Characterization and Analysis of Hes5 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 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 *Hes5-EGFP* strain. **Our analysis suggests that *Hes5-EGFP* transgenic mice may be useful to further studies regarding the developing renal vesicle and S-shaped bodies.**

Hes5 Gene Notes

This gene encodes a member of a family of basic helix-loop-helix transcriptional repressors. The protein product of this gene, which is activated downstream of the Notch pathway, regulates cell differentiation in multiple tissues. Disruptions in the normal expression of this gene have been associated with developmental abnormalities and cancer. In the kidney, *Hes5* has been shown to be expressed in pre-tubular aggregates and comma-shaped bodies and restricted to the middle segment of S-shaped bodies (Piscione *et al*.).

Strain Information

Strain Name: STOCK Tg(Hes5-EGFP)1Gsat/Mmmh
Stock Number: 000316-MU/H
Gene Details:
Promoter: Hes5
Name: hairy and enhancer of split 5 (Drosophila)
Alteration at locus: Transgenic
Reporter: EGFP (Jelly Fish)
Name: Enhanced Green Fluorescent Protein
Alteration at locus: Transgenic

For further information and distribution of transgenic mice, please use the following URL: http://www.mmrrc.org/strains/316/0316.html

Characterization of Hes5 expression in the developing kidney

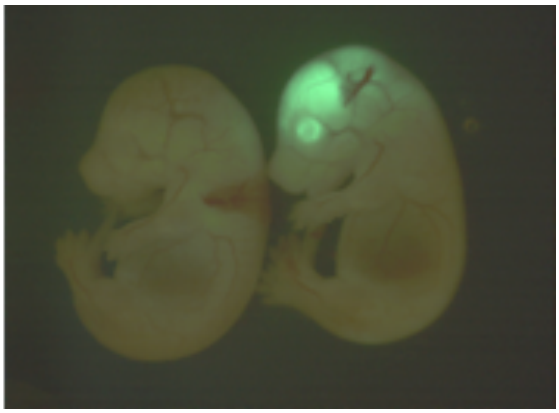

Figure 1. Analysis of *Hes5*-EGFP expression in whole-embryos. Fluorescent image detailing expression of EGFP in E15.5 embryos. The embryo on the left is a non-transgenic littermate, while the embryo on the left is a *Hes5*-EGFP BAC transgenic. Pronounced GFP expression is found in the developing telencelphalon and developing eye.

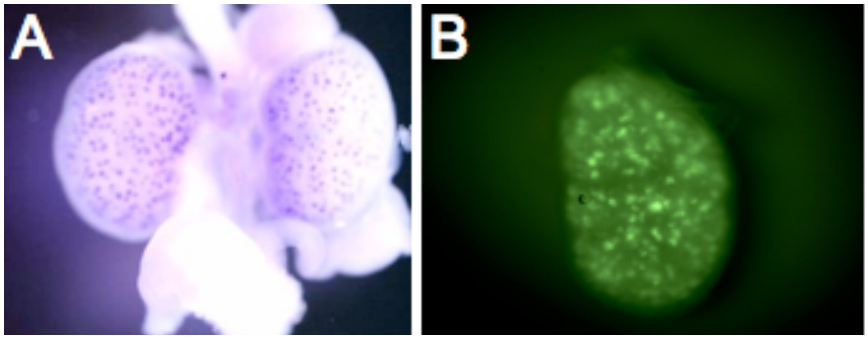

Figure 2. Expression of EGFP in the kidneys of *Hes5*-EGFP BAC transgenic mice. A) Bright field microscopy image detailing the expression of *Hes5* using whole-mount *in situ* hybridization in the kidneys of E15.5 embryos (Image courtesy of GUDMAP, McMahon Group). **B)** Fluorescent microscopy image showing *Hes5*-EGFP expression in the kidney from E15.5 embryos. Note the punctate GFP expression recapitulates the endogenous *Hes5* expression.

