Klf3-GCE Allele Characterization

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Findings:

Both GFP and Cre activity were confirmed in this strain. We conclude this allele does exhibit the expected activity.

Data:

Crosses

The Klf3-GCE strain is a GFP knock-in line. In order to characterize them, two questions were addressed:

1) Is the GFP-Cre-ErT2 cassette (GCE) expressed in the expected Klf3 domain?
2) Does the Cre function as expected in the Klf3 expression domain?

We crossed B6 or Rosa26R$^{lacZ/+}$ (R26R) female mice with Klf3$^{GCE/+}$ males to obtain Klf3-$^{GCE/+}$ embryos and Klf3$^{GCE/+}$; R26R$^{lacZ/+}$ embryos. In order to activate β-gal reporter from the R26R$^{lacZ/+}$ allele, 85ul (10mg/mL) tamoxifen in corn oil was injected into the belly of the pregnant mice at E13.5. The control group from the R26R$^{lacZ/+}$ allele, 85ul corn oil was injected the belly of the pregnant mice at E13.5. The embryos were dissected on E15.5 to collect the urogenital system (UGS). To address the first question assay by GFP and answer the second question assay with β-gal reported.

Two E15.5 litters of B6 were dissected on 3/7/2008 and litters of seven embryos and nine embryos were isolated respectively. Whole embryos as well as dissected UGSs were viewed with a fluorescence microscope to detect GFP. A weak green fluorescence was visible on the back and limbs of the whole embryos as well as the kidneys of the UGSs. The results showed as Fig1 Klf3-GCE wholemount fluorescence.

Five Klf3$^{GCE/+}$; experimentals and four wildtype controls from the first E15.5 litter and one Klf3$^{GCE/+}$; experimentals and two wildtype controls from the second E15.5 litter were embedded for frozen sectioning and immunohistochemistry.

Three E15.5 litters from tamoxifen injected Rosa26R$^{lacZ/+}$ females were dissected on 3/27/2008, 4/11/2008 and 4/18/2008 and litters of nine embryos, eight embryos and eight embryos were isolated respectively. Whole embryos as well as dissected UGSs were viewed with a fluorescence microscope to detect GFP. A weak green fluorescence was visible same as above described. Dissected of each of male and female of Klf3$^{GCE/+}$; Rosa26R$^{lacZ/+}$ UGSs as well as of wildtype littermates UGS from the first litter and dissected of one female of Klf3-$^{GCE/+}$; Rosa26R$^{lacZ/+}$ UGSs as well as one female wildtype littermate UGSs from the second litter were stained with X-gal to assay for β-gal activity, β-gal activity was detected in all of the Klf3$^{GCE/+}$; Rosa26R$^{lacZ/+}$ UGSs. Not in the wildtype control. The results showed as Fig.2 Klf3-GCE wholemount X-gal stain.

Two E15.5 litters of corn oil injected R26R$^{lacZ/+}$ were dissected on 3/7/2008 and 4/11/2008 and litters of four embryos and eight embryos were isolated respectively. Whole embryos as well as dissected UGSs were viewed with a fluorescence microscope and a weak green fluorescence was seen. Three of dissected female UGSs from the second litter were stained with X-gal as a control to assay for β-gal activity, (none of any male of the Klf3-GCE/+ allele were got from the two litters), no β-gal activity was detected in this batch, including two of Klf3$^{GCE/+}$; R26R$^{lacZ/+}$UGSs (determined by PCR genotyping). Xgal staining reveals Tamoxifen induced Cre activity in treated transgenic animals.

Seven Klf3$^{GCE/+}$; R26R$^{lacZ/+}$ experimentals, three Klf3$^{GCE/+}$; R26R$^{lacZ/+}$ corn oil controls, and seven wildtype controls from all of the E15.5 litters 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 (Fig3).

