GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: B6;129X1-*Evl*^{tm1Lfr}/Mmucd **MMRRC #** 000195-UCD

Protocol: (Neo Tcrd Duplex)

Reagent/ Constituent	Volume (µL)
Water	17.675
10x Buffer (contains / without 15mM $MgCl_2$)	2.5
$MgCl_2$ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) *Optional*	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO *Optional*	0.325
Primer 1 (stock concentration is 20µM) Neo TD F	1.0
Primer 2 (stock concentration is 20µM) Neo TD R	1.0
Primer 3 (stock concentration is 20µM) Tcrd F	0.6
Primer 4 (stock concentration is 20µM) Tcrd R	0.6
Taq Polymerase 5Units/µL	0.125
DNA extracted with ☐ NaOH ☒ Proteinase K ☐ Other:	1.0
TOTAL VOLUME OF REACTION:	**25µL**

Comments on protocol:

- This protocol only indicates the presence or absence of internal Neo vector; it does not distinguish heterozygous vs. knockout, nor is it specific to any single construct. TCRD is an internal control to verify DNA is present.
- Betaine/DMSO is standardized due to high GC content in promoter regions and protocol may be tested without. Also, may adjust $MgCl_2$ to increase reaction or decrease non specific amplifications.

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	3:00	**1**
2. Denaturation		94	0:20	
3. Annealing	steps 2-3-4 will cycle in sequence	64	0:30	**12x**
4. Elongation		72	0:35	
5. Denaturation		94	0:20	
6. Annealing	steps 5-6-7 will cycle in sequence	58	0:30	**25x**
7. Elongation		72	0:35	
8. Amplification		72	2:00	**1**
9. Finish		10	∞	n/a

Primers:

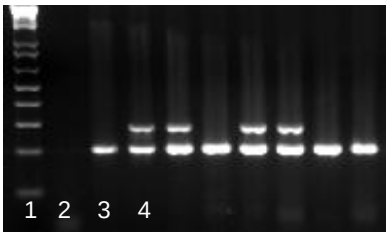
Name	Nucleotide Sequence (5' - 3')
1: Neo TD F	CTT GGG TGG AGA GGC TAT TC
2: Neo TD R	AGG TGA GAT GAC AGG AGA TC
3: Tcrd F	CAA ATG TTG CTT GTC TGG TG
4: Tcrd R	GTC AGT CGA GTG CAC AGT TT

Electrophoresis Protocol:

Agarose: 2% **mV:** 80

Estimated Running Time (min): 90

Expected Bands	Genotype
200 bp	WT +/+
280 bp	Neo +



Lanes:
1. 1Kb+ ladder
2. H_2O
3. Wild-type +/+
4. Neo +