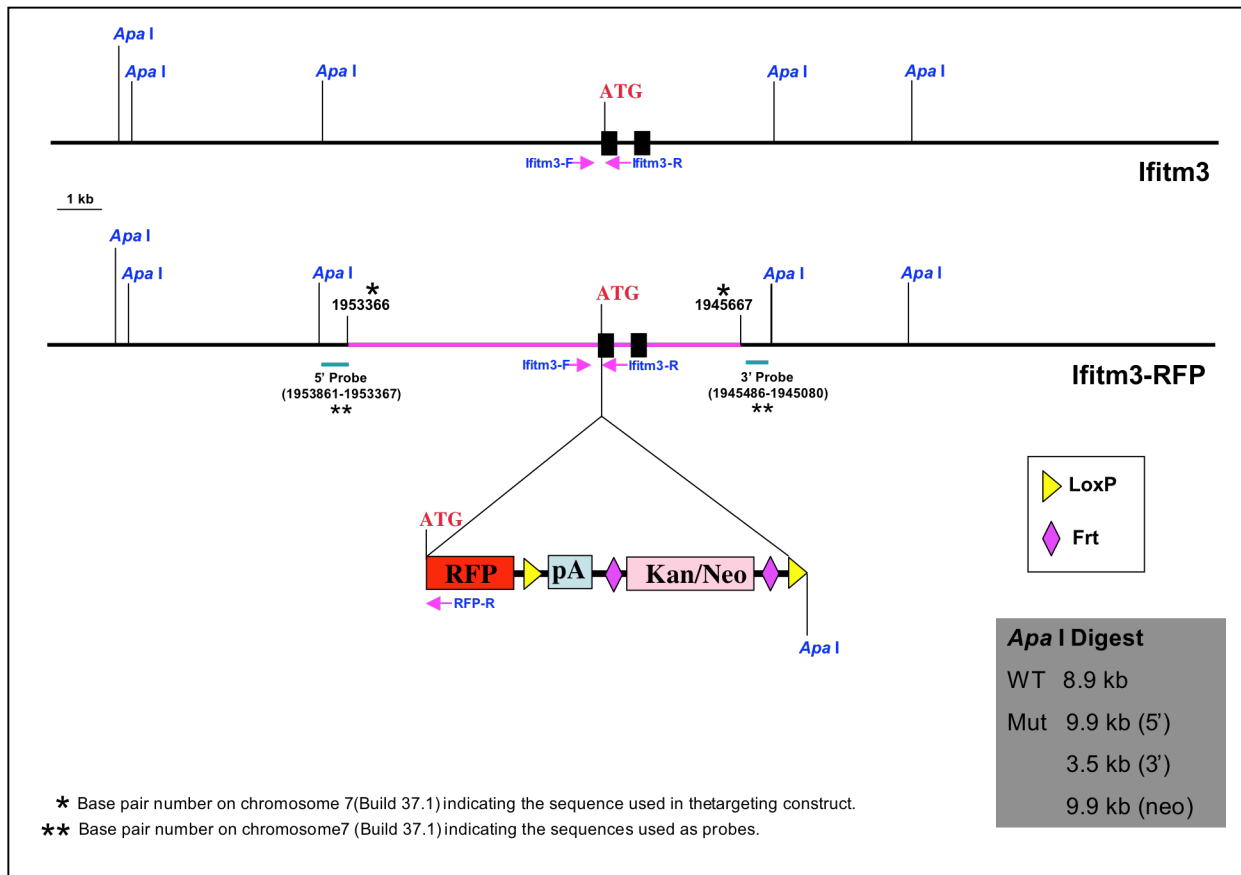


Fragilis (Ifitm3)

