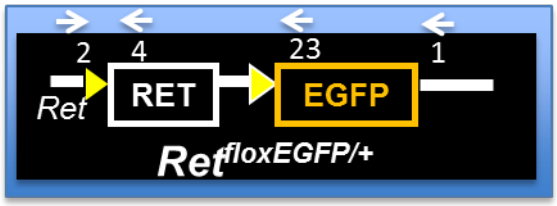
Characterization and Analysis of Conditional Ret-floxEGFP Reporter Mice

(Jain lab, Washington University, St. Louis)

Background. RET is a receptor tyrosine kinase that is activated upon binding to a complex formed by one of the four members of the glial cell line-derived neurotrophic factor family ligands (GFLs: GDNF, NRTN, ARTN, PSPN) and GFRalpha coreceptors (GFRa1-4) [1]. *RET* mutations result in a number of defects and diseases in humans and rodents including maldevelopment of the sympathetic, parasympathetic, sensory and enteric nervous systems, kidneys, ureters and testes. The human developmental diseases with causative *RET* mutations include Hirschsprung disease (intestinal aganglionosis), hereditary oncogenic syndromes (MEN2A, 2B) and CAKUT. CAKUT in mice or humans with RET defects include renal agenesis, hypoplasia, dysplasia, duplication, megaureter and vesicoureteral reflux. In the developing urinary system Ret expression is reported in the Wolffian duct (nephric duct), common nephric duct (CND), ureteric bud, UB tips. In the peripheral nervous system Ret is expressed in sympathetic, parasympathetic, enteric, sensory and motor neurons. Here we describe conditional Ret-floxedEGFP reporter mice where the reporter expression is dependent on Cre-mediated excision of knocked-in RET.

Strain Information.
Strain name: Ret-floxEGFP
Symbol: $Ret^{tm13.1Jmi}$ [2]
MGI ID: 3691588
Background: C57B6
Reporter: enhanced green fluorescent reporter (EGFP)
Locus: Ret
Alteration at locus: knockin of human RET9 isoform at the *Ret* locus

Description: The *Ret-floxEGFP* conditional reporter mice were generated by knocking in floxed hRET9 isoform cDNA followed by EGFP reporter in the first exon of mouse *Ret* locus using homologous recombination [2]. This results in inactivating endogenous mouse Ret expression and instead express hRET. These mice have been used to delete RET in cell types that express Cre and successful inducible deletion is indicated by concomitant expression of the EGFP green fluorescence reporter [2, 3]. When bred with a ubiquitous Cre, EGFP indicates RET expression in all tissues known to express Ret [4]. The Ret-floxEGFP mice were backcrossed with C57B6 mice for more than 15 generations. Selected data are shown below and more details can be found in the above papers.



Genotyping: Ret-floxEGFP mice

CAGCTACCCGCAGCGACCCGGTTC		1		Reverse WT (intron 1)
CAGCGCAGGTCTCTCATCAGTACCGCA		2		Common Forward (Ret promoter)
AGCATCCCTCGAGAAGTAGAGG		4		Reverse Mut (RET knock-in)
Ret conditional KO				
Rxn volume	25			
		1X	4X	
10X Buffer (25 mM MgCl2)		2.5	10	94 hold 4 min
10 mM dNTP (.08 mM final)		0.5	2	
DMSO		1.25	5	94, 45s; 60, 45s; 72, 45s
Primer 2 (10 uM)		2	8	35 cycles
Primer 1 (10 uM)		1	4	
Primer 4 (10 uM)		1	4	72 hold 5 min
Taq		0.2	0.8	
Water		14.55	58.2	Mutant band--200bp
DNA		2		WT band--300bp

CAGCGCAGGTCTCTCATCAGTACCGCA		2		Forward (Ret Promoter)
GCCGTTTACGTCGCCGTCCAGCTCGACCAG		23		Reverse (EGFP cDNA)
EGFP ret removed				
Rxn volume	25			
		1X	4X	
10X Buffer		2.5	10	**94 hold 4 min**
10 mM dNTP		0.5	2	
DMSO		1.25	5	**94, 45s; 60, 45s; 72, 45s**
Primer **2** (10 uM)		1	4	**35 cycles**
Primer **23** (10 uM)		1	4	
Taq		0.2	0.8	**72 hold 5 min**
Water		16.55	66.2	
DNA		2		**Band ~300bp**

Examples of Cre-mediated cell type specific expression using Ret conditional reporter mice

Live EGFP expression in whole Ret-EGFP embryos and sensory and motor neurons in known sites of Ret expression. Live EGFP signal detection in E11.5 Ret-EGFP embryo shows strong EGFP expression (denotes Ret expression) in known sites of Ret expression after crossing Ret-floxEGFP mice with ubiquitous actin Cre mice (Fig. 1, 2).

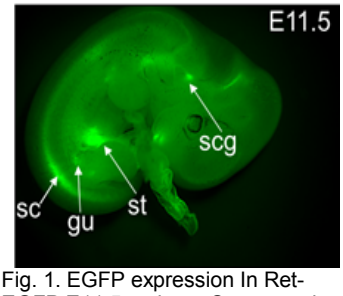


Fig. 1. EGFP expression In Ret-EGFP E11.5 embryo. Scg, superior cervical ganglion; gu, genitourinary; st, stomach; sc, spinal cord.

