

GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

NAME OF PCR: FVB.129X1(B6)-Apoa5^{tm1Hgc}/Mmucd

MMRRC # 011467-UCD

Protocol:

Reagent/Constituent	Volume (μL)
Water	11.275
10x Buffer	2.5
MgCl ₂ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) <i>Optional</i>	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO <i>Optional</i>	0.325
Primer 1 (stock concentration is 20μM) NeoF1	0.5
Primer 2 (stock concentration is 20μM) NeoR1	0.5
Primer 3 (stock concentration is 20μM) mApoAV-F2	0.0
Primer 4 (stock concentration is 20μM) mApoAV-R2	0.0
Taq Polymerase 5Units/μL	0.2
DNA (50-200ng/ μL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	25.000 μL

Comments on protocol:

- Perform PCR for AV gene and Neomycin gene separately.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65°C decreasing in temperature by 1.0°C; next 30 cycles anneal at 55°C.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust MgCl₂ to increase reaction or decrease non-specific amplifications.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	5:00	1
2. Denaturation	94	0:15	
3. Annealing steps 2-3-4 cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	40x
4. Elongation	72	0:40	
5. Amplification	72	5:00	1
6. Finish	15	∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: <u>1.5%</u>	V: <u>90</u>
1. NeoF1	CTTTTGTCAAGACCGACCTG	Estimated Running Time: <u>90</u> min.	
2. NeoR1	AATATCACGGGTAGCCAACGC	Primer Combination	Band
3. mApoAV-F2	ACAGTTGGAGCAAAGGCGTGAT	1 and 2	545 bp
4. mApoAV-R2	CTTGCTCGAAGCTGCCTTTCAG	3 and 4	517 bp
			Genotype
			Mutant
			WT

