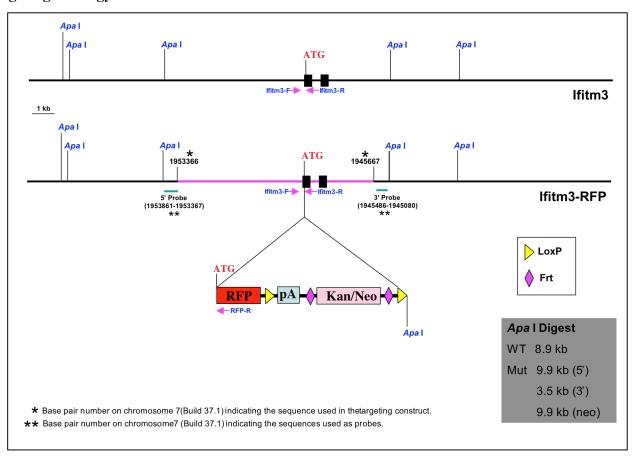
# Fragilis (Ifitm3)

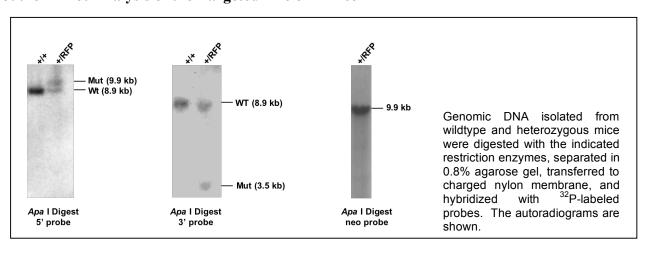
#### A. Rationale

Ifitm3 (Fragilis) is expressed in germ cells during embryonic development. Unlike the germ cell marker Oct4, Ifitm3 is restricted to germ cells making it an ideal marker to examine germ cell development. A strain of mice carrying RFP knocked into Ifitm3 locus was generated by the GUDMAP consortium to investigate germ-cell development.

# **B.** Targeting Strategy



# C. Southern Blot Analysis of the Targeted Allele in Mice



#### **D. PCR Genotyping**

#### a. Primers

Ifitm3-F: 5' gggacaagtcctgacttggg 3'

Ifitm3-R: 5' CCTCTCTGGGCATGTTGATC 3'

RFP-R: 5' cttgatgacgtcctcggagg 3'

## b. Expected Band Sizes

Ifitm3-F + Ifitm3-R: 390 bp Ifitm3-F + RFP-R: 272 bp

# E. Relevant Sequences

# a. Genomic clone used for targeting construct

aaqaqqtaqqqqtqtqqqqtqtqqqatqqqqtqtataqqqcatccacaqataaqtaqqattctqq qcattqcttacctccqqctccatqtccctaqaqtacaqqaaqqqtqcaqaqqcatqtactaqtqaqttttcaqtccaqqqa tagaggaccagcccagggtcctccacttttaggaggaagaatcttgacccagacacgctgtgttcaagaactctatggtg tqaccatqqaatctttqatqctaqqtqccatqttqatccaqqqtattatctctctqtqaqtcctcaqatcctqaaqtqqqq agggtcaggtctggaggggccttggcacccttcattagtctgtcctcaaaattcacctgtgggcatacattttttgcaacc tacttttattaaqqqttcaacctctcctctaqcctaccacctaqcaqaqqtaqtqqaaqaqaqatttattaqqqatatqqqqq aaacqaacttcttcaqcaataqttctttqqqqqtqaqcttqaatcttctttqqcaqcaqttcaqtcctqtaaqcaaacact cagcaagcgacaccaggcacaaaccagcaacagtaactaaatcctgaagaaacctccaagcttgccaacaggcctgaagca agttgaactcagtgaccctatgtaaggagaaccaatacatgtgtgttgttaatgaagaataacgaggcagagcaaaccaaa ccaatgctcagcgctcttctcccactgtccgtggattcatagctacactccttcattaagcactctttcatgtgtttgcta tatccaaacatcctttcacctgtgtctgcttcaggaaaacagtctttcatgtgttttgctttagcaagtcatcatttcacct ctgqatgccctgqaatctgtatttctacctcattttqqqcaccccaaqattacaaqtctttqqaqccctcaqqtqqcactq tctccctqtqttqqtatqtaqqttctaqtcctqqqqtctqaqataatqaqaqqcccccaaaactcatcttqtqaqqctqaq cagtgttcgagatgccctcttcactgaggtccaggcttctgaagcagtagcaattcctttactgtcttgggaagctgagcc catcttaggaagacagaggcctaataccagtggagacaggatcccactgggagagctgctatctttctggaggggagtga qtqqttcaqccaqaccaaqtcctaqqqaqaaatcaaqactttttcaqattqcaccaaqaaqqaacaaqaqqttttttaqaa ggacccagggtggaatttccactaaaactcatggccctggactgaacttcagagtgttcatccttagtcccaggtctgtga ctgaatgaaggtagagatttcaggaggagggtcactgtggccacacttcggtccgttctagtgctttgtatgagcagtgtg tgggcagctatcctgagagtgcagaaacctcgcgtgtgttaggcactggtgcacacagaagccagggcatgatctccagaa cccaccgtggagagtcctggtgctcgcttcccactcaccaaatgtgtaattggagcaggaacaatggaagtcattataact tcccattqtqttqqtqqcctqcaqqqcaaqqttqaqtqqttcqctqqqtqqcaqaaqactccaqqttqttccctatctaat gctagagagtaaacaataacaatgtctcctcctgagaagagagcagagaaaccctgtgctcaggctacaccaagacaatgga ctcctgcaaatccaccattaggaattcatttggagatcagttgcaggtagcagatgctaagtaggtctgaaaggtaaggga tactaaatcagagaacatgggcactggccggaactgtcccctgagaccagagtgaggacagaacctgacaggtaaagtgtc aacagttqtqcatcaqqqqcaqaaqtqqqtcatcctqqqtqqaqcaaaqqctqaccctqacccttqttcttacaqcaqqqc tggtcaatccctgtccctctgatgatccctgggggcagagattgcccttagcacttaacaaagaagatggaaaaatccaaa gactgagtctcaacttcccctgatgtttcatatgggaaggagcgagtcagacaggaagaggtcttctgtccagctcacttg agcctqqqqattqaqtqcatqtqaaqqccttqatatqcaaaqccctcaqqctcatcaaqqccaccaqqqaaataqqatccc tqtqactqaqaaaqqcaqaqaaqtqqccataaaqtttqaqqtqaccactaactttcaqqatqqtctqaqaacatccaqqaq caaatqtqttqqccttcaqqaacqttqqqaqaccataqtaaacaqaqctatqactqaaqttaaatattqttcactatttaa ttcqaqqccaqcctqqtcaacaqaqtqaqttccaqqqcaqccaqqaatacacaqaqaaaccctqtctcqaaaaatcaaaaa caacaacaacaacaacaaaaaaqttataccaaqatccatcattcctgaaaccagaqtgtgttgttgtgaccacaggataacgct aacagaaaccaccactttcaggctccaatcagagtccctgtggccagtccaaagccctacccctgaggcctgaggcctgac tqaqqcctqqaaqqaqqcctctqtqaqqtaqqtacqcccatqqaaaccactctacctaqcttcccaqaqqcaqactttttc

