ALLEN INSTITUTE for BRAIN SCIENCE

Allen Brain Observatory

TECHNICAL WHITE PAPER: PHENOTYPIC CHARACTERIZATION OF TRANSGENIC MICE LINES

OVERVIEW

The Allen Brain Observatory contains data collected via two-photon calcium imaging to quantify neuron activity in the mouse neocortex in response to visual stimuli. This is made possible by the use of animals harboring a genetically encoded calcium sensor, GCaMP6[1], which is imparted by use of the Ai93 line and expressed in subsets of cell populations due to a combinatorial transgenic line breeding strategy. Because the expression of GCaMP6 in each mouse line is mediated by multiple genetic regulatory elements, characterization of the spatial expression of GCaMP6 in the brain is helpful for understanding the anatomical distribution of GCaMP6-expressing cells, and evaluating whether there are other gross phenotypic effects of the breeding strategy.

This document provides a description of the following:

1. An overview of the transgenic strategy used for creating each line.
2. Images of GCaMP6 expression based on 2-photon serial tomography, to detect the native baseline presence of cells harboring GCaMP6-GFP.
3. Phenotypic characteristics of transgenic animals, including summaries of growth.
4. Technical considerations for animal breeding and health.

The following transgenic mouse lines are described:

- Cux2-CreERT2;Camk2a-tTA;Ai93
- Rbp4-Cre;Camk2a-tTA;Ai93
- Rorb-IRES2-Cre;Camk2a-tTA;Ai93
- Scnn1a-Tg3-Cre;Camk2a-tTA;Ai93

[1] Chen TW, Wardill TJ, Sun Y, Pulver SR, Renninger SL, Baohan A, Schreiter ER, Kerr RA, Orger MB, Jayaraman V, Looger LL, Svoboda K and Kim DS. (2013) Ultrasensitive fluorescent proteins for imaging neuronal activity. *Nature* 499(7458):295-300.

Cux2-CreERT2;Camk2a-tTA;Ai93

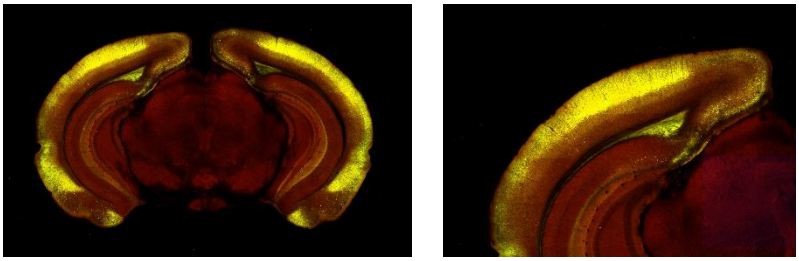
OVERVIEW

Cux2-CreERT2;Camk2a-tTA;Ai93 transgenic mice express a Cre/Tet-dependent, fluorescent calcium indicator GCaMP6f, as well as a transgene directing tetracycline-controlled transactivator protein (tTA) expression in forebrain excitatory neurons. Further specificity is regulated by the tamoxifen-inducible Cux2 promoter, induction of which results in Cre-mediated expression of GCaMP6f in excitatory neurons. Predominant expression is observed in cortical layers 2, 3 and 4 as well as in thalamus, midbrain, pons, medulla, and cerebellum. Calcium influx associated with neural activity results in transient increases in fluorescence of GCaMP6-GFP.

GENETICS

Transgene Expression

Native GCaMP6-GFP expression (yellow), visualized by 2P tomography:



Expressed Gene: GCaMP6f, Genetically encoded calcium indicator
Site of Expression: Expressed under the control of tetO in cells/tissues where cre recombinase is expressed

Expressed Gene: tTA, tetracycline-controlled transactivator
Site of Expression: Expressed in excitatory forebrain progenitors and neurons.

