Spiroplasma MLST

1 DIAGNOSTIC PCR

Pre-heat lid to 95°C
3 min at 95°C

10x cycle of	95°C	for 30 s
	65-56°C	for 30 s
	72°C	for 60 s
25x cycle of	95°C	for 30 s
	55°C	for 30 s
	72°C	for 60 s

6 min at 72°C
Hold at 10°C

Hot start (waiting for the block to reach temperature) is not required, ramp rate 3-5°C/sec

➔ For RpoBF1.ixod / RpoBR1: set elongation time to 120 s in both cycles

2 REAGENTS PER REACTION

5 µl Promega M7822 GoTaq G2 Green Master Mix
2 µl Nuclease free water
1 µl Forward primer at 10 µM
1 µl Reverse primer at 10 µM
1 µl template DNA

3 PRIMERS

3.1 16S

Together, the 16S primers cover regions 1-8 out of 9 variable regions in Spiro 16S

Name	OligoID & length	Sequence	Comments
23F	2366024 23 bp	5‘ CTC AGG ATK AAC GCT GGC GGC AT	
RiboTKSSR	2366025 20 bp	5‘ TAG CCG TGG CTT TCT GGT AA	Product size: ~ 481 bp
16STF1	2366026 23 bp	5‘ GGT CTT CGG ATT GTA AAR GTC TG	
16STR1	2366027 20 bp	5‘ GGT GTG TAC AAG ACC GAG AA	Product size: ~ 975 bp

3.2 ITS

These are improvements of primers SP-ITS-JO4 (5‘ GCC AGA AGT CGG TGT CCT AAC CG) and SP-ITS-N55 (5’ATT CCA AGG CAT CCA CCA TAC M). ITS was not used to calculate phylogenies, as the primer amplified products of different lengths in some samples and the sequencing data was not useable.

Name	OligoID & length	Sequence	Comments
SP-ITS-CVf	2371743 22 bp	5‘ GAT GGA TCA CCT CCT TTC TAT G	
SP-ITS-CVr	2371744 19 bp	5‘ CAG CTC CCC GAA SCT TAT C	Product size: 296 bp

3.3 RpoB

RpoBF1.ixod and RpoR1 produce low yield (weak bands on TBE gel) for some Spiroplasma-positive pea aphid samples and also caused problems during sequencing in the past. Always run RpoBF1.ixod and RpoBR1 with an elongation time of 120s. Product of the two primers overlap.

Name	OligoID & length	Sequence	Comments
RpoB.cv.F2	2375067 20 bp	5‘ TCC ATC ACG TGG AAC TTG AC	
RpoB.cv.R2	2375068 23 bp	5‘ CGG TAA GGT GTT TCA ATA AAA CC	Product size 1‘179 bp
RpoBF1.ixod*	2375069 26 bp	5‘ ATG GAT CAA ACT AAT CCA TTA GCA GA	Requires 120 s elongation step
RpoBR1*	2375070 23 bp	5‘ CCA AAT CTT TGT CCA CCA TTT TG	Requires 120 s elongation step Product size 1‘784 bp

3.4 ApDNAa

Name	OligoID & length	Sequence	Comments
ApDnaA_F1	2429956 23 bp	5‘ ATT CTT CAG TAA AAA TGC TTG GA	
ApDnaA_R1	2429957 23 bp	5‘ ACA CAT TTA CTT CAT GCT ATT GA	Product size 447 bp