

Characterization and Analysis of Notch2 GENSAT BAC transgenic

GENSAT is NIH funded project that was initiated to generate BAC/EGFP transgenic lines with the intention to provide genetic tools that would facilitate the study of the central nervous system (CNS). We have take advantage of the availability of GENSAT transgenic mice to address whether any of the transgenic lines that have been generated would be appropriate to study renal development. The analysis here provides the kidney research community with basic information as to the utility of GENSAT transgenic strains in furthering the study of kidney development. As part of the GUDMAP consortium, we have tested several strains from GENSAT at a single appropriate time point (E15.5) and screened the mice for their ability to aid in the isolation of specific components from the developing kidney for gene expression profiling. Here we report the pattern of EGFP expression in the embryonic day 15.5 kidney of the *Notch2*-EGFP strain. **Our analysis suggests that *Notch2*-EGFP transgenic mice may be useful to further studies regarding the development of the proximal tubule, glomerulus and collecting duct system.**

Notch2 Gene Notes

This gene encodes a member of the Notch family. Members of this Type 1 transmembrane protein family share structural characteristics including an extracellular domain consisting of multiple epidermal growth factor-like (EGF) repeats, and an intracellular domain consisting of multiple, different domain types. Notch family members play a role in a variety of developmental processes by controlling cell fate decisions. Notch2 expression has been observed in epithelial derivatives of the metanephrogenic mesenchyme including the renal vesicles, comma- and S-shaped bodies and in the developing glomeruli (Leimeister *et al.*).

Strain Information

Promoter: Notch2

Name: Notch gene homolog 2 (Drosophila)

Alteration at locus: Transgenic Reporter: EGFP (Jelly Fish)

Name: Enhanced Green Fluorescent Protein

Alteration at locus: Transgenic Transgene: Tg(Notch2-EGFP)DG195Gsat

Name: transgene insertion DG195, GENSAT Project at Rockefeller University

Alteration at locus: Transgenic

For further information and distribution of transgenic mice, please use the following URL: <http://www.mmrrc.org/strains/11558/011558.html>

Characterization of Notch2 expression in the developing kidney

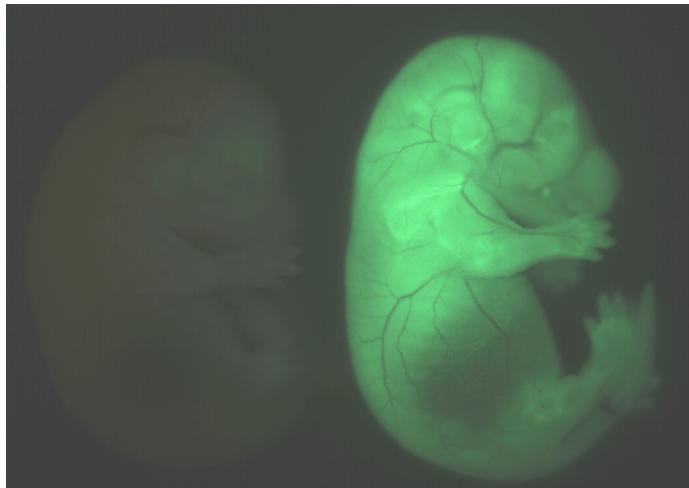


Figure 1. Analysis of *Notch2*-EGFP BAC transgenic mice in E15.5 embryos. Fluorescent image detailing the expression of Notch2 at E15.5. The embryo on the left is a non-transgenic littermate, while the embryo on the right is a *Notch2*-EGFP BAC transgenic. Note the widespread GFP expression in the embryo including skin and skeletal elements.

