Primers and PCR conditions for MLST of *P. multocida*

Gene	Forward primer	Reverse Primer	Amplicon Size (bp)
adk	5'-TTTTTCGTCCCGTCTAAGC -3'	5'-GGGGAAAGGGACACAAG C -3'	570
est	5'-TCTGGCAAAAGATGTTGTC G-3'	5'-CCAAATTCTTGGTTGGTT GG-3'	641
pmi	5'-TGCCTTGAGACAGGGTAAG C-3'	5'-GCCTTAACAAGTCCCATT CG -3'	739
zwf 1	5'-AATCGGTCGTTTGACTGAG C -3'	5'-TGCTTCACCTTCAACTGT GC -3'	808
zwf 2	5'-TGTTAGGTGTGGCAAGAAG AACG – 3'	5' – TTGCAACAAATGGTTTTG GA – 3'	614
mdh	5'-ATTTCGGGATCAGGGTTAG C-3'	5'-GGAAAACCGGTAATGGA AGG	620
gdh	5'-ATCGACTTCTTCCGCAGAC C -3'	5'-GCGGGTGATATTGGTGTA GG -3'	702
pgi	5'-ACCACGCTATTTTTGGTTGC -3'	5'-ATGGCACAACCTCTTTCA CC-3'	784

dNTP (100mM) are diluted to 1.25 mM by adding 5 µl dNTP to 380 µl water.

Taq - 5 U/µl

DNA Preparation

Prepared with Prepman Ultra:

1. Put a loopful of *Pasteurella* from a fresh overnight culture on BA into 200µl Prepman Ultra.
2. Heat @100°C for 10 minutes
3. Cool on ice for 3 minutes
4. Spin for 5 minutes @ 13,000
5. Store the supernatant in the freezer.

Master Mix for 50 µl reaction:

H_2O	31.9 µl	
10 x Roche reaction buffer	5.0 µl	(1.5 mM $MgCl_2$)
Primer F (10 µM)	2.0 µl	(0.4 µM final conc.)
Primer R (10 µM)	2.0 µl	(0.4 µM final conc.)
dNTP mix (1.25 mM)	8.0 µl	(200 µM final conc.)
Roche Taq polymerase	0.1 µl	(0.125 units per reaction)
DNA template	1.0 µl	
Total	50 µl	

Cycles for MLST

Lid 105

1 x cycle	94°C	5 min
30 x cycles	96°C	10 sec
	50°C	5 sec
	60°C	4 min
Hold	4°C	

Electrophoresis

10 µl of PCR product with 2 µl loading buffer are run in 1% DNA-grade agarose. Gel containing ethidium bromide (1 µl/ µg) in 1% TAE buffer at 80 V for 30 min. 100bp DNA ladder is used to determine fragment size.

PCR product clean up

EXD-SAP-IT	1 µl
H_2O	8 µl
PCR product	1 µl

Put in PCR machine and run with lid at 25°C
Cycles:

37°C	30 min and 10 sec
80°C	15 min and 10 sec

Hold at 4°C

Send for sequences in 1.5 ml tube or load a plate

H_2O	4 µl
Primer	4 µl
Clean PCR product	4 µl