GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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DNA Extraction Method: NaOH _____ Proteinase K _X_ Other_____

Protocol: **NAME OF PCR:_LIV-DGAT2 GENOTYPING**_____

Reagent/ Constituent	Volume (uL)
DNA Sample	1
10x Buffer (contains 15mM MgCl2)	5
dNTPs (stock concentration is 25mM)	4
Primer **S7** (stock concentration is 10 uM)	1
Primer **AS4** (stock concentration is 10 uM)	1
Taq Polymerase (LA Taq)	0,25
H2O	37,75
TOTAL VOLUME OF REACTION:	**50 ul**

Comments on protocol *(e.g.,* different concentration of MgCl2, etc): _____

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Strategy:

Steps	Temp (^oC)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [ ]	95	2	**1**
2. Denaturation } steps 2-3-4 will cycle in sequence	95	30 sec	35
3. Annealing	55	30 sec	35
4. Elongation	72	1	35
5. Amplification (i.e., 72^{o}C, 10 min)	72	10	**1**
6. Finish (i.e., 4^{o}C, indefinite)	4	n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5^- - 3^-)
S7:	**AGCTGGTGAAGACACACAACC**
AS4:	**TGATGATAGCATTGCCACTCCC**
3:	
4:	

Electrophoresis Protocol:

% Agarose: _1_ V : _100

Estimated Running Time (min): _45___

Primer combination	Band (bp)	genotype
S7 AS4	~300	transgenic
S7 AS4	~800	WT

PASTE GEL PICTURE HERE, CLEARLY INDICATING LADDER, WATER CONTROL, DNA CONTROL, & DIAGNOSTIC SAMPLES