GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR10719 2610507B11Rik_T1359S (h.KIAA0100) HDR Stock #: 67016

Reagent/Constituent	Volume (µL)
QuantiTect Multiplex PCR Master Mix **Cat No./ID 204541**	5.0
Water	3.4
Target Probe mix	0.3
-21 µM Mutant Forward Primer	
-21 µM Mutant Reverse Primer	
-7 µM Mutant probe	
TCRD (endogenous control) mix	0.3
-21 µM TCRD Forward primer	
-21 µM TCRD Reverse Primer	
-7 µM TCRD probe	
Sample	1.0
TOTAL VOLUME OF REACTION:	**10.00 µL**

Comments on protocol:

Protocol may work with other DNA extraction methods. Reference: ABI User Bulletin #2 and #5 (updated 10/2001) for multiplex in same tube and validation of each assay to match relative efficiencies of reference and target primer/probe combinations. Also reference: Rapid and accurate determination of zygosity in transgenic animals by real-time quantitative PCR. TransRes (2002).

Strategy:

Steps	Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting	95	15:00	**1**
2. Denaturation	95	0:30	**40x**
3. Annealing/Elongation	60	1:00	**40x**
4. To step 2 for 40 cycles			

Primers:

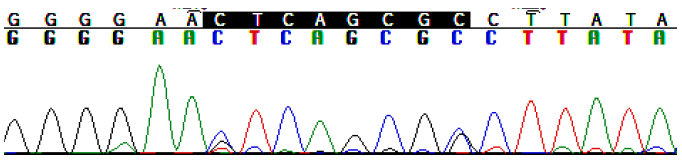
	Name	Nucleotide Sequence (5' - 3')
1.	TCRD Forward Primer	CAGACTGGTTATCTGCAAAGCAA
2.	TCRD Reverse Primer	TCTATGCCAGTTCCAAAAAACATC
3.	TCRD MGB VIC Probe	VIC-ATTATAACGTGCTCCTGG-MGB
4.	TM_2610507B11Rik-F	AGTGCAGTCAGGGCCATGT
5.	TM_2610507B11Rik -R	TGGCCTGACCTTGAGGTATAAGG
6.	2610507B11Rik BHQ-1 FAM Probe	Fam-pdC-GGGGAAG-pdU-pdC-AG-pdC-G-pdC-pdC -BHQ-1

Allele Description: The mouse T1359S model was created using optimized CRISPR Cas9 KI technology utilizing Ribonucleoprotein (RNP) in the presence of a synthetic single strand DNA repair template harboring the desired KI. Zygotes were electroporated and subsequent progeny were screened for the presence of the correctly targeted allele via homology directed repair (HDR). The KI nucleotide is 2 bp from cleavage site and one silent protospacer mutation is engineered into ssODN to protect the HDR derived locus from auto-cutting by Cas9. Key progeny were sequence confirmed.

ttgcccagcaacggggcatccagattgagtgcagtcagggccatgtcttcactcggggaAGTcagcgCcttatacctcaaggtcaggccagagggtctgtttctccta

WT aCt>atC KI
T > S

Sample	ΔCt	Genotype
2610507B11Rik-ntc		No Rxn
2610507B11Rik-wt	1.86	WT
CR10719-33	0.26	Het
CR10719-34	0.25	Het



Het

Note: Homozygous animals will have a deltaCt around -1.0 (1 Ct less than heterozygous animals).

CtcagcgG
WT

GtcagcgC
KI

PCR protocol developed by MMRRC at University of California, Davis

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Alternative Genotyping Protocol
Standard PCR and Sequencing

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

Reagent/Constituent	Volume (µL)
Water	5.0
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM) IVF	0.5
Primer 2. (stock concentration is 20µM) IVR	0.5
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.5
TOTAL VOLUME OF REACTION:	**15.0 µL**

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- When crossing Alg13 and Glt28d2 the Taqman picks up the "other" mutation. Sequencing will be needed if mixed.

Strategy:

Steps		Temp (ºC)	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 (↓1ºC/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:

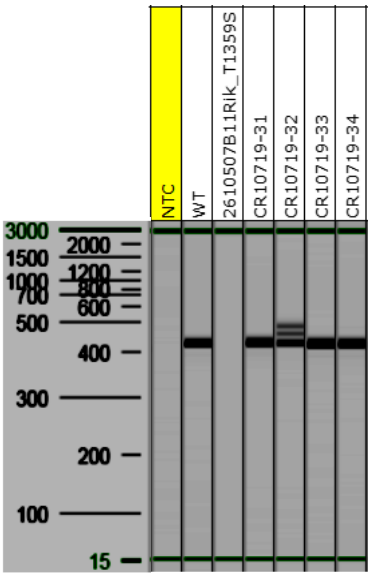
Name	Nucleotide Sequence (5' - 3')
1. CR-26105...Rik_T1359S-IVF	CCTATACTGCACTGTTTCCCCAGCT
2. CR-26105...Rik_T1359S-IVR	GCAGTGGTCAGCGTTCTCTTCAGT

Electrophoresis Protocol:

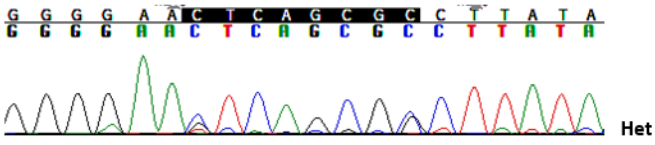
Agarose: **1.5%** : **90**		
Estimated **90** min.		
Primer	**Band** (bp)	**Seq Primer**
1 & 2	499	CR-26105...Rik_T1359S-IVF

Sequencing across Exon 22 to verify the HDR using standard PCR and PCR purification is used to determine the presence of HDR.

Note: Pup #32 was not sequenced as previous TM data indicated that this pup did not have HDR. It is necessary to sequence all WT-sized bands to verify HDR when using only standard PCR.



Sample	Genotype
CR10719-31	Het
CR10719-33	Het
CR10719-34	Het



CtcagcgG
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