Phillippy A, Delcher AL, Smoot M, Shumway M, Antonescu C, Salzberg SL.** 2004. Versatile and open software for comparing large genomes. *Genome Biol* **5**:R12.