

Characterization and Analysis of NRP2 GENSAT BAC transgenic mice

GENSAT is a 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 taken 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 *Nrp2*-EGFP strain. **Our analysis suggests that the *Nrp2*-EGFP transgenic mice may be useful in studying the development of the proximal tubule as well as a subset of cells of the cortical interstitium.**

Nrp2 Gene Notes

Nrp2 is a member of the neuropilin family of receptor proteins, and the encoded transmembrane domain is capable of interacting with proteins such as SEMA3C and SEMA3F. *Nrp2* has been implicated in abnormal lymphatic development, and has been shown to mediate proliferation, survival, and migration of tumor cells (Bagri *et al.*).

Strain Information

Strain Name: STOCK Tg(*Nrp2*-EGFP)CX13Gsat/Mmmh

Stock Number: 000306-MU/H

Gene Details:

Promoter: *Nrp2*

Name: neuropilin2

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

Name: Enhanced Green Fluorescent Protein

Alteration at locus: Transgenic Transgene: Tg(*Nrp2*-EGFP)CX13Gsat

Name: transgene insertion CX13, 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/317/0317.html](http://www.mmrrc.org/strains/317/0317.html)

Characterization of *Nrp2* expression in the developing kidney



Figure 1. Analysis of *Nrp2*-EGFP expression in E15.5 embryos.

Fluorescent image detailing expression of *Nrp2*. The embryo on the left is a non-transgenic littermate, while the embryo on the right is a *Nrp2*-EGFP BAC transgenic. This analysis detected particularly strong expression in the developing facial region and other regions of the embryo.

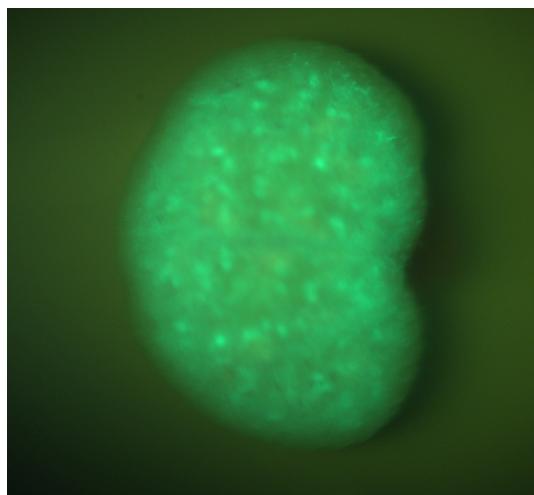


Figure 2. Expression pattern in the kidney of *Nrp2*-EGFP BAC transgenic mice. A fluorescent microscopic image showing *Nrp2*-EGFP expression in the developing kidney at E15.5. Note the punctate pattern of expression.

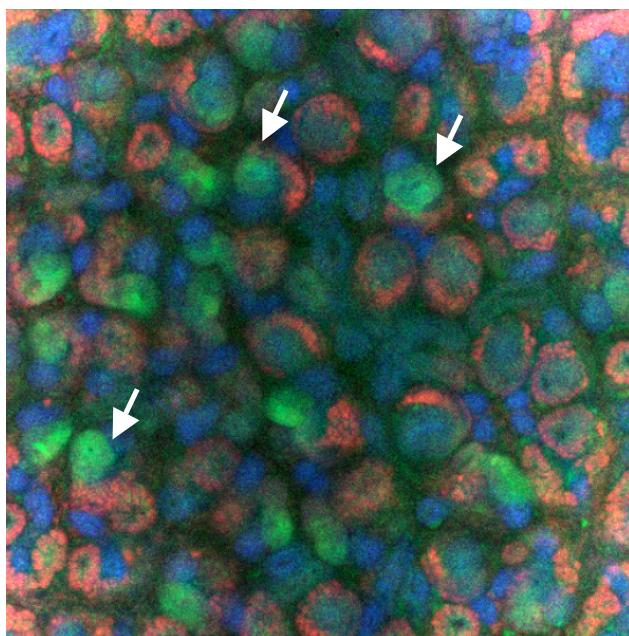


Figure 3. A confocal microscopic analysis showing the expression of the *Nrp2*-EGFP transgene in the developing kidney at E15.5. To further delineate and localize the expression pattern of *Nrp2*-EGFP in the kidney, we performed confocal analysis. This confocal image details the expression of *Nrp2*-EGFP, which can be seen in the developing proximal tubule (arrows). The tubules of the kidney were labeled with E-cadherin, and the mesenchyme and developing glomeruli labeled by WT-1 expression. *Nrp2* (green), E-cadherin (blue), WT-1 (red).

Characterization of *Nrp2* expression in the developing kidney

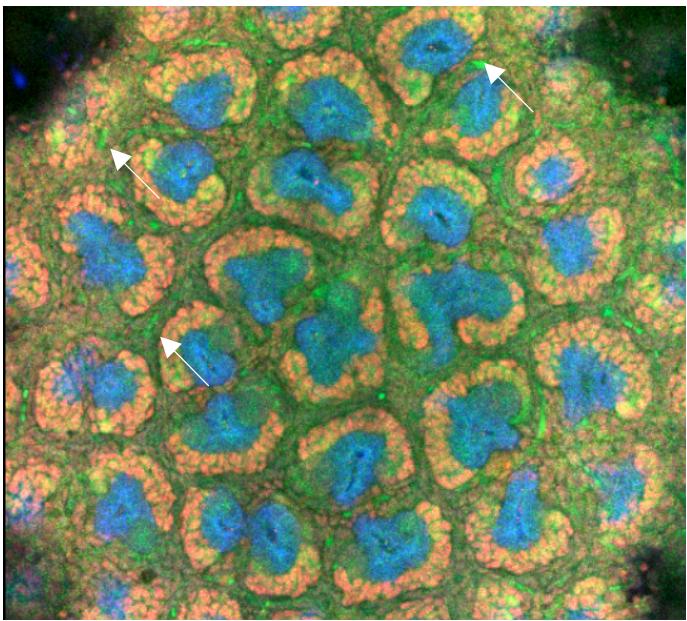


Figure 4. Confocal microscopic analysis of *Nrp2*-EGFP expression in the developing kidney of an E15.5 embryo. To further delineate and localize the expression pattern of *Nrp2*-EGFP in the kidney, we performed a confocal analysis. This confocal image details the expression of *Nrp2*-EGFP, which can be detected in a subset of cells of the cortical interstitium (arrows). The tubules of the kidney were labeled with E-cadherin, and the mesenchyme and developing glomeruli labeled by WT-1 expression. Nrp2 (green), E-cadherin (blue), WT-1 (red).

Confocal movie showing expression of *Nrp2*-EGFP in the developing kidney from E15.5 embryos. To further visualize *Nrp2*-EGFP expression, a file containing a movie that details the expression of *Nrp2*-EGFP is provided. Strong *Nrp2*-EGFP expression can be detected in the developing proximal tubules and a subset of cells in the cortical interstitium. The tubules of the kidney were labeled with E-cadherin, and the mesenchyme and developing glomeruli labeled by WT-1 expression. Sim1 (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

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