

Sox18-GCE Allele Characterization

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Findings: VALIDATED

This allele is expressing GFP-Cre in the expected cell populations at E15.5. Expression is at a low level, but Cre dependent R26R-LacZ expression is observed following Tamoxifen treatment.

Data:

Crosses

The Sox-GCE strain is a GFP knock-in line. In order to characterize this line, two questions were addressed:

- 1) Is the GFP-Cre-ErT2 cassette (GCE) expressed in the expected Sox18 domain?
- 2) Does the Cre function as expected?

We crossed Rosa26R^{lacZ/+} (R26R) female mice with Sox18^{GCE/+} males to obtain Sox18^{GCE/+}; R26R^{lacZ/+} embryos. In order to activate β-gal reporter expression from the R26R^{lacZ/+} allele, 1mg/40g body weight mouse tamoxifen in corn oil was injected into the belly of pregnant E13.5 mice. In the control group from the same crosses the same volume of corn oil was injected into the belly of the pregnant mice at E13.5. Both tamoxifen treated, and corn oil only control Sox18^{GCE/+}; R26R^{lacZ/+} embryos were dissected at E15.5 to collect the urogenital system (UGS).

Three E15.5 litters of tamoxifen injected R26R^{lacZ/+} were dissected on 8/13/2008, 8/15/2008 and 8/27/2008 and twenty-nine embryos were isolated. Whole embryos as well as dissected UGSs were viewed with a fluorescence microscope to detect GFP. No green fluorescence was visible in the whole embryos or the UGSs. Two male and three female Sox18^{GCE/+}; R26R^{lacZ/+} UGSs as well as R26R^{lacZ/+} littermates were stained with X-gal to assay for β-gal activity. β-gal activity was detected in all of the Sox18^{GCE/+}; R26R^{lacZ/+} UGSs, but not in tamoxifen treated R26R^{lacZ/+} controls. These results are shown in Figure 2.

The initial results showed a low frequency of cell labeling with a single dose of Tamoxifen. We therefore decided to dose the pregnant females over four days, E11.5-E14.5 to increase the number for labeled cells for further analysis. One tamoxifen treated and one corn oil control where dissected on 9/12/2008, yielding a litter of seven and ten embryos respectively. Sox18^{GCE/+}; R26R^{lacZ/+} UGSs and control R26R^{lacZ/+} littermates were stained with X-gal to assay for β-gal activity. β-gal activity was detected in the tamoxifen treated Sox18^{GCE/+}; R26R^{lacZ/+} UGSs, but not in the corn oil control Sox18^{GCE/+}; R26R^{lacZ/+} UGSs or the R26R^{lacZ/+} controls. The results are shown in Figure 3.

Remaining Sox18^{GCE/+}; R26R^{lacZ/+} experimentals and Rosa26R^{lacZ/+} littermate controls from the 4-day tamoxifen injected and corn oil control E15.5 litters were embedded for frozen sectioning and immunohistochemistry (See figure 4).

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: 373bp

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

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

Oligonucleotides: for targeted/transgenic allele Size: ~239bp

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

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

Amplifies GFP within GFP-Cre 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	56°C	60sec	35cycles
10uM primer R2	1ul	72°C	90sec	
DMSO	2.5ul	72°C	10min	1 cycle
2-mercaptoethanol	0.125ul			
	0.3ul			
Amplify Taq	(5u/ul)			
5x cresol red dye	2.5ul			
Genomic DNA	1ul			
Total volume	25 ul			

