GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC

Protocol Name: CR10659 Slc1a2 L552Gfs (h.L554fs) HDR

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:

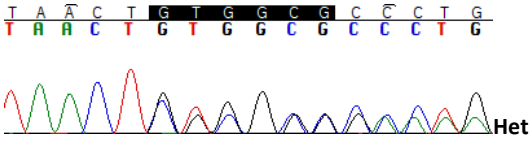
	Name	Nucleotide Sequence (5' - 3')
1.	TCRD Forward Primer	CAGACTGGTTATCTGCAAAGCAA
2.	TCRD Reverse Primer	TCTATGCCAGTTCCAAAAAACATC
3.	TCRD MGB VIC Probe	VIC-ATTATAACGTGCTCCTGG-MGB
4.	TM_Slc1a2-F	GCCTGTAAGTTGGATGTTGATGG
5.	TM_Slc1a2-R	CAAGGTTCTTCCTCAACACTGCA
6.	Slc1a2 MGB FAM Probe	Fam-TTGGCCGCCAGTTAC-MGB

Allele Description: The mouse L552Gfs 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 deletion of CT from L552 (human L554) introduces a frameshift (fs) with a predictable and orthologous early stop. Half of the protospacer was shifted 2nt preventing need of engineered silent mutation to prevent cleavage of HDR allele. Key progeny were sequence confirmed.

aggtgtttcttgtggttcgtggcctgtaagttggatgttgatggtaattgttttcaggtaactggcggccaatggaaagtcagctgactgcagtgttgaggaagaaccttggaa

WT CTG > Ggc KI
L > Gfs

Sample	ΔCt	Genotype
Slc1a2-ntc		No Rxn
Slc1a2-wt	-0.15	WT
CR10659-53	-0.99	Het
CR10685-62	-0.90	Het



tCTggcg
WT

tggcg
KI

Note: Homozygous animals will have a deltaCt around -3.5 (1 Ct less than heterozygous animals).