GENOTYPING BY PCR PROTOCOL FORM

DNA Extraction Method: NaOH _____ Proteinase K __X__ Other_____

Protocol: **NAME OF PCR:_____MC5R Gene PCR_____**

Reagent/ Constituent	Volume (uL)
DNA Sample	5.0
2x Buffer (Proprietary Promega GoTaq Green Mix)	12.5
Primer **1** (stock concentration is 10 uM)	0.5
Primer **2** (stock concentration is 10 uM)	0.5
H20	6.5
Additives if applicable:	
TOTAL VOLUME OF REACTION:	**25 ul**

Comments on protocol *(e.g.,* different concentration of MgCl2, etc): _PCR mix is Promega# M7123____

Strategy:

Steps	Temp (°C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [ ]	95	2:00	**1**
2. Denaturation } steps 2-3-4 will cycle in sequence	95	0:30	**35**
3. Annealing	60	0:30	**35**
4. Elongation	72	1:00	**35**
5. Amplification (i.e., 72°C, 10 min)	72	7:00	**1**
6. Finish (i.e., 4°C, indefinite)	10	n/a	**n/a**

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: MC5R Fwd	**tgg gtc tcg tca gcc tct ta**
2: MC5R Rev	**ggt aat agc ccc ctt cat gct**

Electrophoresis Protocol:

% Agarose: __1.0__ V : __120_

Estimated Running Time (min): ___15___

Primer combination	Band (bp)	genotype
1&2	**580**	MC5R +
	No band	MC5R -

NOTE: For MC5R genotyping you have to run MC5R and NEO PCR reactions. Reactions have to be run separately but can be run on the same block as work with the same reaction conditions.

GENOTYPING BY PCR PROTOCOL FORM

DNA Extraction Method: NaOH _____ Proteinase K __X__ Other_____

Protocol: **NAME OF PCR:_____NEO gene PCR_____**

Reagent/ Constituent	Volume (uL)
DNA Sample	5.0
2x Buffer (Proprietary Promega GoTaq Green Mix)	12.5
Primer **1** (stock concentration is 20 uM)	0.5
Primer **2** (stock concentration is 20 uM)	0.5
H20	6.5
Additives if applicable:	
TOTAL VOLUME OF REACTION:	**25 ul**

Comments on protocol *(e.g.,* different concentration of MgCl2, etc): _adapted from Jackson Lab's Neo genotyping protocol at: http://jaxmice.jax.org/pub-cgi/protocols/protocols.sh?objtype=protocol&protocol_id=701

Strategy:

Steps	Temp (°C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [ ]	95	2:00	**1**
2. Denaturation } steps 2-3-4 will cycle in sequence	95	0:30	**35**
3. Annealing	60	0:30	**35**
4. Elongation	72	1:00	**35**
5. Amplification (i.e., 72°C, 10 min)	72	7:00	**1**
6. Finish (i.e., 4°C, indefinite)	10	n/a	**n/a**

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: Neo 1	**ctt ggg tgg aga ggc tat tc**
2: Neo 2	**agg tga gat gac agg aga tc**

Electrophoresis Protocol:

% Agarose: __1.5__ V : __120____

Estimated Running Time (min): __20___

Primer combination	Band (bp)	genotype
1&2	280	NEO
	none	WT