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

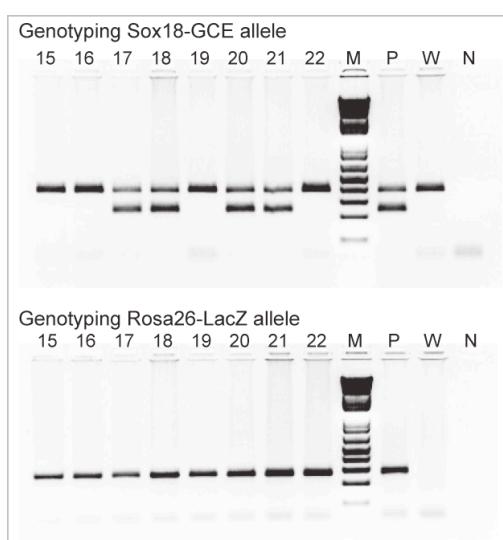


Fig1: No 17, 18, 20 and 21 $\text{Sox18}^{\text{GCE}/+}$, $\text{Rosa26R}^{\text{lacZ}/+}$; No 15, 16, 19 and 22 $\text{Rosa26R}^{\text{lacZ}/+}$; **P:** $\text{Sox18-GCE}/+$ positive control; **W:** Wildtype control; **N:** Negative control.

Native Fluorescence

Whole embryos as well as dissected UGSs were examined with a fluorescent microscope to view GFP expression. However, GFP was not detectable under these conditions.

Cre-recombinase Activity

Dissected UGS samples were stained with X-gal to assay for β -gal activity. Tamoxifen dependent Cre activity was detected in $\text{Sox18}^{\text{GCE}/+}$; $\text{R26R}^{\text{lacZ}/+}$ samples (Fig. 2 and 3).

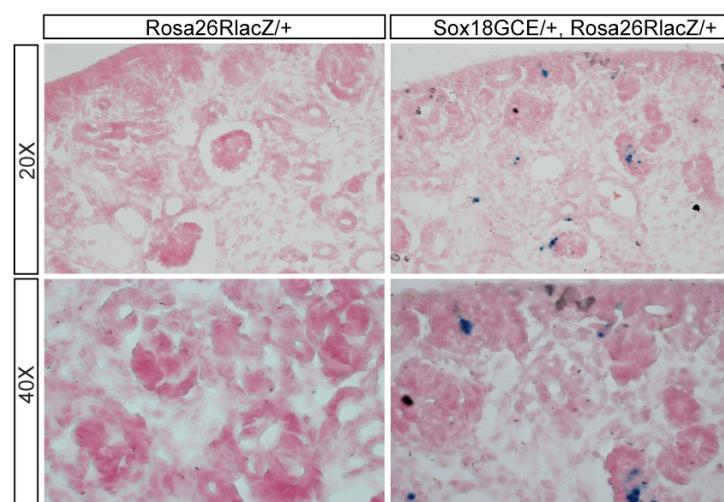
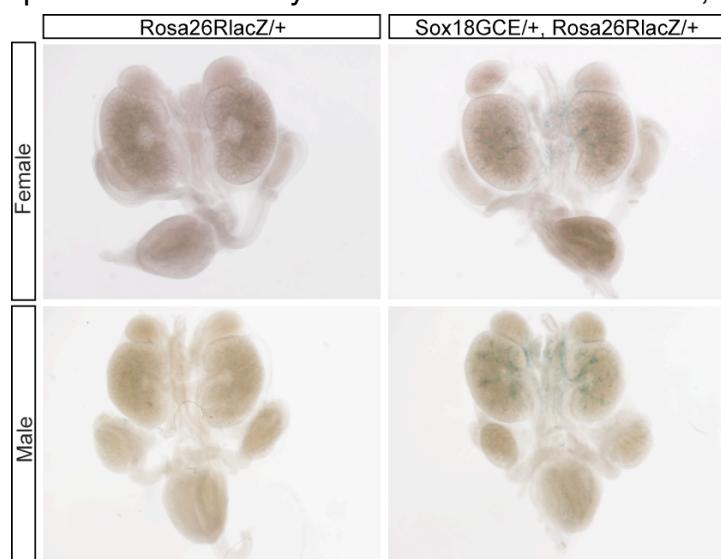


