GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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DNA Extraction Method: Proteinase K

Protocol: **NAME OF PCR: MMRRC#31941/ Mgat5b/ GlcnacT9-CKO**

Reagent/ Constituent	Volume (uL)
DNA Sample	2.0
10x Buffer (contains 15mM MgCl2)	2.5
dNTPs (stock concentration is 25mM)	2.0
Primer 1627 (stock concentration is 20 uM)	0.25
Primer 1628 (stock concentration is 20 uM)	0.25
Taq Polymerase	0.12
H2O	18.6
TOTAL VOLUME OF REACTION:	**25 ul**

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

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Strategy:

Steps	Temp (°C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [ ]	95	5:00	1
2. Denaturation	94	0:30	30
3. Annealing } steps 2-3-4 will cycle in sequence	58	0:30	30
4. Elongation	72	0:30	30
5. Amplification (i.e., 72°C, 10 min)	72	5:00	1
6. Finish (i.e., 4°C, indefinite)	4	∞	∞

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: Primer 1627	**TTGGGGACTTGCTACTCCAGA**
2: Primer 1628	**AGAGCCAGCAAGCGAGAGAA**

Electrophoresis Protocol:

% Agarose: __1.3__ V : ___120___

Estimated Running Time (min): ___90___

Primer combination	Band (BP)	genotype
Primer 1627 & 1628	223	WT
Primer 1627 & 1628	~280	Floxed