

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: **CR11677 Pik3r3 EXDEL**

Stock # 75980

Protocol: **GoTaq® G2 Colorless Master Mix(Promega)**

Reagent/Constituent	Volume (μL)
Water	4.5
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20μM) comF	0.5
Primer 2. (stock concentration is 20μM) wtR	0.5
Primer 3. (stock concentration is 20μM) mutR	0.5
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.5
TOTAL VOLUME OF REACTION:	15.00 μL

Comments on protocol:

- Protocol may work with other DNA extraction methods.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2:00	1x
2. Denaturation	94	0:10	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	10x
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	25x
7. Elongation	68	2:00 (↑20sec/cycle)	
8. Finish	4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5%	V: 90
1. CR_Pik3r3_comF	CAGGCTAGTTCAGCAGACCTTACC	Estimated Running Time: 90 min.	
2. CR_Pik3r3_wtR*	GAAATATCTCCCCAGTACCACTCTGC	Primer Combination	Band (bp)
3. CR_Pik3r3_mutR	GTCTATGGATGTTTGCCTGTATGC	1 & 2, 1 & 3	575, 1255
		1 & 3	696
			wildtype
			mutant

Electrophoresis Protocol:

Allele Description: Exon 2 ENSMUSE00000181454 and flanking splicing regions were constitutively deleted from the Pik3r3 Gene ENSMUSG00000028698 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 559bp deletion from Chr4:116113232-116113790 GRCm39 was screened by PCR analysis. The selected founder animal was backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals for establishing and archiving the line.

*wtR primer untested (ePCR verified)