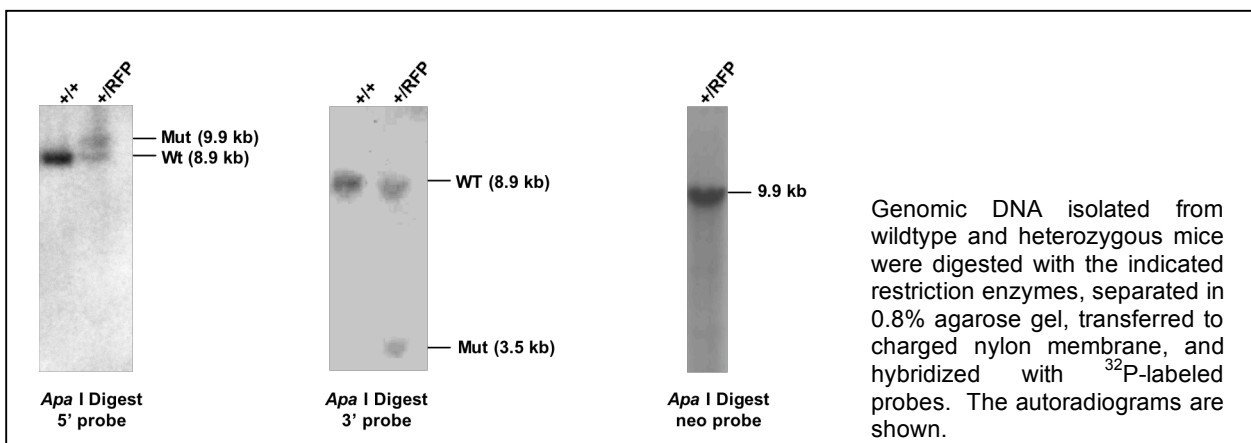
A. Rationale

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



Ifitm3-F

cctgattcacaatccttctctgacatcctgccctggagtaatttgatccgcaggcttttttagatccccgcccctctgaacc
agAGCAGTTTCATCAAGCCAAAATTCAGGAAAAGGAACTTCTGAGAAACCGAAACTGCCGCAGAAAGGGCAGACCCGCAG

Intron/exon junction

CGCGCTCCATCCTTTGCCCTTTTCAGTGCTGCCTTTGCTCCGCACC ATGGCCTCCTCCGAGGACGTCATCAAGGAGTTCA

Start of RFP

RFP-R

TGCGCTTCAAGGTGCGCATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCTACG
AGGGCACCAGACCGCCAAGCTGAAGGTGACCAAGGGCGGCCCTGCCCTTCGCCTGGGACATCCTGTCCCTCAGTTCC
AGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCTTCCCGAGGGCTTCAAGT
GGGAGCGCTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGGCCAGGACTCCTCCCTGCAGGACGGCGAGTTTCATCT
ACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCA
CCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGATGAGGCTGAAGCTGAAGGACGGCGGCCACTACG
ACGCCGAGGTCAAGACCACCTACATGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCTACAAGACCACATCAAGCTGGACA
TCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAGCGCGCCGAGGGCCGCCACTCCACCGGCGCCTAA gaa

End of RFP

ttcctgcagcccaattccgatcatattcaataacccttaat ataacttcgtataatgtatgctatacgaagttat CTGC

LoxP

AGGCGGCCCTAGGGCGGCCGCCACCTCGAGGGGGCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTT

Start of bGH polyA

GTTTGGCCCTCCCGCTGCCTTCCCTGACCCTGGAAGGTGCCACTCCCACTGTCTTTCCTAATAAAAATGAGGAAATTGCA
TCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAAT
AGCAGGCATGCTGGGGATGCGGTGGGCTCGAGATCCACTAGTTCTAGCCTCGAGGCTAGAGCGGCCGCCA ccgcgcgta

End of bGH polyA

gaggatctgttgatcagcagttcaacctgttgatagtagtactaagctctcatgtttcacgtaactaagctctcatgttta
acgtactaagctctcatgtttaacgaactaaaccctcatggctaacgtactaagctctcatggctaacgtactaagctctc
atgtttcacgtaactaagctctcatgtttgaacaataaaattaataataatcagcaacttaaatagcctctaaggttttaag
ttttataagaaaaaaagaatatataaggcttttaagcttttaaggttttaacggttggtggacaacaagccagggatgtaa
cgcactgagaagcccttagagcctctcaaagcaattttcagtgacacaggaacacttaacggctgacagaattagcttcac
gctgccgcaagcactcagggcgcaagggtgctaaaggaagcggaacacgggatccatgcatagatccccctcgaaaacg
ctagcgttaattaa gaagttcctatacttttttagagaataggaacttc AGCTTCTGATGGAATTAGAACTTGGCAAAAC

