

Primers and Protocols for Multi-host *P. multocida* MLST Scheme

Genes

The following seven housekeeping enzyme genes were selected based on their widespread locations around the chromosome and the differing functions of the encoded enzymes.

<i>adk</i>	adenylate cyclase	nucleotide biosynthesis
<i>aroA</i>	3-phosphoshikimate 1-carboxyvinyl transferase	amino acid biosynthesis
<i>deoD</i>	purine nucleoside phosphorylase	nucleotide biosynthesis
<i>gdhA</i>	glutamate dehydrogenase	amino acid biosynthesis
<i>g6pd</i>	glucose-6-phosphate 1-dehydrogenase	energy metabolism: pentose phosphate pathway
<i>mdh</i>	malate dehydrogenase	energy metabolism; TCA cycle
<i>pgi</i>	phosphoglucose isomerase	energy metabolism; glycolysis

PCR Amplification

Chromosomal DNA was prepared from 1.0 ml volumes of overnight cultures grown in brain heart infusion broth with the InstaGene Matrix (Bio-Rad) according to the manufacturer's instructions. PCR fragments of the seven housekeeping enzyme genes were amplified from chromosomal DNA with a *Taq* DNA polymerase kit (Boehringer Mannheim) according to the manufacturer's instructions. Each 50 µl amplification reaction mixture comprised 5.0 µl 10x PCR buffer (100 mM Tris-HCl, 15 mM MgCl₂, 500 mM KCl, pH 8.3), 4.0 µl dNTP solution (Promega UltraPure dNTP set adjusted to 1.25 mM each dNTP), 4.0 µl of each forward and reverse primer (25 pmol/µl), 2 µl chromosomal DNA (5-20 ng/µl) and 1 U *Taq* DNA polymerase. PCRs were carried out in a Perkin Elmer 480 DNA thermal cycler using the following amplification parameters: denaturation at 94°C for 45 s, annealing at 56 to 60°C (see below) for 45 s, and extension at 72°C for 2 min. Thirty cycles were performed, and a final extension step of 72°C for 10 min was used. Production of a PCR amplicon of the expected size was confirmed by agarose gel electrophoresis and the DNA purified with a QIAquick PCR purification kit (Qiagen, Chatsworth, Calif.). The DNA was finally eluted in 30 µl of sterile, distilled H₂O and stored at -20°C. One microlitre of purified DNA was

analysed by agarose gel electrophoresis to confirm purification and for estimation of DNA concentration.

PCR Primers

Locus	Primer sequence (5' to 3')		Annealing temperature (°C)
	Forward	Reverse	
<i>adk</i>	AAGGBACWCAAGCVCAAT	CACTTTTTKYGTMCCGTC	57
<i>aroA</i>	TTTACCDGGYTCYAAAAG	CTTTYACVCGCCAGTTAT	56
<i>deoD</i>	GTGCATTTGTCYGATGTTG	TGSYGTKGTTTGTTCGTG	60
<i>gdhA</i>	YTTAGTTGARCCTGAACG	CTTGACCTTCAATYGTGC	57
<i>g6pd</i>	CHGGYGAYYTMACTYATCG	TTTBGCGATBARTTTRTCRGC	56
<i>mdh</i>	AAGTTGCWGTWYTAGGTG	CCTAATTCAATATCYGCACG	57
<i>pgi</i>	GCCWGTGYTKGTTGATGG	TTGKGCTGGCGCRATRAA	60

Sequencing Reactions

Sequencing was carried out in both directions using the primer pairs shown below. The same amplification and sequencing primers were used for *adk* and *deoD*; new internal forward and reverse sequencing primers were designed for *g6pd*, *gdhA* and *pgi*; the forward amplification primer and a new internal reverse primer were used for *aroA*; and the reverse amplification primer and a new internal forward primer were used for *mdh*. The most informative regions of the larger genes were selected for sequencing based on preliminary studies. Sequence reactions were performed with the ABI Prism™ Big Dye Terminator cycle sequencing kit (Applied Biosystems) in a GeneAmp PCR System 9700 (Applied Biosystems) thermal cycler according to the manufacturer's instructions. Each 10 µl sequencing reaction mixture comprised 4.5 µl DNA template (10-20 ng/µl), 1.5 µl forward or reverse primer (2 pmol/µl) and 4 µl Terminator reaction mix. The following cycle sequencing parameters were used: 25 cycles of 10 s at 96°C, 5 s at 50 °C and 4 min at 60 °C. Sequence analysis was carried out with an Applied Biosystems 377 DNA Sequencer (University of Glasgow Sequencing Service). The sequenced regions between the primers were trimmed so that the sequences were "in frame". The trimmed sequences varied in length from 514 to 652 bp.

