Ai32 mice express an improved channelrhodopsin-2/EYFP fusion protein following exposure to Cre recombinase. These mice can be used in optogenetic studies for rapid in vivo activation of excitable cells by illumination with blue light (450-490 nm).

A C57BL/6J congenic version of this strain is available as Stock No. 024. Donating Investigator Hongkui Zeng, Allen Institute for Brain Science.

Typically mice are recovered in 10-14 weeks. Contact Customer Service to place an order or for more information.
Ai32 mice homozygous for the Rosa-CAG-LSL-ChR2(H134R)-EYFP-WPRE conditional allele are viable and fertile. A 
\textit{loxP}-flanked STOP cassette prevents transcription of the downstream ChR2(H134R)-EYFP fusion gene. Because this CAG promoter driven reporter construct was targeted for insertion into the \textit{Gt(ROSA)26Sor} locus, ChR2(H134R)-EYFP expression is determined by which tissue(s) express Cre recombinase.

When bred to mice that express Cre recombinase, the resulting offspring will have the STOP cassette deleted in the \textit{cre}-expressing tissues; resulting in expression of the ChR2(H134R)-EYFP fusion protein. ChR2(H134R)-EYFP expression following exposure to \textit{cre} can be detected by EYFP fluorescence (and presumably by mRNA \textit{in situ} hybridization) and antibody staining \textit{immunohistochemistry}; although this was not tested by the donating investigator).

The donating investigator reports Ai32 mice have no significant expression of ChR2(H134R)-EYFP prior to introduction of Cre recombinase. Importantly, very low levels of ChR2(H134R)-EYFP expression may be present before Cre recombination - but the ChR2(H134R)-EYFP expression levels after Cre recombination are significantly greater than those baseline levels. As such, it is recommended that researchers include Cre-negative Ai32 controls to establish the baseline ChR2(H134R)-EYFP levels in their experiments.

For characterization information, see images at the Allen Institute for Brain Science website (Ai32 images).

Of note, the \textit{FRT} sites flanking the mutation allow for additional targeted replacement of the reporter sequences through \textit{Flp}-mediated recombination if so desired. Similarly, the \textit{attB}/\textit{attP}-flanked selection cassette may be removed by introduction of the site-specific bacteriophage PhiC31 integrase if so desired.

The ChR2(H134R)-EYFP fusion protein is composed of a \textit{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. 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. 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.
Genotyping Protocols
Standard PCR: Gt(ROSA)26Sor(COP4-EGFP)
Genotyping resources and troubleshooting

Breeding Considerations
When maintaining a live colony, homozygous mice may be bred together.

Additional Breeding and Husbandry Support
Mating System
Homozygote x Homozygote

Citation
When using the Ai32 or Ai32(RCL-ChR2(H134R)/EYFP) mouse strain in a publication, please cite the originating article(s) and include JAX stock #012569 in your Materials and Methods section.

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

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

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### CRYORECOVERY - DOMESTIC NOT-FOR-PROFIT & ACADEMIC PRICING

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<td>Heterozygous or wildtype for Gt(ROSA)26Sor&lt;tm32.1(CAG-COP4*H134R/EYFP)Hze&gt;</td>
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### RELATED PRODUCTS AND SERVICES

| Frozen Mouse Embryo | B6;129S-Gt(ROSA)26Sor<tm32(CAG-COP4*H134R/EYFP)Hze>/J Frozen | $2595.00 |

### 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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