Id3-RFP Allele Characterization

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Findings:

RFP is detected in the expected tissues. We conclude this allele does exhibit the expected activity.

Data:

Crosses

The ID3-RFP strain is a RFP knock-in line. To characterize this line, we examined RFP expression for the correct spatial pattern at E15.5.

We crossed wildtype B6 female mice with ID3-RFP males to obtain ID3-RFP/+ embryos. The embryos were dissected on E15.5 to collect the urogenital system (UGS) and examined for RFP expression. Two E15.5 litters were dissected and eleven embryos isolated. Whole embryos as well as dissected UGSs were viewed with a fluorescence microscope to detect RFP. A strong red fluorescence was visible in whole embryos as well as the kidneys of the UGSs (Fig2). Seven ID3-RFP/+ embryos and four wildtype control littermates were embedded for frozen sectioning and immunohistochemistry.

Genotyping

Tail samples of the embryos were collected and incubated in tail digestion buffer overnight at 55ºC. 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: 351bp
DNA sequence (forward): 5' `tcctcggtatcagcgcttcc` 3'
DNA sequence (reverse1) 5' `caatggccaggctacgttcc` 3'

Oligonucleotides: for targeted/transgenic allele — Size: 234bp
DNA sequence (forward): 5' `tcctcggtatcagcgcttcc` 3'
DNA sequence (reverse 2) 5' `cttgatgacgtcctcggagg` 3'

Rxn Buffer and Conditions: (25µl reaction)

10X PCR	2.5ul			
1.25mM dNTP	4ul	94ºC	3min	1 cycle
10uM primer F	1ul	94ºC	30sec	
10uM primer R1	1ul	65ºC	45sec	35cycles
Amplify Taq	0.3ul (5u/ul)	72ºC	60sec	
5x cresol red dye	2.5ul	72ºC	10min	1 cycle
Genomic DNA	1ul			
Total volume	**25 ul**			

10X PCR Buffer
500 mM KCl
100 mM Tris-HCl, pH 8.4
15 mM MgCl2
200ug/ml gelatin (Fluka # 48322)

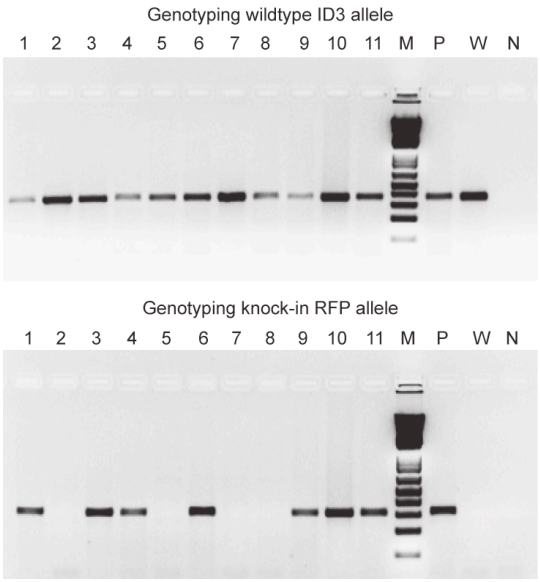

Figure 1. ID3-RFP genotyping. No 1, 3, 4, 6, 9,10 and 11 $ID3^{RFP/+}$, No 2, 5, 7 and 8 Wildtype. P respective positive controls W: Wildtype control; N: Negative control

Native Fluorescence

Whole embryos as well as dissected UGSs were viewed with a fluorescence microscope to detect RFP. At E15.5, RFP was expressed in the ID3 domain in the kidney.