Sequencing primers

Locus	Primer sequence (5' to 3')		Sequence length (bp)
	Forward	Reverse	
<i>adk</i>	AAGGBACWCAAGCVCAAT	CACTTTTTKYGTMCCTGTC	531
<i>aroA</i>	TTTACCDGGYTCYAAAAG	TGCATCATCTTAAGGGTG	558
<i>deoD</i>	GTGCATTTGCGYCATGTTG	TGSYGTKGTTTGTTCGTG	576
<i>g6pd</i>	GATGCTGCCGATTATGG	CAAGACTTTTGCCACTTC	513
<i>gdhA</i>	CGCGTTAACCACATTACC	CCCTTCAGCCACTAATTG	651
<i>mdh</i>	TGTCCAAAAGCTTGTGTG	CCTAATTCAATATCYGCACG	552
<i>pgi</i>	GTGATTTCTGGTGAATGG	GGAAATACGCTGCAAAAC	609

Locations of primers within housekeeping enzyme genes

NB: red highlights PCR amplification/sequencing primers; green highlights internal sequencing primers; underscore highlights trimmed “in frame” sequences used for database and concatenation.

adk

ATGAAAATTATCCTATTAGGTGCACCGGGTGCAGGGGAAAGGGACACAAGCGCAATTTATTATGAATAAAATTTGGC
ATTCCACAAATTTCAACGGGTGATATGTTGCGTGGTGCATCAAAGCAGGGACAGACTTAGGTAAACAAGCAAAA
ACCTTAATGGATGCGGGTCAATTAGTGCAGATGATTTAATCATTTTCATTAGTGAAAGAACGTGTTGCACAAGCC
GATTTGTGCCAAAGGTTTCTTATTAGACGGCTTCCCACGCACCATTCACCAAGCGGATGCATTAATAAACAGTCGGT
ATCCAAATTGACTACGTTTTAGAGTTTTGATGTGCCTGATGAAGTGATCGTAGAACGTATGAGTGGTGCCTGTGA
CACCAAGCCTCAGGTGCTACTTATCACGTCGTTTTACAACCCACCAAAAAGTAGAAGGGAAAGATGACGTGACTGGC
GAAGACTTAATCATCCGTGCCGATGATAAACCTGAAACTGTATTAGATCGCTTAAAAGTGTATCACTCCACCACG
AAACCATTAGTGGACTACTACCAAGCAGAAGCGAAAAGCGGGTAAACCAAGTACTTCCGCTTAGACGGGACGAAA
AAAGTCGAAGAAGTGAGTCAAGAATTAGATACTATTTTTAGCGTAA

