

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

Protocol Name: B6N.129S5-*Il1f5*^{tm1Lex}/Mmucd MMRRC: 032394-CD

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) <i>Optional</i>	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO <i>Optional</i>	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 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.

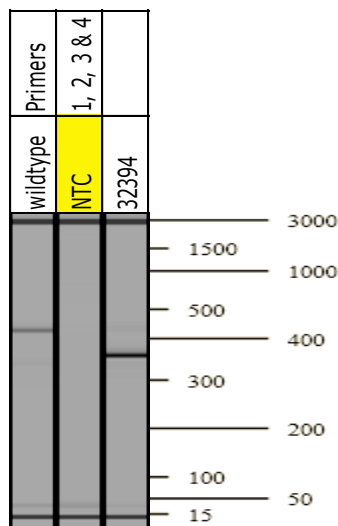
Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	5:00	1
2. Denaturation	94	0:15	
3. Annealing steps 2-3-4 cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	40x
4. Elongation	72	0:40	
5. Amplification	72	5:00	1
6. Finish	15	∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. Neo3a	GCAGCGCATCGCCTTCTATC	Estimated 9 min.		
2. UNQ1896-16	GAGCTCCATGATGTTCACTGG	Primer	Band (bp)	Genotype
3. UNQ1896-7	GAGCTGCATCCTGTCTATCG	1 & 2	384	mutant
4. UNQ1896-8	CCTCTAGCTGATATACATCC	3 & 4	448	wildtype



PCR protocol developed by MMRRC at University of California, Davis