

PCR for allele-specific restriction analysis (ASRA) of *pfdhps* codons 436 and 437

PRIMARY PCR

Sense primer PS400-A **GGGGTATTAAATGTTAATTATGATTCT**

Antisense primer PS400-B **GGGGTCACATTTAACAATTTTATT**

Product size: 249 bp

PCR PROGRAM (ASRA-1)

Primary Denaturing: 95,5 min

Denaturing: 92, 30 sec

Annealing: 45, 30 sec

Extension: 65, 45 sec

Cycles: 45

Final Extension: 72, 15 min

SECONDARY PCR

Sense primer PS400-D1

TGTTCAAAGAATGTTTGAAATGA

Antisense primer PS400-D2

CCATTCCTTTTGAAATAATTGTAAT

Product size: 148 bp

PCR PROGRAM (ASRA-2)

Primary Denaturing: 95,5 min

Denaturing: 92, 30 sec

Annealing: 45, 30 sec

Extension: 65, 30 sec

Cycles: 15-25

Final Extension: 72, 15 min

RESTRICTION DIGEST

Digests for each are performed separately

Analyze products on 2% agarose gel

437 codon

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

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

Cleaved product sizes: 69 and 79 bp

436 codon

Enzyme: MapA1 I

Codon cleaved: Ala (African variant) and not wild (Ser) or mutant (Phe)

Approx cleaved product sizes: 69 and 79bp

PCR for allele-specific restriction analysis (ASRA) of *pfdhps* codons 540 and 581

PRIMARY PCR

Sense primer PS500-A **GGGCCCAAACAAATTCTATAGTG**

Antisense primer PS500-B **GGCCGGTGGATACTCATCATATA**

Product size: 256 bp

PCR PROGRAM (ASRA-1)

Primary Denaturing: 95,5 min

Denaturing: 92, 30 sec

Annealing: 45, 30 sec

Extension: 65, 45 sec

Cycles: 45

Final Extension: 72, 15 min

SECONDARY PCR

Sense primer PS500-D1

GCGCGCGTTCTAATGCATAAAAAGAGG

Antisense primer PS500-D2

CCCGGGTAAGAGTTTAATAGATTGATCAGCTTTCTTC

Product size: 201 bp

PCR PROGRAM (ASRA-2)

Primary Denaturing: 95,5 min

Denaturing: 92, 30 sec

Annealing: 45, 30 sec

Extension: 65, 30 sec

Cycles: 15-25

Final Extension: 72, 15 min

RESTRICTION DIGEST

Digests for each are performed separately

Analyze products on 2% NuSeive gel

540 codon

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

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

Cleaved product sizes: 56 and 145bp

581 codon

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

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

Cleaved product sizes: 36 and 154bp

PCR for allele-specific restriction analysis (ASRA) of *pfdhps* codon 613

PRIMARY PCR

Sense primer PS613-A **GGGAAGAAACATGATCAATCTAT**

Antisense primer PS613-B **GGGACCTGAAAAGAAATACATAA**

Product size: 384 bp

PCR PROGRAM (ASRA-1)

Primary Denaturing: 95,5 min

Denaturing: 92, 30 sec

Annealing: 45, 30 sec

Extension: 65, 45 sec

Cycles: 45

Final Extension: 72, 15 min

SECONDARY PCR

Sense primer PS613-D1

AGTATCCACTTTTTATTGGATATTCAAGAAAAAGAATTAGT

Antisense primer PS613-D2

ACTTTTGTATACTCATTGAATTTT

Product size: 254 bp

PCR PROGRAM (FR100-2)

Primary Denaturing: 95,5 min

Denaturing: 92, 30 sec

Annealing: 42, 30 sec

Extension: 65, 45 sec

Cycles: 15-25

Final Extension: 72, 15 min

RESTRICTION DIGEST

Digests for each are performed separately

Analyze products on 2% NuSeive gel

613 codon

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

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

Approx cleaved product sizes: 42 and 212 bp

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

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

Approx cleaved product sizes: 42 and 212 bp