Sequence length = 533 bp; trimmed length = 531 bp

aroA

GTGATAAAAGATGCGACCGCTATTACTCTCAATCCCATCAGCTATATTGAAGGCGAGGTGCGTTTACCGGGCTCC
AAAAGCTTTATCCAATCGCGCACTCTTACTTTCCGCATTAGCTAAAGGAAAAACAACATTAACCAATCTGTTAGAT
AGTGATGATGTGCGCCATATGTTAAATGCGTTAAAAGAACTTGGCGTGACTTATCAACTCTCAGAAGACAAATCC
GTCTGTGAAATTGAAGGCTTAGGACGTGCTTTTGAATGGCAAAGTGGCTTAGCTTTATTTTTGGGCAATGCAGGG
ACGGCGATGCGTCCCTTGACTGCCGCGCTTTGTTTATCTACACCGAACCAAGGAAGGCAAAAATGAAATAGTCTTG
ACTGGCGAACCTCGTATGAAAGAACGCCAATACAACATTTAGTTGATGCATTATGTCAAGCTGGCGCAGAAATT
CAGTATTTAGAACAAGAAGGTTACCCACCTATCGCCATTTCGAAATACCGGACTCAAAGGCGGACGAATACAAATT
GATGGGTGAGTTTCTTCTCAATTTTTGACCGCACTTTTAAATGGCTGCCCGATGGCAGAGGCGGATACGGAAATT
GAAATCATCGGTGAGCTGGTTTTCCAAACCTTACATTGATATCACCCTTAAGATGATGCAAACCTTTGGCGTTGAA
GTTGAAAACCAAGCCTATCAACGCTTTTTTGGTGAAAGGTCATCAGCAATACCAATCACCACACAGGTTTCTAGTA
GAAGGCGATGCCTCTTCTGCTTCTTATTTTTCTTGCCGCGGCAGCAATCAAGGGAAAAAGTAAAAGTACAGGCGTC
GGTAAAAATAGCATTCAAGGGGATCGTCTGTTTTCGGATGTGCTAGAAAAAATGGGGGCGCATATCACTTGGGCG
GACGATTTTATTCAAGTGGAAGGCAACCTCAAAGGCATCGATGGATATGAACCATATTTCCCGATGCGGCA
ATGACCATTGCCACCACAGCGCTTTTTGCAGAAGGTGAAACGGTCATTTCGTAATATTTATAACTGGCGCGTAAAA
CAAAATGATCGCTTGACCGCGATGGCGACCGAACTGCGTAAGGTGGGGCGGAAGTGGAAGAAGGCGAAGATTTTT
ATTCGTATCCAGCCATTGAATCTGGCGCAATTTCAACATGCTGAAATTTGAAACATACAATGATCACCGCATGGCG
ATGTGCTTTGCTTTAATCGCATTGTGCGAAACGTGCGTACGATTTTAGACCCGAGCTGTACCAGCAAAAACGTTT
CCTACGTTTTTTGATACTTTTTTACGCTTAAACACACGCAGAAAAGTTAG

Sequence length = 561 bp; trimmed length = 558 bp

deoD

ATGACTCCACATATTAACGCACCTGCCG **GTGCATTTGCCGATGTTG** TTTTAATGCCGGGTGACCCATTACGTGCC
AAATACATCGCAGAAACCTTCTTACAAGATGTGAAAGAAATCACGAATGTCCGTAATATGCTTGGGTTACAGGGA
ACCTATAAAGGCCGCAAAATTTCTGTTATGGGACATGGTATGGGGATCCCATCTTGTTCGATTTACACAAAAGAA
TTGATTACGGAATATGGCGTGAAGAAAATTATCCGTGTTGGTTCCTTGGCGTGCTGTCCGTATGGATGTCAAATTA
CGTGATGTGGTGATTGGATTTGGTGCCTGTACCGATTCCAAAAGTCAACCGTATCCGTTTAAAAACCATGACTTT
GCTGCGATCGCGGACTTTGACATGACAATGGCGGCAGTGAAGCGGCAAAAGCAAAAGGCTTAAATGTCCATGTT
GGTAACTTATTCTCTGCAGACTTATTCTATACACCAGATGTGGAAAATGTTTGGTGTAAATGGAAAAATATGGCATC
TTAGGTGTTGAAATGGAAGCTGCGGGAATTTATGGTGTGGCCGAGAATTTGGTGCCAAAGCCTTAACAATCTGT
ACTGTGTCTGATCACATTCGTACA **CACGAACAAAACAACCA** GAAGAACGTCAATTAACATTTAATGATATGATT
GAAATCGCATTAGAATCCGTTCTAATTGGCGATAACGCGTAA