Fig2. Cre-dependent β -gal activity in $\text{Sox18}^{\text{GCE}/+}$; $\text{R26R}^{\text{lacZ}/+}$ UGSs. Single dose tamoxifen resulted in a low percentage of labeled cells in transgenics. Controls were

negative for Bgal activity. *Upper two rows:* $\text{Sox18}^{\text{GCE}/+}$; $\text{R26R}^{\text{lacZ}/+}$ and $\text{R26R}^{\text{lacZ}/+}$ control tamoxifen injected of UGSs were stained with X-gal, (X-gal Blue). (Wholemount X-gal stain). *Lower two rows:* $\text{Sox18}^{\text{GCE}/+}$; $\text{R26R}^{\text{lacZ}/+}$ and $\text{R26R}^{\text{lacZ}/+}$ control tamoxifen injected kidneys were stained with X-gal,(X-gal blue). (Section X-gal staining)

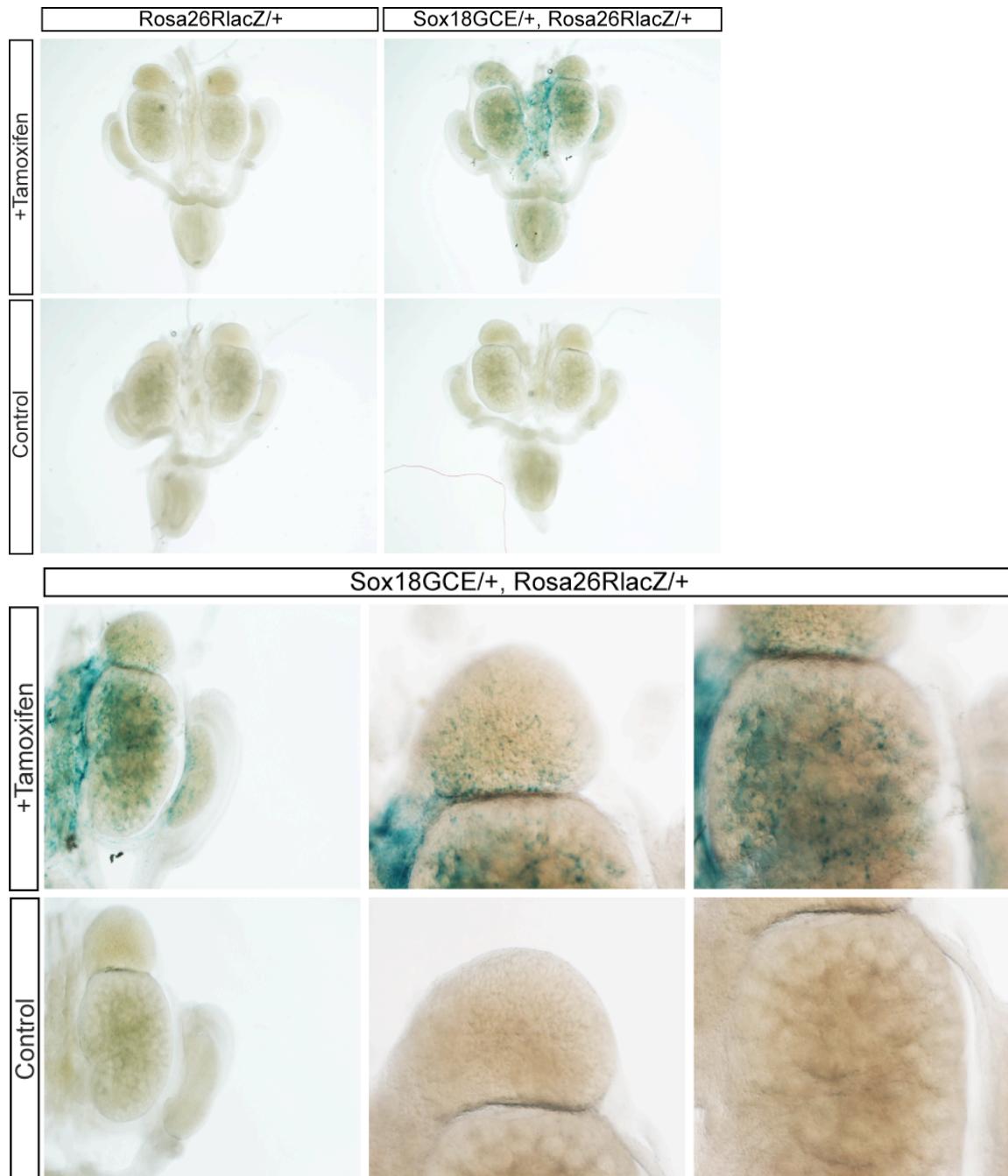


Fig3. Tamoxifen 4-day treatment. β -gal activity was detected in the $\text{Sox18}^{\text{GCE}/+}$; $\text{R26R}^{\text{lacZ}/+}$ tamoxifen injected UGSs. Not in $\text{Sox18}^{\text{GCE}/+}$; $\text{R26R}^{\text{lacZ}/+}$ corn oil control UGSs or the $\text{R26R}^{\text{lacZ}/+}$ control (consecutive tamoxifen injections). *Upper panel:* $\text{Sox18}^{\text{GCE}/+}$; $\text{R26R}^{\text{lacZ}/+}$ tamoxifen injected and corn oil control UGSs and $\text{R26R}^{\text{lacZ}/+}$ were stained with

X-gal, (X-gal Blue). Lower two rows: Sox18^{GCE/+}; R26R^{lacZ/+} tamoxifen injected and corn oil control kidneys were stained with X-gal, (X-gal blue).

Immunohistochemistry

