

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR11531 Amdhd2 EXDEL

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

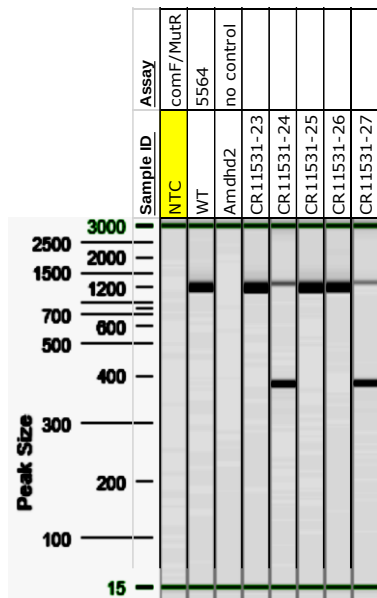
Primers:

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5%	V: 90
1. CR_Amdhd2_comF	CGACTCACGATGCGTAGC	Estimated Running Time: 90 min.	
2. CR_Amdhd2_wtR*	CCACACACCCACGATAGTCC	Primer Combination	Band (bp)
3. CR_Amdhd2_mutR	GCAGGTTAGGGAGGAAGAGC	1 & 2, 1 & 3	232, 992
		1 & 3	375
			wildtype
			mutant

Electrophoresis Protocol:

Allele Description: Exon 2-3(ENSMUSE00001262581, ENSMUSE00001218513) and flanking splicing regions were constitutively deleted from the Amdhd2 Gene ENSMUSG00000036820 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 617bp deletion from Chr 17: 24381888 - 24382504 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)



PCR protocol developed by MMRRC at University of California, Davis