Expressed Gene: cre, cre recombinase
Site of Expression: Tamoxifen-inducible expression in cortical layers 2/3/4, thalamus, midbrain, pons, medulla and cerebellum.

Native reporter expression:

in situ hybridization of *Cux2* (NCBI Accession: NM_007804.2) in C57BL/6J
http://mouse.brain-map.org/experiment/show/72128748

Ai93: *in situ* hybridization of *Camk2a* (NCBI Accession: NM_009792.3) in C57BL/6J
http://mouse.brain-map.org/experiment/show/79490122

PHENOTYPE

Growth and Development

Mean (±SD) Weight at weaning (~p21):

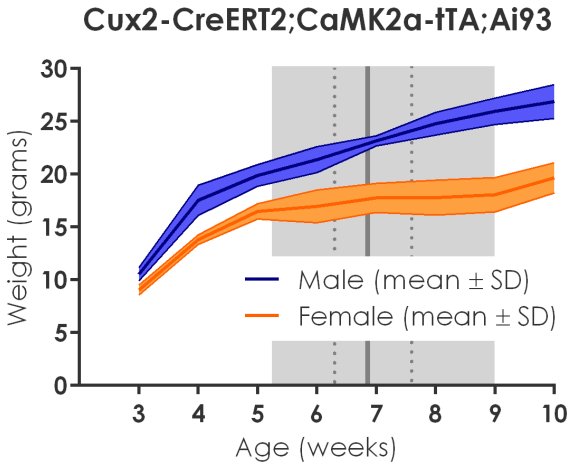
Male: 8.39 ± 1.56

Female: 8.13 ± 1.46

Mean (±SD) Weight at surgery (~p40):

Male: 18.65 ± 2.31

Female: 16.38 ± 1.28



Coat Color: Black or Agouti

Mean (±SD) litter size: 5.3 ± 2.45 (4 breeding sets)

Post-weaning viability: 99.2%

TECHNICAL

Mating System & Breeding Considerations

Breeding sets (pairs and trios) consisted only of tTA heterozygous crosses as homozygous crosses for tTA caused undesirable and/or lethal phenotypes. Both parent mice were homozygous for Cre and GCaMP6f, although heterozygous mice were used when available.

Litter composition

[50% +/+/+], [50% +/-/+] (Cre, tTA, GCaMP6f)

Health and care notes

- **Inducible transgene induction procedure**: Transgene induction with Tamoxifen is required. Adult mice (>p21) received 5 daily doses of Tamoxifen (200mg/kg, oral gavage) and allowed at least 1 week to recover prior to surgical procedures.
- **Dietary needs**: No special dietary needs. Standard chow regimen included breeder chow (PicoLab High Energy Mouse Diet (5LJ5), LabDiet) during the two weeks post-weaning followed by regular chow (PicoLab Laboratory Rodent Diet (5L0D), LabDiet). Additionally, during recovery from surgery mice received a flavor-enhanced hydration/nutrition gel (DietGel (76A), ClearH2O).
- **General observations**: No abnormal phenotype or behavior

REFERENCES

Cux2-ERT2

Source: https://www.mmrrc.org/catalog/sds.php?mmrrc_id=32779

Originating Lab: Ullrich Mueller

Franco SJ, Gil-Sanz C, Martinez-Garay I, Espinosa A, Harkins-Perry SR, Ramos C, Müller U. (2012) Fate-restricted neural progenitors in the mammalian cerebral cortex. *Science* Aug 10;337(6095):746-9.

Camk2a-tTA

Source: https://www.mmrrc.org/catalog/sds.php?mmrrc_id=31781

Originating Lab: Mark Mayford & Ullrich Mueller

Mayford M; Bach ME; Huang YY; Wang L; Hawkins RD; Kandel ER. 1996. Control of memory formation through regulated expression of a CaMKII transgene. *Science* 274(5293):1678-83. PubMed: 8939850MGI: J:37107

Ai93

Source: https://www.jax.org/strain/024103

Originating Lab: Allen Institute for Brain Science
Madisen L; Zwingman TA; Sunkin SM; Oh SW; Zariwala HA; Gu H; Ng LL; Palmiter RD; Hawrylycz MJ; Jones AR; Lein ES; Zeng H. 2010. A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat Neurosci* 13(1):133-40.

