

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: CR10742 Ms4a4a iDex

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. Ms4a4a-cond-IV5F	GGTTCTAGCATATGTAAGTCACTCCTATCC	Estimated Running Time: 90 min.
2. Ms4a4a-cond-IV5R	CACACAGACTCACTGTGAAACCCTT	Primer Combination Band (bp) Genotype
3. Ms4a4a-cond-IV3R	CTCAAGAGCAGCACGGAGTTGGA	1 & 2, 1 & 3 703, 3584 wildtype
		1 & 3 993 mutant

Electrophoresis Protocol:

Allele Description: 2591bp were deleted from the 46th coding nucleotide of Exon 2 ENSMUSE00001333641 to the 64th coding nucleotide of Exon 3 ENSMUSE00001334957 from the Ms4a4a gene [ENSMUST00000188995.1](#) using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

*wtR primer untested (ePCR verified)