Frt

End of Kan^R

AATACTGAGAATGAAGTGTATGTGGAACAGAGGCTGCTGATCTCGTTCTTCAGGCTATGAAACTGACACATTTGAAACCA
CAGTACTTAGAACACAAAGTGGGAATCAAGAGAAAAACAATGATCCCACGAGAGATCCATGCATAGATCTTAATTAATTAG
AAAACTCCATCGAGCATCAAATGAAACTGCAATTTATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTT
CTGTAATGAAGGAGAAAACCTCACCGAGGACGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCC
AACATCAATACAACCTATTAATTTCCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATC
CGGTGAGAATGGCAAAAGCTTATGCATTTCTTTCCAGACTTGTTC AACAGGCCAGCCATTACGCTCGTCATCAAAACTACT
CGCATCAACCAAACCGTTATTCATTCGTGATTGCGCCTGAGCGAGACGAAATACGCGATCGCTGTTAAAAGGACAATTACA
AACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTCACCTGAATCAGGATATTTCTTCTAA
TACCTGGAATGCTGTTTCCCGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGT
CGGAAGAGGCATAAATCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTGGCAACGCTACCTTTGCCATG
TTTCAGAAACAACTCTGGCGCATCGGGCTTCCCATACAATCGATAGATTGTGCGCACCTGATTGCCCGACATTATCGCGAGC
CCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCTCGAGCAAGACGTTTCCCGTTGAATATGGCT
CATATGAAACGATCCTGTCTCTTGATCAGATCTTGATCCCCTGCGCCATCAGATCCTTGGCGGCAAGAAAGCCATCCAGTT
TACTTTGCAGGGCTTCCCAACCTTACCAGAGGGCGCCCCAGCTGGCAATTCGGTTCGCTTGCTGTTAATTAAGCGGCCGC

TCTAGCCTCGAGGCTAGAACTAGTGGATCTCGAGCCCACCGCATCCCCAGCATGCCTGCT ATTGTCTTCCCAATCCTCCC

aatcattcatctcatagctgcctttccaagggaaacttatttctggttcccagaggcaagcacctcaaaagtagcctggatc
tatgggactccgtttgcctcctaaatcattgtggtggggtttaatgtcaccatcatacccagggcagcacgggtggatct
gaagtgccttggtttagtggaagtcgctgtagtgggtgcagccaggaatttgggagtgggtgtgggtgggtagaaaggggtg
ggggcacttttgcaagttaaagggtttgggtttcttatccaggggtgcagagcccaacttgttccccagtcocggattctg
acagctgacctgccagcggggaatttaaccaccagagggaaacaaaaggcagcttcaagggatgtgcctagcgtcttct
caccggcacaggatgtcaggctccctgggaggggtgtgtgaaagaaggattgagcaatggggtttataaactagctctgag
gtatggaacaagattcccctgctggtggggagccttctctccgcccaagcagctatggtttcccagaacacactttttttt
ttttttggtttttgcgagacagggtttctctgtatagccctggctatcctggaactcactttgttagaccaggtggcctcgaa
ctcagaaatccgcctgccctgctcccagtgagaaacacacttttataaaggaatcttgtcttattggggattggctgag
tactaagaggatacaaatgaagcaggatagagatgggaaattaggaggacatgggaaggaagaagccccgtcctggcatct
agattgtcttcttactgactgagagtcaagaaacttgataaagccaaaattcccctccatgtctgtc

c. 5' probe

caatcccattgcttcaaccgcccaagattctgaacatccttgctctggtcatctcccctcatcttctacatcatgctta
tcgttttatacagctttaacttactaggtaaccaagataatagaaccactagttaggtactaactagtttagttagctaat
tattaattaactaaactagtaccgaatttagtatctttagtacctattagataactaaggggtgggactccatgcactgtcaa
agtcaatggactactaaattcttccatgcactgtcttcgtgaagaagcattcatgtgctctgccctcagagttgccct
taccctggccagagggccctaactgtcactctaccctcctgtaaagtttatcctcatgcatatgttcacagtggttcaa
taaagtgcacgtggttagcattgtggtgagacttccctatcccctgactgctccctactgagcttactcagagtttccctgg
cttctccttgttg

d. 3' probe

ctaactgtttggcgctgtcttgtcctgacatttttctggttatcaagggtaaacccttacctcctttatagaagag
cttaaggggaaggagcctctctgggcaccaagggcagtgctaggcatcggctggctcccttactgctgcagctctgatgg
aaggaatccgagggccacattaccagggcagggatagcaggaatttctggggaggcaaattttctgtccaagcctctag
ttaagaattgacctacccacaaacctgttagggaaacgggtactggagctttttgtggacttgggaagtcttttcgactga
ggtaaaactgtcatagcacctgatgattcttgtcctcagaaagcggtagccagggccccgcacagcctccttcatgcctaga
gaacctc