

PROTOCOL FOR *spa* typing
CRL course Copenhagen 20-22 April 2009

21st April (afternoon)

Use same lysates used before for PCR

Controls:
S aureus

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
SPA 1095F new	1794	5'-AGACGATCCWTCAGTGAGC-3'
SPA extend:f	1827	5'-TAATCCACCAAATACAGTTGTACC-3'

Run then 5µl 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: *spa* should be amplified in all *Staphylococcus aureus* strains but the length of the amplified fragment is variable.

For *spa* 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 using a specific sequence analysis software (Bionumerics *spa* Typing plugin, for example) to attribute the *spa* type corresponding to the number and sequence of repeats present.

References:

Shopsin B, Gomez M, Montgomery SO, Smith DH, Waddington M, Dodge DE, Bost DA, Riehman M, Naidich S, Kreiswirth BN. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. J Clin Microbiol. 1999 Nov;37(11):3556-63.

PCR: SPA

Dato: 20-03-2009

Init:

Projektnr.:
Primer 1: 1794 SPA 1095F new
Primer 2: 1827 SPA extend_f
DNA polymerase: Amplicon taq
PCR produkt: Varierende længde
Bemærkninger: 2 µl template Evt. positiv kontrol: S. aureus.

Antal prøver	1	5	1.	5	min ved	94	°C
PCR H ₂ O	41	205	2.	35	Cykler		
10xPCR buffer	5	25		45	sek ved	94	°C
dNTP	0,5	2,5		45	sek ved	62	°C
25 mM MgCl ₂	0	0		1½	Min ved	72	°C
Primer 1	0,5	2,5	3.	10	min ved	72	°C
Primer 2	0,5	2,5	4.		Pause ved	4	°C
Taq polymerase	0,5	2,5					
Total volumen	48	240					

M:	100bp Ladder
1	1
2	2
3	3
4	4
5	5
6	H2O
7	Pos control
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	

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