DTU Food
National Food Institute

Community Reference Laboratory Antimicrobial Resistance

PROTOCOL FOR MLST (Multi locus sequence typing)

Use extracted DNA or boiling lysates for PCR

Controls:
None needed (these are housekeeping genes and should be present in all *S. aureus* isolates)

PCR amplification of 7 housekeeping genes
Set up and run each one of the PCR reactions according to the conditions described in the PCR sheets (contains PCR mix and conditions).

Primers

Primer name	Sequence
arc up MLST_Sa	5'-TTGATTCACCAGCGCGTATTGTC-3'
arc dn MLST_Sa	5'-AGGTATCTGCTTCAATCAGCG-3'
aro up MLST_Sa	5'-ATCGGAAATCCTATTTCACATTC-3'
aro dn MLST_Sa	5'-GGTGTTGTATTAATAACGATATC-3'
glp up MLST_Sa	5'-CTAGGAACTGCAATCTTAATCC-3'
glp dn MLST_Sa	5'-TGGTAAAATCGCATGTCCAATTC-3'
gmk up MLST_Sa	5'-ATCGTTTTATCGGGACCATC-3'
gmk dn MLST_Sa	5'-TCATTAACTACAACGTAATCGTA-3'
pta up MLST_Sa	5'-GTTAAAATCGTATTACCTGAAGG-3'
pta dn MLST_Sa	5'-GACCCTTTTGTTGAAAAGCTTAA-3'
tpi up MLST_Sa	5'-TCGTTCATTCTGAACGTCGTGAA-3'
tpi dn MLST_Sa	5'-TTTGCACCTTCTAACAATTGTAC-3'
yqi up MLST_Sa	5'-CAGCATACAGGACACCTATTGGC-3'
yqi dn MLST_Sa	5'-CGTTGAGGAATCGATACTGGAAC-3'

Run then 5µl of each PCR product on a 1,5% agarose gel for 25 min at about 130V. with a 100bp Ladder molecular weight marker. Stain the gel in Ethidium bromide about 20-30min.
Destain briefly in milliQ water.
Take a photo in the GelDoc.
Observe the bands: each PCR reaction should have a good yield of amplification.
For MLST typing the amplified PCR products need to be purified using a common purification kit and sequenced or sent for sequencing.

Sequencing results will then be interpreted by submitting the 7 obtained sequences to the MLST server www.mlst.net to obtain the corresponding sequence type (ST type).