

Fragilis-RFP Allele Characterization

Authors: Jinjin Guo, Jill McMahon, M. Todd Valerius, and Andrew P. McMahon

Created: 30 April 2010
Updated: 24 May 2010
Submitted: 6 June 2010

Version: final
Tags: &kmap&mousestrains&gudmap&characterization &Fragilis-RFP

Findings: VALIDATED

Our analysis confirms the expected expression of RFP in the expected Fragilis expression domain, within E-Cadherin germ cells of both male and female gonads.

Data:

Crosses

The Fragilis-RFP strain was generated by homologous recombination in ES cells. The integration of the Fragilis-RFP DNA into the Fragilis domain disrupts the allele. Two male founders, 588 and 589 were crossed to C57Bl6 females and the urogenital system (UGS) was collected from 4 litters of 15.5 dpc embryos. Both founder males transmitted the recombined allele. The RFP reporter was expressed at high levels in the ovaries of female Fragilis^{RFP/+} embryos and in the testis of male Fragilis^{RFP/+} embryos.

Genotyping

Tail samples of the embryos were collected and incubated in tail digestion buffer overnight at 55°C. PCR was performed as per the protocol below and the PCR products were run on a 1.5% agarose gel (Fig1).

Oligonucleotides: for Wt allele Size: 390bp

DNA sequence (forward): 5'- gggacaaggcttgacttggg -3'

DNA sequence (reverse 1) 5'- cctctctggcatgttgc -3'

Oligonucleotides: for targeted/transgenic allele Size: 272bp

DNA sequence (forward): 5'- gggacaaggcttgacttggg 3'

DNA sequence (reverse 2) 5'- cttgatgacgtcctcgagg -3'

Amplifies 5' arm into RFP sequence within RFP region.

Rxn Buffer and Conditions: (25μl reaction)

10X GSB	2.5ul			
25mM dNTP	1ul	94°C	3min	1 cycle
10uM primer F	1ul	94°C	30sec	
10uM primer R1	1ul	58.5°C	30sec	35cycles
10uM primer R2	1ul	72°C	45sec	
DMSO	2.5ul	72°C	10min	1 cycle
2-mercaptoethanol	0.125ul			
Amplify Taq	0.3ul (5u/ul)			
5x cresol red dye	5ul			
Genomic DNA	1ul			

10X Gitschier Buffer (GSB):
670 mM Tris, pH 8.8
166 mM Ammonium Sulfate
65 mM MgCl₂
0.1% gelatin

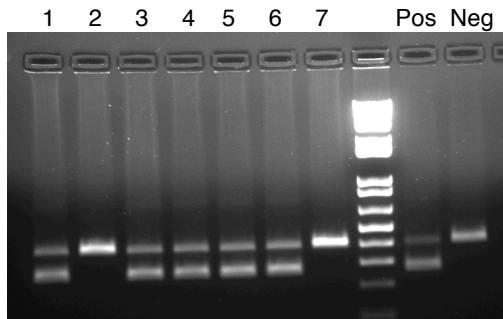


Fig1: Fragilis-RFP genotyping. Tail samples from embryos dissected at 15.5dpc for UGS collection: M588 Fragilis^{RFP/+} X female C57Bl6 (lanes 1-7). Fragilis^{RFP/+} embryos: 1, 3, 4, 5, 6. Pos: Fragilis^{RFP/+} DNA, Neg: wildtype DNA.

Native Fluorescence

Whole embryos as well as dissected UGSs where examined with a fluorescent microscope to view RFP expression.