Immunohistochemistry was performed to examine if the GCE allele was expressed in the expected Sox18 domain. Three of Sox18^{GCE/+}; R26R^{lacZ/+}, and R26R^{lacZ/+} UGSs were assayed. GFP protein was examined by staining with chicken-anti-GFP. To test for Cre function, three of Sox18^{GCE/+}; R26R^{lacZ/+} experimentals, one of Sox18^{GCE/+}; R26R^{lacZ/+} corn oil control and R26R^{lacZ/+} UGSs were assayed, β-gal and GFP expression were examined by double staining with rabbit anti-β-gal and chicken-anti-GFP; β-gal expression were examined by double staining with rabbit anti-β-gal and rat-anti-PECAM or rabbit anti-β-gal and rat-anti-Flk1.

Whole UGSs were fixed in 4% paraformaldehyde at 4°C 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 stained with rabbit-anti-β-gal/chicken-anti-GFP/mouse-anti-Cytokeratin, rabbit-anti-β-gal/rat-anti-PECAM/mouse-anti-Cytokeratin, rabbit-anti-β-gal/rat-anti-Flk1/mouse-anti-Cytokeratin, respectively. GFP (Chicken, Aves Labs, Inc, GFP-1020, 1:500); beta-gal (Rabbit, MP Biomedicals, LLC, 55976, 1: 20000); Flk1 (Rat, BD Pharmingen, 555307, 1:1000); PECAM (Rat, BD Pharmingen, 553370, 1:1000); 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.

GFP protein was detected in both Sox18^{GCE/+}; R26R^{lacZ/+} tamoxifen injected and corn oil control kidneys and ovary, no GFP was detected in R26R^{lacZ/+} control kidneys; LacZ protein was detected only in Sox18^{GCE/+}; R26R^{lacZ/+} tamoxifen injected kidneys and ovary, but no in both Sox18^{GCE/+}; R26R^{lacZ/+} corn oil control and R26R^{lacZ/+} control kidneys.

