B6.Cg-<sup>Trp<sub>34.1(tetO·COP4<sup>4H134R/EYFP)Hze</sub></sup>/J

Stock No: 031334 | Ai134(TITL-ChR2H134R-EYFP)-D, Ai134D, Ai134(TITL-ChR2-Y

- Congenic, Targeted Mutation

CRYO RECOVERY

PLACE ORDER

Typically mice are recovered in 10-14 weeks. Contact Customer Service to place an order or for more information.
Also Known As: Ai134(TITL-ChR2H134R-EYFP)-D, Ai134D, Ai134(TITL-ChR2-YFP)-D

Ai134(TITL-ChR2H134R-EYFP)-D mice (also called Ai134D or Ai134(TITL-ChR2-YFP)-D) are a Cre/Tet-dependent, optogenetic line - created by targeted insertion at the \(\text{Igs7}\) 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 an improved channelrhodopsin2/EYFP fusion protein (ChR2(H134R)-EYFP). Subsequent illumination of ChR2(H134R)-expressing (EYFP fluorescent) cells with blue light leads to reversible photostimulation of action potential firing/neural activity in these cells.

Donating Investigator

Hongkui Zeng, Allen Institute for Brain Science
Ai134(TITL-ChR2H134R-EYFP)-D mice (also called Ai134D or Ai134(TITL-ChR2-YFP)-D) 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). Ai134D mice harbor the TIGRE-Ins-TRE-LSL-ChR2(H134R)/EYFP conditional allele, designed with a modified Tet response element (TRE or tetO) and loxP-flanked STOP cassette upstream of the ChR2(H134R)/EYFP fusion protein (see detailed description below). When bred with other mice expressing Cre recombinase and tetracycline-controlled transactivator protein (tTA) or reverse tetracycline-controlled transactivator protein (rtTA), ChR2(H134R) 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 ChR2(H134R) expression (EYFP fluorescence), illuminating neurons with blue light (~450-490 nm) leads to rapid and reversible photostimulation of robust action potential firing activity in these cells.

Specifically, the donating investigator reports Ai134D mice have no reported levels of EYFP fluorescence in absence of Cre and tTA. When bred with tTA-driver and Cre-driver lines to create triple transgenic animals (ChR2(H134R)/EYFP+/Cre+/tTA+), cells expressing both tTA and Cre exhibit robust/bright fluorescence. Light-induced expression of the activation opsin occurs at levels sufficient to effectively depolarize/activate cortical neurons. ChR2(H134R)/EYFP expression/function in tissues other than brain has not yet been evaluated (January 2018).

For example, combining Ai134 with the moderately-expressing tTA-driver line ROSA26-ZtTA (see Stock No. 012266) and the cortical layer 4 Cre driver line Scnn1a-Tg3-Cre (Stock No. 009613) generates triple transgenic Scnn1a-Tg3-Cre;ROSA26-ZtTA;Ai134 animals. Those mice show good ChR2(H134R) expression and measurable photo-responses. The same may be expected for Ai134D. Additionally, the donating investigator advises against using very strong tTA drivers (e.g., Camk2a-tTA) in these triple transgenics - suggesting that the resulting high ChR2(H134R) expression levels lead to fluorescent aggregates in cells and/or aberrant morphology of labeled cells.

Ai134D heterozygotes are viable and fertile with no reported gross physical or behavioral abnormalities. To date (January 2018), it has not been attempted to make this strain homozygous.

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 ChR2(H134R)-EYFP fusion protein is composed of a Chlamydomonas reinhardtii-derived channelrhodopsin-2 that harbors a gain-of-function H134R substitution fused in-frame with an enhanced yellow fluorescent protein. The ChR2(H134R) is designed to cause larger stationary photocurrents compared to ChR2. This ChR2(H134R) functions as a blue light-driven cation channel that depolarizes the cell and causes action potentials. As such, illuminating ChR2(H134R)-expressing cells with blue light (450-490 nm) leads to rapid and reversible photostimulation of action potential firing activity in these cells.

Development

Expression Data
Breeding Considerations
When maintaining a live colony, heterozygous mice may be bred together, to wildtype mice from the colony or to C57BL/6J inbred mice (Stock No. 000664). To date (January 2018), it has not been attempted to make this strain homozygous.

Additional Breeding and Husbandry Support
Mating System
Heterozygote x Wild-type
Wild-type x Heterozygote

Citation
When using the Ai134(TITL-ChR2H134R-EYFP)-D, Ai134D, Ai134(TITL-ChR2-YFP)-D mouse strain in a publication, please cite the originating article(s) and include JAX stock #031334 in your Materials and Methods section.

Production of mice from cryopreserved embryos or sperm occurs in a maximum barrier room, G200

Genotyping Protocols
Standard PCR: \( Igs7^{tm134.1(tetO-COP4*H134R/EYFP)Hze} \)

Genotyping resources and troubleshooting
Cryorecovery - Domestic Not-For-Profit & Academic Pricing

<table>
<thead>
<tr>
<th>SERVICE</th>
<th>GENOTYPE</th>
<th>PRICE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryo Recovery</td>
<td>Heterozygous or wildtype for lgs7&lt;tm134.1(tetO-COP4*H134R/EYFP)Hze&gt;/J</td>
<td>$2,854.50</td>
</tr>
</tbody>
</table>

We will fulfill your order by providing at least two carriers for each strain ordered. The total number, sex, and genotypes provided will vary, although typically 8 or more animals are provided. Please check genotypes which will be recovered. While the genotypes of all animals produced will be communicated to you prior to scheduling shipment, the genotypes of animals provided may not reflect the mating scheme and genotypes described in the strain description. Animals are typically ready to ship in 11-14 weeks. If a second recovery is required to produce the minimum number of animals, then delivery time would increase to approximately 25 weeks. If we fail to produce animals of the correct genotype, you will not be charged. We cannot guarantee the reproductive success of mice shipped to your facility. If the mice are lost after the first three days (post-arrival) or do not produce progeny at your facility, a new order and fee will be necessary.

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