Supporting information for Mehlotra *et al*. (2001) *Proc. Natl. Acad. Sci. USA* **98** (22), 12689–12694. (10.1073/pnas.221440898)

Table 3. *pfcrt* PCR primers and SSOPs

Nest1 primers region I (5′UTR - intron 2)			
23327up*	5′-CAT TGT CTT CCA CAT ATA TGA CAT AAA-3′		
24076dn	5′-TTG GTA GGT GGA ATA GAT TCT CTT-3′		
Nest2 primers region I (5′UTR - intron 2)			
23402up†	5′-CCG TTA ATA ATA AAT ACA CGC AG-3′		
24011dn‡	5′-AAT TTC CCT TTT TAT TTC CAA ATA AGG-3′		
23939dn	5′-CGG ATG TTA CAA AAC TAT AGT TAC C-3′		
Reverse transcriptase-PCR primers (exon1/2 - exon 2/3)			
23561up	5′-ATT TAG TAC AAG AAG GAA GTA TGG CTC-3′		
24197dn	5′-AAA GCT TCG GTG TCG TTT TTT G-3′		
Probes for region I		**Wash stringency**	
CVMNK§	5′-TA TGT GTA ATG AAT AAA-3′	0.8× SSC	37°C
CVIET	5′-TA TGT GTA ATT GAA ACA-3′	0.8× SSC	37°C
SVMNT	5′-TA AGT GTA ATG AAT ACA-3′	0.8× SSC	37°C
SVMNT*	5′-TA TCT GTA ATG AAT ACA-3′	0.8× SSC	37°C
Nest1 primers region II (intron 3/exon 4–exon 8/intron 9)			
24511up	5′-CTC CTT TTT AGA TAT CAC TTA TAC-3′		
25460dn	5′-GCT TCT TAC CCA TGC TCC GT-3′		
Nest2 primers region II (intron 3/exon 4–exon 8/intron 9)			
24541up†	5′-CTC GGA GCA GTT ATT ATT GTT G-3′		
25145dn‡	5′-ATA TAT ATA TAT ATG GGC ACA TTC A-3′		
Nest1 primers region III (exon 8/intron 9–intron 11)			
25441up	5′-ACG GAG CAT GGG TAA GAA GC-3′		
26220dn	5′-ACG AAC AAG CCA TTT GAT ATT AC-3′		
Nest2 primers region III (exon 8/intron 9–intron 11)			
25588up†	5′-GAA AAC CTT CGC ATT GTT TTC C-3′		
26132dn‡	5′-ATT TCA CAC TTA CCA AAG TTA CG-3′		

PCR amplification of three *pfcrt*-specific sequences were performed with primers identified in Table 3. Nomenclature for PCR primers is based on nucleotide sequence numbering derived from GenBank accession no. AF030694 (1). For *pfcrt* region I, nest 1 reactions used 23327up and 24076dn and nest 2 reactions used 23402up and 23939dn (sequence-specific oligonucleotide probe hybridization assays) or 24011dn (direct DNA sequencing) covering polymorphic codons 72-76 and 97 in exon 2. For *pfcrt* region II, nest 1 reactions used 24511up and 25460dn and nest 2 reactions used 24541up and 25145dn (direct DNA sequencing) covering polymorphic codons 220 and 271 in exons 4 and 6, respectively. For *pfcrt* region III, nest 1 reactions used 25441up and 26220dn and nest 2 reactions used 25588up and 26132dn (direct DNA sequencing) covering polymorphic codons 326 and 371 in exons 9 and 11, respectively. Codon 356, which

shows polymorphism in only some of the CQR strains (2), was not analyzed in this study. Nest 1 amplification conditions were: 94°C for 3 min (1x); 94°C for 30 sec, 56°C for 30 sec, 60°C for 1 min (45x); 60°C for 3 min (1x). Nest 2 amplification conditions were: 94°C for 3 min (1x); 94°C for 30 sec, 56°C for 30 sec, 60°C for 1 min (25x); 60°C for 3 min (1x). UTR, untranslated region.

*Nucleotide coordinates based on GenBank accession no. AF030694.

†M13 Forward primer sequence (5′-GTT TTC CCA GTC ACG ACG TTG TAA AAC GAC GGC CAG-3′) added as a 5′ extension.

‡M13 Reverse primer sequence (5′-TGA GCG GAT AAC AAT TTC ACA CAG GAA ACA GCT ATG AC-3′) added as a 5′ extension.

§Probe nomenclature based on polymorphic *Pfcrt* amino acid haplotype spanning residues 72-76.

References:

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