PCR and allele-specific restriction analysis (ASRA) of *pfcrt*codon 76

PRIMARY PCR

5 ul of slide/filter-paper extract in total 25 ul PCR

Pfcrt 76 sense primer 76-A 5'**GCGCGCGC**ATGGCTCACGTTTAGGTGGAG3'

Pfcrt76 antisense primer 76-B 5' **GGGCCCGG**CGGATGTTACAAAACTATAGTTACC3'

Product size: 206 bp

PCR PROGRAM (ASRA-1)

SECONDARY PCRs

1-2 ul of primary reaction in total 25 ul secondary PCR

Pfcrt 76 sense primer 76-D1 5' TGTGCTCATGTGTTTAAACTT3'
Pfcrt 76 antisense primer 76-D2 5'CAAAACTATAGTTACCAATTTTG3'

Product size: 145 bp

PCR PROGRAM (ASRA-2)

RESTRICTION DIGEST

5-8 ul of PCR was digested with 1 unit enzyme for 5 hours at 50^0C in a total 20 ul reaction using New England Biolabs #3 buffer. Products were examined by agarose electrophoresis on 2% agarose (Life Technologies, Grand Island, NY), with an "uncut" (no enzyme) digest run alongside.

Enzyme: Apo I (New England Biolabs, Beverly, MA)

Codon cleaved: wild (Lys) and not mutant (Thr)

Cleaved product sizes: 99 and 46 bp

PCR and allele-specific restriction analysis (ASRA) of *pfcrt*codons 72, 74 and 75

PRIMARY PCR

Same as for *pfcrt* 76

SECONDARY PCRs

For 72 codon

0.2 ul of primary reaction in total 25 ul secondary PCR
(note: more primary inoculate will result in nonspecific bands)

CRT72MS 5'TTTATATTTTAAGTATTATTTATTTAAGTG**G**A3'

76-D2 5'CAAAACTATAGTTACCAATTTTG3'

Product size: 93 bp

For 74 and 75 codons

0.2 ul of primary reaction in total 25 ul secondary PCR
(note: more primary inoculate will result in nonspecific bands)

CRT745MS TAAGTATTATTTATTTAAGTGTATGTGT**C**AT

76-D2 5'CAAAACTATAGTTACCAATTTTG3'

Product size: 84 bp

PCR PROGRAM (7245)
Primary Denaturing: 95^0C,5 min
Denaturing: 92^0C, 30 sec
Annealing: 42^0C, 20 sec
Extension: 65^0C, 20 sec
Cycles: 20-30
Final Extension: 72^0C, 15 min

RESTRICTION DIGESTS

72 codon

72 codon

5-8 ul of CRT72MS/76-D2 PCR was digested with 1 unit enzyme overnight at 37^0C in a total 20 ul reaction using New England Biolabs #4 buffer. Products were examined by agarose electrophoresis on 2% agarose (Life Technologies, Grand Island, NY), with an "uncut" (no enzyme) digest run alongside.

Enzyme: Fok I and Mbo I (New England Biolabs, Beverly, MA)

Codon cleaved by Fok I: wild(C) and not 7G8 mutant (S-AGT) or DIV mutant (S-TCT)

Codon cleaved by Mbo I: DIV mutant (S-TCT) and not wild (C) or 7G8 mutant (S-AGT)

Cleaved product sizes (approximate): 55 and 38 bp

74 codon

5-8 ul of CRT745MS /76-D2 PCR was digested with 1 unit enzyme overnight at 37^0C in a total 20 ul reaction using New England Biolabs #4 buffer. Products were examined by agarose electrophoresis on 2% agarose (Life Technologies, Grand Island, NY), with an "uncut" (no enzyme) digest run alongside.

Enzyme: Nla III (New England Biolabs, Beverly, MA)

Codon cleaved: wild (M) and not mutant (I), regardless of codon 75 identity

Cleaved product sizes (approximate): 53 and 31 bp

75 codon

5-8 ul of CRT745MS /76-D2 PCR was digested with 1 unit enzyme overnight at 37^0C in a total 20 ul reaction using New England Biolabs #4 buffer. Products were examined by agarose electrophoresis on 2% agarose (Life Technologies, Grand Island, NY), with an "uncut" (no enzyme) digest run alongside.

Enzyme: BspHI (New England Biolabs, Beverly, MA)

Codon cleaved: 74 (M) only if codon 75 is wild (N) and not mutant (E)

Cleaved product sizes (approximate): 53 and 31 bp