Methods to evaluate native gene expression:
Specimen preparation and serial two-photon tomography:
Refer to the “Overview” whitepaper in the Documentation section of the Allen Mouse Brain Connectivity Atlas for detailed descriptions.

Rbp4-Cre;Camk2a-tTA;Ai93

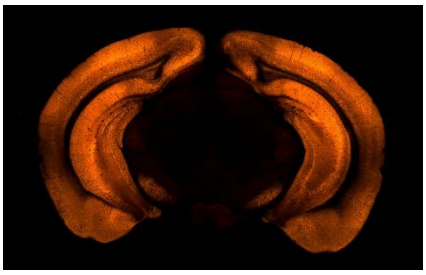
OVERVIEW

Rbp4-Cre;Camk2a-tTA;Ai93 mice have a Cre/Tet-dependent, fluorescent calcium indicator GCaMP6f, as well as a transgene directing tetracycline-controlled transactivator protein (tTA) expression in forebrain excitatory neurons. Further specificity is achieved by the *Rbp4* promoter that results in Cre-mediated expression of GCaMP6f in excitatory neurons in cortical layer 5 as well as dentate gyrus. Calcium influx associated with neural activity results in transient increases in fluorescence of GCaMP6.

GENETICS

Transgene Expression

Native GCaMP6-GFP expression (orange), visualized by 2P tomography:



Expressed Gene: GCaMP6f, Genetically encoded calcium indicator
Site of Expression: Expressed under control of tetO in cells/tissues where cre recombinase is expressed

Expressed Gene: tTA, tetracycline-controlled transactivator
Site of Expression: Expressed in excitatory forebrain progenitors and neurons.

Expressed Gene: cre, cre recombinase
Site of Expression: Expressed throughout cortex; enriched in cortical layer 5 and dentate gyrus.

Native reporter expression:

in situ hybridization of *Rbp4* (NCBI Accession: NM_011255.1) in C57BL/6J
http://mouse.brain-map.org/experiment/show/71016583

Ai93: *in situ* hybridization of *Camk2a* (NCBI Accession: NM_009792.3) in C57BL/6J
http://mouse.brain-map.org/experiment/show/79490122

PHENOTYPE

Growth and Development

Mean (±SD) Weight at weaning (~p21):

Male: 8.39 ± 1.56

Female: 8.13 ± 1.46

Mean (±SD) Weight at surgery (~p40):

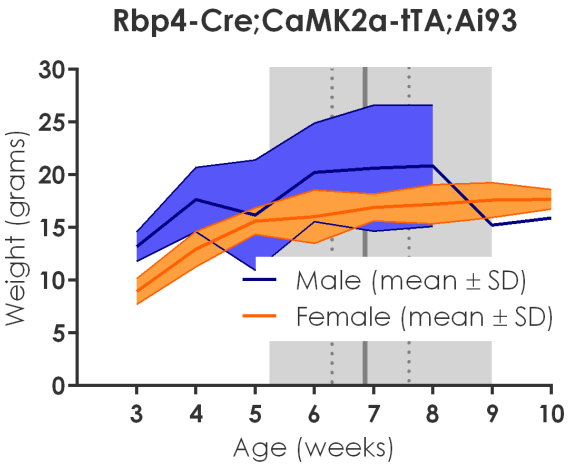
Male: 18.65 ± 2.31

Female: 16.38 ± 1.28

Coat Color: Black or Agouti

Mean (±SD) litter size: 5.71 ± 1.58 (9 breeding sets)

Post-weaning viability: 98.9%



TECHNICAL

Mating System & Breeding Considerations

Breeding sets (pairs and trios) consisted only of heterozygous crosses of Cre-/tTA+ and Cre+/tTA-, as homozygous crosses for either Cre or tTA caused undesirable and/or lethal phenotypes. Both parent mice were homozygous for GCaMP6f, although heterozygous GCaMP6f mice were used when available.

