

1 **Additional File 2: Table. Primers developed and used in this study.** Primers were designed  
 2 for the *Sii*/SBSEC MLST scheme of housekeeping genes and identification based on *groEL*, *lacS*  
 3 and *lacZ* genes.

Primer name	sequence (5'-3') <sup>1)</sup>	target gene	amplicon size [bp]	gene used as target in MLST of other streps
ddl-fw ddl-rev	CTT ACG TGA CTT ATG TTG AAG G CCA AAG AAG TGG ATA CAT TGA C	D-alanine-D-alanine ligase	522	<i>S. uberis</i>
gki-fw2 gki-rev gki-fw6.2-ala <sup>2)</sup> gki-rev1.2-ala <sup>2)</sup>	TGA ACT GGG CAC ACA CTG CTG GGT TYA AGA TGT TTG CG GGG MTC WCC TGG AGC TGT PGC AGC WGA HAC DCC ACC	glucokinase	521	<i>S. pneumoniae</i>
glnA-fw glnA-rev	GCT GTT GCA GGT TTG ATC TG TAC AGT GCA TAC CTG AAC CG	glutamine synthetase	478	group B streptococci
groEL-fw groEL-rev	GGT TCA CCA CTT ATC ACW AAT G ATC ACC GTC AAG TTC AAG TTC	<i>groEL</i> heat shock protein <i>cpn60/hsp60</i>	1167	only used for (sub)species identification
lacZ-6.2 lacZ-17rev	TTC CTC AAG AAT CAA ATG CTG CCA CAA GAC CAA ATG ATA ACA C	$\beta$ -galactosidase <i>lacZ</i>	1027	only used for <i>lacZ</i> amplification and sequencing
lacS-8 lacS-18.1rev	GCG TGA CGT GCT TCA GTC GAT TGA ATA CAG TTG TTG GTT TG	lactose symporter/antipporter <i>lacS</i>	501	only used for <i>lacS</i> amplification and sequencing
mutS-fw mutS-rev	GTG CCA GAT TAY TTC TTC CG GCT TCC ATC ATT TCC ACC AT	DNA mismatch repair ATPase	581	<i>S. suis</i>
mutS2-fw mutS2-rev	CCA AAT ACT GGT GGT AAG AC TCA AAC GCT CTG CTT CAT TG	mismatch repair ATPase	529	<i>Sii</i>
pheS-fw pheS-rev	GCT AAA TTG GCT GAA ATG CG ACA CGY CCT GGT GAR ATC AT	phenylalanyl tRNA synthetase	557	group B streptococci
proS-fw proS-rev2	CTG CTA ACC TTG AAA TGG CA AAC GTA TTC CGC TTC AAA ATC	prolyl tRNA synthetase	455	<i>Sii</i>
pyrE-fw pyrE-rev	AGC ATC ACA AAT TGC ATC AG ACA TCA GCA CCT TCA CGT TC	orotate phosphoribosyl transferase	426	<i>S. salivarius</i>
thrtRNA-syn-fw thrtRNA-syn-rev	CCA CCG TAA ACT TGG TAA AG TGW GCA TTT TCC CAC ATT TC	threonyl tRNA synthetase	606	<i>S. salivarius</i>
tpi fw3 tpi-rev	ATC GCT GGT AAC TGG AAA ATG CGA TAG CCC AGA TTG GTT CG	triosephosphate isomerase	410	<i>S. zooepidemicus</i>

4 <sup>1)</sup> mixed base sites according to IUB code <sup>2)</sup> modified primers to amplify the same gene region in  
 5 *S. alactolyticus*.