

	Forward RH Primers Gen 2	Tm (°C)
L38I Wt	5' -GAAAGACAAAAGAGGGTGTTrCT/iSpC3/iSpC3/CG-3'	55.8
L38I	5' -GAAAGACAAAAGAGGGTGTTrAT/iSpC3/iSpC3/CG-3'	53.8
S42Y Wt	5' -AGGGTGTTCCTCTATGTAGGCTrCC/iSpC3/iSpC3/AT-3'	59.2
S42Y	5' -AGGGTGTTCCTCTATGTAGGCTrAC/iSpC3/iSpC3/AT-3'	57.6
H50N Wt	5' -AAACCAAGGAGGGAGTGGTGrCA/iSpC3/iSpC3/TT-3'	61.1
H50N	5' -AAACCAAGGAGGGAGTGGTGrAA/iSpC3/iSpC3/TT-3'	58.9
A53E Wt	5' -GGAGTGGTGCATGGTGTGGrCA/iSpC3/iSpC3/AT-3'	62.5
A53E	5' -GGAGTGGTGCATGGTGTGGrAA/iSpC3/iSpC3/AT-3'	60.3
T72K Wt	5' -TGTTGGAGGAGCAGTGGTGA rCG/iSpC3/iSpC3/GT-3'	64.1
T72K	5' -TGTTGGAGGAGCAGTGGTGA rAG/iSpC3/iSpC3/GT-3'	61.7
T75K Wt	5' -AGCAGTGGTGACGGGTGTGA rCA/iSpC3/iSpC3/GT-3'	64.8
T75K	5' -AGCAGTGGTGACGGGTGTGA rAA/iSpC3/iSpC3/GT-3'	62.8
S129Y Wt	5' -CAATGAGGCTTATGAAATGCCTTrCT/iSpC3/iSpC3/GC-3'	58.4
S129Y	5' -CAATGAGGCTTATGAAATGCCTTrAT/iSpC3/iSpC3/GC-3'	56.7
	Reverse Primers	
RP#1	5' -TCTTGCCCCAACTGGTCCTTT-3'	59.2
RP#2	5' -TCCACAGGCATATCTTCCAGAAT-3'	58.3

Amplicon size (bp)
216 with RP#1 265 with RP#2
204 with RP#1 253 with RP#2
180 with RP#1 229 with RP#2
169 with RP#1 218 with RP#2
113 with RP#1 162 with RP#2
104 with RP#1 153 with RP#2
92 with RP#3 219 with RP#4
with RP#1 plus 49