Characterization and Analysis of Crym 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. We have utilized *Crym-EGFP* transgenic mice to examine the transcription profile of cells at E15.5 as well as several other developmental time points including P0, P1, P2, P3 and P4. Here we report the pattern of EGFP expression in the developing kidney of the *Crym-EGFP* strain. **Our analysis suggests that the *Crym-EGFP* transgenic mice may be a useful tool to study the development of the cap mesenchyme and renal vesicle.**

Crym Gene Notes

The crystallins are family of proteins that have been determined to have multiple roles. One family member encodes the major proteins of vertebrate eye lens and maintains the transparency and refractive index of the lens. Additional members do not perform structural roles in lens tissue, but instead bind to thyroid hormone for possible regulatory or developmental roles (Graw).

Strain Information

Strain Name: STOCK Tg(Crym-EGFP)82Gsat/Mmcd
Stock Number: 012003-UCD
Promoter: Crym
Name: crystallin, mu
Alteration at locus: Transgenic
Reporter: EGFP (Jelly Fish)
Name: Enhanced Green Fluorescent Protein
Alteration at locus: Transgenic

Genetic Alterations:
Genotype modified to contain multiple copies of a modified BAC in which EGFP reporter gene is inserted immediately upstream of the coding sequence of the targeted gene.

For further information and strain distribution please use the following URL: http://www.mmrrc.org/strains/12003/012003.html

Characterization of Crym expression in the developing kidney

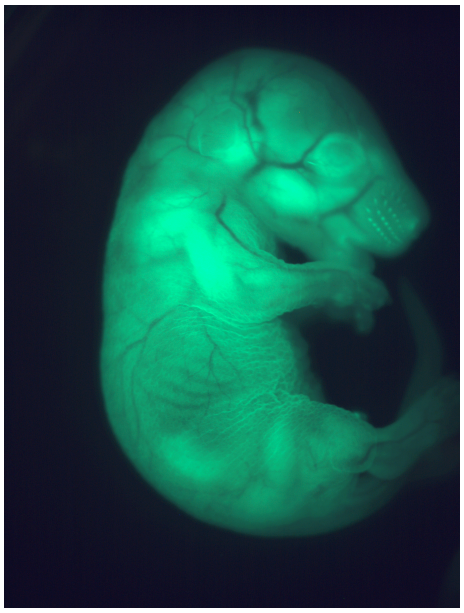
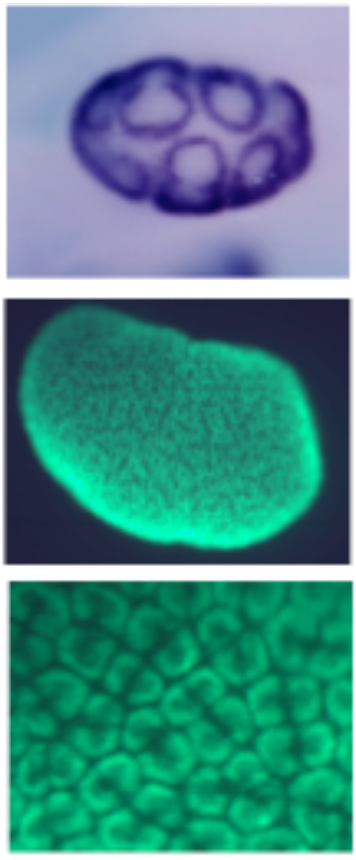


Figure 1. Analysis of *Crym*-EGFP expression in whole-embryos. Fluorescent image detailing expression of EGFP in an E16.5 embryo. This analysis detected wide spread GFP expression in the developing embryo including the skin, muscle, skeletal elements and other regions of the embryo.

Figure 2. Expression of EGFP in the kidneys of *Crym*-EGFP BAC transgenic mice. Top) Bright field microscopy image detailing the expression of *Crym* using whole-mount *in situ* hybridization in the kidneys of E12.5 embryos (Image courtesy of GUDMAP, Little Group). **Middle)** Fluorescent microscopy image showing *Crym*-EGFP expression in the kidney from E18.5 embryos. Note the expression of GFP expression in the cap mesencyme. **Lower)** Image represents a close-up detailing GFP expression in the developing cap mesenchyme.



Characterization of Crym expression in the developing kidney

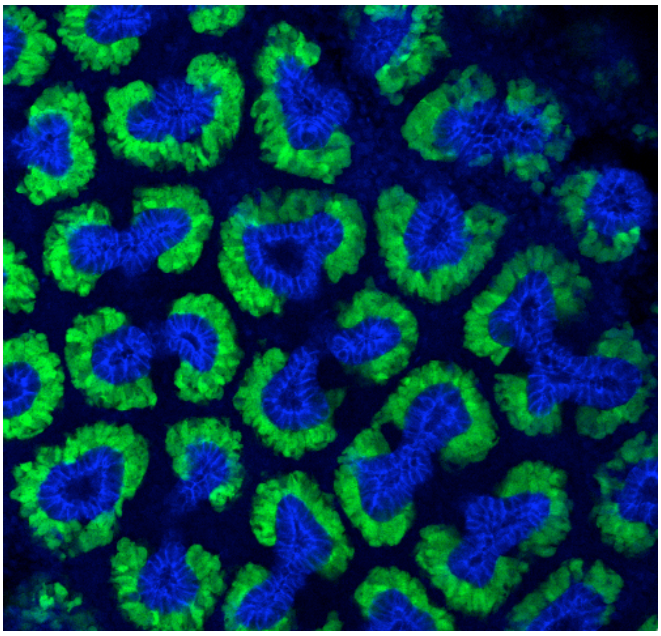


Figure 3. Confocal analysis of *Crym*-EGFP expression in the developing kidney. To further delineate and localize the expression pattern of *Crym*-EGFP in the kidney of E18.5 embryos, we performed confocal analysis. This image details the expression of *Crym*-EGFP, which can be seen in the cap mesenchyme. The tubules of the kidney were labeled with E-cadherin. Crym (green) and E-cadherin (blue).

Confocal movie showing expression of *Crym*-EGFP in the developing kidney of E18.5 embryos. To further visualize *Crym*-EGFP expression, a file containing a movie that details the expression of *Crym*-EGFP is provided. Strong *Crym*-EGFP expression can be detected in the developing cap mesenchyme. The tubules of the kidney were labeled with E-cadherin Crym (green) and E-cadherin (blue). The confocal images are available as a movie 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-Uvomorulin (E-cadherin, Sigma). The secondary antibodies was 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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