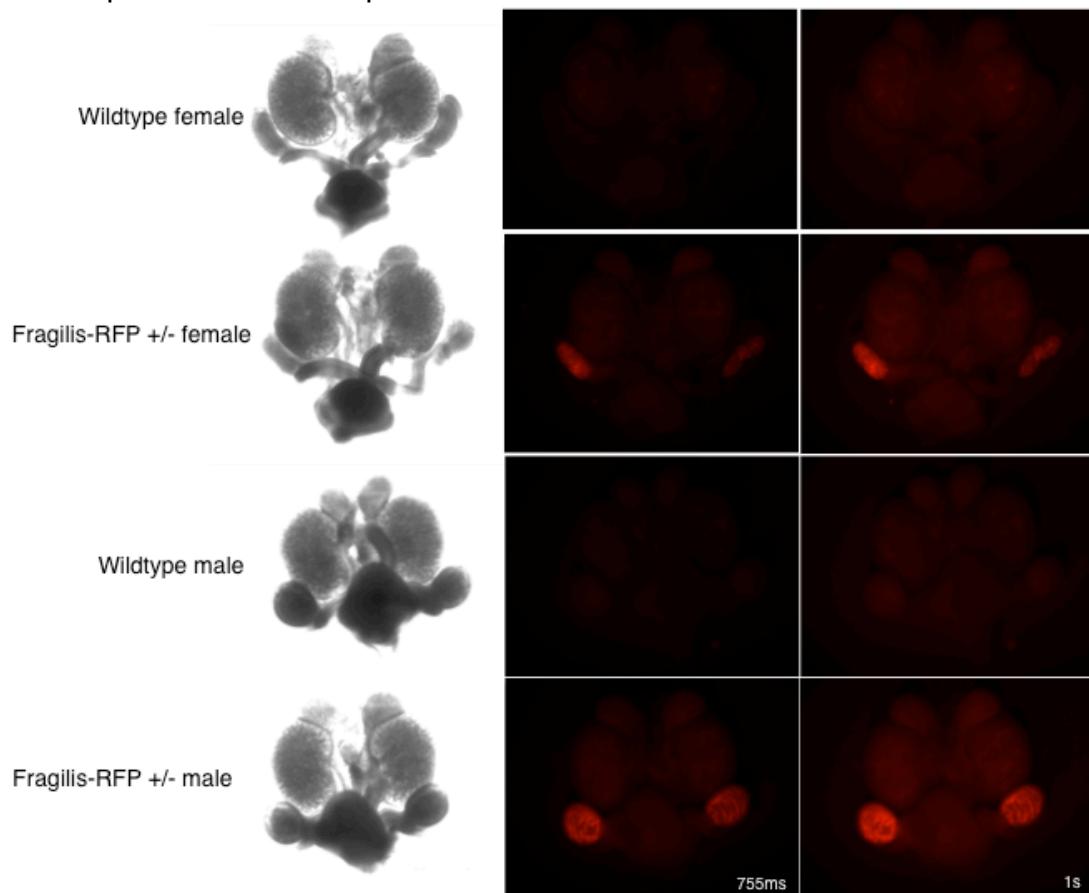


Fig 2. Wholemount RFP detection in 15.5 dpc *Fragilis*^{RFP/+} UGS samples. Strong RFP fluorescence was visible in dissected UGSs. Expression was limited to the Fragilis domain.

Immunohistochemistry

Immunohistochemistry was performed to examine whether RFP was expressed in cells within the expected Fragilis domain. Frozen sections of *Fragilis*^{RFP/+} and wildtype 15.5 dpc UGS probed with anti-RFP and anti-E-Cadherin (germ cells) antibodies confirms co-localization of RFP expressing cells and E-Cadherin positive cells.

Whole UGSs were fixed in 4% paraformaldehyde at 4°C for 2 hours, washed 3 times in PBS, equilibrated in 30% sucrose overnight and then embedded in OCT and flash frozen on dry-ice. The UGSs were sectioned at 16um and probed with either rabbit-anti-RFP/rat-anti-E-Cadherin /chicken-anti-Laminin or rabbit-anti-RFP/rat-anti-Flk1/mouse-anti-Cytokeratin antibodies. Anti-RFP (Rabbit, MBL PM 005 1:1000); anti-E-Cadherin (Rat, Sigma, U3254, 1:1000); anti-Flk1 (Rat, BD Pharmingen, 555307, 1:1000); anti-Laminin (Chicken, Abcam, ab14055, 1:500); anti-Cytokeratin (Mouse IgG1, Sigma, C 2562, 1:500) were incubated overnight at 4°C and detected with secondary antibodies Alexafluor 488, 568, 633, and 647 (Molecular probes) as indicated in the figure.

