GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC

NAME OF PCR: B6;129S4-*Neurod1*^{tm1Jle}/Mmucd **MMRRC #** 000367-UCD

Protocol:

Reagent/ Constituent	Volume (µL)
Water	10.775
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) NDW-1	0.5
Primer 2 (stock concentration is 20µM) NDW-2 (JL-25)	0.5
Primer 3 (stock concentration is 20µM) NDW-3	0.5
Taq Polymerase 5Units/µL	0.2
DNA extracted with ☒ NaOH ☐ Proteinase K ☐ Other:	1.0
TOTAL VOLUME OF REACTION:	**25µL**

Comments on protocol:

- 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/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	5:00	**1**
2. Denaturation		94	0:15	
3. Annealing	steps 2-3-4 will cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	**10x**
4. Elongation		72	0:40	
5. Denaturation		94	0:15	
6. Annealing	steps 5-6-7 will cycle in sequence	55	0:30	**30x**
7. Elongation		72	0:40	
8. Amplification		72	5:00	**1**
9. Finish		15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: NDW-1	ACC ATG CAC TCT GTA CGC ATT
2: NDW-2 (JL-25)	GAG AAC TGA GAC ACT CAT CTG
3: NDW-3	AAA CGC CGA GTT AAA GCC ATC

Electrophoresis Protocol:

Agarose: 2% **V:** 100 **Estimated Running Time:** 60 **min**

Expected Bands	Genotype
433 bp	WT +/+
643 / 433 bp	HET +/-
643 bp	KO -/-