MLST sequencing of *C. trachomatis* Uppsala scheme

PCR amplification

PCR amplification of the five MLST regions is performed using the HotStarTaq DNA Polymerase (Qiagen, Hilden, Germany) with the primer pairs shown in table 1 and the reaction components concentrations as seen in table 2. The cycling program is shown in table 3.
5 µl of extracted DNA samples are added to each PCR reaction with a total volume of 25 µl. To visualize the PCR products, agarose gel electrophoresis (1%, 5 Vcm^{-1} for 45 min) is performed on 5 µl of each sample.
If necessary, a second step of the PCR is performed with an inner primer pair.
The *ompA* gene is amplified under the same conditions as the MLST regions.

Table 1 Primer pairs used for PCR amplification and sequencing of the five MLST regions and *ompA*.

Region	Name	Function	Sequence
***hctB* (CT046)**	hctB39F	Outer primer	**5´-CTCGAAGACAATCCAGTAGCAT-3´**
	hctB794R	Outer primer	**5´-CACCAGAAGCAGCTACACGT-3´**
	CT046 NF	Inner primer	**5´-AACTCCAGCTTTTACTGCTA -3´**
	CT046 NR3	Inner primer	**5´-CCCCAAATATGCAACAGGAT-3´**
CT058	CT222F	Outer primer	**5´-CTTTTCTGAGGCTGAGTATGATTT-3´**
	mod CT058IR	Outer primer	**5´-AATCYTCCTTRGCCTCTCTT-3´**
	CT058 NF	Inner primer	**5´-AGGTGGCTGCGTTAAGATAACT-3´**
	mod CT058 NR	Inner primer	**5´-AAATTVGCCTGAAGTAGAGACA-3´**
CT144	CT144:248F	Outer primer	**5´-ATGATTAACGTGATTTGGTTTCCTT -3´**
	CT144:1046R	Outer primer	**5´-GCGCACCAAAACATAGGTACT-3´**
	CT144 NF	Inner primer	**5´-CGAAATCGGATATCTCTTTT-3´**
	CT144 NR	Inner primer	**5´-CCTAAACATACGGCTATTCC-3´**
CT172	CT172 OR	Outer primer	**5´-GATCAAGCCATCTTAGACATGC-3´**
	Four610R	Outer primer	**5´-CGTCATTGCTTGCTCGGCTT-3´**
	CT172 NF	Inner primer	**5´-AGGTCGCCCAAATTCCATGT-3´**
	CT172 NR	Inner primer	**5´-GCTCCGGCTATTTTGTTTAGGA-3´**
***pbpB* (CT682)**	pbpB1F	Outer primer	**5´-TATATGAAAAGAAAACGACGCACC-3´**
	pbpB1OR	Outer primer	**5´- AAGAACCTTCCATCTCCTGAAT -3´**
	CT682 NF	Inner primer	**5´-TCATCACTTTGCGTATATGGCA-3´**
	mod CT682NR	Inner primer	**5´-AAAAGCTTRCKTACTTGATCGA-3´**
ompA	118F	Outer primer	**5´-ATTGCTACAGGACATCTTGTC-3´**
	1163R	Outer primer	**5´-CGGAATTGTGCATTTACGTGAG-3´**
	ctr200F	Sequencing	**5´-TTAGGIGCTTCTTTCCAATAYGCTCAATC-3´**
	ctr254R	Sequencing	**5´-GCCAYTCATGGTARTCAATAGAGGCATC-3´**
	MOMP87	Inner primer	**5´-TGAACCAAGCCTTATGATCGACGGA-3´**
	RVS1059	Inner primer	**5´-GCAATACCGCAAGATTTTCTAGATTTCATC-3´**

Table 2 Concentrations of the components in each PCR reaction using the HotStarTaq DNA Polymerase system for amplification of the five MLST target regions.

Reagent	Concentration
Forward primer	0.4 µM
Reverse primer	0.4 µM
dents	0.2 mm
$MgCl_2$	2 mm
DNA Polymerase (e.g. HotStarTaq polymerase or high fidelity polymerase)	0.5 U per reaction
Total volume	25 µl

Table 3 Cycling program used for PCR amplification of the MLST target regions.

Temperature	Time	Cycles	Purpose
95 °C	15 min	1x	Initial denaturation
94 °C	45 s	40x	Denaturation
60 °C	45 s		Annealing
72 °C	90 s		Elongation
72 °C	10 min	1x	Final elongation
4 °C	∞		Cooling

Sequencing

Prior to sequencing the amplification products are purified using the ExoSapIt kit (Amersham Bioscience) according to the manufacturer's instructions. Alternative purification systems can be used. Purified PCR products are sent to a commercial service for sequence determination. The amplification primers are also used as sequencing primers. Additional sequencing primers are needed to achieve the complete sequence of the longer *ompA* fragment. These are also listed in table 1.

Obtained sequences are aligned and the obtained consensus sequence of each region represents the genotype (allele type) for each sample.

New allele types are confirmed by repeated PCR amplification and sequence determination.

Information

The original MLST scheme is described in Klint M, Fuxelius HH, Goldkuhl RR, Skarin H, Rutemark C, Andersson SG, Persson K, Herrmann B. High-resolution genotyping of Chlamydia trachomatis strains by multilocus sequence analysis. J Clin Microbiol. 2007;45:1410-4.

The protocol above is a modified and currently used version described in Bom RJ, Christerson L, Schim van der Loeff MF, Coutinho RA, Herrmann B, Bruisten SM. Evaluation of high-resolution typing methods for Chlamydia trachomatis in samples from heterosexual couples. J Clin Microbiol. 2011;49:2844-53.

An overall, but not updated, view of results from this MLST-scheme is available in Herrmann B, Isaksson J, Ryberg M, Tångrot J, Saleh I, Versteeg B, Gravningen K, Bruisten S. Global Multilocus Sequence Type Analysis of Chlamydia trachomatis Strains from 16 Countries. J Clin Microbiol. 2015;53:2172-9.

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