Litter composition

[25% +/+/+], [25% -/+/+], [25% +/-/+], [25% -/-/+] (Cre, tTA, GCaMP6f)

Health and care notes

- **Inducible transgene induction procedure**: Not applicable
- **Dietary needs**: No special dietary needs. Standard chow regimen included breeder chow (PicoLab High Energy Mouse Diet (5LJ5), LabDiet) during the two weeks post-weaning followed by regular chow (PicoLab Laboratory Rodent Diet (5L0D), LabDiet). Additionally, during recovery from surgery mice received a flavor-enhanced hydration/nutrition gel (DietGel (76A), ClearH2O).
- **General observations**: A small fraction (7.2%) of Rbp4-Cre;Camk2a-tTA;Ai93 mice exhibited a tremulous phenotype and were therefore excluded from experimental use.

REFERENCES

Rbp4-Cre

Source: https://www.mmrrc.org/catalog/sds.php?mmrrc_id=31125

Originating Lab: Nathaniel Heintz & Charles Gerfen

Gong S, Doughty M, Harbaugh CR, Cummins A, Hatten ME, Heintz N, Gerfen CR. Targeting Cre recombinase to specific neuron populations with bacterial artificial chromosome constructs. *J Neurosci* 2007 Sep 12;27(37):9817-23.

Camk2a-tTA

Source: https://www.mmrrc.org/catalog/sds.php?mmrrc_id=31781

Originating Lab: Mark Mayford & Ullrich Mueller

Mayford M; Bach ME; Huang YY; Wang L; Hawkins RD; Kandel ER. 1996. Control of memory formation through regulated expression of a CaMKII transgene. *Science* 274(5293):1678-83. PubMed: 8939850MGI: J:37107

Ai93

Source: https://www.jax.org/strain/024103

Originating Lab: Allen Institute for Brain Science
Madisen L; Zwingman TA; Sunkin SM; Oh SW; Zariwala HA; Gu H; Ng LL; Palmiter RD; Hawrylycz MJ; Jones AR; Lein ES; Zeng H. 2010. A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat Neurosci* 13(1):133-40.

Methods to evaluate native gene expression:
Specimen preparation and serial two-photon tomography
Refer to the “Overview” whitepaper in the Documentation section of the Allen Mouse Brain Connectivity Atlas for detailed descriptions.

Rorb-IRES2-Cre;Camk2a-tTA;Ai93

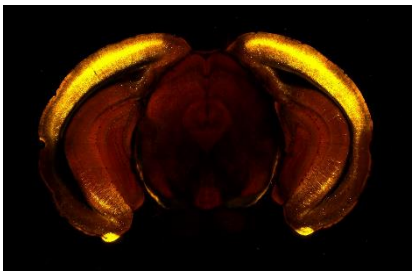
OVERVIEW

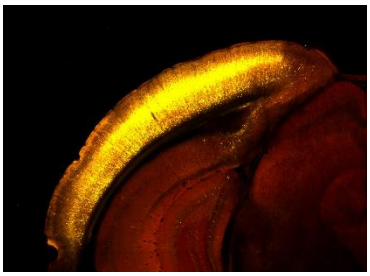
Rorb-IRES2-Cre;Camk2a-tTA;Ai93 mice have Cre/Tet-dependent, fluorescent calcium indicator GCaMP6f, and a transgene directing tetracycline-controlled transactivator protein (tTA) expression in forebrain excitatory neurons. The *Rorb* promoter confers further specificity, resulting in Cre-mediated expression of GCaMP6f in excitatory neurons in cortical layer 4 (dense) and layers 5/6 (sparse), as well as in superior colliculus and thalamus. Calcium influx associated with neural activity results in transient increases in fluorescence of GCaMP6.