Sequence length = 578 bp; trimmed length = 576 bp

g6pd

ATTGAAGCAGACAACAATTGTATTGTAATTTTTGGTGCAT **CAGGTGATTTAACTTATCG** TAAATTGATTCCTGCA
CTGTATAACTTATATAAAATCGGTCGTTTACTGAGCATTCTCCGTGTTAGGTGTGGCAAGAACGGAATTAAGT
GATGAGGGTTTCCGTGAAAAAATGCGCCAAGCGTTGATCAAAAGTGAAAAAGCGAATGGCGAAACACTCGATCAA
TTTTGTAGCCACCTTTATTATCAGGCATTAATAACCGCG **GATGCTGCCGATTATGG** CAAGTTAATTCCTCGTCTT
GATGATTTACATGATAAATATCAAACCTGTGGTAAACACACTTTACTATTTATCCACGCCGCCAAGTCTTTATGGT
GTGATTCCAGAATGTCTTGGCGCACATGGGTTAAATACTGAAAGTTTGGCTGGAAAGCGGTTAATTGTGGAAAA
CCGTTTGGTTATGATATCCGCACGGCAAAAAGAACTCGATATTCAAATTCACCGTTTCTTTGATGAACACCAAAT
TATCGTATTGACCACTATCTTGGTAAAGAAACGGTTCAAAATCTGCTCGTGTGCGTTTTTCTAATGGATGGTTT
GAACCACTCTGGAACCGTAATTTTATTGATTATATTGAAATCACGGGCGCAGAATCTATCGGTGTAGAAGAGCGT
GGTGGTTATTACGATGATTCTGGCGCAATGCGTGATATGTTCCAAAACCATTTGTTGCAAGTGTAGCCATGGTT
GCGATGGAGCCACCGGCAATTATTAATGCTGACTCAATGCGTGAT **GAAGTGGCAAAAAGTCTTG** TATTGTTTACAT
CCATTAAGTGAGGATGACTTAGAAAATCATTTAGTCTTAGGGCAATATACGGCAGGCACAGTTGAAGGTGAAGCA
GTTAAGGGCTACTTACAGGAAAAAGGTGTACCGGCAGAGTCTAATACGGAAACTTACATGGCATTACGCTGTGAA
ATTGACAACCTGGCGTTGGGCGGGTGTGCCATTTTATGTGCGTACTGGGAAACGGTTACCAAGTCGAGTGACCGAA
ATTGTGATTCATTTCAAACACACACATCCGGTATTTAGCCAAAAAGCACCAGAAAACAAATTAATTTATCCGT
ATTCAACCCGATGAAGCGATTTTCGATGCGTTTTGGTTTTGAAAAAACCGGAGCAGGTTTTGAAGCAAAAGAAGTG
TCGATGGATTCCGTTATGCGGATCTTGCCACCAAGCTTACTGACCGCTTATGAGCGTTTATTATTGGATTCT
ATGAAAGGCGATGCCACTTTGTTTGC CGCTACTGATGCGGTACATGCCTGTTGGCAGTTTGTGGAGCCGATTTA
CAATATAAAGCACAAAATGGCGGTGTTTATGAGTATGAAGCCGCTACTTGGGGACCGACAGAA **GCCGACAAACTG**
ATCGCGAAA ACGGGTCTGTTTTGGCGTAAACCAAGTGGATTAATGAAAAAGAAAGTGTAA

Sequence length = 514 bp; trimmed length = 513 bp

gdhA

ATGTCTCAAGTTGCAACATTAGATGCGTTTTTTAGAAAAGAGTCGCACAACGCGACGGTACGCAACCTGAATTTTTTA
CAGGCAGTACGTGAAGTATTTACCTCTATCTGGCCTTTTTTTAGAAAGCTAACC AAAATATCGTTACAAAGCCCTA
CTCGAACG **CTTAGTTGAACCTGAACG** TGTATTTCAGTTCGCGTAGCATGGACAGATGATCAAGGTCAAACCCAA
GTTAACCGTGCAATCCGCGTGCAATACAACAGTGCAATCGGTCTTTCAAAGGAGGAATGCGCTTCCATCCTTCT
GTTAATCTTTCCATTTTAAAATTTAGGTTTTGAACAAATCTTCAAAA **CGCGTTAACCACATTAC** AATGGGG
GGCGGCAAAGGCGGTTCCGATTTTGTATCCTAAAGGAAAAATCTGACGGTGAAGTCATGCGTTTTCTGCCAAGCTTTA
ATGGCAGAATTATACCGTCATGTTGGCGCCGATACCGATGTGCTGCGGGTGATATTGGTGTAGGAGGACGTGAA
GTCGGCTATCTAGCAGTTATATGAAAAAATATCTAACCAAGCCGCTTGCCTGTTTACCGGTCGTGGCTTGTCA
TTCGGTGGCAGTTTAAATTCGCCCAGAAGCAACCGGTTATGGTCTAGTCTATTTCCGCCAAGCGATGCTCGCTGAA
AAAGGGCAAAGCTTTGCCGTTAAAACGGTGTCTGTTTCAGGTTCCGGTAACTGGCGCAATATGCCATTGAAAAA
GCGCTGCAATTGGGGGCAAAAAGTCGTAACCTGTTCTGACTCATCTGGTTATGTTTATGATGAAGCCGGTTTTACA
ACAGAAAAATGGCTGCATTAATGGAATTGAAAAACGAAAAACGCGGTCGTGTGGAAGAAATACGCGAAACAAATC
GGTTTAAAATACGTAGCAGGCAAAACGCCTTGGGAAAGTCAAAGTTGATATTGCACTGCCCTTGTGCGACACAAAAC
GAATTAGACTTAGCGTCTGCTAATACCTAATTGCCAATGGCGTA **CAATTAGTGGCTGAAGGG** GCTAACATGCCA

