GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC

Protocol Name: **B6N.129S5-*Layn*^{tm1Lex}/Mmucd** **MMRRC:** **032426-UCD**

Protocol:

Reagent/Constituent	Volume (µL)
Water	9.775
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
	24.5

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. DNA026-15	TAGGCAAACTCAGAATCTCCC
2. GT IRES	CCCTAGGAATGCTCGTCAAGA
3. DNA026-5	TTTGGCTAAGCAGAGGAGC
4. DNA026-6	CAGACTGCCAAGAGAAAGC

Electrophoresis Protocol:

Argarose: **1.5%** V: **90**

Estimated **9** min.

Primer	**Band** (bp)	**Genotype**
1 & 2	572	mutant
3 & 4	271	wildtype