

COLLEGEN TYPE 1 RECEPTOR (CD36)

TAQMAN

Location: Chromosome 7q11.2.
 Polymorphism: 15 bp Insertion/Deletion.

PCR COCKTAIL:

REAGENT	[STOCK]	[FINAL]	AMT. PER SAMPLE
dd H ₂ O	----	----	7.72 µL
CD36 FAM probe (I)	10.0 µM	0.2 µM	0.44 µL
CD36 VIC probe (D)	10.0 µM	0.2 µM	0.44 µL
CD36-F primer	100 µM	0.9 µM	0.20 µL
CD36-R primer	100 µM	0.9 µM	0.20 µL
Universal Master Mix (ABI)	2X	1X	11.00 µL
DNA (Proteinase K treated)	15 ng/µL	30 ng	<u>2.00 µL</u>
			22.00 µL

Probe Sequences: (Applied Biosystems)

CD36 FAM (I): 5'-6FAM-AATAGCACAATAAAGCACTTGTGCC-TAMRA-3'

CD36 VIC (D): 5'-VIC-ACCATTGTAACAATAGTGTGCCAAA-TAMRA-3'

NOTE: Designed TURBO probes.

Primer Sequences: (Genosys)

CD36-F: 5'-CGC AGA TCA CTA AAG TAT ATC TTT AAT TCT G -3'

CD36-R: 5'-AAT GAA CCA GTC AAT TTT GGA CAA C -3'

PCR CYCLE:

TEMPERATURE	TIME	CYCLES
50 ° C	2 min	1 cycle
95 ° C	10 min	1 cycle
95 ° C	15 sec	40 cycles
62 ° C	1 min	

Length of PCR fragment: 125 bp Insertion / 110 bp Deletion.

REFERENCES:

GenBank: Z32764.

Protocol designed by ARIC DNA Laboratory.

FOR PUBLICATION:

Genotyping of a 15 bp insertion/deletion in the Collagen Type I Receptor (CD36) gene was performed using the TaqMan assay (Applied Biosystems). A maximum 125-bp product was amplified utilizing 0.9 μ M each of the forward primer 5'-CGCAGATC ACTAAAGTATATCTTTAATTCTG-3' and the reverse primer 5'-AATGAACC AGTCAATTTTGGACAAC-3', 30 ng DNA, 5.0 mM MgCl₂, and 1X TaqMan Universal PCR Master Mix containing AmpliTaq Gold DNA Polymerase in a 22 μ l reaction volume. After an initial step of 2 min at 50°C and 10 min at 95°C to activate the AmpliTaq Gold, the products were amplified using 40 cycles of 15 s at 95°C and 1 min at 62°C. A total of 0.2 μ M of each of the sequence-specific probes 5'-6FAM-AATAGCACAAATAAAGCACTTGTGCC-TAMRA-3' and 5'-VIC-ACCATTG TAACAATAGTGTGCCAAA-TAMRA-3' was used in the allele discrimination assay, and allele detection and genotype calling were performed using the ABI 7700 and the Sequence Detection System software (Applied Biosystems).