GENETICS

Transgene Expression

Native GCaMP6-GFP expression (yellow), visualized by 2P tomography:





Expressed Gene: GCaMP6f, Genetically encoded calcium indicator
Site of Expression: Expressed under the control of tetO in cells/tissues where cre recombinase is expressed.

Expressed Gene: tTA, tetracycline-controlled transactivator
Site of Expression: Expressed in excitatory forebrain progenitors and neurons.

Expressed Gene: cre, cre recombinase
Site of Expression: Dense, patchy expression in cortical layer 4; sparse in layers 5/6. Sub-cortical expression in superior colliculus and thalamus.

Native reporter expression:

in situ hybridization of *Rorb* (NCBI Accession: NM_146095.1) in C57BL/6J
http://mouse.brain-map.org/experiment/show/79556597

Ai93: *in situ* hybridization of *Camk2a* (NCBI Accession: NM_009792.3) in C57BL/6J
http://mouse.brain-map.org/experiment/show/79490122

PHENOTYPE

Growth and Development

Mean (±SD) Weight at weaning (~p21):

Male: 7.89 ± 1.52

Female: 8.92 ± 1.44

Mean (±SD) Weight at surgery (~p40):

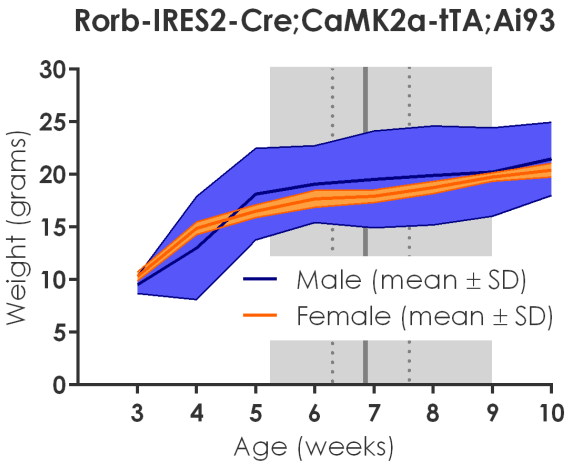
Male: 19.49 ± 1.67

Female: 16.7 ± 1.56

Coat Color: Black

Mean (±SD) litter size: 7.7 ± 3.47 (6 breeding sets)

Post-weaning viability: 99.4%



TECHNICAL

Mating System & Breeding Considerations

Breeding sets (pairs and trios) consisted only of heterozygous crosses of Cre-/tTA+ and Cre+/tTA-, as homozygous crosses for either Cre or tTA caused undesirable and/or lethal phenotypes. Both parent mice were homozygous for GCaMP6f, although heterozygous GCaMP6f mice were also used in these studies.

Litter composition

[25% +/+/+], [25% -/+/+], [25% +/-/+], [25% -/-/+] (Cre, tTA, GCaMP6f)

Health and care notes

- **Inducible transgene induction procedure**: Not applicable
- **Dietary needs**: No special dietary needs. Standard chow regimen included breeder chow (PicoLab High Energy Mouse Diet (5LJ5), LabDiet) during the two weeks post-weaning followed by regular chow (PicoLab Laboratory Rodent Diet (5L0D), LabDiet). Additionally, during recovery from surgery mice received a flavor-enhanced hydration/nutrition gel (DietGel (76A), ClearH_2O).
- **General observations**: No abnormal phenotype or behavior

REFERENCES

Rorb-IRES2-Cre

Source: https://www.jax.org/strain/023526

Originating Lab: Allen Institute for Brain Science

Harris JA, Hirokawa KE, Sorensen SA, Gu H, Mills M, Ng LL, Bohn P, Mortrud M, Ouellette B, Kidney J, Smith KA, Dang C, Sunkin S, Bernard A, Oh SW, Madisen L, Zeng H (2014) Anatomical characterization of Cre driver mice for neural circuit mapping and manipulation. *Front Neural Circuits* 8:76.

