

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

Protocol Name: MMRRC 71888 C57BL/6N-Ace2em1(ACE2)MbpTmprss2em1(TMPRSS2)MbpSlc6a20bem1Mbp/Mmucd

Protocol: *GoTaq® G2 Colorless Master Mix(Promega)*

Reagent/Constituent	Volume (µL)
Water	4.0
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM) IVF	0.5
Primer 2. (stock concentration is 20µM) IVR	0.5
Primer 3. (stock concentration is 20µM) kiR	0.5
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.5
TOTAL VOLUME OF REACTION:	15.0 µL

Comments on protocol:

- Protocol may work with other DNA extraction methods.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2:00	1x
2. Denaturation	94	0:10	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	10x
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	25x
7. Elongation	68	2:00 (↑20sec/cycle)	
8. Finish	4	∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5% V: 90			
1. C19-Ace2-IVF	CTGTTTACATATCTGTCCTCTCCAGG	Estimated Running 90 min.			
2. C19-Ace2-IVR	GCTACAGAGGCAGTCACTCATCCTC	Primer Combination		Band (bp)	Genotype
3. C19-hAce2-kiR	CCTCAGATCTCCAGCTTTCCCAA	1 & 2		522	wildtype
		1 & 3, 1 & 2		723, 3757	mutant

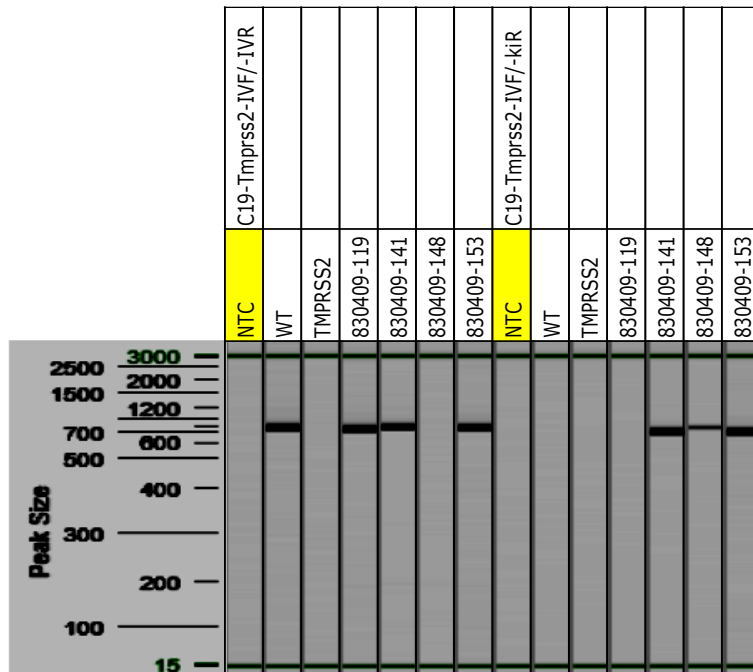
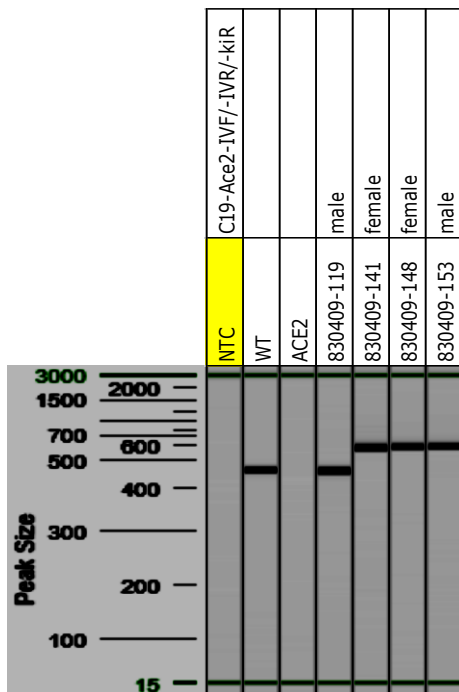
Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5% V: 90			
1. C19-Tmprss2-IVF	AGGTTCTCTGTACCTCAGAGGAGGA	Estimated Running 90 min.			
2. C19-Tmprss2-IVR	CCTGTCTCACCCTTTCCAACATAACC	Primer Combination		Band (bp)	Genotype
3. C19_Tmprss2-kiR	ACCTGAGGAGTCGCACTCTATCC	1 & 2		721	wildtype
		1 & 3, 1 & 2		643, 4001	mutant

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	ACE2	TMPRSS2
#119	wt	wt
#141	hom	het
#148	hom	hom
#153	hemi	het

*** Note: Separate WT and Mut rxn required for Qiaxcel imaging (TMPRSS2).

Large mutant band is not observed with these PCR protocols.

