GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC

Protocol Name: **CR10000 Slc22a29 EXDEL**

Reagent/Constituent	Volume (µL)
QuantiTect Multiplex PCR Master Mix Cat No./ID 204541	5.0
Water	3.4
Fam Probe mix	0.3
-21 µM Forward primer	
-21 µM Reverse primer	
-7 µM probe	
Yak Probe mix	0.3
-21 µM Forward primer	
-21 µM Reverse primer	
-7 µM probe	
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
	10.0 µL

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- WT (LOA) primers have a background due to Slc22a27 limited reactivity. Therefore, the WT should just be considered an endogenous control reaction in analysis.

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.	CR_Slc22a29-LoA Primer F	GAGGCAGAAAGTTCCCTTC
2.	CR_Slc22a29-LoA Primer R	GCATCACATCTACTCAGTTCAG
3.	CR_Slc22a29-LoA Probe	/5YAKYEL/AGGCACATGGCTCCCTTCCTC/3IABKFQ/
4.	CR_Slc22a29-Mut Primer F	CCTTAGATACAGAGAGTTTGATTAAG
5.	CR_Slc22a29-Mut Primer R	GCATCACATCTACTCAGTTCAG
6.	CR_Slc22a29-Mut Probe	/56-FAM/AGAGAAAGG/ZEN/CACATGGCTCCCTTC/3IABKFQ/

Allele Description: Exon 2 ENSMUSE00001079800 and flanking splicing regions were constitutively deleted from the Slc22a29 gene ENSMUSG00000075044 using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

	Sample ID	Δ Ct	Genotype
0.1	NTC		no rxns
0.1	WT Control	16.60	WT
0.1	Het Control	2.64	Het
0.2	CR10000-109	17.72	WT
0.2	CR10000-110	17.86	WT
0.2	CR10000-111	1.67	Hom
0.2	CR10000-112	1.70	Hom
0.2	CR10000-113	11.45	WT
0.2	CR10000-114	2.53	Het
0.2	CR10000-115	11.40	WT
0.2	CR10000-116	2.64	Het
0.2	CR10000-117	2.59	Het
0.2	CR10000-118	16.99	WT