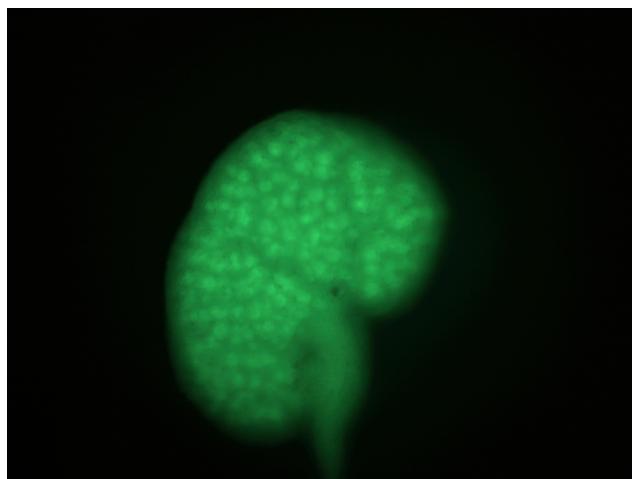


Figure 2. Expression of GFP in the kidneys of *Notch2*-EGFP BAC transgenic mice. Fluorescent microscopic image detailing *Notch2*-GFP expression in the developing kidney at E15.5. Note the punctate GFP expression found in the developing renal vesicle and S-shape bodies.

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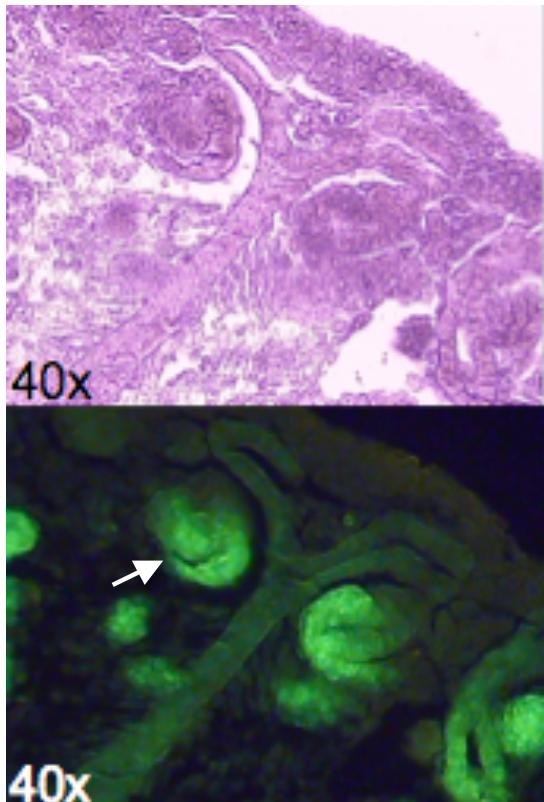


Figure 3. Expression of GFP in *Notch2*-EGFP BAC transgenic mice. The top image is from a bright-field microscopic image of a cryo-section ($8\mu\text{M}$) through an E15.5 kidney. The bottom image represents a fluorescent microscopic detailing the expression of GFP in the developing kidney. Note the expression of GFP in the developing S-shape body (arrow).

