GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: **B6.129X1-*Ntrk2*^{tm1Lfr}/Mmucd** **MMRRC #** **000187-UCD**

Protocol:

Reagent/ Constituent	Volume (µL)
Water	17.6
10x Buffer (contains 15mM $MgCl_2$)	2.5
$MgCl_2$ (stock concentration is 25mM)	1.7
dNTPs (stock concentration is 10mM)	0.5
Primer 1 (stock concentration is 25µM) TrkB n2	0.5
Primer 2 (stock concentration is 25µM) TrkB c8	0.5
Primer 3 (stock concentration is 25µM) TrkB c7	0.5
Taq Polymerase 5Units/µL	0.2
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	**25µL**

Comments on protocol:

TrkB is an older synonym of the gene ***Ntrk2***. Use a simplex reaction with TrkB n2 and TrkB c7 to amplify only the transgenic allele.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	3:00	**1**
2. Denaturation	94	1:00	
3. Annealing } steps 2-3-4 will cycle in sequence	63.5	1:30	} **35x**
4. Elongation	72	1:30	
5. Amplification	72	7:00	**1**
6. Finish	4	∞	n/a

Primers:

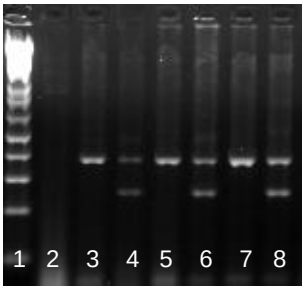
Primer Name	Nucleotide Sequence (5' - 3')
1: TrkB n2	ATG TCG CCC TGG CTG AAG TG
2: TrkB c8	ACT GAC ATC CGT AAG CCA GT
3: TrkB c7	GAT GAT TTC TAG CCT TTT CTG G

Electrophoresis Protocol:

% Agarose: 2 **mV:** 80

Estimated Running Time (min): 90

Expected Bands	Genotype
369 bp	WT +/+
245 bp	Tg/+



Lanes:
1. 1Kb+ ladder
2. H_2O
3. Wild-type +/+ sample
4. Tg, hemizygous sample
5 & 7. WT
6 & 8. Tg