

## Characterization and Analysis of *Irx3* GENSAT BAC transgenic mice

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 *Irx3-EGFP* strain. **Our analysis suggests that the *Irx3-EGFP* transgenic mice may be useful in studying a subset of cells from the cortical interstitium, but this transgene does not recapitulate the known expression pattern of *Irx3* in the developing S-shape body.**

### **Irx3 Gene Notes**

Mammalian homologues of the *Drosophila Iroquois* homeobox gene complex represent a conserved family of homeodomain-containing transcription factors. This gene family has been shown to regulate diverse cellular and developmental processes including patterning and regionalization of differentiation in both vertebrates and invertebrates. In the mouse, *Irx3* is weakly expressed in the medial region of the developing S-shape body (Reggiani *et al.*).

### **Strain Information**

Promoter: *Irx3*

Name: Iroquois related homeobox 3 (*Drosophila*)

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

Name: Enhanced Green Fluorescent Protein

Alteration at locus: Transgenic Transgene: Tg(*Irx3-EGFP*)BW61Gsat

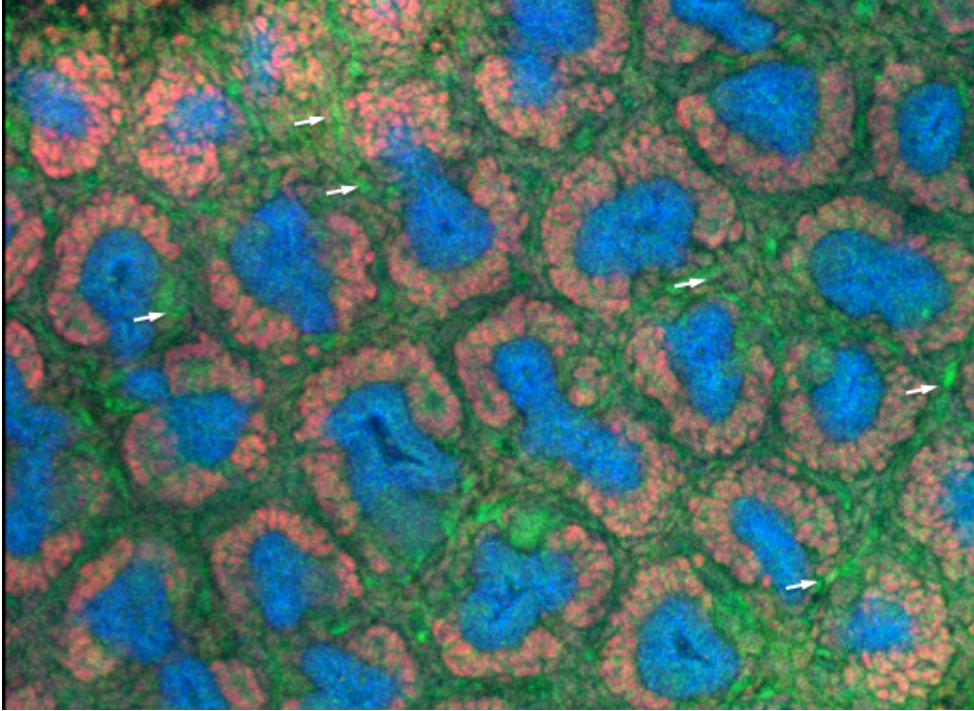
Name: transgene insertion BW61, GENSAT Project at Rockefeller University

Alteration at locus: Transgenic

For further information and strain distribution please use the following URL:

<http://www.mmrrc.org/strains/245/0245.html>

## Characterization of *Irx3* expression in the developing kidney



**Figure 1. Confocal analysis of *Irx3*-EGFP expression in the developing kidney of E15.5 embryos.** To further delineate and localize the expression pattern of *Irx3*-EGFP in the kidney, we performed confocal analysis. This confocal image details the expression of *Irx3*-GFP, which can be detected in a subset of cortical interstitial cells (arrows). The tubules of the kidney were labeled with E-cadherin, and the mesenchyme and developing glomeruli labeled by WT-1 expression. *Irx3* (green), E-cadherin (blue), WT-1 (red).

**Confocal movie showing expression of *Irx3*-EGFP in the developing kidney.** To further visualize *Irx3*-EGFP expression, a file containing a movie further detailing the expression of *Irx3*-EGFP is provided. Weak *Irx3*-EGFP expression can be in a subset of cells of the cortical interstium. The tubules of the kidney were labeled with E-cadherin, and the mesenchyme and developing glomeruli labeled by WT-1 expression. *Irx3* (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  $\mu$ L 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  $\mu$ m thick optical sections were obtained every 5  $\mu$ m to a depth of at least 80  $\mu$ m. 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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Hartman HA, Lai, HL, Patterson LT. Cessation of renal morphogenesis in mice. *Dev Biology*. 2007 310:379-387

Reggiani L, Raciti D, Airik R, Kispert A, Brändli AW. The prepattern transcription factor *Irx3* directs nephron segment identity. *Genes Dev*. 2007 Sep 15;21(18):2358-70.