TH-EGFP Allele Characterization

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FINDINGS: VALIDATED

Our analysis identified expression of TH-EGFP in 15.5 dpc urogenital tract and structures in anatomical locations consistent with developing peripheral nervous system innervation. TH-EGFP BAC transgene expression was comparable to whole mount in situ hybridization for Tyrosine hydroxylase.

Source of Transgenic Line

TH-EGFP mice (Strain Name: Tg(Th-EGFP)DJ76Gsat/Mmnc; Stock Number: 000292-UNC) were obtained from Mutant Mouse Regional Resource Center. Animals were maintained by breeding on the FVB/N strain background.

Genotyping Tail biopsies were collected and incubated in tail digestion buffer overnight at 55ºC and then DNA was extracted by phenol/chloroform using routine methods. PCR was performed as described below and the PCR products were separated on 10% vertical polyacrylamide gels (see gel images below). Oligonucleotides used in the genotyping reactions to identify the transgenic allele included:

DNA sequence (forward): 5'-cctacggcgtgcagtgcttcagc-3'
DNA sequence (reverse): 5'-cggcgagctgcacgctgcgtcctc-3'

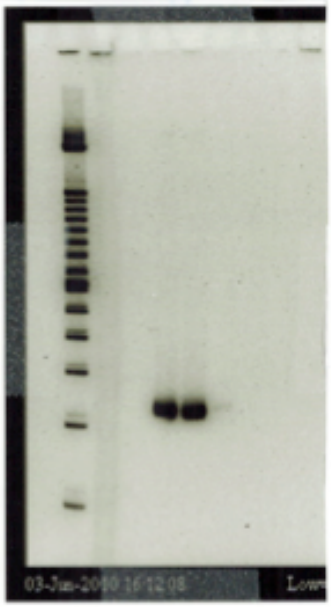
Rxn Buffer and Conditions: (20µl reaction)

10X PCR B2.5	2 ul	
10mM dNTP	0.4ul	94°C 5min 1 cycle
6.6uM primer F	0.75ul	94°C 30sec
6.6uM primer R	0.75ul	55°C 30sec 34 cycles
Taq Polymerase	0.1ul (5u/ul)	72°C 30sec
Genomic DNA	3ul (diluted 1:25)	72°C 10min 1 cycle
Total rxn volume	**20 ul**	

NOTE: 10x PCR B2.5 reaction buffer consists of: 100mM Tris pH 8.3, 500mM KCL, 20mM MgCl2
Expected PCR product band: ~300bp

EGFP PCR ~ 300bp

0. 100bp Ladder
1. H20
2 .WT DNA
3 .Positive Control (DNA from previous litter used)
4. 2321C1 – Positive
5. 2321C2 – Negative
6. 2321C3 – Negative
7. 2321C4 – Negative
8. 2321C5 – Negative



Analysis of TH-EGFP BAC transgene Expression in the Urogenital Tract

Expression was examined in whole embryos and in dissected tissues viewed by direct fluorescence on an upright microscrope at 15.5 days post coitus (dpc).

