

GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
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 530-754-MMRRC

NAME OF PCR: B6.129X1-*Ntrk3*^{tm1(cre)Lfr}/Mmcd **MMRRC #** 000364-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
Water	13.5
10x Buffer (contains 15mM MgCl ₂)	2.5
5x Q Solution	5.0
dNTPs (stock concentration is 10mM)	0.5
Primer 1 (stock concentration is 10μM) Trkc2B-F (common forward)	0.5
Primer 2 (stock concentration is 10μM) Trkc2-R (wild-type reverse)	0.5
Primer 3 (stock concentration is 10μM) IRES2-R (mutant reverse)	0.5
Taq Polymerase 5 Units/μL	0.5
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	25μL

Comments on protocol:

Q Solution is found in Qiagen PCR Reagent Kit. Can use two separate reactions if multiplex is problematic.
 For simplex Wild-type PCR, knockout may show a 2256 bp band.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? CHECK HERE ☐	94	5:00	1
2. Denaturation	94	0:30	} 30x
3. Annealing	60	1:00	
4. Elongation	72	1:00	
5. Amplification	72	5:00	1
6. Finish	25	∞	n/a

Primers:

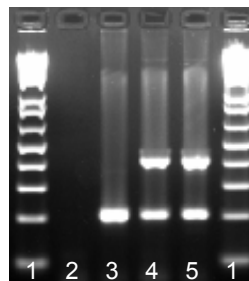
Primer Name	Nucleotide Sequence (5' - 3')
1: Trkc2B-F	TCC TCC ATG CTT TGG GGA AGG CCA C
2: Trkc2-R	CAG TGT TGT ATG TGT AGC AGG CAC T
3: IRES2-R	TGC CGC CTT TGC AGG TGT GTC TTA C

Electrophoresis Protocol:

% Agarose: 2 mV: 110

Estimated Running Time (min): 60

Expected Bands	Genotype
204 bp	WT +/+
204 /407 bp	Het +/-
407 bp	Hom -/-



Lanes:
 1. 1Kb+ ladder
 2. H₂O control
 3. B6 WT +/+ sample
 4-5. Het +/- Tici TK

Homozygous sample not available for photo purposes