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

---

**GENETIC OVERVIEW**

**Genetic Background**

<table>
<thead>
<tr>
<th>Allele Type</th>
<th>Gene Symbol</th>
<th>Gene Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Targeted (Conditional ready (e.g. floxed), Reporter)</td>
<td>Gt(ROSA)26Sor</td>
<td>gene trap ROSA 26, Philippe Soriano</td>
</tr>
</tbody>
</table>

**RESEARCH APPLICATIONS**

Neurobiology Research
Research Tools
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{tm35.1(CAG-aop3/GFP)Hze}
Genotyping resources and troubleshooting

Breeding Considerations
Homozygous mice are viable and fertile. 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 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.
Production of mice from cryopreserved embryos or sperm occurs in a maximum barrier room, **G200**

---

### Pricing & Availability

**Cryo Recovery**

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

#### Domestic

Pricing effective for USA, Canada and Mexico shipping destinations

<table>
<thead>
<tr>
<th>SERVICE/PRODUCT</th>
<th>DESCRIPTION</th>
<th>PRICE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryo Recovery</td>
<td>Heterozygous or Wild-type for Gt(ROSA)26Sor&lt;tm35.1(CAG-AOP3/GFP)Hze&gt;</td>
<td>$2,854.50</td>
</tr>
</tbody>
</table>

---

### RELATED PRODUCTS AND SERVICES

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

---

### THE JACKSON LABORATORY'S GENOTYPE PROMISE

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.
Terms Of Use

TERMS OF USE

General Terms and Conditions

ADDITIONAL USE RESTRICTIONS APPLY

NOT AVAILABLE TO COMPANIES OR FOR COMMERCIAL USE
Use of MICE only available to non-profit entities.

LICENSING INFORMATION

Phone: 207-288-6470
Email: TechTran@jax.org

Related Strains

- All
- By Allele
- By Gene
- By Collection