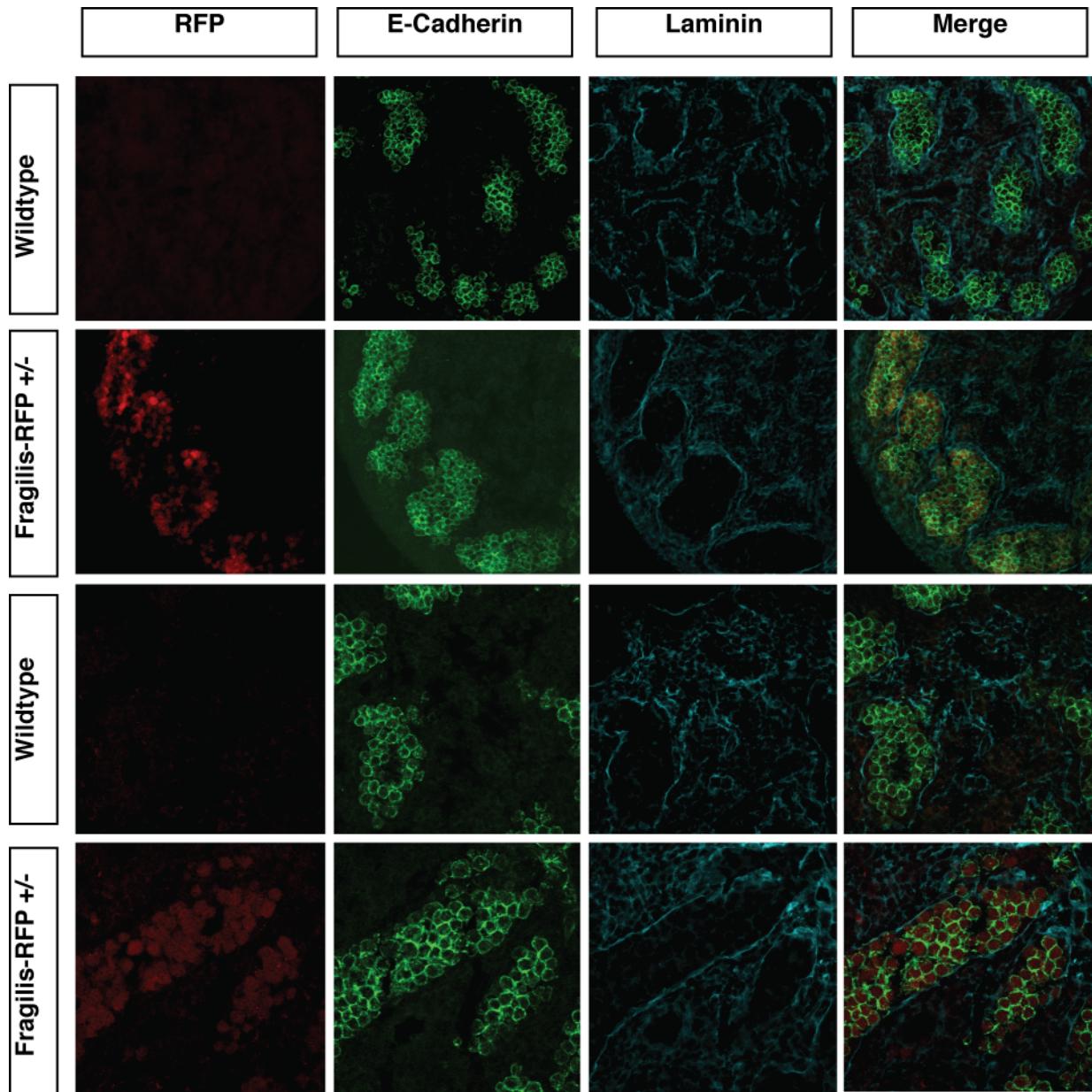


Fig 4. RFP is detected in germ cells within the testis of Fragilis^{RFP/+} embryos.
Fragilis^{RFP/+} and wildtype 15.5 dpc UGS probed with anti-RFP, anti E-Cadherin (germ cells) and anti-Laminin (basal lamina) indicates that Fragilis-RFP localizes within E-Cadherin positive germ cells in the testis.