ACTACTATTGAAGCAACAGATGCCTTTTTAGACGCGGGCGTGCTCTTTGGTCCGGGTAAAGCGGCGAATGCTGGC
GGTGTAGCAACCTCAGGACTAGAAATGACGCAAAGTTTACAACGTTTATACTGGTCTGCGGAAGAAGTCGATAAA
AAACTCCACAGTATCATGTTAGATATTCACGCGAACTGTAAAAAATACG**GCACGATTGAAGGTCAAG**CCAACATT
AATTATGTCGTTGGTGCAAACGTTGCGGGATTGTCAAAGTCGCCGATGCGATGTTAGCGCAAGGTGTTTACTAA

Sequence length = 652 bp; trimmed length = 651 bp

mdh

ATGA**AAGTTGCAGTTCTAGGTG**CCGCAGGTGGTATTGGTCAAGCGTTAGCATTATTATTGAAATTACAATTACCA
GCCGGTTTCAGAGTTATCATTATATGATATTGCCCCCGTGACACCAGGTGTTGCTGCGGATGTCAGCCATATTCCA
ACAGCTGTAAAAGTGCAAGGATTTGCCGGTGAAGATCCAACACCTGCATTACAAGGGGCTGATGTAGTATTAATT
TCTGCAGGTGTTGCACGTAAACCGGGTATGGATCGTTCTGATTTATTTAATATTAATGCCGGTATCGTGCGTAAC
TTAATTGAGAAAGTTGCGCAAGTT**FGTCCAAAAGCTTTGTGTG**GGGATTATTACTAATCCTGTGAATACGACAGTA
GCAATTGCGGCTGAAGTATTGAAAAAGCGGGTGTTCAGATAAGCGTAAATTAATCGGTGTAACCACATTAGAT
ATTATTCTGTTCTGAAACCTTTGTATCAGAATTGAAAAATTTAGAGCCAGCGCGCACGACAGTGCCTGTGATTGGT
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TCCATGGCACAAGCCGAGCACGTTTTGCACTTTCTTTAGTTAAAGCTTAAATGGGGAAAAATGTAGTTGAATGT
ACTTATGTGGAAGGTGATGGCAAATATGCCCGTTTCTTTGCTCAGCCTGTTTCGTTTAGGTAAAGAAGCGCTTGAA
GAAATTCTACCATTAGGGTCATTGAGTGCATTTGAACAACATGCATTAGATGCAATGTTAGAGACATTA**CGTGCG**
GATATTGAATTAGCCGAGAAATTTGTCAATCAATAAATGAAAAGTTGCAGTTCTAGGTGCCGAGGTGGTATTGGT
CAAGCGTTAGCATTATTATTGAAATTACAATTACCAGCCGGTTCAGAGTTATCATTATATGATATTGCCCCCGTG
ACACCAGGTGTTGCTGCGGATGTCAGCCATATTCCAACAGCTGTAAAAGTGCAAGGATTTGCCGGTGAAGATCCA
ACACCTGCATTACAAGGGGCTGATGTAGTATTAATTTCTGCAGGTGTTGCACGTAAACCGGGTATGGATCGTTCT
GATTTATTTAATATTAATGCCGGTATCGTGCGTAACTTAATTGAGAAAAGTTGCGCAAGTTTGTCCAAAAGCTTGT
GTGGGATTATTACTAATCCTGTGAATACGACAGTAGCAATTTGCCGCTGAAGTATTGAAAAAGCGGGTGTTTAC
GATAAGCGTAAATTAATTCGGTGTAAACCACATTAGATATTATTTCGTTCTGAAACCTTTGTATCAGAATTGAAAAAT
TTAGAGCCAGCGCACGACAGTGCCTGTGATTGGTGGGCATTCTGGTGTCAACAT

