# Characterization and Analysis of Meis1 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 *Meis-EGFP*strain. **Our analysis suggests that the *Meis1-EGFP* transgenic mice may be useful in studying the development of the cortical interstitium**

## Meis1 Gene Notes

Meis1 belongs to the TALE (three amino acid loop extension) family of homeodomain-containing proteins and is most closely related to the PBX family of homeodomain proteins. Meis1 has been shown to act as a transcriptional activator and complex with PBX1 or PBX2. In addition, Meis1 has been show to be required for hematopoiesis, megakaryocyte lineage development and vascular patterning (Hisa *et al*.).

## Strain Information

Strain Name: STOCK Tg(Meis1-EGFP)FO156Gsat/Mmcd
Stock Number: 000306-MU/H
Promoter: Meis1
Name: Meis homeobox 1
Alteration at locus: Transgenic Reporter: EGFP (Jelly Fish)
Name: Enhanced Green Fluorescent Protein
Alteration at locus: Transgenic Transgene: Tg(Meis1-EGFP)FO156Gsat
Name: transgene insertion FO156, 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/11189/011189.html

# Characterization of Meis1 expression in the developing kidney

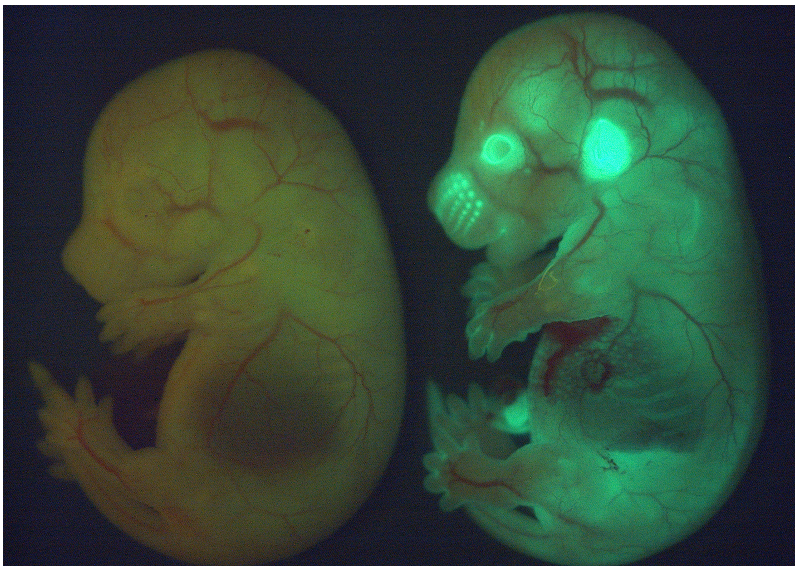

**Figure 1. Analysis of *Meis1*-EGFP expression in E15.5 embryos.** Fluorescent microscopic image detailing expression of *Meis1* at E15.5. The embryo on the left is a non-transgenic littermate, while the embryo on the left is a *Meis1*-EGFP BAC transgenic. Note the widespread GFP expression in the embryo including, vibrissae, eye and skin.

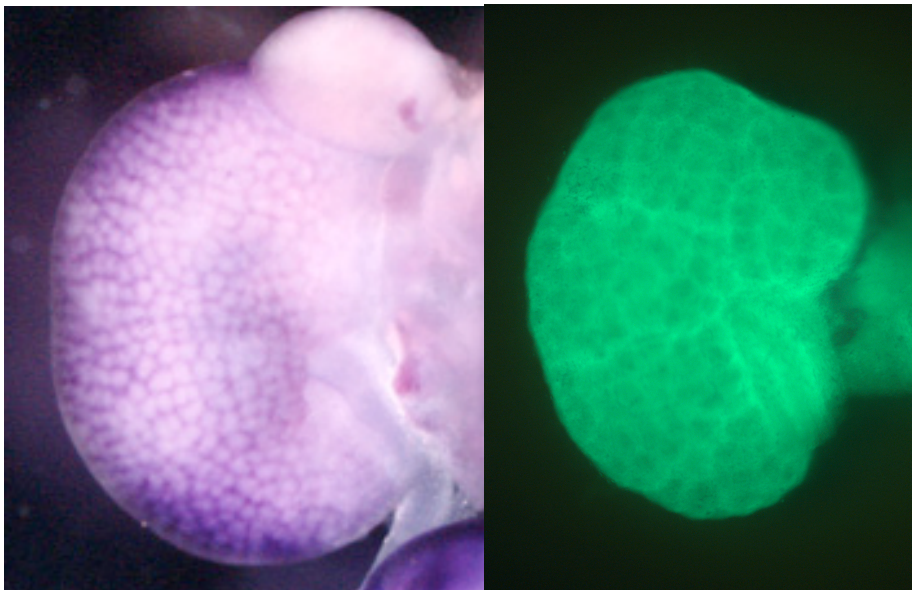

**Figure 2. Expression of *Meis1* in the kidneys from E15.5 embryos.** The image on the left details the expression of Meis1 in an E15.5 detected using whole-mount *in situ* hybridization analysis (Image courtesy of GUDMAP, McMahon lab). The fluorescent microscopic image on the right details GFP expression in a *Meis1*-EGFP transgenic mouse in the developing kidney. Note the "honeycomb" GFP expression.

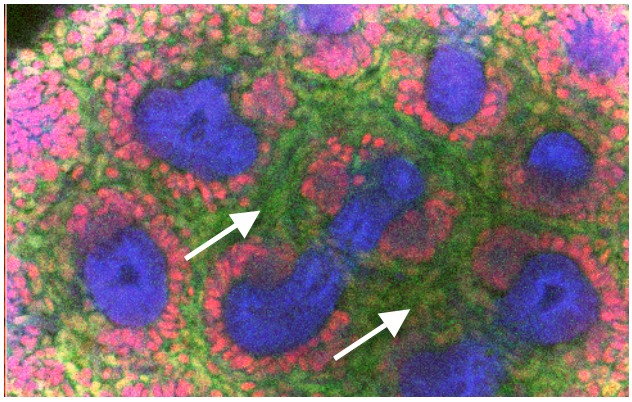

**Figure 3. Confocal analysis of *Meis1*-EGFP expression in the developing kidney from a e15.5 embryo**. To further delineate and localize the expression pattern of *Meis1*-EGFP in the kidney, we performed confocal analysis. This confocal image details the expression of *Meis1*-EGFP, which can be seen in the cells of the cortical interstitium (arrows). The tubules of the kidney were labeled with E-cadherin, and the mesenchyme labeled by WT-1 expression. Meis1 (green), E-cadherin (blue), WT-1 (red).

**Confocal movie showing expression of *Meis1*-EGFP in the developing kidney at e15.5**. To further visualize *Meis1*-EGFP expression, a file containing a movie that details the expression of *Meis1*-EGFP is provided. Again, strong *Meis1*-EGFP expression can be detected in the developing cortical interstitium. The tubules of the kidney were labeled with E-cadherin, and the mesenchyme and developing glomeruli labeled by WT-1 expression. Meis1 (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.

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)."

Hartman HA, Lai, HL, Patterson LT. Cessation of renal morphogenesis in mice. Dev Biology. 2007 310:379-387

Hisa T, Spence SE, Rachel RA, Fujita M, Nakamura T, Ward JM, Devor-Henneman DE, Saiki Y, Kutsuna H, Tessarollo L, Jenkins NA, Copeland NG. Hematopoietic, angiogenic and eye defects in Meis1 mutant animals. EMBO J. 2004 Jan 28;23(2):450-9. Epub 2004 Jan 8.