Oligonucleotides: for Wt allele Size: 437 bp
DNA sequence (forward): 5'- `cagttagtcctgcccgggag` -3'
DNA sequence (reverse1) 5'- `agatcggcctacggttgacc` -3'

Oligonucleotides: for targeted/transgenic allele Size: ~276bp
DNA sequence (forward): 5'- `cagttagtcctgcccgggag` -3'
DNA sequence (reverse 2) 5'- `gtccagctcgaccaggatgg` -3'
Amplifies GFP within tTA-GFP-Cre region

Rxn Buffer and Conditions: (25μl reaction)

10X GSB	2.5ul
25mM dNTP	1ul
0.25ug/ul primer F	1ul
0.25ug/ul primer R1	1ul
0.25ug/ul primer R2	1ul
DMSO	2.5ul
2-mercaptoethanol	0.125ul
Amplify Taq	0.3ul(5u/ul)
5x cresol red dye	2.5ul
Genomic DNA	1ul
Total volume	**25 ul**

94ºC	3min	1 cycle
94ºC	30sec	35cycles
60ºC	60sec	
72ºC	90sec	
72ºC	10min	1 cycle

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

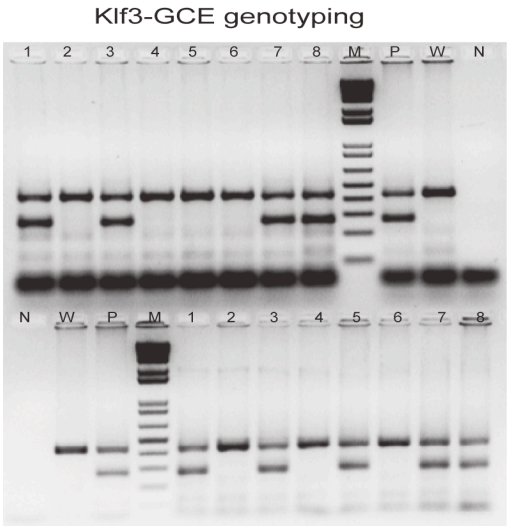
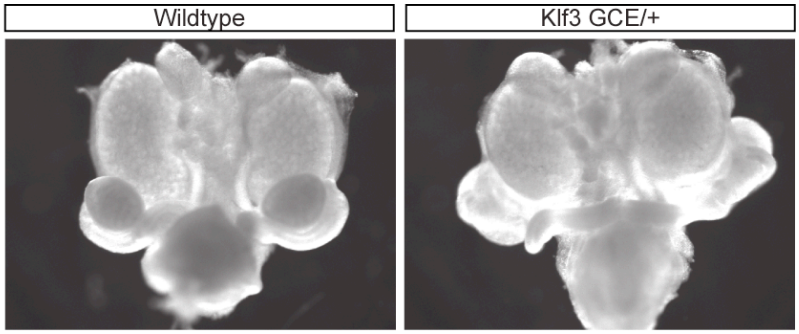


Figure 1. Klf3-GCE genotyping.
Upper row 1,3, 7 and 8 Klf3$^{GCE/+}$, Rosa26R$^{lacZ/+;}$, 2,4,5 and 6wildtype P Klf3-GCE/+ positive control; W: Wildtype control; N: Negative control. Lower row 1,3,5,7 and 8 Klf3$^{GCE/+}$, Rosa26R$^{lacZ/+;}$, 2,4 and 6wildtype P Klf3-GCE/+ positive control; W: Wildtype control; N: Negative control

Native Fluorescence

Whole embryos as well as dissected UGSs were viewed with a fluorescence microscope to detect GFP. Klf3$^{GCE/+}$ transgenic UGSs showed clear GFP expression.



