GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC

Protocol Name: **MMRRC 71887 C57BL/6N-Ace2em1(ACE2)MbpTmprss2em1(TMPRSS2)MbpFurinem1(FURIN)Mbp/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 (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. 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. C19-Furin-IVF	ATCAGTGTGTGGCTGAGAGGACTG
2. C19-Furin-IVR	CTGCTGCATGGTTTGAGAGTCTCT
3. C19-Furin-kiR	GCTGTTCCAGCCACTGTACTTGAG

Agarose: **1.5%** **V:** **90**
Estimated Running **90** min.

Primer Combination	**Band** (bp)	**Genotype**
1 & 2	477	wildtype
1 & 3, 1 & 2	506, 4371	mutant

Allele Description: Generation of a humanized ACE2 (ENSG00000130234.6) mouse line. CRISPR RNP was utilized to assist with homologous recombination using dsDNA repair template in ES cells. The mouse exon two 5' UTR-ATG is replaced with human exon two 5' UTR/CDS/m3'UTR. The expression of the human CDS will be controlled by the mouse regulatory systems (including 3'UTR) as the expression pattern is similar between species and specifically highly expressed in the lung. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

Allele Description: Generation of a humanized TMPRSS2 (ENSG00000184012.7) mouse line. Human cDNA consisting of exons 3-14 will be fused to the mouse exon 2 ENSMUSE00000641779. CRISPR RNP will be used to assist with homologous recombination using dsDNA repair template in ES cells. The mouse exon 2 splice acceptor (SA) and the guide protospacer nt 1-17 is removed from the HR template to prevent potential downstream splicing or RNP re-cleavage. The expression of the human CDS will be controlled by the mouse regulatory systems (including 3'UTR) as the expression pattern is similar between species and specifically highly expressed in the lung. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

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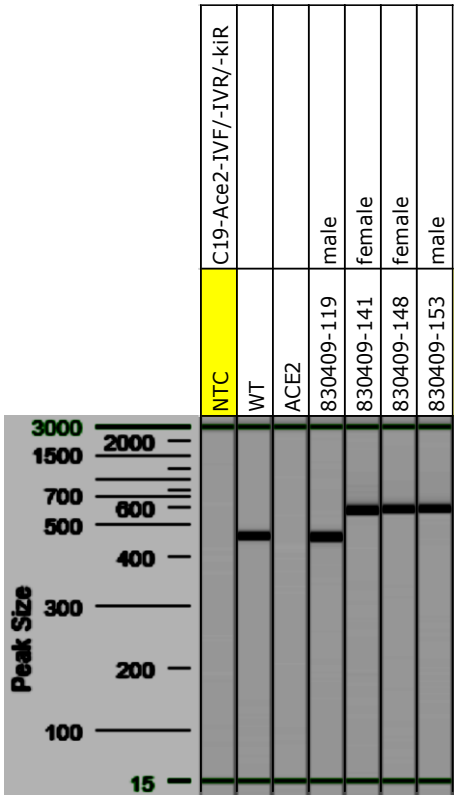
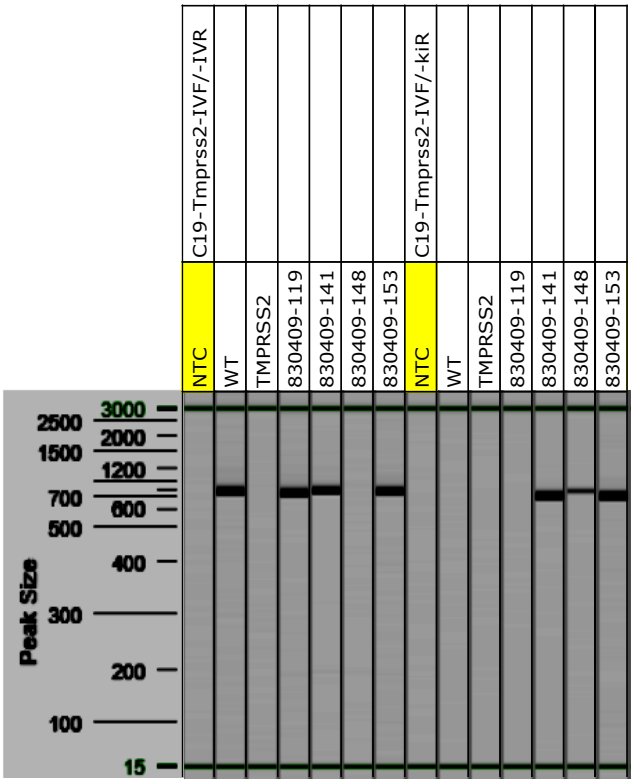
Allele Description: Generation of a humanized FURIN (ENSG00000140564.6) mouse line Human cDNA consisting of exons 2-16 will replace mouse exon 1 ATG. CRISPR RNP will be used to assist with homologous recombination using dsDNA repair template in ES cells. The mouse exon 2 ATG replaced with human exon 2 CDS/m3'UTR in the HR template to prevent potential downstream splicing or RNP re-cleavage (2 engineered silent mutations in hCDS). The expression of the human CDS will be controlled by the mouse regulatory systems (including 3'UTR) as the expression pattern is similar between species and specifically high in the lungs. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.





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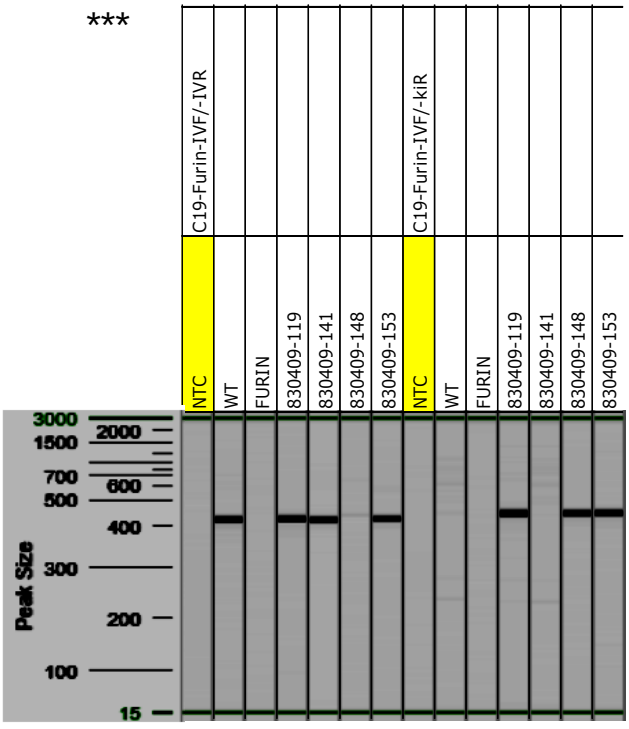

	ACE2	TMPRSS2	FURIN
#119	wt	wt	het
#141	hom	het	wt
#148	hom	hom	hom
#153	hemi	het	het

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

Large mutant band is not observed with these PCR protocols.