

ROR gamma

Primers:

ROR1 tgt ggg gac aag tca tct gg
ROR2 gcc cag agc cag gca ctt ct
003-2 caa cca tgg gat cgg cca ttg aac
003-3 gcc gct cag aag aac tcg tca ag

Reaction:

10.0 uL : DNA/H2O in thin-wall tube

Add Master Mix (40 uL per sample)

5.0 uL : 10xBuffer
1.0 uL : 10 mM dNTP
2.5 uL : [20 uM] ROR1/003-2
2.5 uL : [20 uM] ROR2/003-3
0.5 uL : Taq (Fisher)
28.5 uL : H2O

50.0 uL Total

Cycling:

step 1: 94, 3 min
step 2: 1.5 degrees/sec to 55
step 3: 55, 45 sec
step 4: 72, 1 min
step 5: 94, 45 sec
step 6: 1.5 degrees/sec to 55
step 7: 55, 45 sec
step 8: 72, 1 min
step 9: goto step 5, 35 cycles
step 10: 72, 5 min
step 11: 4, for ever
step 12: end

Wild-type: 300 bp
Knock-out: 500 bp

*note - while these primers may be multiplexed to accommodate a single PCR reaction, it is advisable to set up 2 separate reactions. One for the ROR set and one for the NEO set.