

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR10587 Pde6c R565Q 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 (°C)	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	TCTATGCCAGTTCCAAAAACATC
3. TCRD MGB VIC Probe	VIC-ATTATAACGTGCTCCTGG-MGB
4. TM_Pde6c-F	ACCGTCCTGTACCTACCA
5. TM_Pde6c-R	TCAATGGCTTCCAGCATCAGTA
6. Pde6c MGB FAM Probe	Fam-AACTGGCAACACGGA-MGB

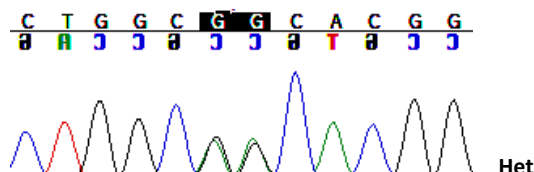
Allele Description: The mouse R565Q model was created using optimized CRISPR Cas9 KI technology utilizing Ribonucleoprotein (RNP) in the presence of a synthetic single strand DNA repair template harboring the desired KI. Zygotes were electroporated and subsequent progeny were screened for the presence of the correctly targeted allele via homology directed repair (HDR). The KI nucleotides disrupt the PAM site (nGG) and are 4 and 5 bp from cleavage site and engineered into ssODN to protect the HDR derived locus from auto-cutting by Cas9. Key progeny were sequence confirmed.

gttcttacaaggtggatgtacacagtcggaagggtaccgtcctgtcacctaccacaactggcAAcacggattcaacgtggggcagaccatgttcactttgttaa

WT cGG>cAA KI

R>Q

Sample	ΔCt	Genotype
Pde6c-ntc		No Rxn
Pde6c-wt	16.67	WT
CR10587-42	-0.51	Hom
CR10587-43	0.39	Het



cGG
WT

cAA
KI