## PCR and allele-specific restriction analysis (ASRA) of *pfcrt*codons 220 and 271

PRIMARY PCR

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

Primary sense primer: CRT2a ACAATTATCTCGGAGCAGTT

Primary antisense primer: CRT2b CCCAAGAATAAACATGCGAAAC

Product size (bp): 720

*PCR PROGRAM (CRT-DA)*
Primary Denaturing: 95, 5 min
Denaturing: 92, 30 sec
Annealing: 46, 1 min
Extension: 65, 90s
Cycles: 45
Final Extension: 72, 15 min

SECONDARY PCRs

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

220 codon

Allele-specific primer: *220-BG*: TATTTATTTATTTATATATTTTGTTTTCTT**G**C**C**ATTAAGG

Common primer: *220C:*TATTGTTGTAACAATAGC

Product size (bp): 132

*PCR PROGRAM (CRT-DB)*
Primary Denaturing: 95, 5 min
Denaturing: 92, 30 sec
Annealing: 46, 30 sec
Extension: 65, 30 sec
Cycles: 20
Final Extension: 72, 15 min

271 codon

Allele-specific primer:*271-XM:*GGCACATTCATTTATTTATTTTTTCTTTCCTAATTAAT**GAA**TACGTT

Common primer: *271C:* GGCTATGGTATCCTTTTTCC

Product size (bp): 121

*PCR PROGRAM (CRT-DB)*

## RESTRICTION DIGEST

### 220 codon

5-8 ul of PCR was digested with 1 unit enzyme overnight at $37^0$C 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: Bgl I (New England Biolabs, Beverly, MA)

Codon cleaved: wild (Ala) and not mutant (Ser)

Cleaved product sizes: 95 and 37 bp

### 271 codon

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

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

Codon cleaved: mutant (Glu) and not wild (Gln)

Cleaved product sizes: 78 and 43 bp