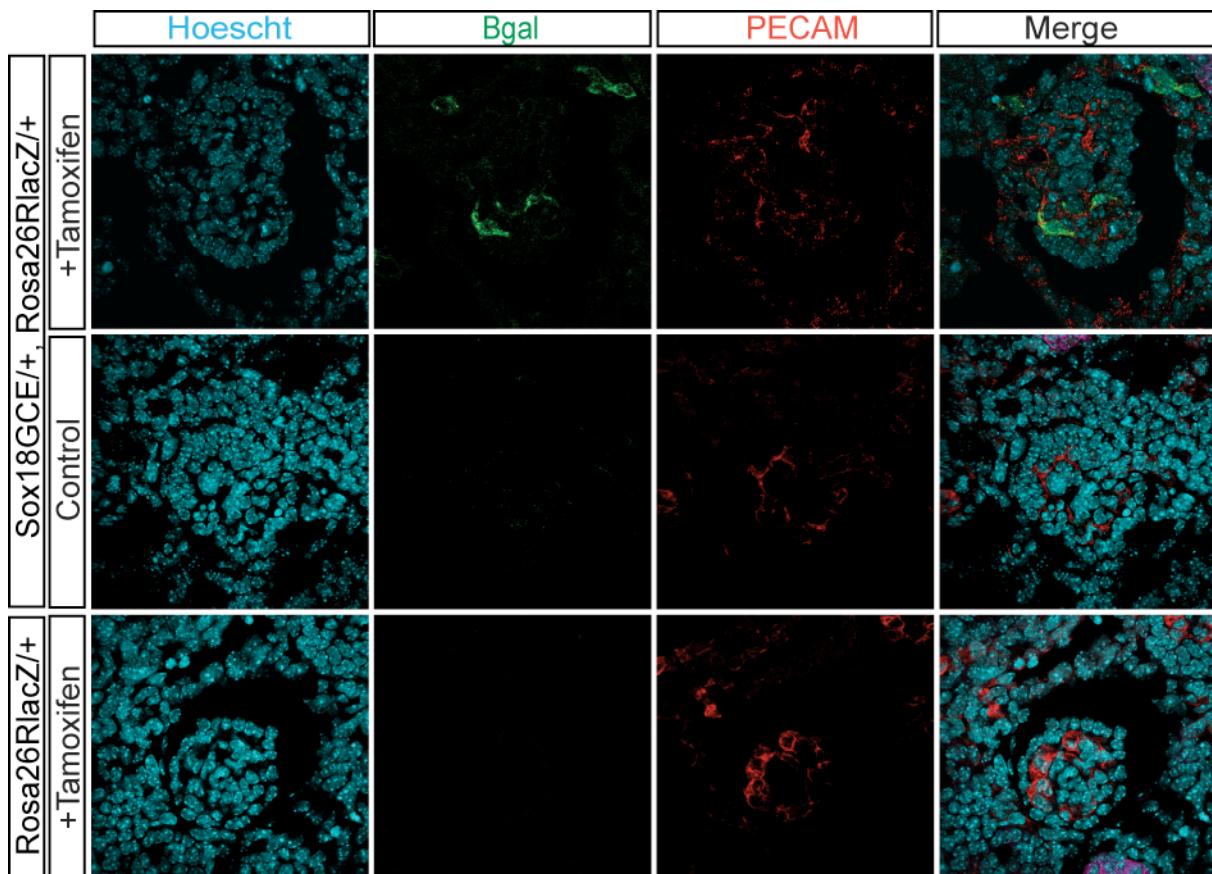


Fig4. Beta-galactosidase detected in endothelial cells of the glomerulus in Sox18^{GCE/+}; R26R^{lacZ/+} tamoxifen injected kidneys. Sox18^{GCE/+}; R26R^{lacZ/+} and R26R^{lacZ/+} kidneys are stained with anti-β-gal and anti-PECAM (CD31), (b-gal green, PECAM red). Co-localization of PECAM staining and Tamoxifen-dependent B-gal expression is detected in endothelial cells of the glomerulus.