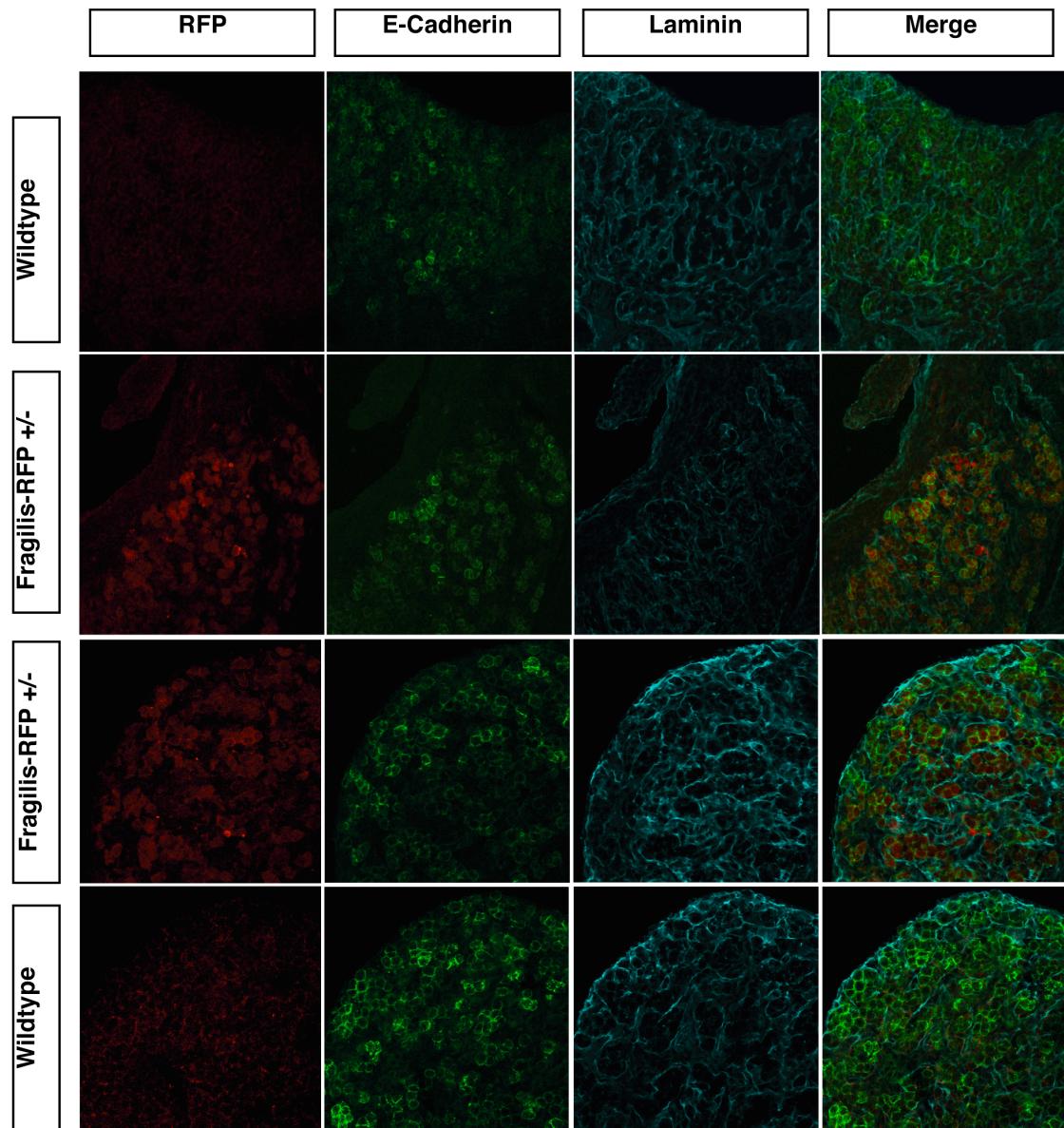


Fig 5. RFP is detected in germ cells in the ovaries of *Fragilis*^{RFP/+} embryos.
Fragilis^{RFP/+} and wildtype 15.5 dpc UGS probed with anti-RFP, anti-E-Cadherin (germ cells) and anti-Laminin (basal lamina) indicates that *Fragilis*-RFP localizes within E-Cadherin positive germ cells in the ovary.