DTU Food
National Food Institute

PROTOCOL FOR PCR AMPLIFICATION OF *CAMPYLOBACTER JEJUNI AND C. COLI* RECOMMENDED BY THE EURL-AR

2ND VERSION - NOVEMBER 2013

Changes from previous version:

In sample sheet: Primer mix collected in forward and reverse primers, respectively.

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).

This protocol can be used for the EURL-AR EQAS and for monitoring programs.

Protocol

DNA extraction (boiling lysates):

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

PCR Controls:

Campylobacter jejuni ATCC 33560 (CCUG 11284)

Campylobacter coli ATCC 33559 (CCUG 11283)

DTU Food
National Food Institute

Primers used in this PCR:

Target gene	Primer name (internal EURL no.)	Sequence	Expected amplicon size
mapA$_{C.jejuni}$	MDmapA1 (3034)	5'-CTA TTT TAT TTT TGA GTG CTT GTG-3	589 bp
mapA$_{C.jejuni}$	MDmapA2 (3035)	5'-GCT TTA TTT GCC ATT TGT TTT ATT A-3'	
ceuE$_{C.coli}$	COL3 (3036)	5'-AAT TGA AAA TTG CTC CAA CTA TG -3'	462 bp
ceuE$_{C.coli}$	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. At the EURL-AR, this PCR 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. If you need assistance with the optimization, you are welcome to contact the EURL-AR.

- DreamTaq Green PCR Master Mix (2x)
- Primer, MDmapA1
- Primer, MDmapA2
- Primer, COL3
- Primer, MDCOL2
- Primer, 16S primer 804 RX
- Primer, 16S primer 10FX
- Destillled mili-Q water

Template:

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

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:

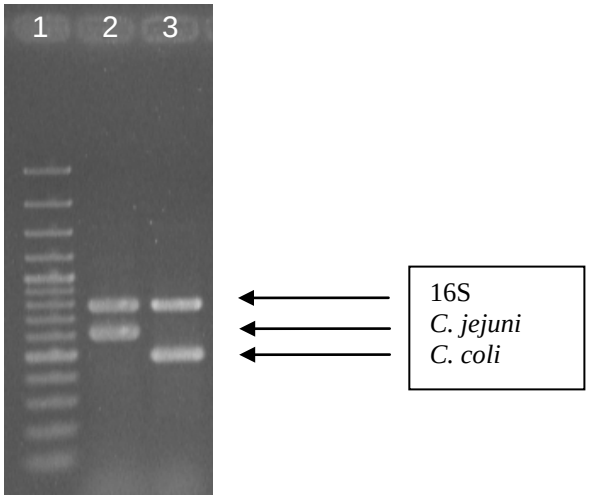

Figure 1. Multiplex PCR for detection of *C. jejuni and C. coli*

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

DTU Food
National Food Institute

European Union Reference Laboratory Antimicrobial Resistance

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 **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

No. of reactions	1	**10**
PCR H_2O	9,5	**95**
2xGreen PCR Master Mix	12,5	**125**
dNTP	0	**0**
25 mM $MgCl_2$	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:	
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	

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

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