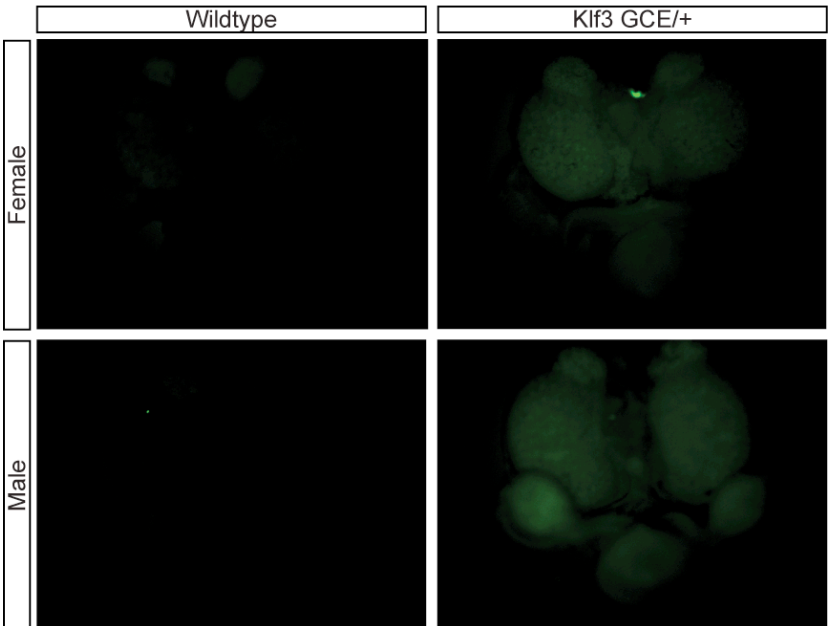


Figure 2. Klf3-GCE Fluorescence. Brightfield and darkfield images showing native GFP expression in freshly dissected UGSs.

Immunohistochemistry

Immunohistochemistry was performed to examine if the GCE allele was expressed in the expected Klf3 domain. A $Klf3^{GCE/+}$; $R26R^{lacZ/+}$ experimental, a $Klf3^{GCE/+}$; $R26R^{lacZ/+}$ corn oil control and a wildtype UGSs were examined by double staining with mouse anti-β-gal and chicken-anti-GFP.

For the chicken-anti-GFP antibody, $Six2TGC^{tg/+}$ embryos were employed as a positive control, and the anti-Pax2 staining were done to verify tissue quality for immunohistochemistry. We cut 20um sections and stained with mouse-anti-b-gal/chicken-anti-GFP, chicken-anti-GFP, and chicken-anti-GFP/rabbit-Pax2, respectively.GFP (Chicken, aves LABS, INC, GFP-1020, 1:500), beta-gal (Mouse IgG1, DSHB, 40-1a-s, 1:20) and Pax2(Rabbit, Covance, PRB-276P, 1:250) for overnight at 4ºC. The secondary antibodies were Alexafluor 488 and 568 (Molecular probes).

