

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: CR10865 Mirlet7i KO

Protocol:

Reagent/Constituent	Volume (μL)
Water	11.275
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
Taq Polymerase 5Units/μL	0.2
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	25.000 μL

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? <input type="checkbox"/>	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')	Agarose: 1.5% V: 90		
1. CR_Mirlet7i_ivqcF3	CAGCTCCGCTCGGGCAGA	Estimated Running Time: 90 min.		
2. CR_Mirlet7i_ivqcR2	ATGCGGGGAGCTAACGGTTTC	Primer Combination	Band (bp)	Genotype
3.		1 & 2	348	wildtype
		1 & 2	176	mutant

Allele Description: Exon 1 [ENSMUSE00000522631](#) flanking splicing regions were constitutively deleted from the Mirlet7i gene [ENSMUSG00000065406.3](#) using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

