Htr3a-EGFP Allele Characterization

Authors: Elaine K. Ritter, E. Michelle Southard-Smith
Generated: 23 April 2015 Version 2
Updated: 23 April 2015 Submitted: 23 April 2015

FINDINGS: VALIDATED

Our analysis identified expression of Htr3a-EGFP in 14.5 dpc fetal mice in anatomical locations consistent with developing peripheral nervous system elements. Htr3a-EGFP BAC transgene expression was comparable to whole mount in situ hybridization for Tyrosine hydroxylase.

Source of Transgenic Line

Htr3a-EGFP mice (Strain Name: Tg(Htr3a-EGFP)DH30Gsat/Mmnc; Stock Number: 000273-UNC-RESUS) were obtained from Mutant Mouse Regional Resource Center. Animals were maintained by breeding on the Swiss Webster outbred 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:

EGFP Genotyping: 310 bp PCR product
Forward Primer: 5' – CCTACGGCGTGCAGTGCTTCAGC – 3'
Reverse Primer: 5' – CGGCGAGCTGCACGCTGCGTCCTC – 3'

Rxn Buffer and Conditions: (20µl reaction)

10X PCR B2.5	2 ul
10mM dNTP	0.4ul
6.6uM primer F	0.75ul
6.6uM primer R	0.75ul
Taq Polymerase	0.1ul (5u/ul)
Genomic DNA	3ul (diluted 1:25)
Total rxn volume	**20 ul**

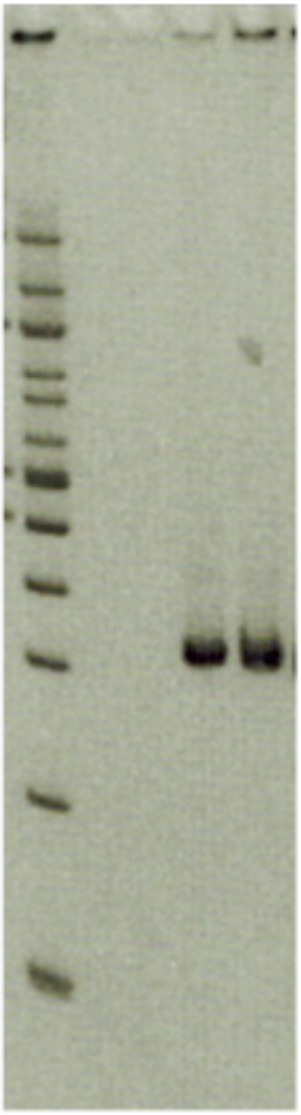
PCR Thermal Cycling Conditions:

94C 5min 1 cycle		
94C 30sec		
Ramp 0.5/sec to	94C	
72C 30sec		35 cycles
72C 30sec		
Ramp 0.5/sec to	72C	
72C 10min 1 cycle		
10C hold indefinitely		

NOTE: 10x PCR B2.5 reaction buffer consists of: 100mM Tris pH 8.3, 500mM KCL, 20mM MgCl2

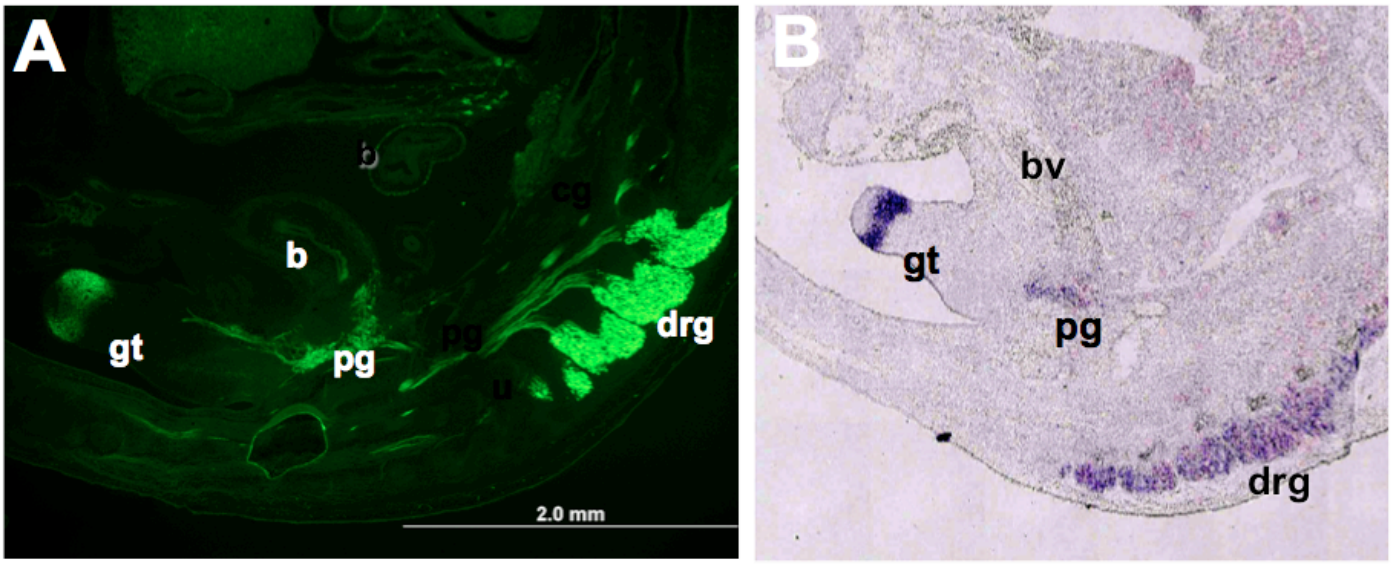
Expected PCR product band: 310 bp

0. 100bp Ladder
1. H20 Negative Control
2. Wild Type DNA
3. Positive Control Parent
4. Transgenic Tail offspring



Comparison of Htr3a BAC transgenic expression with Htr3a In situ

Ventrolateral view of Htr3a-EGFP expression compared to sectional in situ for Htr3a gene at 14.5dpc.



(A) Mid-sagital image captured from direct fluorescence of sectioned urogenital tract from 14.5dpc fetal Htr3a-EGFP transgenic mouse. EGFP fluorescence is dorsal root ganglia (drg), pelvic ganglia (pg), and peripheral nerve fibers running between these ganglia. Non-neuronal expression is also observed in the distal tip of the genital tubercle (B) Sagital *in situ* sectional image from Eurexpress.org (image euxassay_008385_12) with contrast enhanced to reveal transcription of the Htr3a gene in pelvic ganglia and dorsal root ganglia. Blood vessel flanking the bladder (bv) and genital tubercle (gt).