

GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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 530-754-MMRRC

NAME OF PCR: B6.129S-Il21^{tm1Lex}/Mmucd

MMRRC # 032800-UCD

Protocol: Run as Simplex, Multiplex may not work.

Reagent/ Constituent	Volume (µL)
Water	11.475
10x Buffer (without MgCl ₂)	2.5
MgCl ₂ (stock concentration is 25mM)	1.75
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) Neo3a	0.4
Primer 2 (stock concentration is 20µM) 0767-R	0.4
Primer 3 (stock concentration is 20µM) 0767-2	0.0
Primer 4 (stock concentration is 20µM) 0767-3	0.0
Taq Polymerase 5Units/µL	0.15
DNA extracted with <input checked="" type="checkbox"/> NaOH <input type="checkbox"/> Proteinase K <input type="checkbox"/> Other:	1.0
TOTAL VOLUME OF REACTION:	25.000µL

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 MgCl₂ to increase reaction or decrease non specific amplifications.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input type="checkbox"/>	94	5:00	1
2. Denaturation	94	0:15	} 10x
3. Annealing	65 to 55 (↓1°C/cycle)	0:30	
4. Elongation		0:40	
5. Denaturation	94	0:15	
6. Annealing	55	0:30	
7. Elongation		0:40	
8. Amplification	72	5:00	1
9. Finish	15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: Neo3a	GCAGCGCATCGCCTTCTATC
2: 0767-R	ACCATCTACTGACTTGTTAGACTC
3: 0767-2	GGAGACTCAGTTCTGGTGG
4: 0767-3	GGAGCTGATAGAAGTTCAGG

Electrophoresis Protocol:

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

Primer Combination	Band	Genotype
1 and 2	230 bp	KO
3 and 4	305 bp	WT

