

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Primer Type
oIMR8365	-	GCA AGT TTC TAT ATG CAT TCT C	-	Mutant Forward
oIMR8366	-	CCT CCA TTT CCA ATA GGT AG	-	Mutant Reverse
oIMR8367	-	ATA TGC ATG ATC AAC ACC ACA G	-	Wild type Forward
oIMR8368	-	TTT CCA TTG CTG CCC TAT AG	-	Wild type Reverse

Number of Reactions

Reaction/Components

Reaction Component	Volume (µl)	Final Concentration	Total Volume (µl)
ddH2O	3.56	-	14.24
10 X AB PCR BufferII	1.20	1.00 X	4.80
25 mM MgCl2	0.96	2.00 mM	3.84
2.5 mM dNTP	0.96	0.20 mM	3.84
20 uM oIMR8365	0.20	0.33 uM	0.80
20 uM oIMR8366	0.20	0.33 uM	0.80
20 uM oIMR8367	0.60	1.00 uM	2.40
20 uM oIMR8368	0.60	1.00 uM	2.40
5 mM DNA Loading Dye	1.66	0.69 mM	6.64
5 U/ul Taq DNA Polymerase	0.06	0.03 U/ul	0.24
DNA	2.00	-	8.00

Cycling

Step #	Temp °C	Time	Note
1	94	3 min	-
2	94	30 sec	-
3	55	1 min	-
4	72	1 min	repeat steps 2-4 for 35 cycles
5	72	2 min	-
6	10	-	hold