

PROTOCOL FOR PCR for *mecA*, *nuc* and 16S
CRL course Copenhagen 20-22 April 2009

21st April (afternoon)

Use pre-prepared cultures on blood agar to prepare DNA lysates for PCR

Preparation of boiling lysates (normal protocol):

Take a loopful of culture and suspend in 1ml PBS
Centrifuge 5min at 20000g
Take out the supernatant and resuspend in 100 µl TE10:1 Buffer
Boil 10 min
Cool on ice
Dilute 1:10 in TE buffer

Preparation of boiling lysates (short protocol):

Take a loopful of culture and suspend in 100 µl TE10:1
Boil 10 min
Cool on ice

PCR Controls (pre-prepared DNA samples):

S aureus 50A247 *mecA*+ *nuc*+ 16S +
S aureus 23-88.10019 *mecA*- *nuc*+ 16S + (MSSA)
S epidermidis OXA 1.5 *mecA*- *nuc*- 16S + (coagulase negative)

PCR

Set up and run the PCR according to the conditions described in the PCR sheet (contains PCR mix and conditions).

Primers

Primer name	Primer #	Sequence
MECUP1	Primer 1523	5'-GGGATCATAGCGTCATTATTC-3'
MECUP2	Primer 1524	5'-AACGATTGTGACACGATAGCC-3'
NUCPCR1	Primer 1525	5'-TCAGCAAATGCATCACAAACAG-3'
NUCPCR2	Primer 1526	5'-CGTAAATGCACTTGCTTCAGG-3'
16SUP1	Primer 1527	5'-GTGCCAGCAGCCGCGGTAA-3'
16SUP2	Primer 1528	5'-AGACCCGGGAACGTATTCAC-3'

Run then 8µl of the 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: *mecA* 527bp; *nuc* 255bp and 16S 886bp.

Interpret the results

Reference:

Poulsen AB, Skov R, Pallesen LV. 2003. Detection of methicillin resistance in coagulase-negative staphylococci and in staphylococci directly from simulated blood cultures using the EVIGENE MRSA Detection Kit. *J Antimicrob Chemother.* Feb;51(2):419-21.