

GENOTYPING BY PCR PROTOCOL
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 530-754-MMRRC

NAME OF PCR: FVB/N-Tg(APOA1-Scarb1)1Hgc/Mmucd MMRRC # 009982-UCD

DNA Extraction Method: ☐ NaOH ☒ Proteinase K ☐ Other: _____

Protocol:

Reagent/ Constituent	Volume (μL)
Water	14.75
10x Buffer (contains 15mM MgCl ₂)	2.5
dNTPs (stock concentration is 10mM)	0.5
Primer 1 (stock concentration is 25μM) ApoA1-341F	0.5
Primer 2 (stock concentration is 25μM) SRB1-177R	0.5
Taq Polymerase (5Units/μL)	0.25
DNA Sample	5.0
TOTAL VOLUME OF REACTION:	25μL

Comments on protocol:

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☒	95	15:00	1
2. Denaturation	94	0:45	} 30x
3. Annealing	60	1:00	
4. Elongation	72	1:00	
5. Amplification	72	5:00	1
6. Finish	4	∞	n/a

Primers:

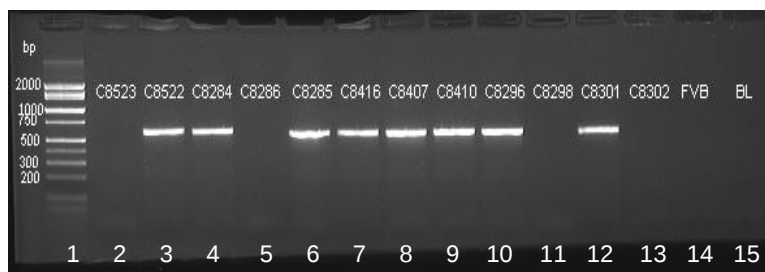
Name	Nucleotide Sequence (5' - 3')
1: ApoAv Fwd	TAT TTC AAG CAG AGG GAA GCC TGT A
2: ApoAv Rev	GCC CAT TTA ATT CAT CCT CCA CTG T

Electrophoresis Protocol:

% Agarose: 2 V: 160

Estimated Running Time (min): _____

Expected Bands	Genotype
no band	WT +/+
~600 bp	Transgene



Lanes:
 1. 1Kb+ ladder
 2, 5, 11, 13. Samples, WT +/+
 3, 4, 6-10, 12. Samples, Tg/+
 10. Wild-type control +/+
 15. BL-no DNA control