GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: C57BL/6JApb-*Ncaph2*^{nes}/Mmcd, (nessy) **MMRRC #** 031692-UCD

Protocol:

Reagent/ Constituent	Volume (µL)
Water	12.6
10x Buffer (contains 15mM $MgCl_2$) Use Platinum Pfx 10x buffer	1.5
dNTPs (stock concentration is 10mM)	0.6
Primer 1 (stock concentration is 20µM) Forward	0.15
Primer 2 (stock concentration is 20µM) Reverse	0.15
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	**15µL**

Comments on protocol:

We have used other primer sequences successfully, so primers can be modified. Please note that kleisin b has two very similar pseudogenes on chromosome 15. These need to be considered when designing primers. This is the major reason we have not used ms PCR.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	95	3:00	**1**
2. Denaturation	95	0:30	
3. Annealing } steps 2-3-4 will cycle in sequence	55	0:30	} **40x**
4. Elongation	68	1:00	
5. Amplification	68	10:00	**1**
6. Finish	4	∞	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: Foward	CCG CTA ACC ACC TAG AAA CTT AGT C
2: Reverse	AAA CGG AAA TAA TGT ACT TGT TGG A

Restriction Digest Cutting overnight at 37 °C

Steps	Volume (µL)
PCR Product	2.5
Water	19.25
NEB Buffer I	2.5
100x BSA } supplied with enzyme	1.25
BccI Enzyme (NEB)	1.5
TOTAL VOLUME:	**27µL**

Electrophoresis Protocol:

% Agarose: 1.5 **V:** _____

Expected Band (bp)	Genotype
300 / 321	WT +/+
621	Hom -/-
321 / 621	Het +/-