GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: CR10541 Oxct1 R285W 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 WT Forward primer	
-21 µM WT Reverse Primer	
-7 µM WT probe	
Sample	1.0
TOTAL VOLUME OF REACTION:	**10.00 µL**

Comments on protocol:

Protocol may work with other DNA extraction methods. WT Vic probe may be substituted for WT Orange 540 probe. 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:

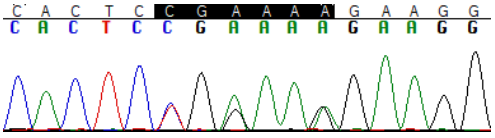
	Name	Nucleotide Sequence (5' - 3')
1.	TM_Oxct1_WT-F	CAGACCCTAACCACAGGTACTTTG
2.	TM_Oxct1_WT-R	GATTTGCCTTTTCCATCTCCTTCC
3.	Oxct1-WT Orange 560 BHQ-1 Probe	Orange 560-AG-pdC-G-pdU-pdU-pdU-A-pdU-pdC-A-pdC-pdU-pdC-pdC-GAAAGGA-BHQ-1
4.	TM_Oxct1_KI-F	CAGACCCTAACCACAGGTACTTTG
5.	TM_Oxct1_KI-R	GGATTTGCCTTTTCCATCTCCTTCT
6.	Oxct1-KI Fam BHQ-1 Probe	Fam-AAG-pdC-G-pdU-pdU-pdU-A-pdU-pdC-A-pdC-pdU-pdC-pdU-GGAAAGA-BHQ-1

Allele Description: The mouse R285W 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 nucleotides are next to the cleavage site (1 bp 5' and 1/2 bp 3'), and one silent PAM mutations was engineered into ssODN to prevent cleavage of the KI allele by Cas9. Key progeny were sequence confirmed.

gtgaaaagcagaccctaaccacaggtactttgtttctgcttactgggaagcgtttatcactcTGGaaAgaaggagatggaaaaggcaaatccggtaagcctggaggcgat

WT CGA > TGG KI

Sample	ΔCt	Genotype
Oxct1-ntc		No Rxn
Oxct1-WT	3.57	WT
CR10541-63	0.04	Het
CR10541-64	-0.73	Hom



Het

CgAaaG
WT
TgGaaA
KI

PCR protocol developed by MMRRC at University of California, Davis