GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC

Protocol Name: **MMRRC 71891 hACE2/hTMPRSS2/ACE2_D355N**

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:

Name	Nucleotide Sequence (5' - 3')
1. C19-Ace2-IVF	CTGTTTACATATCTGTCCTCTCCAGG
2. C19-Ace2-IVR	GCTACAGAGGCAGTCACTCATCCTC
3. C19-hAce2-kiR	CCTCAGATCTCCAGCTTTCCCAA

Electrophoresis Protocol:

Agarose: **1.5%** **V:** **90**

Estimated Running **90** min.

Primer Combination	**Band** (bp)	**Genotype**
1 & 2	522	wildtype
1 & 3, 1 & 2	723, 3757	mutant

Name	Nucleotide Sequence (5' - 3')
1. C19-Tmprss2-IVF	AGGTTCTCTGTACCTCAGAGGAGGA
2. C19-Tmprss2-IVR	CCTGTCTCACCCTTTCCAACATAACC
3. C19_Tmprss2-kiR	ACCTGAGGAGTCGCACTCTATCC

Agarose: **1.5%** **V:** **90**

Estimated Running **90** min.

Primer Combination	**Band** (bp)	**Genotype**
1 & 2	721	wildtype
1 & 3, 1 & 2	643, 4001	mutant

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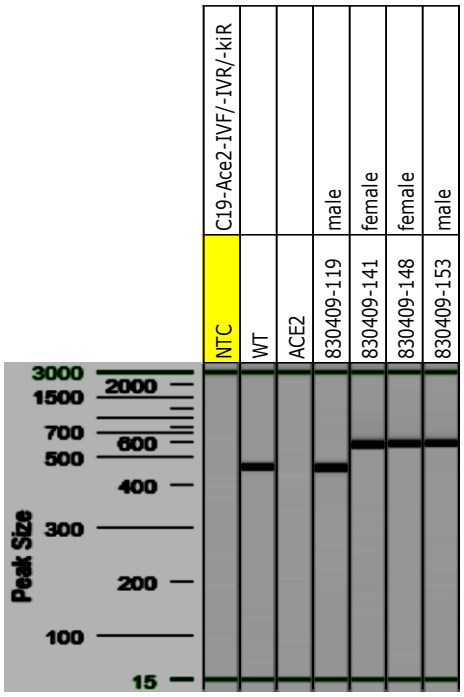

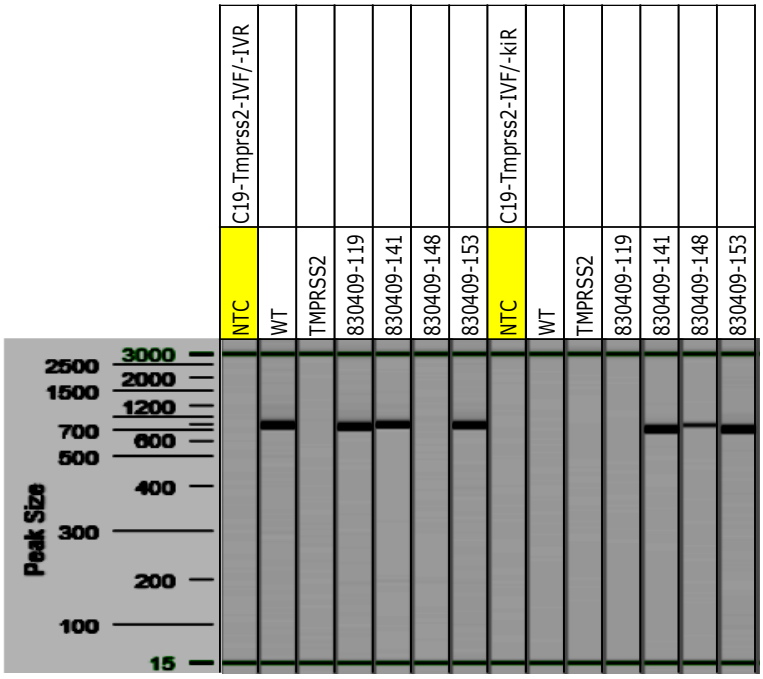

	ACE2	TMPRSS2
#119	wt	wt
#141	hom	het
#148	hom	hom
#153	hemi	het

***** 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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Protocol Name: **MMRRC 71891 hACE2/hTMPRSS2/ACE2_D355N**

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_hACE2_D355N-F	CCAGGAAATGTTCAGAAAGCAG
5.	TM_ hACE2_D355N-R	GTCAGGAAGTCGTCCATTGT
6.	hACE2_D355N-FAM MGB-NFQ Probe	FAM/TGAAATTGC/ZEN/CCTTCC/MGB-NFQ/

ATGTTCAGAAAGCAGTCTGCCATCCCACAGCTTGGGACCTGGGGAAGGGC**AAT**TTCAGGATCCTTATGTGCACAAAGGTGACAATGGACGACTTCCTGACAGC

WT GAC > AAT KI (**D > N**)

Sample	ΔCt	Genotype
hACE2_D355N		
WT	14.82	WT
CR11432-132	-0.82	Hom
CR11432-134	-0.07	Hemi
CR11432-140	0.10	Het

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Alternative Genotyping Protocol

Standard PCR and Sequencing – Tyk2_P1124A

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 (ºC)	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 (↓1ºC/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:

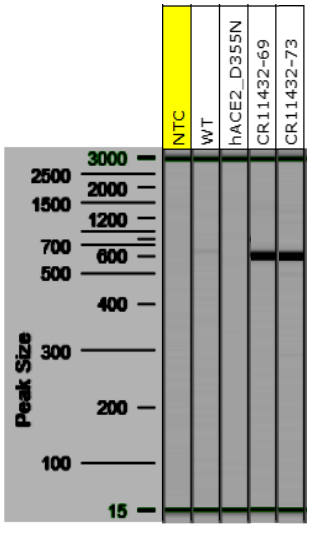
Name	Nucleotide Sequence (5' - 3')
1. hACE2_ivqcF	CCCTGCTCATTTGCTTGGTG
2. hACE2_ivqcR	TCCCAACAATCGTGAGTGCTTG

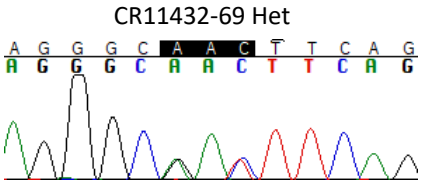
Electrophoresis Protocol:

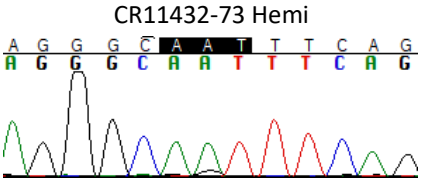
Agarose: **1.5%** : **90**		
Estimated **90** min.		
Primer	**Band** (bp)	**Seq Primer**
1 & 2	560	hACE2_ivqcF

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.







cGaCt
WT

cAaTt
KI

PCR protocol developed by MMRRC at University of California, Davis