GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: B6;129S5-*Kl*^{tm1Lex}/Mmucd **MMRRC: 011732-UCD**

Protocol:

Reagent/Constituent	Volume (µL)
Water	10.275
10x Buffer	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)	0.5
Primer 2. (stock concentration is 20µM)	0.5
Primer 3. (stock concentration is 20µM)	0.5
Primer 4. (stock concentration is 20µM)	0.5
Taq Polymerase 5Units/µL	0.2
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
	TOTAL VOLUME
	25

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65ºC decreasing in temperature by 1.0ºC; next 30 cycles anneal at 55ºC.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust $MgCl_2$ to increase reaction or decrease non-specific amplifications.

Strategy:

Steps		Temp (ºC)	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 cycle in sequence	65 to 55 (↓1ºC/cycle)	0:30	**40x**
4. Elongation		72	0:40	
5. Amplification		72	5:00	**1**
6. Finish		15	∞	n/a

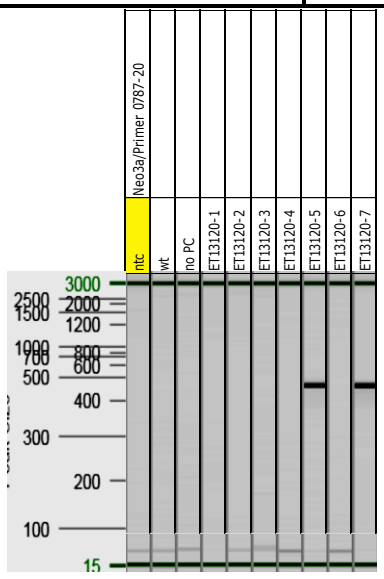
Primers:

Name	Nucleotide Sequence (5' - 3')
1. Primer 0787-12	GATGGGGTCGACGTCA
2. Primer 0787-13	TAAAGGAGGAAAGCCATTGTC
3. Primer Neo3a	GCAGCGCATCGCCTTCTATC
4. Primer 0787-20	ATGCTCCAGACATTCTCAGC

Electrophoresis Protocol:

Agarose :1.5% V:90
Running Time: 90 min.

Primer	Band (bp)	Genotype
1 & 2	186	wildtype
3 & 4	455	mutant



PCR protocol developed by MMRRC at University of California, Davis