Camk2a-tTA

Source: https://www.mmrrc.org/catalog/sds.php?mmrrc_id=31781

Originating Lab: Mark Mayford & Ullrich Mueller

Mayford M; Bach ME; Huang YY; Wang L; Hawkins RD; Kandel ER. 1996. Control of memory formation through regulated expression of a CaMKII transgene. *Science* 274(5293):1678-83. PubMed: 8939850MGI: J:37107

Ai93

Source: https://www.jax.org/strain/024103

Originating Lab: Allen Institute for Brain Science
Madisen L; Zwingman TA; Sunkin SM; Oh SW; Zariwala HA; Gu H; Ng LL; Palmiter RD; Hawrylycz MJ; Jones AR; Lein ES; Zeng H. 2010. A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat Neurosci* 13(1):133-40.

Methods to evaluate native gene expression:
Specimen preparation and serial two-photon tomography
Refer to the “Overview” whitepaper in the Documentation section of the Allen Mouse Brain Connectivity Atlas for detailed descriptions.

Scnn1a-Tg3-Cre;Camk2a-tTA;Ai93

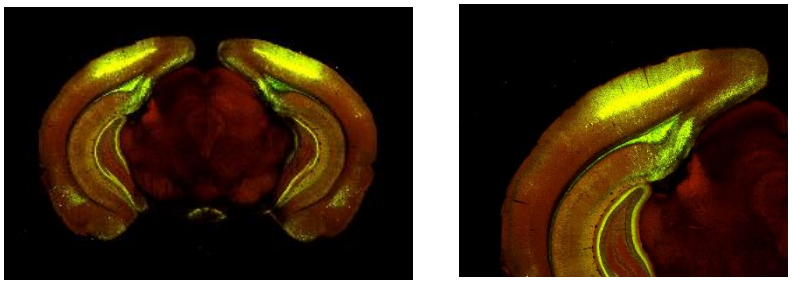
OVERVIEW

Scnn1a-Tg3-Cre;Camk2a-tTA;Ai93 mice have a Cre/Tet-dependent, fluorescent calcium indicator GCaMP6f, and a transgene directing tetracycline-controlled transactivator protein (tTA) expression in forebrain excitatory neurons. The *Scnn1a* promoter confers further specificity, resulting in mice that exhibit GCaMP6f in excitatory neurons in cortical layer 4 and in restricted populations within the cortex, thalamus, and in cerebellum. Calcium influx associated with neural activity results in transient increases in fluorescence of GCaMP6.

GENETICS

Transgene Expression

Native GCaMP6-GFP expression (yellow), visualized by 2P tomography:



Expressed Gene: GCaMP6f, Genetically encoded calcium indicator
Site of Expression: Expressed under the control of tetO in cells/tissues where cre recombinase is expressed.

Expressed Gene: tTA, tetracycline-controlled transactivator
Site of Expression: Expressed in excitatory forebrain progenitors and neurons.

Expressed Gene: cre, cre recombinase
Site of Expression: Expressed in cortical layer 4 and in restricted populations within cortex, thalamus, and in cerebellum.