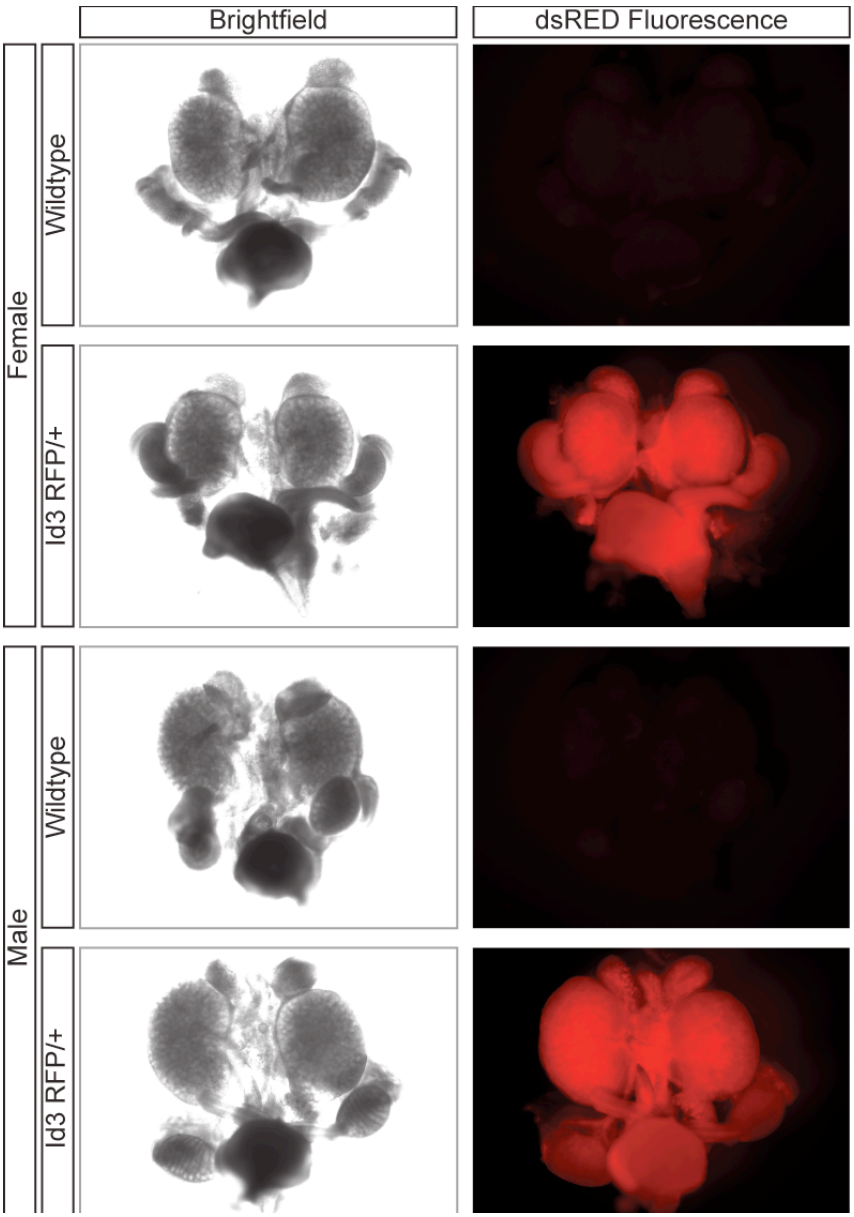

Figure 2. RFP is detected in transgenic E15.5 whole UGS. Broad, intense fluorescence is easily viewable in transgenic tissues.

Immunohistochemistry

Immunohistochemistry was performed to examine if the RFP allele was expressed in the expected ID3 domain. Two of each ID3-RFP/+; and wildtype UGSs (male and female) were assayed. RFP protein were assayed by staining with Rabbit-anti-RFP.

Whole UGSs were fixed in 4% paraformaldehyde at 4ºC 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 20um and stained with Rabbit-anti-RFP,

(Rabbit, MBL,PM005 1:1000),for overnight at 4ºC. The secondary antibodies were Alexafluor 488 (Molecular probes).

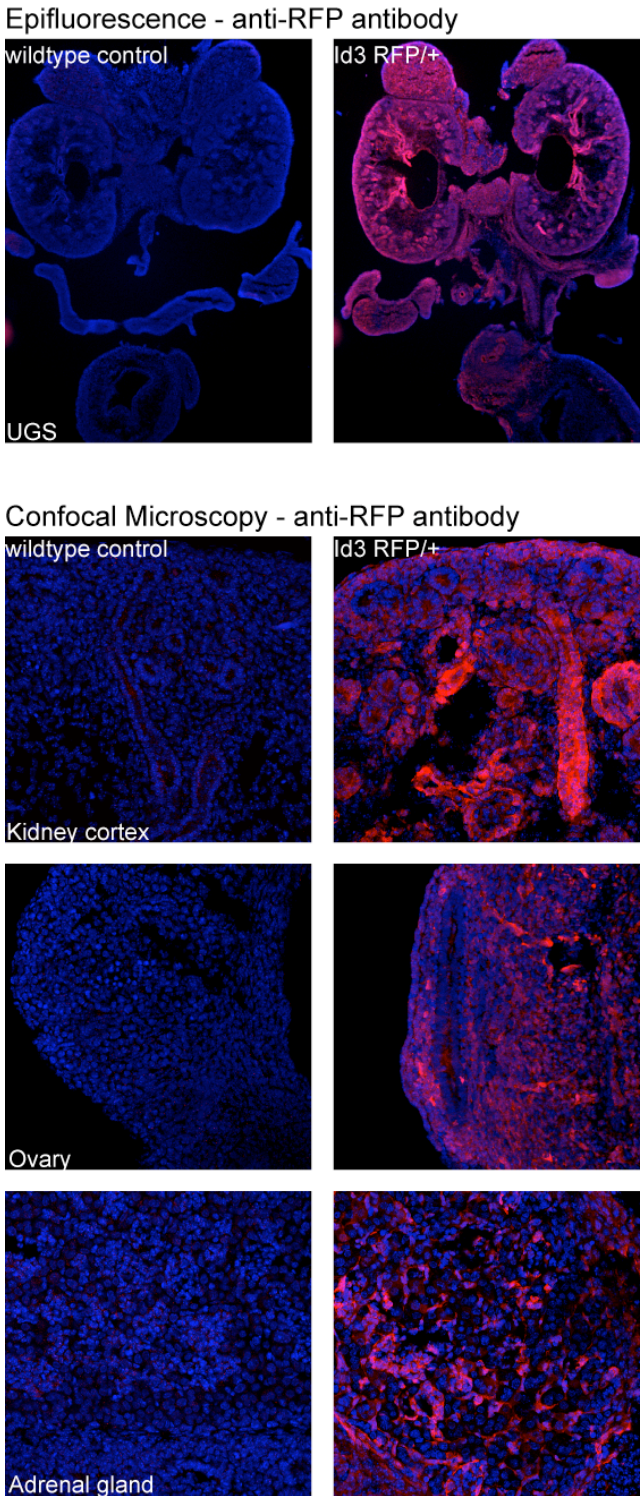

Figure 3. Immunohistochemistry shows RFP expression in several tissue populations. RFP is detected in the stalk of the collecting duct but not the tips. Signal is also seen in the cortical mesenchyme, the capsule, and the interstitial mesenchyme.