

## SUPPLEMENTARY INFORMATION

*Enrichment Free qPCR for Rapid Identification and Quantification of *Campylobacter jejuni*, *C. coli*, *C. lari* and *C. upsaliensis* in Chicken Meat Samples by a New Couple of Primers*

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### Summary

1. Table S-1 reports the microorganisms used in the work.
2. Table S-2. reports the accession numbers of sequences used in the work.
3. Table S-3. rapports data obtained with chicken meat samples artificially spiked with *Campylobacter jejuni*

**Table S-1.** List of the microorganisms and DNAs used in the work.

	Microorganisms	Collection code
<b>Positive controls</b>		
	<i>Campylobacter jejuni</i> subsp. <i>jejuni</i>	DSM 4688 <sup>A</sup>
	<i>C. coli</i>	DSM 24155 <sup>A</sup>
	<i>C. lari</i> subsp. <i>lari</i>	DSM 11375 <sup>A</sup>
	<i>C. upsaliensis</i>	DSM 5365 <sup>A</sup>
<b>Negative controls</b>		
	<i>C. fetus</i>	DSM 5361 <sup>A</sup>
	<i>C. cryaerophila</i>	DSM 7289 <sup>A</sup>
	<i>Helicobacter pylori</i> DNA	DSM 7492 <sup>B</sup>
	<i>H. pylori</i>	ICS <sup>C</sup>
	<i>H. suis</i>	DSM 19735 <sup>A</sup>
	<i>Arcobacter butzleri</i> DNA	DSM 8739 <sup>B</sup>
	<i>Listeria monocytogenes</i>	ATCC 7644 <sup>D</sup>
	<i>L. innocua</i>	DSM 20649 <sup>A</sup>

<i>L. seeligeri</i>	DSM 20751 <sup>A</sup>
<i>L. marthii</i>	DSM 23813 <sup>A</sup>
<i>L. welshimeri</i>	DSM 15452 <sup>A</sup>
<i>L. ivanovii</i>	DSM 52491 <sup>A</sup>
<i>Staphylococcus aureus</i>	DI4A <sup>E</sup>
<i>Bacillus cereus</i>	DSM 4282 <sup>A</sup>
<i>B. cereus</i>	DI4A RC3 <sup>E</sup>
<i>B. subtilis</i>	DSM 4181 <sup>A</sup>
<i>Salmonella enterica</i>	DSM 9378 <sup>A</sup>
<i>Escherichia coli</i>	DISTAM <sup>F</sup>
<i>Lactobacillus plantarum</i>	ATCC RAA 793 <sup>D</sup>
<i>Saccharomyces cerevisiae</i>	ATCC 36024 <sup>D</sup>

<sup>A</sup>DSM: Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany); <sup>B</sup>DNAs from DSM collection; <sup>C</sup>ICS: Isolated from Clinical samples (Hospital of Udine, Italy); <sup>D</sup>ATCC: American Type Culture Collection (Manassas, VA, USA); <sup>E</sup>DI4A: Department of Agricultural, Food, Environmental and Animal Sciences (Udine, Italy); <sup>F</sup>DISTAM: Department of Food technologies and Microbiology Science (Milan, Italy).

**Table S-2.** Accession number of the sequences analyzed in silico to design CampyPFW and CampyPRV primers.

BACTERIA SEQUENCES (16S and 16S-23S ribosomal RNA gene)					
N	Accession number	Genus, species	N.	Accession number	Genus, species
1	EF373994.1	<i>Aeromonas sobria</i>	15	AB089244.1	<i>Morganella morganii</i>
2	AM062666.1	<i>Bacillus cereus</i>	16	AF405374.1	<i>Pediococcus pentosaceus</i>
3	EF205020.1	<i>B. subtilis</i>	17	FJ518598.1	<i>Proteus vulgaris</i>
4	AF047423.1	<i>Citrobacter freundii</i>	18	JN418884.1	<i>Pseudomonas aeruginosa</i>
5	EF527445.1	<i>Escherichia coli</i>	19	AF268968.1	<i>Ps. brennerii</i>
6	FJ410387.1	<i>Enterobacter aerogenes</i>	20	AM086254.1	<i>Ps. brennerii</i>
7	AF047426.1	<i>E. aerogenes region</i>	21	EF198908.1	<i>Ps. fluorescens</i>
8	EU078570.1	<i>E.cloacae</i>	22	KF857261.1	<i>P. migulae</i>
9	AY277975.1	<i>Helicobacter ganmani</i>	23	AF046822.1	<i>Salmonella enterica</i>
10	DQ399570.1	<i>Klebsiella pneumoniae</i>	24	KX696458.1	<i>Shigella sonnei</i>
11	AB092638.1	<i>Lactobacillus plantarum</i>	25	U11784.1	<i>Staphylococcus aureus</i>
12	AF000655.1	<i>Legionella pneumophila</i>	26	AY531067.1	<i>Vibrio parahaemolyticus</i>
13	AB295116.1	<i>Leuconostoc lactis</i>	27	FJ429988.1	<i>Weissella cibaria</i>
14	AY684791.1	<i>Listeria monocytogenes</i>	28	AF293850.1	<i>Yersinia enterocolitica</i>