Sequence length = 552 bp; trimmed length = 552 bp

pgi

ATGAAAAACATCAATCCTACAACAACAAACCGCGTGGAAAAGCCTTACAACAACATCATAAAACGCAAAGTGCGGTG
ACAATTCAACAACCTTTTTGCGCAAGAAAAAGATCGTTTTACGGATTATTCCCTTGTCTTTTAACAAATGAAGTGTTA
GTGGACTTCTCCAAAAACAATGTGACAAAAGAAACCTTAGGATTATTGCGCCAGTTAGCGCAAGAAATGTCATTG
TCTGAGGCAGTGACGCGATGTTTAGTGGGGCAAAAATCAATAAAACGGAAGATCGCGCCGTTTACATACCACA
CTTCGTAATCGTTCTAATTC**SCCTGTGTTGGTTGATGG**GAAAGATGTGATGCCAGAAGTGAATGCGGTTTTAGCC
AAAATGAAAGATTTTGGCATCGT**GTGATTTCTGGTGAATGG**AAAGGTTATACTGGTAAAGCGATTACAGATGTG
GTGAACATCGGAATTTGGTGGGCTGATTTAGGACCTTATATGGTAAACAGAAAGCCTTACGTCCTTATAAAAAATCAT
TTGAATCTACATTTTCGTGTCTAACGTAGATGGTACACATATTGCGGAGACCTTAAAGAAAAGTTAATCCAGAAACC
ACGCTATTTTTGGTTGCGTCAAAAACCTTTACCACACAAGAACTATGACCAATGCGCATTTCTGCGCCTAATTGG
TTCTTAGCCACGGCAAAAAGACGAAAAGTCATGTGGCAAAAACCTTTGACGCACTGTCCACAAAACAGTAAAGCCGTT
GCTGAATTTGGTATTGATACGAATAATATGTTTGAATTTTGGGATTGGGTAGGGGGTCGTTATTTCATTATGGTCA
GCGATTGGTTTATCTATTGCCCTTTCAATCGGTTTTGAGCATTTTGAAGCGCTTTTAGCGGGTGCACACGAAATG
GATAACATTTCCGTAAGTCCCAATTTAGCAAAAATTTCCGACCACATTAGCGTTAATTTGGTTTGTGGAATACC
AATTTCTTAGGTGCACAAACCGAAGCGATTCTACCTTATGATCAATATTTACACC**GTTTTTGCAGCGTATTTCCAA**
CAAGGCAATATGGAATCCAATGGGAAATATGTTGATCGTAATGGCGAAGTGTATCGATAACTACCAAACGGGACCA
ATTATTTGGGGCGAACCTGGTACCAATGGTCAACATGCGTTCTATCAATTAATTCACCAAGGCACTACCTTGATT
CCTTGTGAT**ITCATCGCGCCAGCACAA**ACCATAATCCATTAGCGGATCATCATGAAAAATTACTTTCAAACCTT
TTTGACAAAACCGAAGCGTTGGCTTTTGGTAAAAACGAAAAGAGAGGTAGAAGCAGAATTCGTGAAAGCAGGTA
TCTTTAGATGAGGTGAAAGAGGTTGTGCCATTTAAAAGTGTTTACGGGAAAATAAACAACCAACTCGATTTTGGT
CAAAAATTTACCATTTACTTTAGGTGCTCTCATTGCAATGTACGAACACAAGATTTTGTACAAGGGGTAATG
TTAATATTTATAGCTTTGACCAATGGGGCGTTGAGTTGGGCAACAGCTTGCGAATCGTATTTTGCAGAGCTA
GCAAACCGGAAACAATTACAACCTCATGACAGTTCAACTAATGGCTTAATTAACCAATATAAACAATGGCGTTAA

Sequence length = 613 bp; trimmed length = 609