GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: CR10641 Sap30l E155D (h.SAP30L E156D) 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:

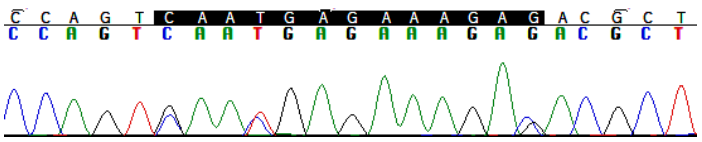
	Name	Nucleotide Sequence (5' - 3')
1.	TCRD Forward Primer	CAGACTGGTTATCTGCAAAGCAA
2.	TCRD Reverse Primer	TCTATGCCAGTTCCAAAAAACATC
3.	TCRD MGB VIC Probe	VIC-ATTATAACGTGCTCCTGG-MGB
4.	TM_Sap30l-F	GACTGTGAGCCGACACTTCAG
5.	TM_Sap30l-R	CATGTAGATGAAATAGGCAAGCGTG
6.	Sap30l BHQ-1 FAM Probe	Fam-A-pdC-pdC-AG-pdU-pdC-AA-pdC-GAGAAAGA-pdC-A-pdC-BHQ-1

Allele Description: The mouse E155D 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 11 bp from cleavage site and two silent protospacer mutations are engineered into ssODN to protect the HDR derived locus from auto-cutting by Cas9. Key progeny were sequence confirmed.

agggactttggtcttttctcctgtgtagactgtgagccgacacttcaggaacataccagtCaaCgagaaaGACacgcttgcctatttcatctacatggtgaagagtaacagga

WT gaG>gaC KI
E > D

Sample	ΔCt	Genotype
Sap30l-ntc		No Rxn
Sap30l-wt	16.73	WT
CR10673-34	1.09	Het
CR10673-35	0.92	Het



Het

GaaTgagaaagaG
WT

CaaCgagaaagaC
KI

Note: Homozygous animals will have a deltaCt around -1.7 (1 Ct less than heterozygous animals).