Ai90(TITL-Chronos)-D (also called Ai90D) mice are a Cre/Tet-dependent, optogenetic line, created by targeted insertion at the lgs7 locus (TIGRE; an intergenic region on mouse chromosome 9 that allows reporter expression to be tightly regulated). Following Cre-mediated removal of the STOP cassette, they may be used to generate Tet-Off/Tet-On mutant animals with conditional (inducible/reversible) expression of the channelrhodopsin/EGFP fusion protein (Chronos/EGFP). Subsequent illumination of Chronos-expressing (EGFP fluorescent) cells with blue-to-green light leads to reversible photostimulation of action potential firing/neural activity in these cells.

Donating Investigator
Hongkui Zeng, Allen Institute for Brain Science

GENETIC OVERVIEW

<table>
<thead>
<tr>
<th>Genetic Background</th>
<th>Generation</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

\( lgs7^{tm90.1(tetO-COP4*/EGFP)Hze} \)

<table>
<thead>
<tr>
<th>Allele Type</th>
<th>Gene Symbol</th>
<th>Gene Name</th>
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<tbody>
<tr>
<td>Targeted (Conditional ready (e.g. floxed), Reporter, Inducible)</td>
<td>lgs7</td>
<td>intergenic site 7</td>
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</tbody>
</table>

RESEARCH APPLICATIONS

Research Tools
Neurobiology Research
Ai90(TITL-Chronos)-D mice are a Cre/Tet-dependent, optogenetic line, created by targeted insertion at the Igs7 locus (TIGRE; an intergenic region on mouse chromosome 9 that allows reporter expression to be tightly regulated). Ai90D mice harbor the TIGRE-Ins-TRE-LSL-Chronos conditional allele, designed with a modified Tet response element (TRE or tetO) and loxP-flanked STOP cassette upstream of an improved Chronos/EGFP fusion protein (see detailed description below). When bred with other mice expressing Cre recombinase, tetracycline-controlled transactivator protein (tTA) and/or reverse tetracycline-controlled transactivator protein (rtTA), Chronos expression in cells/tissues where the expression patterns of the individual promoters driving Cre and tTA/rtTA overlap can be regulated with tetracycline or its analog doxycycline (dox). Following induction of Chronos expression (EGFP fluorescence), illuminating neurons with blue-to-green light leads to reversible photostimulation of action potential firing/neural activity in these cells.

Specifically, the donating investigator reports Ai90D mice have no reported levels of EGFP fluorescence in absence of Cre and tTA. When Ai90D mice are bred with tTA-driver and Cre-driver lines to create triple transgenic animals (Chronos+/tTA+/Cre+), cells expressing both tTA and Cre exhibit robust fluorescence. Light-induced expression of the activation opsin is expected to occur at levels sufficient to effectively depolarize/activate cortical neurons. Chronos expression/function in tissues other than brain has not yet been evaluated by the donating investigator (March 2014).

Heterozygous Ai90D mice are viable and fertile with no reported gross physical or behavioral abnormalities. The donating investigator has not attempted to generate homozygous Ai90D mice to date (March 2014).

The bacterial opsins are retinal-binding proteins that combine a light-sensitive domain with an ion channel or pump; providing light-dependent ion transport, membrane potential alteration, and sensory functions to bacteria. The *Stigeoclonium helveticum*-derived channelrhodopsin ShChR (Chronos) is a blue-to-green light-driven cation channel that depolarizes the cell and causes action potentials. As such, illuminating Chronos-expressing cells with blue-to-green light (~470-530 nm) leads to rapid and reversible photostimulation of robust action potential firing activity in these cells. Chronos has high light sensitivity and faster kinetics than previous channelrhodopsins. The action spectrum of Chronos is shifted to longer wavelengths compared to ChR2. In addition, Chronos is especially well suited to be paired with a red-shifted channelrhodopsin in order to allow for two light color activation of independent populations of neurons. The Chronos/EGFP fusion gene is composed of a mammalian codon-optimized Chronos fused in-frame to the N-terminus of EGFP.
Breeding Considerations

When maintaining a live colony, heterozygous mice may be bred to wildtype mice from the colony or to C57BL/6J inbred mice (Stock No. 000664). The donating investigator has not attempted to generate homozygous mice to date (March 2014).

Additional Breeding and Husbandry Support

Citation

When using the Ai90(TITL-Chronos)-D or Ai90D mouse strain in a publication, please cite the originating article(s) and include JAX stock #024100 in your Materials and Methods section.
Pricing & Availability

Cryo Recovery

Typically mice are recovered in 10-14 weeks. Contact Customer Service to place an order or for more information.

<table>
<thead>
<tr>
<th>SERVICE/PRODUCT</th>
<th>DESCRIPTION</th>
<th>PRICE</th>
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<tbody>
<tr>
<td>Cryo Recovery</td>
<td>Heterozygous or Wildtype for Igs7&lt;tm90.1(tetO-COP4*/EGFP)Hze&gt;</td>
<td>$2,854.50</td>
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<table>
<thead>
<tr>
<th>RELATED PRODUCTS AND SERVICES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frozen Mouse Embryo</td>
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</table>

PAYMENT TERMS AND CONDITIONS

Terms are granted by individual review and stated on the customer invoice(s) and account statement. These transactions are payable in U.S. currency within the granted terms. Payment for services, products, shipping containers, and shipping costs that are rendered are expected within the payment terms indicated on the invoice or stated by contract. Invoices and account balances in arrears of stated terms may result in The Jackson Laboratory pursuing collection activities including but not limited to outside agencies and court filings.

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The Jackson Laboratory has rigorous genetic quality control and mutant gene genotyping programs to ensure the genetic background of JAX® Mice strains as well as the genotypes of strains with identified molecular mutations. JAX® Mice strains are only made available to researchers after meeting our standards. However, the phenotype of each strain may not be fully characterized and/or captured in the strain data sheets. Therefore, we cannot guarantee a strain's phenotype will meet all expectations. To ensure that JAX® Mice will meet the needs of individual research projects or when requesting a strain that is new to your research, we suggest ordering and performing tests on a small number of mice to determine suitability for your particular project. We do not guarantee breeding performance and therefore suggest that investigators order more than one breeding pair to avoid delays in their research.
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Email: TechTran@jax.org

Related Strains

All
By Allele
By Gene
By Collection