GENOTYPING PROTOCOL
MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: **MMRRC 71890 C57BL/6N-Ace2em1(ACE2)Mbp Tmprss2em1(TMPRSS2)Mbp Plscrem1Mbp/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:

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

Name	**Nucleotide Sequence (5' - 3')**
1. CR-Plscr1-IVF	TTGAGCCACCATATGATTGCTG
2. CR-Plscr1-IVR	GACTCTAGACAGCCACTGATAAGG
3. Post PCR digest w/BtsI-v2	

Agarose: **1.5%**	V: **90**	
Estimated Running **90** min.		
Primer Combination	**Band** (bp)	**Genotype**
1 & 2	437	
Digest	159/278	wildtype

PCR protocol developed by MMRRC at University of California, Davis

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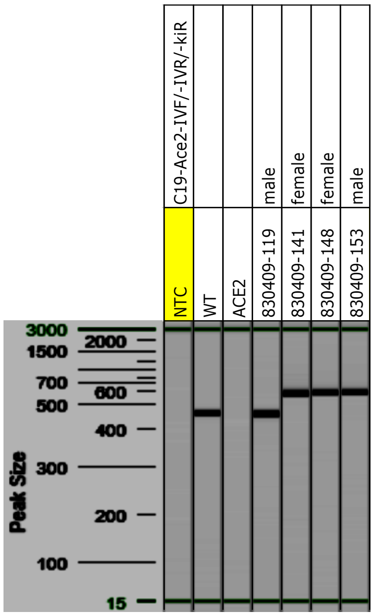

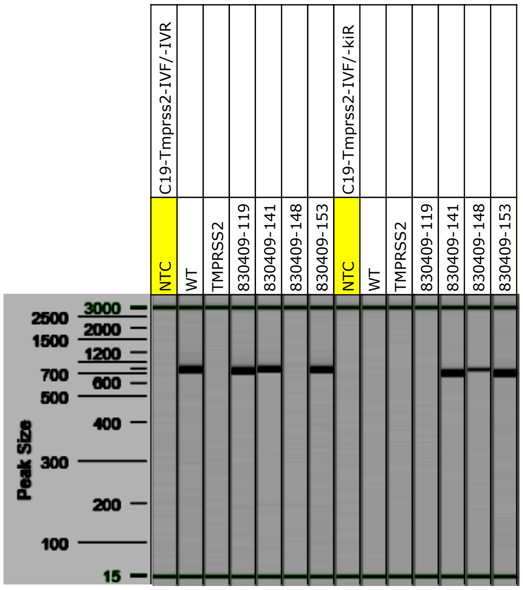

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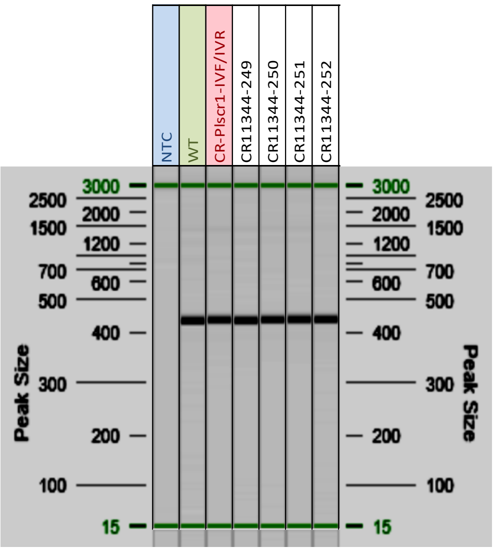
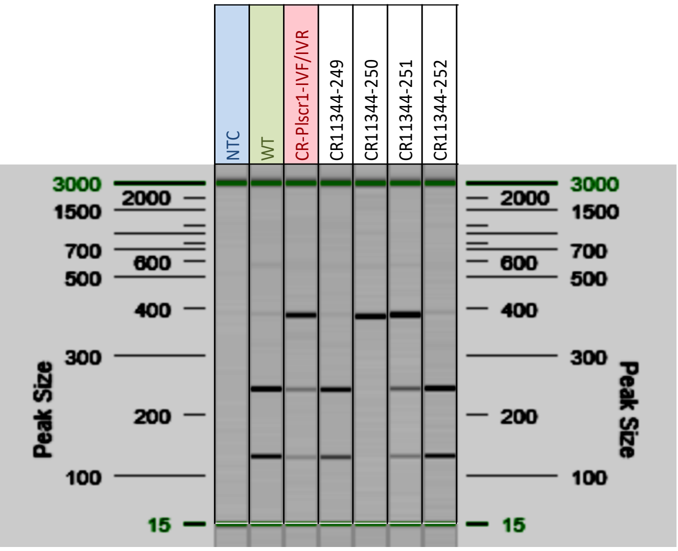
	ACE2	TMPRSS2
#119	wt	wt
#141	hom	het
#148	hom	hom
#153	hemi	het



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

Large mutant band is not observed with these PCR protocols.

BtsI-v2 Restriction Enzyme Digest (NEB)



BtsI-v2 Restriction Enzyme Digest (NEB)

BtsI-v2 Order R0667

Restriction Enzyme Digestion

Steps

1. Set up reaction as follows:

COMPONENT	50 µl REACTION
DNA	1 µg
10X rCutSmart Buffer	5 µl (1X)
BtsI-v2	1.0 µl (10 units)†
Nuclease-free Water	to 50 µl

2. Incubate at 37°C for 5–15 minutes as BtsI-v2 is Time-Saver qualified.

	Plscr1_Q271Y
#249	wt
#250	hom
#251	het
#252	wt

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