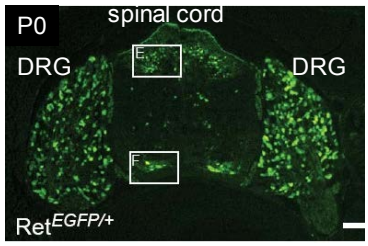


Fig. 2. EGFP expression In all sensory neurons in dorsal root ganglia (DRG) and their projections in the spinal cord (E, white box) and in motor neurons (F, white box).

Conditional expression of EGFP reporter in a cell type specific manner shows Ret excision and EGFP expression. Shown are results from crosses of two different tissues specific Cre recombinase deletor strains with Ret-floxEGFP mice (Fig. 3, 4) [2, 3].

Example of successful complete conditional deletion of Ret using Ret-floxEGFP mice and dopaminergic neuron specific Cre mice (Fig. 5). Dat-Cre mice were used to delete Ret in dopaminergic neurons by breeding with $Ret^{floxEGFP/-}$ mice (one allele is null). Green fluorescence shows deletion of Ret (Fig. 5). Immunostaining with antiRet antibody confirms absence of Ret. Immunostaining with antiTH antibodies shows presence of dopaminergic neurons and nerve fibers indicating that neurons are still present but lack Ret [2].

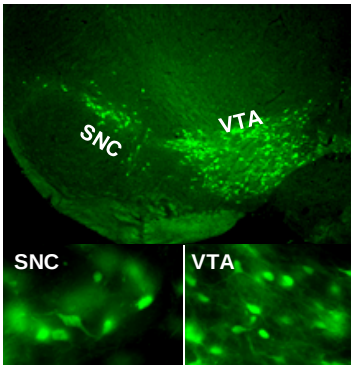


Fig. 3. Cre expressed by *Dat locus* excises RET in the midbrain dopaminergic neurons as detected by EGFP expression ($Ret^{floxEGFP/floxEGFP}$ x $Dat^{Cre/+}$). SNC, substantia nigra compacta; VTA, ventral tegmental area.

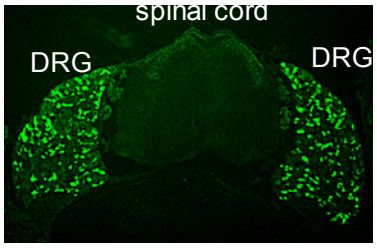


Fig. 4. Cre expressed by *NaV1.8 locus* excises RET in the nociceptive DRG sensory neurons as detected by EGFP expression ($Ret^{floxEGFP/floxEGFP}$ x $NaV1.8^{Cre/+}$). Note green staining in dorsal spinal cord only (DRG projections) and not in other areas of Ret expression (ventral spinal cord, contrast with Fig 2).

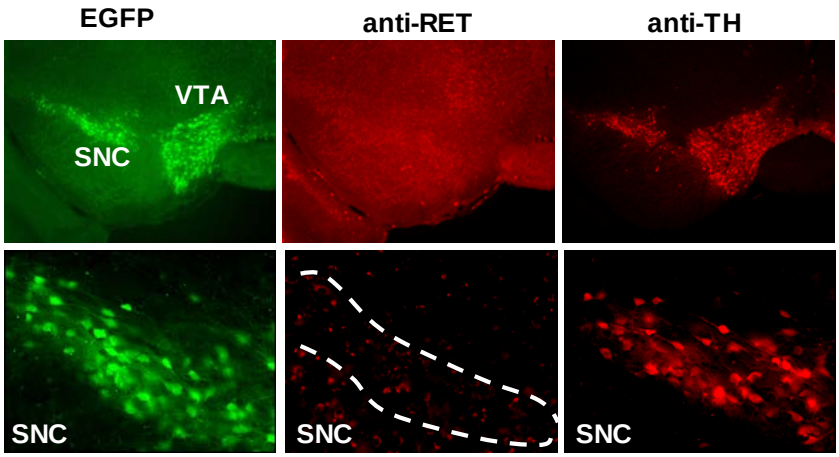


Fig. 5. Conditional deletion of Ret in midbrain dopaminergic neurons using DatCre mice ($Ret^{floxEGFP/-}$ x $Dat^{Cre/+}$). SNC, substantia nigra compacta; VTA, ventral tegmental area.

REFERENCES

1. Jain, S., *The many faces of RET dysfunction in kidney.* Organogenesis, 2009. **5**(4): p. 1-14.
2. Jain, S., et al., *RET Is Dispensable for Maintenance of Midbrain Dopaminergic Neurons in Adult Mice.* J. Neurosci., 2006. **26**(43): p. 11230-11238.
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4. Hoshi, M., et al., *Novel mechanisms of early upper and lower urinary tract patterning regulated by RetY1015 docking tyrosine in mice.* Development, 2012. **139**(13): p. 2405-15.