Native reporter expression:

in situ hybridization of *Scnn1a* (NCBI Accession: NM_011324.1) in C57BL/6J; a subset of Scnn1a-positive neurons are represented in Tg3 strain.
http://mouse.brain-map.org/experiment/show/70562125

Ai93: *in situ* hybridization of *Camk2a* (NCBI Accession: NM_009792.3) in C57BL/6J
http://mouse.brain-map.org/experiment/show/79490122

PHENOTYPE

Growth and Development

Mean (±SD) Weight at weaning (~p21):

Male: 8.99 ± 1.27

Female: 8.76 ± 0.99

Mean (±SD) Weight at surgery (~p40):

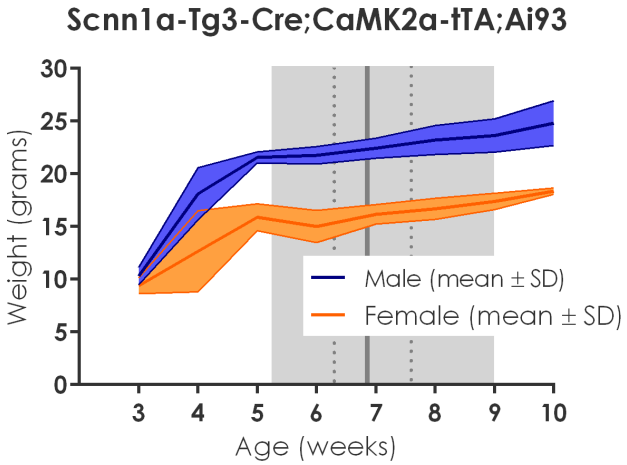
Male: 19.85 ± 2.29

Female: 17.02 ± 1.38

Coat Color: Black

Mean (±SD) litter size: 7.33 ± 3.39 (3 breeding sets)

Post-weaning viability: 98.8%



TECHNICAL

Mating System & Breeding Considerations:
Breeding sets (pairs and trios) consisted only of heterozygous crosses of Cre-/tTA+ and Cre+/tTA-, as homozygous crosses for either Cre or tTA caused undesirable and/or lethal phenotypes. Both parent mice were homozygous for GCaMP6f, although heterozygous GCaMP6f mice were also used in these studies.

Litter composition:
[25% +/+/+], [25% -/+/+], [25% +/-/+], [25% -/-/+] (Cre, tTA, GCaMP6f)

Health and care notes

- **Inducible transgene induction procedure**: Not applicable
- **Dietary needs**: No special dietary needs. Standard chow regimen included breeder chow (PicoLab High Energy Mouse Diet (5LJ5), LabDiet) during the two weeks post-weaning followed by regular chow (PicoLab Laboratory Rodent Diet (5L0D), LabDiet). Additionally, during recovery from surgery mice received a flavor-enhanced hydration/nutrition gel (DietGel (76A), ClearH_2O).
- **General observations**: No abnormal phenotype or behavior

REFERENCES

Scnn1a-Tg3-Cre
Source: https://www.jax.org/strain/009613
Originating Lab: Allen Institute for Brain Science
Madisen L; Zwingman TA; Sunkin SM; Oh SW; Zariwala HA; Gu H; Ng LL; Palmiter RD; Hawrylycz MJ; Jones AR; Lein ES; Zeng H. 2010. A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat Neurosci* 13(1):133-40.

Camk2a-tTA
Source: https://www.mmrrc.org/catalog/sds.php?mmrrc_id=31781
Originating Lab: Mark Mayford & Ullrich Mueller
Mayford M; Bach ME; Huang YY; Wang L; Hawkins RD; Kandel ER. 1996. Control of memory formation through regulated expression of a CaMKII transgene. *Science* 274(5293):1678-83. PubMed: 8939850MGI: J:37107

Ai93

Source: https://www.jax.org/strain/024103

Originating Lab: Allen Institute for Brain Science
Madisen L; Zwingman TA; Sunkin SM; Oh SW; Zariwala HA; Gu H; Ng LL; Palmiter RD; Hawrylycz MJ; Jones AR; Lein ES; Zeng H. 2010. A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat Neurosci* 13(1):133-40.

Methods to evaluate native gene expression:
Specimen preparation and serial two-photon tomography
Refer to the “Overview” whitepaper in the Documentation section of the Allen Mouse Brain Connectivity Atlas for detailed descriptions.