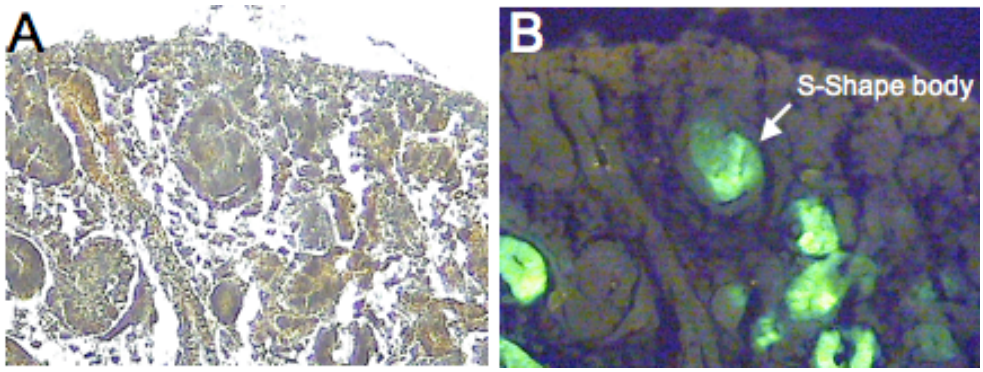

Figure 3. Expression pattern *Hes5*-EGFP transgenic mice. A) Bright-field microscopy image showing a cryo-section (8μM section) through an E15.5 kidney. **B)** Fluorescent microscopy image showing *Hes5*-EGFP expression in the developing kidney. Note the expression of GFP in the developing S-shape body.

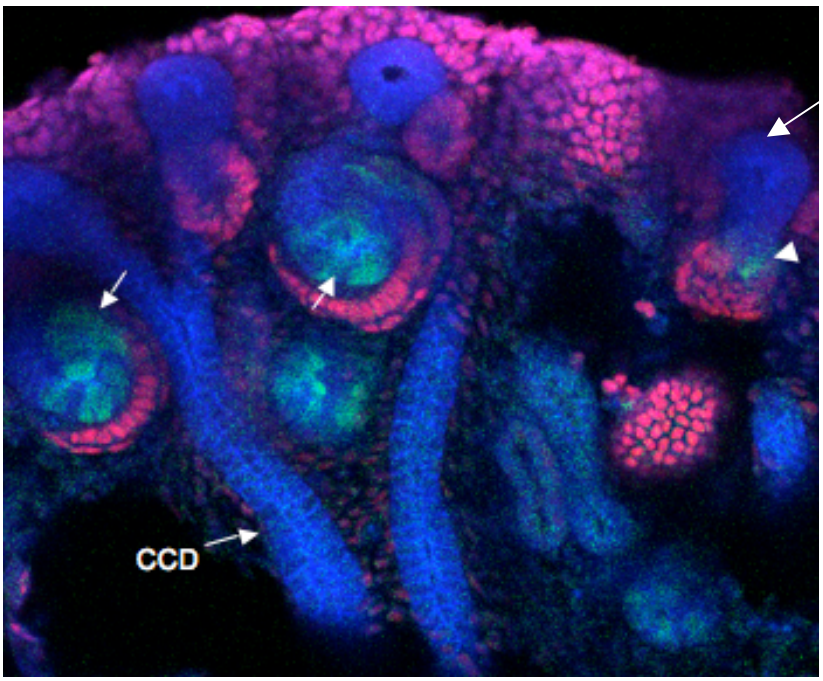

Figure 4. Confocal analysis of *Hes5*-EGFP expression in the developing kidney. To further delineate and localize the expression pattern of *Hes5*-EGFP in the kidney, we performed confocal analysis. This image details the expression of *Hes5*-EGFP, which can be seen in the medial region of the developing S-shape body. This is consistent with the known expression pattern of *Hes5* in the kidney. Arrows indicate the expression of *Hes5* in the S-shape body. The tubules of the kidney were labeled with E-cadherin, and the mesenchyme and developing glomeruli labeled by WT-1 expression. Hes5 (green), E-cadherin (blue), WT-1 (red).

Confocal movie showing expression of *Hes5*-EGFP in the developing kidney. To further visualize *Hes5*-EGFP expression, a file containing a movie that details the expression of *Hes5*-EGFP is provided. Again, strong *Hes5*-EGFP expression can be detected in the in the medial region of the developing S-shape body. In addition, the expression of *Hes5* can also be sub-region of the renal vesicle. The expression from *Hes5*-GFP transgenic mice is consistent with the known expression pattern of *Hes5* in the kidney. The tubules of the kidney were labeled with E-cadherin, and the mesenchyme and developing glomeruli labeled by WT-1 expression. Hes5 (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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GENSAT Project, Howard Hughes Medical Institute, The Rockefeller University, 1230 York Avenue, Box 260, New York 10021, USA."The Gene Expression Nervous System Atlas (GENSAT) Project, NINDS Contracts N01NS02331 & HHSN271200723701C to The Rockefeller University (New York, NY)."

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