MLST scheme *Streptococcus gallolyticus* subsp. *gallolyticus*

The *Streptococcus gallolyticus* subsp*. gallolyticus* MLST scheme uses internal fragments of the following seven housekeeping genes:

aroE (SGGBAA2069_c13440)

glgB (SGGBAA2069_c07540)

nifS (SGGBAA2069_c13360)

p20 (SGGBAA2069_c04560)

tkt (SGGBAA2069_c21090)

trpD (SGGBAA2069_c05200)

uvrA (SGGBAA2069_c18560)

Primers used for amplification and sequencing

gene	forward primer 5'-3'	reverse primer 5'-3'	amplified size [bp]	compared size [bp]
aroE	CCTACGCTTGTAGCATTG	CTTAGCTGCGGTTGTTG	596	458
glgB	CAGCAGCAGTTCTTACAG	ACCGTGAACCACTTCATC	950	491
nifS	GATTCGGACAGCTGATTG	GTCTGGTGGTACAGAAAG	859	738
p20	TATTTACGCCCACGTCTG	CATAGCGCAATAGGTCAC	493	394
tkt	GTCAAACGGTGGATACTC	CCGAATACGGTCATACTG	550	441
trpD	CGACGCCATGTGTAATTG	AAGGTAAGGGCTAGGTTC	643	418
uvrA	CTCGCAAGGTACGTAAAC	GGCAACACCTTGATTGTC	675	517

PCR protocol

reagent	initial concentration	volume [µl]
HotMaster *Taq*-buffer (5Prime)	10 x	5
primer forward	20 µM	1
primer reverse	20 µM	1
dNTP (Fermentas)	5 mM	2
HotMaster *Taq*-polymerase (5Prime)	5 U/µl	0.25
dest. H_2O	-	35.75
template DNA	-	5

	T [°C]	t [sec]	No. of cycles
initial denaturation	95	120	1
denaturation	95	60	
annealing	56	30	30
elongation	72	30	
final elongation	72	120	1

Purification of PCR products

reagent	volume [µl]
Exonuclease I solution[1]	1
Shrimp Alkaline Phosphatase (USB, Cleveland, USA)	1
PCR product	5

[1] Exonuclease I solution: 10 µl glycerine, 80 µl TE-buffer, 10 µl Exonuclease I (NEB, Frankfurt am Main, Germany)

T [°C]	t [sec]
37	1800
80	900

Sequencing protocol

reagent	initial concentration	volume [µl]
premix [1]	10 x	2
BigDye sequencing buffer [1]	5 x	2
primer forward/reverse [2]	20 µM	1.5
purified PCR product	-	2
dest. H2O	-	12.5

[1] BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Darmstadt, Germany)
[2] For sequencing reactions PCR primers were used.

	T [°C]	t [sec]	No. of cycles
initial denaturation	95	120	1
denaturation	95	10 sec	25
annealing and elongation	60	240 sec	

Excess dye terminators and primers were removed by centrifugation using a spin column prepared with sephadex-G-50 (Amersham, Braunschweig, Germany). Finally, a denaturation step for 120 sec at 95°C was performed. The sequences of both strands were determined with a 3500 Genetic Analyzer DNA-sequencer (Applied Biosystems, Darmstadt, Germany).