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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:

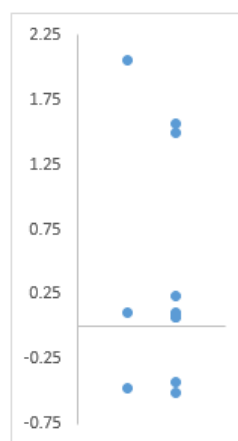
Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	95	15:00	1
2. Denaturation	95	0:30	40x
3. Annealing/Elongation	60	1:00	40x
4. To step 2 for 40 cycles			

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_Slc6a20b_UTR-F	CAAAGGTTAGTGGGAGGATCTAAA
5. TM_Slc6a20b_UTR-R	CAGGACTCTGGAATCAGCTTTC
6. Slc6a20b_UTR -FAM MGB-NFQ	FAM/TGGCCAACCT/ZEN/GTGAGG/MGB-NFQ

TGTTGGTTCCTTCTTGACCACTGCTTCCTCGCTGCCTCAACCTCACAGTGGCCAGTTAGATCCTCCCACTAACCTTTGGCCACATTCTTTCTGTGT
WT AGGTG > AGTTG KI

Sample ID	Δ Ct	genotype
Slc6a20-ntc		
WT DNA	2.05	WT
Slc6a20-het control	0.11	Het
Slc6a20-hom control	-0.47	Hom
CR11365-260	0.11	Het
CR11365-261	-0.50	Hom
CR11365-262	0.10	Het
CR11365-263	0.08	Het
CR11365-264	-0.42	Hom
CR11365-265	1.49	WT
CR11365-266	1.57	WT
CR11365-267	0.24	Het
CR11365-268	0.07	Het



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Alternative Genotyping Protocol

Standard PCR and Sequencing – Slc6a20 5UTR G>T

Protocol: *GoTaq® G2 Colorless Master Mix(Promega)*

Reagent/Constituent	Volume (μL)
Water	5.5
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20μM) IVF	0.5
Primer 2. (stock concentration is 20μM) IVR	0.5
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	15.0 μL

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- When crossing Alg13 and Glt28d2 the Taqman picks up the "other" mutation. Sequencing will be needed if mixed.

Strategy:

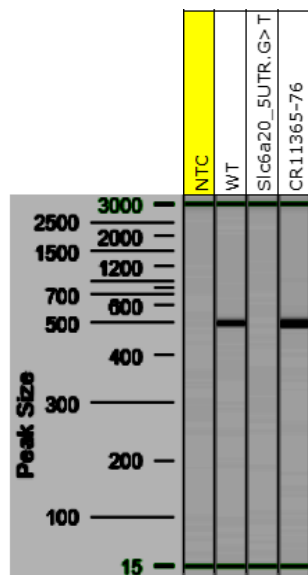
Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2:00	1x
2. Denaturation	94	0:10	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	10x
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	25x
7. Elongation	68	2:00 (↑20sec/cycle)	
8. Finish	4	∞	n/a

Primers:

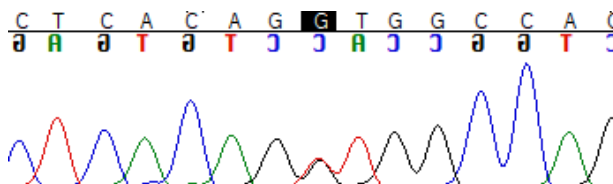
Name	Nucleotide Sequence (5' - 3')	Electrophoresis Protocol:		
1. CR-Slc6a20-IVF	CCAAGATAATGGCATCCTGTTTCAC	Agarose: 1.5%	:	90
2. CR-Slc6a20-IVR	GCATGTGCTGAAGGTGATTCCAT	Estimated	90	min.
		Primer	Band (bp)	Seq Primer
		1 & 2	507	hACE2_ivqcF

Sequencing across Exon 9 to verify the HDR using standard PCR and PCR purification is used to determine the presence of HDR.

Note: It is necessary to sequence all WT-sized bands to verify HDR when using only standard PCR.



CR11365-76 Het



agGtg
WT

agTtg
KI