

GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
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 530-754-MMRRC

NAME OF PCR: FVB/N-Tg(APOA5)1Hgc/Mmcd **MMRRC #** 009981-UCD

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

Protocol:

Reagent/ Constituent	Volume (µL)
Water	14.75
10x Buffer (contains 15mM MgCl ₂)	2.5
MgCl ₂ (stock concentration is 25mM)	1.0
dNTPs (stock concentration is 10mM)	0.5
Primer 1 (stock concentration is 25µM) ApoAv Fwd	0.5
Primer 2 (stock concentration is 25µM) ApoAv Rev	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	} 35x
3. Annealing } steps 2-3-4 will cycle in sequence	60	1:00	
4. Elongation	72	1:00	
5. Amplification	72	5:00	1
6. Finish	4	∞	n/a

Primers:

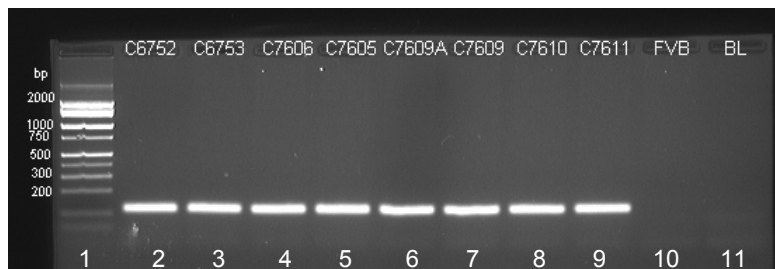
Name	Nucleotide Sequence (5' - 3')
1: ApoAv Fwd	TAT TTC AAG CAG AGG GAA GCC TGT A
2: ApoAv Rev	GCC CAT TTA ATT CAT CCT CCA CTG T

Electrophoresis Protocol:

% Agarose: 2 **V:** 160

Estimated Running Time (min): _____

Expected Bands	Genotype
no band	WT +/+
~100 bp	Transgene



Lanes:
 1. 1Kb+ ladder
 2-9. Samples, Tg/+
 10. Wild-type +/+
 11. BL-no DNA control