

## PCR and allele-specific restriction analysis (ASRA) of *pfmdr1* codons 86 and 1246

### PRIMARY PCRS

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

Pfmdr 86 sense primer MDR-A 5'**GCGCGCGTTGAACAAAAAGAGTACCGCTG**3'

Pfmdr 86 antisense primer MDR-B 5'**GGGCCCTCGTACCAATTCCTGAACTCAC**3'

Product size: 450 bp

Pfmdr 1246 sense primer 1246-A 5'**GGGGGATGACAAATTTTCAAGATTA**3'

Pfmdr 1246 antisense primer 1246-B 5'**GGGGGACTAACACGTTTAAACATCTT**3'

Product size: 295 bp

*PCR PROGRAM (ASRA-1)*

### SECONDARY PCRS

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

Pfmdr 86 sense primer MDR-D1TTTACCGTTTAAATGTTTACCTGC

Pfmdr 86 antisense primer MDR-D2CCATCTTGATAAAAAACACTTCTT

Product size: 291 bp

Pfmdr 1246 sense primer 1246-D1 AATGTAAATGAATTTTCAAACC

Pfmdr 1246 antisense primer 1246-D2 CATCTTCTCTTCCAAATTTGATA

Product size: 203 bp

*PCR PROGRAM (ASRA-2)*

### RESTRICTION DIGESTS

5-8 ul of PCR was digested with 1 unit enzyme overnight at 37°C in a total 20 ul reaction using New England Biolabs #3 or 4 buffer. Products were examined by agarose electrophoresis on 2% agarose (Life Technologies, Grand Island, NY), with an "uncut" (no enzyme) digest run alongside.

#### 86 codon

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

Codon cleaved: mutant (Tyr) and not wild (Asn)

Cleaved product sizes: 126 and 165bp

#### 1246 codon

Enzyme: Bgl II (New England Biolabs, Beverly, MA)

Codon cleaved: wild-type (Asp) and not mutant (Asp)

Cleaved product sizes: 113 and 90bp