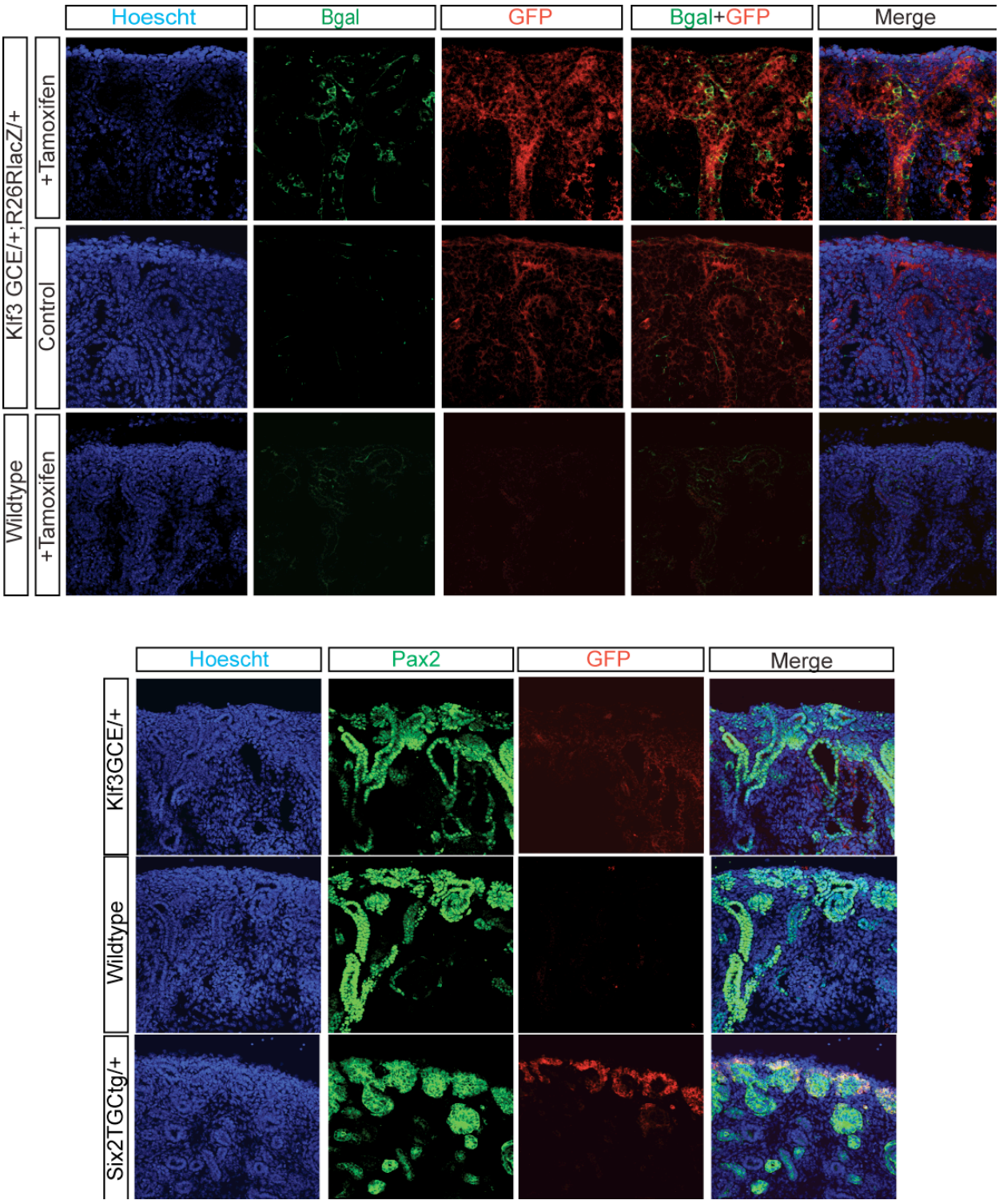


Figure 3. GFP protein detected by antibody staining. GFP protein was detected in both Klf3$^{GCE/+}$; R26R$^{lacZ/+}$ tamoxifen injected and corn oil control kidneys, no GFP was detected in wildtype control kidneys; Bgal protein was detected only in Klf3$^{GCE/+}$; R26R$^{lacZ/+}$ tamoxifen injected kidneys, but not in either Klf3$^{GCE/+}$; R26R$^{lacZ/+}$ corn oil control and wildtype control kidneys.

Upper three rows: Klf3$^{GCE/+}$ and wildtype kidneys are stained with anti- b-gal and anti-GFP, (b-gal green, GFP red).

Lower three rows: Controls. Six2TCG$^{tg/+}$, Klf3$^{GCE/+}$ and wildtype kidneys were stained with anti-Pax2 and anti-GFP, Pax2 protein was detected in all Six2TCG$^{tg/+}$ and Klf3$^{GCE/+}$ kidneys and wildtype, and serves as a tissue control. GFP protein was detected in Six2TCG$^{tg/+}$ and Klf3$^{GCE/+}$ kidneys, indicating the GFP antibody is detecting GFP protein as expected.

Cre-recombinase activity

All five dissected UGSs from the first litter were stained with X-gal to assay for β-gal activity. No β-gal activity was detected in this batch (data not shown), including two Klf3$^{GCE/+}$; Rosa26R$^{lacZ/+}$ UGSs (determined by PCR genotyping).