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Ifitm3-R

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Intron/exon junction

CGCGCTCCATCCTTTGCCCTTTTCAGTGCTGCCTTTGCTCCGCACC ATGGCCTCCTCCGAGGACGTCATCAAGGAGTTCA

Start of RFP

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End of RFP

ttcctgcagcccaattccgatcatattcaataacccttaat ataacttcgtataatgtatgctatacgaagttat CTGC

LoxP

Start of bGH polyA

End of bGH polyA

Frt End of Kan<sup>R</sup>

# Start of Kan<sup>R</sup> End of bGH polyA

# Start of bGH polyA End of Neo

Start of Neo End of PGK promoter

Start of PGK promoter

tcga gaagttcctatactttttagagaataggaacttc gatcc ataacttcgtataatgtatgctatacgaagttat

Frt LoxP

taggtccctcgaggggatccac AACCACACTTCTCAAGCCTTCATCACCGCTGCCAGTGGAGGACAGCCCCCAAACTACG

Ifitm3 continues

 ${\tt AAAGAATCAAGGAAGAATATGAGGTGGCTGAGATGGGGGCACCGCACGGATCGGCTTCTGTCAGAACTACTGT{\tt GATCAACA}}$ 

Ifitm3-R

TGCCCAGAGAGGTGTCGGTGCCTGACCATGTGGTCCTGGTCCCTGTTCAATACACTCTTCATGAACTTCTGCTGCCTGGGCT

#### Exon/intron junction

agctccatctacccagatggcaccgaggtatgagtgtggggtcctttgtttaaaataagtttgtgttcattcttctgtggt gtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtacacgtgtaggcttaaaggctctctggggggcagacacagggtgaggctg  $\verb|cctggggattctggagtcagactcttccaatgcagagatgcctatagcaccttcactcaacagactgggattctagcctgt|$ gtggaaaatgttcaggagattcatggggaacaatagcctacagtggggtgaacaacaagtgctagggcctcctggatgatc agaggatgtaaaatgttcctgacctaagatggggagatgcagtccacagggagtacgtcaggaaaactgtcagagcatctgcctatcagctccagggccactgaccgtgtctatctgttctgctccagTCTAGGGATCGGAAGATGGTGGTGATGTGACTG GAGCCCAGGCCTACGCCTCCACTGCTAAGTGCCTGAACATCAGCACCTTGGTCCTCAGCATCCTGATGGTTGTTATCACCA TTGTTAGTGTCATCATCATTGTTCTTAACGCTCAAAACCTTCACACTTAATAGAGGATTCCGACTTCCGGTCCTGAAGTGC ATAAAGTGCACTTGATAACCACCatcctatgactgcttttattcctcccacccctctatcttccttgctctgaggtggaca tgtaggttatcctcaatttggcagtagtctctggcctgctgttgtggacatttgctggccaaagtcctggatggtatttgg ctgcttcaggcagggagaactcaagcttcaactggccctggccccacatttccagggcacccagtccagttcagaagtgga  $\tt ggaccaggtctgggtccgtattcttctggacgaatactctgaaccagacaagatggtgtcaaaagttccatgcctgcttcc$ cgggggaaccctgggctgatcctgtccaggatttgctgggtgctgtggggggcctggtgtcccctactggtcccacagc agcgatgcagttcattccttgtcgccctggagcctagaatgaccttttaaggctcaaagtcgctgccctggcgccccctgc ggtaagggagagcgccaatcacgccttcttgtgccggggtatgcaggactcacctctctggtcgggatcctagtacccacg 

#### c. 5' probe

#### d. 3' probe