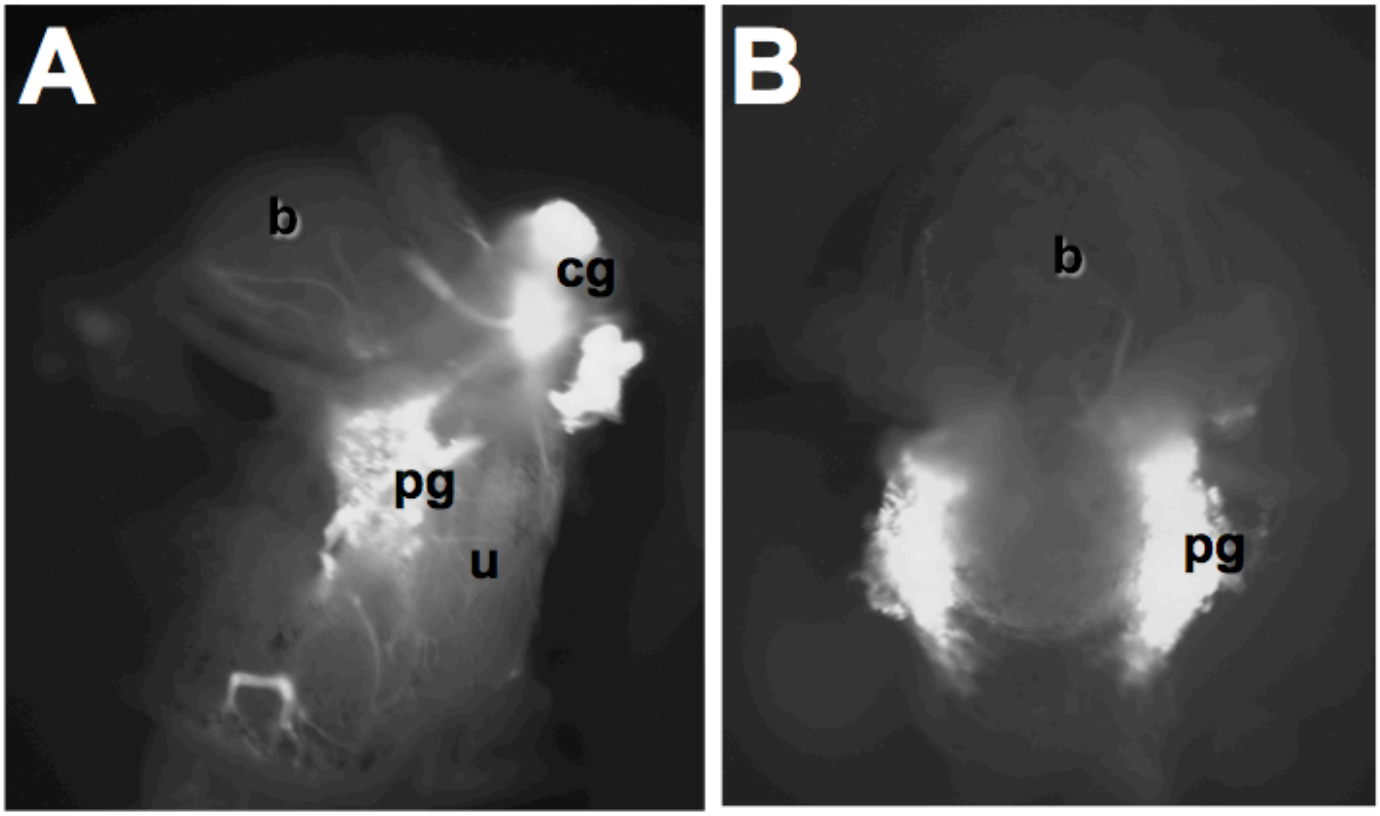
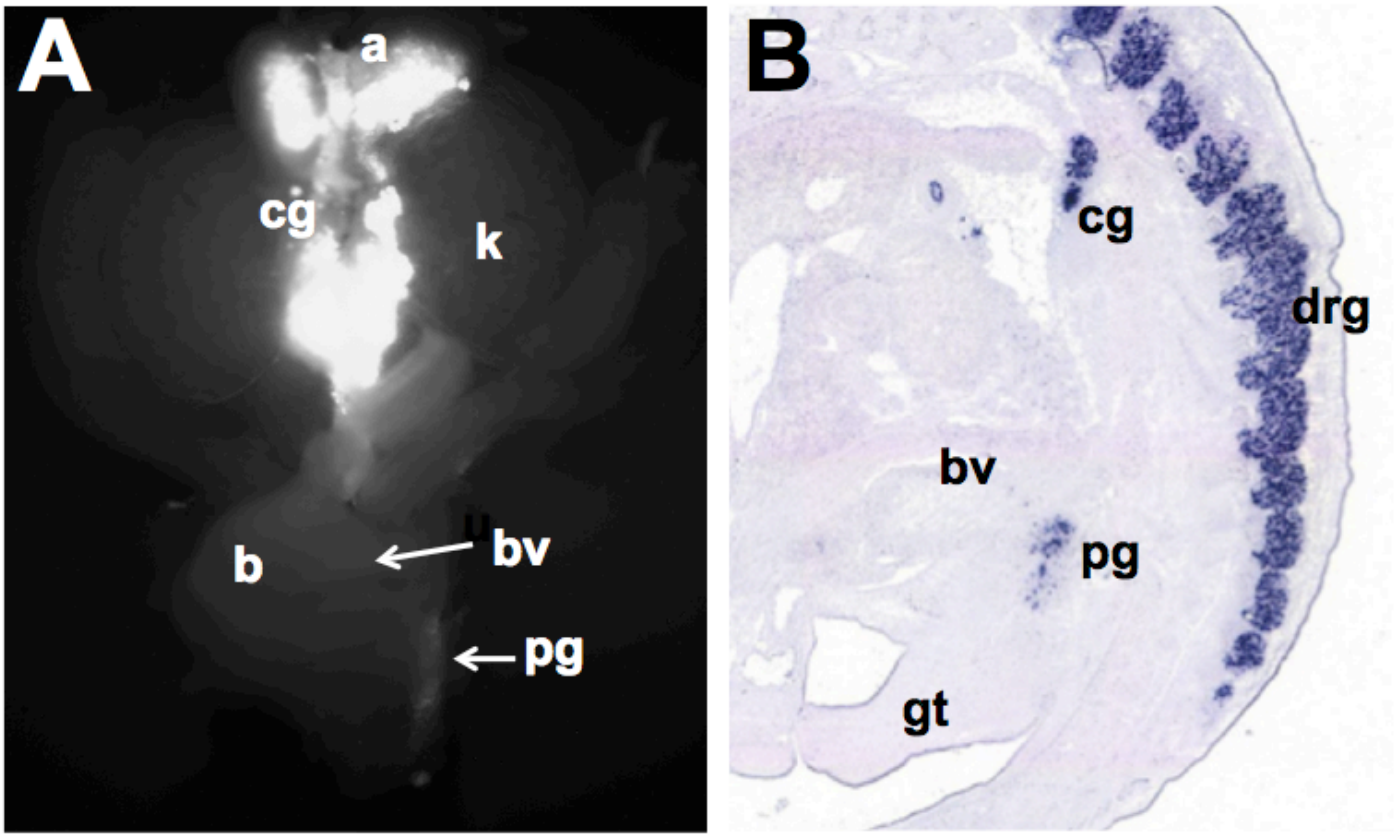


