

GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: C57BL/6-Unc13d^{jinx}/Mmcd, (jinx) MMRRC # 016137-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
Water	12.675
10x Buffer (contains 15mM MgCl ₂)	2.5
Betaine (stock concentration is 5M) <i>Optional</i>	6.5
dNTPs (stock concentration is 25mM)	0.5
DMSO <i>Optional</i>	0.325
Primer 1 (stock concentration is 20μM) jinx PCR F1	0.5
Primer 2 (stock concentration is 20μM) jinx PCR R1	0.5
Taq Polymerase	0.5
DNA sample extracted with ☐ NaOH ☐ Proteinase K ☒ Other: Any	1.0
TOTAL VOLUME OF REACTION:	25μL

Comments on protocol:

- PCR products are verified to contain the correct amplicon size by running ~10μl of the reaction on a gel and the remaining 15μl purified via column based PCR purification method for sequencing.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65° C decreasing in temperature by 1.0 ° C; next 30 cycles anneal at 55° C.
- Betaine and DMSO have been standardized due to high GC content. 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	5:00	1
2. Denaturation	94	0:15	} 10x
3. Annealing } steps 2-3-4 will cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	
4. Elongation	72	0:40	} 30x
5. Denaturation	94	0:15	
6. Annealing } steps 5-6-7 will cycle in sequence	55	0:30	
7. Elongation	72	0:40	
8. Amplification	72	5:00	1
9. Finish	15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: jinx PCR F1	CTA CAT GAA CAC CAA CCT GGT CCA GGA G
2: jinx PCR R1	GGA TCA TGA AGA GGA AGG AGA TGC AGT TAG G
3: jinx_Seq (F)	TAC ATG AAC ACC AAC CTG GTC C-
4: jinx_Seq (R)	AGA GGA AGG AGA TGC AGT TAG G

Electrophoresis Protocol:

Agarose: 2% mV: 80 Estimated Running Time: 90 min

Primer Combination	Band	Genotype
1 and 2	775 bp	<i>jinx</i>
SNP found at position ~ 96 of sequencing		

Mutation site (red) and flanking sequence:

WT cacgagt**C**agtggcatgttg

jinx cacgagt**A**agtggcatgttg