

Supplementary 1.

Reference sequence detail for ACE gene 600 bps within rs4343 (gen bank, NCBI databased). Ref|NC_000017.11|:63488370-63488970 Homo sapiens chromosome 17, GRCh38.p14 Primary Assembly. SNP rs4343 located at: Chr17: 63.488.670 (located at 301 base sequences in this figure which represent G allele)

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1  aaaaaaaaaa aaggagagga gagagactca agcacgcccc tcacaggact gctgaggccc
61  tgcaggtgtc tgcagcatgt ggccccaggc cggggactct gtaagccact gctggagagc
121 cactcccata ctttctccca tttctctaga cctgctgcct atacagtcac ttttatgtgg

181 tttcgccaat tttattc cag ctctgaaatt ctctgagctc cccttacaag
    cagaggtgag

241 ctaagggctg gagctcaagg cattcaaacc cctac cagat ctgacgaatg
    tgatggccac

SNP rs4343
301 gtcccgaaa tatgaagacc tgttatgggc atgggagggc tggcgagaca
    aggcggggag

361 agccatcctc cagttttacc cgaaatacgt ggaactcadc aaccaggctg ccgggctcaa

421 tggtagagtc ctgctgccaa catcactggc acttggtgtec cttcattttc
    ctcaaagagg

481 tgctgtgaaa ccccaagcct aggaaaaggt agatccctgg aggaggcagg taatgtggtg
541 ttgggagagc ctggctgtgt ccctctgtga ggctatgtag atgcagggga ctgctggagg
601 t
  
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Diagram illustrating the reference sequence detail for the ACE gene 600 bps within rs4343 (gen bank, NCBI databased). The sequence is shown with various primers and mismatches highlighted. The SNP rs4343 is located at position 301, where the G allele (G) is present. The sequence is divided into two main regions: the 5' region (left) and the 3' region (right). The 5' region contains the primer FO (forward outer) and the 3' region contains the primer RO (reverse outer). The primer Fi (A allele) is located at position 241, and the primer Ri (G allele) is located at position 301. The sequence is shown with various mismatches highlighted in green and red. The sequence is also shown with various primers and mismatches highlighted in green and red.

Primer design for this study

Fi: 5' CATATTTGACGAATGTGATGGCCCA 3' (276-301 forward inner primer for A allele with 5 base mismatch from gen bank reference sequences, colored in green letter).

Ri: 5' CCAATAACAGGTCTTCATATTTCCGGTAC 3' (301-330 reverse inner primer for G allele, with 2 base mismatch from gen bank sequences, colored in green letter)

FO: 5' CATTTCTGAAATTCTCTGAGCTCCCT 3' (198-224 forward outer primer with 2 base mismatch from gen bank reference sequences in 3' side, colored in green letter)

RO: 5' TTAGGAAAATGAAGGGACCCAAGTGC 3' (449-476 reverse outer primer with 1 base mismatch from gen bank reference sequences in 5' side, colored in green letter)

Product size for A allele: 202 (located at 276-476; Fi-RO)

Product size for G allele: 134 (located at 198-330; FO-Ri)

Product size of two outer primers: 280 (located at 198-476; FO-RO)

Tetra Primer specific for SNP rs4343 ACE gene used in TETRA ARMS PCR, anneal in reference sequences and the PCR product size.