Figure 1. Distribution of TH-EGFP+ neuronal progenitors in fetal mouse lower urinary tract. (A) Dorsolateral view of TH-EGFP transgene expression observed under direct fluorescence illumination in 15.5 dpc pelvic ganglia. Neuronal processes are seen as fine tendrils extending from the pelvic ganglia out into the bladder body. Intense fluorescence is also seen in celiac ganglia above the urethra. (B) In anterior views of micro-dissected bladder, TH-EGFP expression is seen in pelvic ganglia (pg), the bladder wall (b) and urethra (u), celiac ganglia (cg).

Comparison of TH BAC transgenic expression with TH In situ

Ventrolateral view of TH-EGFP expression compared to sectional *in situ* for TH gene at 14.5dpc.



(A) Whole mount direct fluorescence image of micro-dissected urogenital tract from 14.5dpc fetal TH-EGFP transgenic mouse. EGFP fluorescence is visible in adrenal (a), celiac ganglia (cg), and pelvic ganglia (pg) (B) Sagital *in situ* sectional image from Eurexpress.org (image euxassay_007178_18) reveals transcription of TH gene in pelvic ganglia, celiac ganglia, and dorsal root ganglia (drg). No DRG are visible in the whole mount image on the left as these structures are removed during micro-dissection. Blood vessel flanking the bladder (bv), kidney (k), genital tubercle (gt).