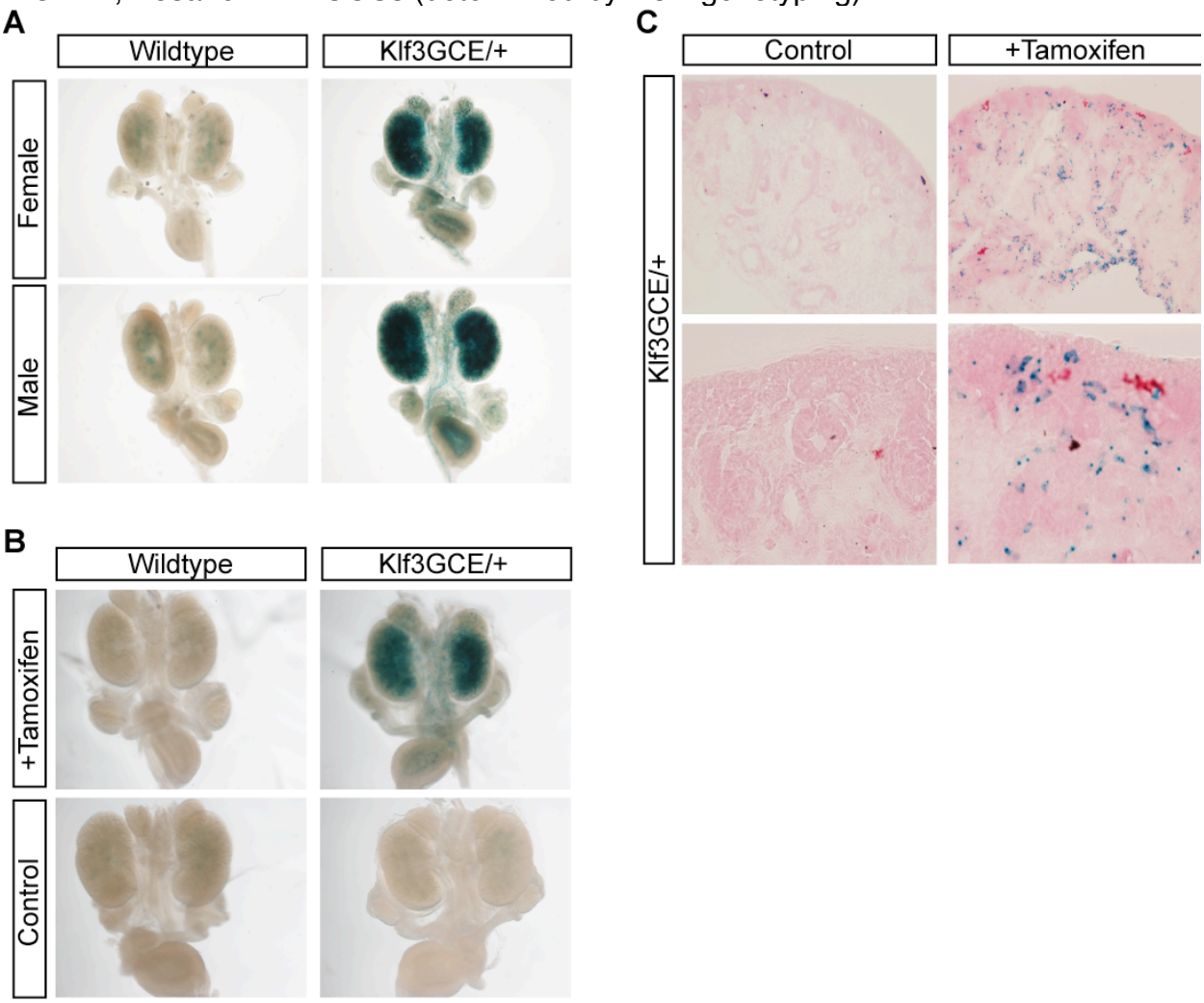


Figure 4. Cre-dependent Bgal activity. β-gal activity was detected in all of the Klf3$^{GCE/+}$; Rosa26R$^{lacZ/+}$ tamoxifen injected UGSs. Not in Klf3$^{GCE/+}$ Rosa26R$^{lacZ/+}$ corn oil control UGGs and the wildtype control.

A: Klf3$^{GCE/+}$; R26R$^{lacZ/+}$ and wildtype control tamoxifen injected of UGSs were stained with X-gal, (X-gal Blue).

B: $Klf3^{GCE/+}$; $R26R^{lacZ/+}$ tamoxifen injected and corn oil control UGSs as well as wildtype control UGSs were stained with X-gal,(X-gal blue).
C: $Klf3^{GCE/+}$; $R26R^{lacZ/+}$ tamoxifen injected and corn oil control kidneys were stained with X-gal (X-gal blue).