The Ai35D (or Ai35Δneo) allele has a floxed-STOP cassette preventing transcription of the downstream Arch-GFP fusion gene. Exposure to Cre recombinase that deletes the STOP cassette results in Arch-GFP expression. These Ai35D mice are useful for optogenetic studies to express an inhibitory opsin that effectively silences the activity of cortical neurons (and perhaps other excitable cell types such as muscle cells and immune cells).

Of note, the similarly-designed Ai40D mouse line (Stock No. 021188) has an ArchT/EGFP fusion protein with improved light sensitivity, and is on a C57BL/6J genetic background.

Donating Investigator

Hongkui Zeng, Allen Institute for Brain Science
Ai35D (or Ai35Δneo) mice homozygous for the Rosa-CAG-LSL-Arch-GFP-WPRE conditional allele are viable and fertile. A loxP-flanked STOP cassette prevents transcription of the downstream Arch-GFP fusion gene (see below for detailed description of Arch-GFP). Because the CAG promoter driven reporter construct was targeted for insertion into the Gt(ROSA)26Sor locus, Arch-GFP 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 cre-expressing tissues, resulting in expression of the Arch-GFP fusion protein. The donating investigator reports that Ai35D mice do not express Arch-GFP prior to introduction of Cre recombinase. Fusion protein expression following exposure to cre can be detected by GFP fluorescence and mRNA (in situ hybridization) [and presumably by antibody staining (immunohistochemistry); although this was not tested by the donating investigator]. Following exposure to Cre recombinase, illuminating Arch-expressing neurons with yellow-green light (~575 nm) leads to reversible photoinhibition of action potential firing/neural activity in these cells. The donating investigator specifically reports that expression of the inhibitory opsin occurs at levels sufficient to effectively silence the activity of cortical neurons. Fusion protein expression in tissues other than brain has not yet been evaluated by the donating investigator (April 2011). Unlike the Ai35 mice from which they were derived, these Ai35D mice no longer harbor the downstream FRT site or attB/attP-flanked selection cassette.

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. Archaerhodopsin-3 (Arch, aR-3, or aop3) is a yellow-green light-driven (~575 nm) outward proton pump that causes hyperpolarization and prevents action potentials. Unlike light-driven chloride pumps that enter long-lasting inactive states in response to light, Arch spontaneously recovers from light-dependent inactivation. Arch is capable of generating photocurrents at several hundred picoamps (pA) even at low light powers. For example, illumination of Arch-expressing cells leads to reversible photoinhibition of action potential firing/neural activity in these cells.

The Arch-GFP fusion protein is composed of the Halorubrum sodomense-derived Archaerhodopsin-3 (Arch, aR-3, or aop3) fused in-frame with a green fluorescent protein (GFP). The Arch-GFP fusion protein in these mice has small domain modifications designed to facilitate correct processing and localization of newly synthesized Arch-GFP protein (including a signal sequence and an endoplasmic reticulum exporting sequence).

For characterization information, see images at the Allen Institute for Brain Science website (Ai35 images).

Of note, the similarly-designed Ai40D mouse line (Stock No. 021188) has an ArchT/EGFP fusion protein with improved light sensitivity, and is on a C57BL/6J genetic background.
Genotyping Protocols
Standard PCR: Gt(ROSA)26Sor\textsuperscript{m35.1(CAG-aop3/GFP)Hze}
Genotyping resources and troubleshooting

Breeding Considerations
Homzygous mice are viable and fertile. When maintaining a live colony, homzygous mice may be bred together.

Additional Breeding and Husbandry Support
Mating System
Homozygote x Homozygote

Citation
When using the Ai35D or Ai35(RCL-Arch/GFP) mouse strain in a publication, please cite the originating article(s) and include JAX stock #012735 in your Materials and Methods section.
Animal Health Reports
Facility Barrier Level Descriptions

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.

Domestic
Internation

Pricing effective for USA, Canada and Mexico shipping destinations

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RELATED PRODUCTS AND SERVICES

| Frozen Mouse Embryo | B6;129S-Gt(ROSA)26Sor<tm35.1(CAG-aop3/GFP)Hze>/J Frozen Embryo | $2595.00 |

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