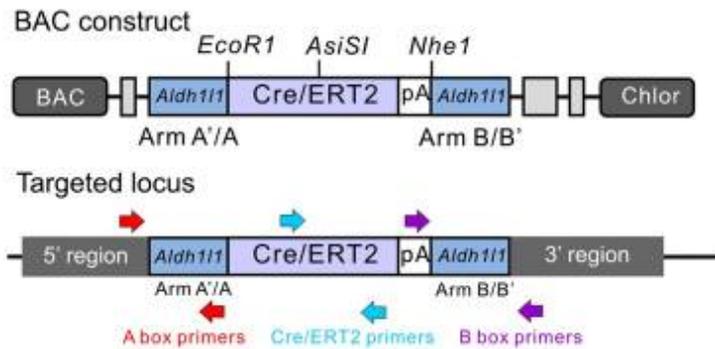


PCR Genotyping Aldh111-BAC-CreERT2 (Tg(Aldh111-cre/ERT2)^{1Khakh}; Aldh111-Cre/ERT2 BAC)

MGI ID#: 5806568

REFERENCE: Srinivasan R; Lu TY; Chai H; Xu J; Huang BS; Golshani P; Coppola G; Khakh BS (2016). New transgenic mouse lines for selectively targeting astrocytes and for studying calcium signals in astrocyte processes *in situ* and *in vivo*. *Neuron*. PMID: [27939582](https://pubmed.ncbi.nlm.nih.gov/27939582/)



A 228 kb mouse BAC (RP23-7M9) containing the 48 kb mouse Aldh111 coding region, a ~48 kb 5' flanking region and ~130 kb 3' flanking region was identified through a database search and obtained from the BACPAC Resource Center (Oakland Children's Hospital, Oakland, CA). Benefitting from an established protocol (Yang and Gong, 2005), the Cre/ERT2 cDNA (from Addgene plasmid #14797) was inserted into exon 1 of the Aldh111 gene immediately preceding the translation initiation codon. Cloning steps for construction of the modified Aldh111 BAC targeting construct were essentially as described (Yang and Gong, 2005). Once completed, BAC purification, pronuclear injections and transgenic mouse generation was done at the University of California, Davis Mouse

Biology Program (UC Davis MBP). Briefly, the modified Aldh111 BAC construct was purified and then injected into the pronucleus of fertilized oocytes. Injected oocytes were implanted into female FVB mice to generate pups. Founder pups were identified by PCR amplification of the Cre/ERT2 cassette. All founder pups were created in an FVB background and then backcrossed to C57/Bl6N mice till a 100% congenic C57/Bl6N strain expressing Aldh111-Cre/ERT2 was achieved. The Aldh111-Cre/ERT2 mice were backcrossed with C57/Bl6N mice for > 5 generations, i.e. to ~97% C57/Bl6N. The mice that are to be deposited at JAX have been backcrossed for 8 generations (i.e. to ~99% C57/Bl6N).

From Jax:

oJDW 1334 (31091 / Aldh1 FWD, in intron 1/exon 2): 5'- CTT CAA CAG GTG CCT TCC A

oJDW 1335 (30308 / Cre REV): 5'-GGC AAA CGG ACA GAA GCA

Tg = 180 bp band

If you use JDW 83 (CreERT2 rev) with the FWD it will be ~432 bp

Thermocycler Conditions (from Jax)

- 1-94°C-2:00
- 2-94°C-0:20
- 3-65°C-0:15
- 4-68°C-0:10
- 5-Repeat step 2 - 4 for 10 cycles, -0.5°C per cycle
- 6-94°C-0:15
- 7-60°C-0:10
- 8-72°C-0:10
- 9-Repeat steps 6-8 for 28 cycles
- 10-72°C-2:00
- 11-16°C-forever

