

GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: B6;129X1-Itga8^{tm1Lfr}/Mmucd, MMRRC # 000193-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) <i>Optional</i>	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO <i>Optional</i>	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 MgCl₂ 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	} 12x
3. Annealing	64	0:30	
4. Elongation	72	0:35	
5. Denaturation	94	0:20	} 25x
6. Annealing	58	0:30	
7. Elongation	72	0:35	
8. Amplification	72	2:00	1
9. Finish	10	∞	n/a

Primers:

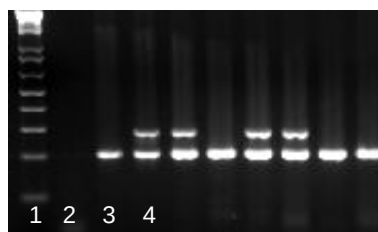
Name	Nucleotide Sequence (5' - 3')
1: Neo TD F	CTT GGG TGG AGA GGC TAT TC
2: Neo TD R	AGG TGA GAT GAC AGG AGA TC
3: Tcrd F	CAA ATG TTG CTT GTC TGG TG
4: Tcrd R	GTC AGT CGA GTG CAC AGT TT

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. H₂O
3. Wild-type +/-
4. Neo +