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

mmrrc@ucdavis.edu 530-754-MMRRC

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) 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
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.

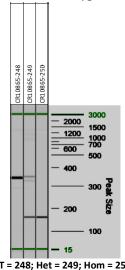
Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	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.



WT = 248; Het = 249; Hom = 250