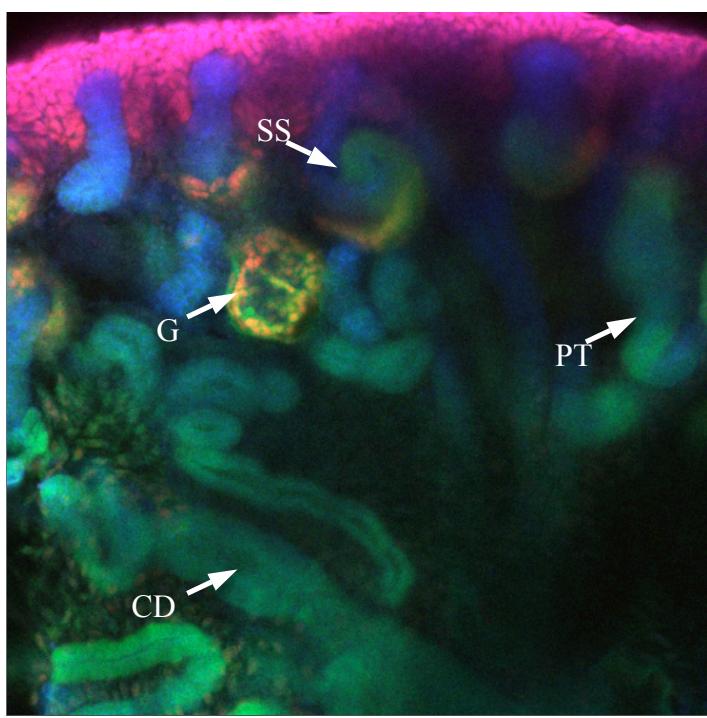


Figure 4. Confocal analysis of *Notch2*-EGFP expression in the developing kidney. To further delineate and localize the expression pattern of *Notch2*-EGFP in the kidney, we performed confocal analysis. This image is from a confocal analysis of a bisected E15.5 kidney and details the expression of the *Notch2*-EGFP transgene. GFP expression is noted in several regions of the kidney including the glomerulus (G), developing S-shape body (SS), proximal tubule (PT) and collecting duct (CD). This expression is consistent with the known expression of *Notch2* in the kidney. The tubules of the kidney were labeled with E-cadherin, and the mesenchyme and developing glomeruli labeled by WT-1 expression. Notch2 (green), E-cadherin (blue), WT-1 (red).

Confocal movie showing expression of *Notch2*-EGFP in the developing kidney.

To further visualize *Notch2*-EGFP expression, a file containing a movie is provided. Strong *Notch2*-EGFP expression can be detected in the proximal region of the developing S-shape body. In addition, the expression of *Notch*-EGFP is also present in the proximal tubule, glomerulus and collecting duct. The expression from *Notch2*-EGFP transgenic mice is consistent with the known expression pattern of *Notch2* in the kidney. The tubules of the kidney were labeled with E-cadherin, and the mesenchyme and developing glomeruli labeled by WT-1 expression. *Notch2* (green), E-cadherin (blue), WT-1 (red). The confocal images are available as movies and can be downloaded from <http://www.gudmap.org/Resources/MouseStrains/index.html>.

Methods

Tissue processing for confocal microscopy

Kidneys were dissected in phosphate buffered saline (PBS). The kidneys or the organ explants were rocked for 1–2 h in 2% paraformaldehyde in PBS, washed twice with PBS, and then rocked for 1–2 h in 100% methanol. The tissues were washed twice with cold PBS containing 0.05% Tween-20 (PBT). Kidneys were bisected. Primary antibodies, diluted to 1:250 to 1:400, were added to the tissues in 400 µL of PBT containing 2% goat serum and incubated overnight with rocking. Tissues were washed with 5 exchanges of PBT over 8 h with rocking. The secondary antibodies, diluted to 1:400 in PBT containing 2% goat serum, were added and incubated overnight. The tissues were again washed with 5 exchanges of PBT over 8 h. The tissue was washed for 5–10 min and mounted in a depression slide in PBT before they were examined by confocal microscopy. The entire procedure was performed at 4 °C with pre-cooled reagents.

The following primary antibodies were utilized: anti-WT1 (c-19, Santa Cruz), anti-Uvomorulin (E-cadherin, Sigma). The secondary antibodies were Alexa 555-conjugated anti-rabbit and Alexa 633-conjugated anti-rat secondary antibodies (Molecular Probes).

Confocal imaging

The tissues were imaged with a Zeiss LSM510 equipped with an Argon (488 nm) and two HeNe lasers (543 nm and 633 nm). We used a multi-track configuration, refractive index correction, and automatic gain control. Approximately 2 µm thick optical sections were obtained every 5 µm to a depth of at least 80 µm. The sections began at the surface of the kidney and were on a plane tangential to it.

References

Gong S, Zheng C, Doughty ML, Losos K, Didkovsky N, Schambra UB, Nowak NJ, Joyner A, Leblanc G, Hatten ME, Heintz N. A gene expression atlas of the central nervous system based on bacterial artificial chromosomes Nature. 2003 Oct 30;425(6961):917-25.

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Leimeister C, Schumacher N, Gessler M. Expression of Notch pathway genes in the embryonic mouse metanephros suggests a role in proximal tubule development. Gene Expr Patterns. 2003 Oct;3(5):595-8.