BACTERIAL WHOLE DNA					
N.	Accession number	Genus, species	N.	Accession number	Genus, species
1	kv861263.1	<i>Aeromonas sobria</i>	16	Ivwz01000001.1	<i>Pseudomonas brenneri</i>

2	ap007209.1	<i>Bacillus cereus</i>	17	NZ_LDET01000015.1	<i>Ps. fluorescens</i>
3	nz_cp011534.1	<i>Bacillus subtilis</i>	18	fnty01000001.1	<i>Ps. migulae</i>
4	CP016762.1	<i>Citrobacter freundii</i>	19	acfI01000033.1	<i>Saccharomyces cerevisiae</i>
5	fm991728.1	<i>Helicobacter pylori b38</i>	20	NC_003197.2	<i>Salmonella enterica</i> <i>subsp. Enterica</i>
6	ba000007.2	<i>Escherichia coli O157:H7</i>	21	nz_lfwb01000312.1	<i>Morganella morganii</i>
7	NZ_LYDO01000004.1	<i>Enterobacter aerogenes</i>	22	nz_lyem01000481.1	<i>Shigella sonnei</i>
8	nz_cp011798.1	<i>E. cloacae</i>	23	ap008934.1	<i>Staphylococcus saprophyticus</i> <i>subsp. saprophyticus</i>
9	nz_cp010435.1	<i>Helicobacter pylori</i>	24	kn150745.1	<i>Proteus vulgaris</i> strain
10	fo203501.1	<i>Klebsiella pneumoniae</i>	25	nz_lirr01000034.1	<i>Vibrio parahaemolyticus</i>
11	nz_cp012650.1	<i>Lactobacillus plantarum</i>	26	nz_cp012873.1	<i>Weissella cibaria</i>
12	nz_cp011105.1	<i>Legionella pneumophila</i>	27	am286415.1	<i>Yersinia enterocolitica</i> <i>subsp. enterocolitica</i>
13	nz_cp011105.1	<i>Legionella pneumophila</i>	28	cp007224.1	<i>Pseudomonas aeruginosa</i>
14	ae005176.1	<i>Lactococcus lactis</i> subsp. <i>lactis</i>	29	cp015918.1	<i>Pediococcus pentosaceus</i>
15	nz_bbrp01000009.1	<i>Listeria monocytogenes</i>			

**CAMPYLOBACTER spp. (16S and 16S-23S gene)****ANIMALS (16S gene)**

N.	Accession number	Genus, species	N.	Accession number	Genus, species
1	KP064555.1	<i>Campylobacter fetus</i>	1	KP721213.1	<i>Sus scrofa domesticus</i>
2	GQ167709.1	<i>C. concisus</i>	2	DQ334849.1	<i>Meleagris gallopavo</i>
3	JX912515.1	<i>C. gracilis</i>	3	AB489247.1	<i>Gallus gallus</i>
4	L04322.1	<i>C. concisus,</i>			
5	AB301966.1	<i>C. fetus</i> subsp. <i>fetus</i>			
6	M65011.1	<i>C. fetus</i> subsp. <i>venerealis</i>			
7	NZ_FPBB01000005.1	<i>C. hyoilectinalis</i>			
8	nz_jhqq01000009.1	<i>C. mucosalis</i>			
9	nz_jmti01000045.1	<i>C. sputorum</i> biovar <i>sputorum</i>			

**4. Section SI3 reports an additional test acrried out on four chicekn meat samples.****Anlysis of hicken meat samples artificially spiked with *Campylobacter jejuni***

Serial decimal dilutions of *C. jejuni* overnight culture (BHI, microaerophilic conditions) containing approximately  $10^8$  cells/mL, were inoculated into meat samples (named SC) to reach final concentrations of  $10^7$ ,  $10^5$ ,  $10^3$ , and 0 cell/g of meat. A plate count was performed on the spiked samples to obtain the correspondent CFU/g value. DNA of SCs was extracted at  $t_0$  from the homogenization Stomacher bags as reported in Section 2.3 of Materials a Methods and used for qPCR. Results of the analyses are reported in the Table S-3 below.

**Table S-3. Mean Ct values with standard deviation (SD), cell quantification expressed in cell/mL and by plate count method evaluation (CFU/g).**

SC Samples	<b>mean Ct ± SD</b>	qPCR	Plate count method (mCCDA)
		<b>cells/ mL*</b>	<b>CFU/g</b>
1 SC	21.37 ± 0.47	5.10E+04	1.16 × 10 <sup>7</sup>
2 SC	26.50 ± 0.19	1.05E+03	1.07 × 10 <sup>5</sup>
3 SC	32.16± 1.16	1.43E+01	1.22 × 10 <sup>3</sup>
4 SC	32.40 ± 0.29	1.20E+01	< 20

\* DNA used as a template in the test was diluted 1:1000