

Service contract for the provision of EU networking and support for public health reference laboratory functions for antimicrobial resistance in *Salmonella* species and *Campylobacter* species in human samples

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## Multiplex PCR for differentiation of *C. coli* and *C. jejuni*.

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## 1. BACKGROUND

Speciation of *Campylobacter* strains is important for the strain characterization which also allows for selecting the right interpretative criteria for the correct categorization of the antimicrobial susceptibility profile. The primer sets in this multiplex PCR protocol target the identification of *Campylobacter jejuni* and *Campylobacter coli* based on the amplification of the two genes, *mapA* *C. jejuni* and *ceuE* *C. coli*. In addition, a 16S primer set has been included as quality assurance of the DNA-preparation and analysis (internal control).

## 2. PROTOCOL

### 2.1. DNA extraction (boiling lysates):

- In an eppendorf tube, dissolve a single colony into 100µl TE/MQ 10:1
- Denature the DNA by boiling the tube for 10 minutes
- Centrifuge the tube at 20.000 g (4°C) for 5 minutes
- Dilute the sample 1:10 in TE/MQ 10:1
- Store DNA samples at -20°C

### 2.2. PCR Controls:

*Campylobacter jejuni* ATCC 33560 (CCUG 11284)

*Campylobacter coli* ATCC 33559 (CCUG 11283)

### Primers used in this PCR:

Target gene	Primer name (internal EURL no.)	Sequence	Expected amplicon size
<i>mapA</i> <i>C.jejuni</i>	MDmapA1 (3034)	5'-CTA TTT TAT TTT TGA GTG CTT GTG-3'	589 bp
<i>mapA</i> <i>C.jejuni</i>	MDmapA2 (3035)	5'-GCT TTA TTT GCC ATT TGT TTT ATT A-3'	
<i>ceuE</i> <i>C.coli</i>	COL3 (3036)	5'-AAT TGA AAA TTG CTC CAA CTA TG -3'	462 bp
<i>ceuE</i> <i>C.coli</i>	MDCOL2 (3037)	5'-TGA TTT TAT TAT TTG TAG CAG CG-3'	
16S	16S primer 804 RX (442)	5'-GAC TAC CNG GGT ATC TAA TCC-3'	800 bp
16S	16S primer 10FX (444)	5'-AGA GTT TGA TCC TGG CTN AG-3'	

### Preparation of primers:

Primers are diluted to a concentration of 130 mg/mL

### Reaction mix:

Prepare the following mixture in a microcentrifuge tube (for a 25µl reaction). Prepare additionally one blank reaction without template DNA as negative control.

This PCR protocol is optimized for the Dream Taq Green PCR master mix, which can be replaced by another polymerase, although the protocol might need some optimization to adjust for the particular conditions at your laboratories.

□ DreamTaq Green PCR Master Mix (2x)

- Primer, MDmapA1
- Primer, MDmapA2
- Primer, COL3
- Primer, MDCOL2
- Primer, 16S primer 804 RX
- Primer, 16S primer 10FX
- Distilled milli-Q water

**Template:**

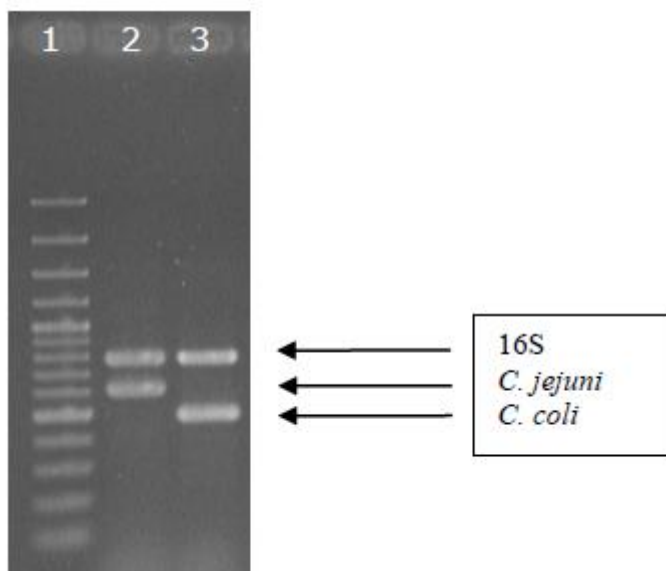
As template for the PCR we recommend to use 0.5µl of the above prepared DNA in a 25µl PCR reaction.

**2.3. Electrophoresis:**

Run 5-8µl of the PCR-mixture in parallel with a 100bp ladder molecular weight marker on a 1.5% agarose gel in TBE 1X. Run the gel for 45 min at about 100V. Stain the gel in ethidium bromide circa 20-30 minutes.

De-stain briefly in milli-Q water.

Take a picture in the transilluminator under UV light. Observe the bands and interpret the results according to the description below and Figure 1:



**Figure 1.** Multiplex PCR for detection of *C. jejuni* and *C. coli*

Lane 1: 100 bp ladder

Lane 2: *Campylobacter jejuni* ATCC 33560 (CCUG 11284)

Lane 3: *Campylobacter coli* ATCC 33559 (CCUG 11283)

**Reference:**

**M. Denis, C. Soumet, K. Rivoal, G. Ermel, D. Blivet, G. Salvat and P. Colin.** Development of a m-PCR assay for simultaneous identification of *Campylobacter jejuni* and *C. coli*. Letters in Applied Microbiology 1999, 29, 406–410

## 2.4. Example for set-up

### PCR SAMPLE SHEET (Example for set-up)

Primer F (forward): 442+3034+3036
Primer R (reverse): 444+3035+3037
DNA polymerase: Dream Taq Green PCR master mix
PCR products: 16S – 800 bp; mapA – 589 bp; ceuE – 462 bp
Remarks: 0.5µl of the DNA template. Run: 1.5% agarose gel run at 100V for 45 minutes
<b>Reference: M. Denis, C. Soumet, K. Rivoal, G. Ermel, D. Blivet, G. Salvat and P. Colin.</b> Development of a m-PCR assay for simultaneous identification of <i>Campylobacter jejuni</i> and <i>C. coli</i> . Letters in Applied Microbiology 1999, 29, 406–410

No. of reactions	1	10		
PCR H <sub>2</sub> O	9,5	95		
2xGreen PCR Master Mix	12,5	125		
dNTP	0	0		
25 mM MgCl <sub>2</sub>	0	0		
Primer F (0,50 µl of each)x3	1,5	15		
Primer R (0,50 µl of each)x3	1,5	15		
Taq polymerase	0	0		
Total volume	25	250		
1.	10	min at	95	°C
2.	30	Cycles		
	30	sec at	94	°C
	90	sec at	59	°C
	60	sec at	72	°C
3.	10	min at	72	°C

M:		M:		M:	
1		1		1	
2		2		2	
3		3		3	
4		4		4	
5		5		5	
6		6		6	
7		7		7	
8		8		8	
9		9		9	
10		10		10	
11		11		11	
12		12		12	