DSLT protocol for Pseudomonas aeruginosa

A detailed protocol can be requested at contact@dlst.org.

The DLST is based on the analysis of 400bp and 350bp of the two *Pseudomonas aeruginosa* highly variable loci, *ms172* and *ms217*.

Chromosomal DNA extraction of *P. aeruginosa*

The DNA extraction protocol is based on a simple heating process in a 96-wells plate: bacterial isolates are incubated overnight at 37°C in 5ml of LB medium and crude DNA extracts are prepared by mixing 10µl of this broth with 90µl of TE and heating this solution at 95°C for 10 minutes. This reduces time and cost per DNA extraction. However, traditional DNA extraction methods for *P. aeruginosa* are also fully appropriate for DLST.

PCR amplification of the variable region of the *ms172* and *ms217* loci

This PCR protocol is used to amplify highly polymorphic regions of the *ms172* and *ms217* loci. The priming regions are localized on conserved regions outside of the polymorphic regions. The forward and reverse primers are:

ms172.forward (5'-3')	GGATTCTCTCGCACGAGGT
ms172.reverse (5'-3')	TACGTGACCTGACGTTGGTG
ms217.forward (5'-3')	TTCTGGCTGTCGCGACTGAT
ms217.reverse (5'-3')	GAACAGCGTCTTTTCCTCGC

PCR amplification of each loci (*ms172* and ms 217 separately) were carried out in 22µl reaction volumes containing 2µl of the DNA extract solution, 1U of *Taq* DNA polymerase (Invitrogen), 1X Taq Reaction Buffer, 1.5mM MgCl2, 0.3µM of each primer, and 0.2mM of each dNTP. Cycling conditions were 5 min of initial denaturation at 94°C; 35 cycles consisting of 30s at 94°C, 30s at 60°C (*ms172*) or 64°C (*ms217*), 45s at 72°C; and a final extension for 10min at 72°C. Some optimization may be required according to the material used.

Sequencing of the reverse fragment

DNA fragments of 400bp for *ms172* and 350bp for *ms217* have been selected for the *P. aeruginosa* DLST typing method and the protocol has been adapted for the 3' sequencing (i.e. using the reverse primer) of these fragments. Annealing temperature for the sequencing reaction is 60 °C for the *ms172* and 64°C for the *ms217*.

Trimming patterns

Both sequences must be trimmed after a start signature that can be recognized on the beginning of the sequence:

Locus	start signature	length
ms172	ACGATGCTGGADCCA	400bp
ms217	CAGCATGGCG	350bp

For shorter alleles (sequence repeatedly shorter than 400bp or 350bp), please contact the curator of the website.

Allele and DLST type assignment

Once you obtained a sequence for both *ms172* and *ms217*, it is possible to obtain the allelic number corresponding to your sequence using the following options:

Submission of trace files.

A trace file can be submitted for the *ms172* and *ms217* loci. For both loci, the software will look for start signatures and will trim the sequences to their expected length (400 and 350bp). The sequences are then compared to the *ms172* and *ms217* database of alleles. If alleles that are already known are found, the corresponding alleles are given. . If one or two of the sequences are not known, the quality of the trace file is checked. A new allele number is given only if the quality of the trace file is good enough and if you have requested the permission to add new alleles using the contact form. If the quality is not good enough, a message indicate that a good quality trace file should be entered.

Submission of fasta files.

Fasta files with record named *ms172* and *ms217* for one isolate at a time can be submitted. The fasta file must be in the following form:

>ms172
Sequence of 400bp
>ms217
Sequence of 350bp

It will soon be possible to submit fasta files with batches of isolates.

Submission of plain text sequences.

Plain text sequences can be entered for the ms172 and ms217 loci. These sequences must start with the corresponding start signature and must be trimmed at the correct length.