ALLEN INSTITUTE for BRAIN SCIENCE

Allen Brain Observatory

TECHNICAL WHITE PAPER: PHENOTYPIC CHARACTERIZATION OF TRANSGENIC MOUSE 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. The use of a fluorescent calcium indicator, GCaMP6f, was used to register neural activity in the visual cortex of transgenic mice exposed to various visual stimuli. Calcium influx associated with neural activity results in transient increases in fluorescence of GCaMP6-GFP. These experiments use the transgenic mouse line Ai93, in which GCaMP6f expression is dependent on the activity of both Cre recombinase and the tetracycline-controlled transactivator protein (tTA). Triple transgenic mice (Ai93, tTA, Cre) were generated by first crossing Ai93 mice with Camk2a-tTA mice, which preferentially express tTA in forebrain excitatory neurons. Double transgenic mice were then crossed with a Cre driver line to generate mice in which GCaMP6f expression is induced in the specific populations of neurons that express both Cre and tTA.

Under the *Transgenic Characterization* tab of the Allen Brain Observatory, data is available to view the native GCaMP6 fluorescence for each line using 2-photon serial tomography as performed on animals that have not undergone surgery (described in the "Visual Coding Overview" whitepaper in Documentation).

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:

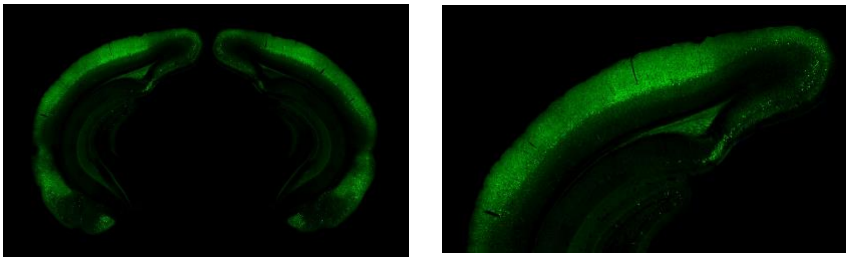
- Cux2-CreERT2;Camk2a-tTA;Ai93(TITL-GCaMP6f)
- Emx1-IRES-Cre;Camk2a-tTA;Ai93(TITL-GCaMP6f)
- Nr5a1-Cre;Camk2a-tTA;Ai93(TITL-GCaMP6f)
- Rbp4-Cre_KL100;Camk2a-tTA;Ai93(TITL-GCaMP6f)
- Rorb-IRES2-Cre;Camk2a-tTA;Ai93(TITL-GCaMP6f)
- Scnn1a-Tg3-Cre;Camk2a-tTA;Ai93(TITL-GCaMP6f)

Cux2-CreERT2;Camk2a-tTA;Ai93(TITL-GCaMP6f)

Overview
Cux2-CreERT2;Camk2a-tTA;Ai93(TITL-GCaMP6f) 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 (green), 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.

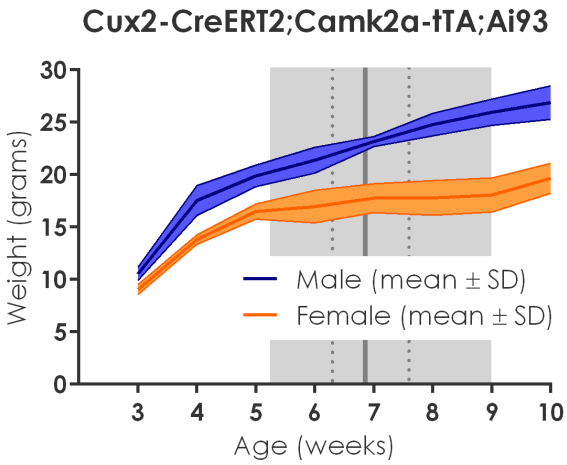
Native gene expression:

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

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. Although the desirable genotype for experimental animals was heterozygous for all three transgenes, in some instances, animals homozygous for Cre and/or GCaMP6f were included. Actual genotypes of individual animals can be found in the Genotyping whitepaper located in Documentation.

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.jax.org/strain/007004
Originating Lab: Mark Mayford
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(TITL-GCaMP6f)
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.

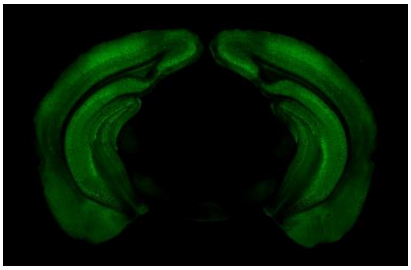
Emx1-IRES-Cre;Camk2a-tTA;Ai93(TITL-GCaMP6f)

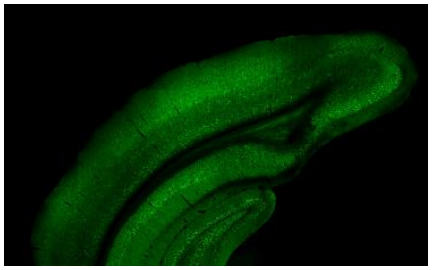
Overview

Emx1-IRES-Cre;Camk2a-tTA;Ai93(TITL-GCaMP6f) 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 Emx1 promoter, resulting in mice that exhibit GCaMP6f in excitatory neurons across layers of cortex as well as in hippocampus. Calcium influx associated with neural activity results in transient increases in fluorescence of CGaMP6-GFP.

Genetics
Transgene Expression

Native GCaMP6-GFP expression (green), 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 cortex (all layers) and hippocampus.

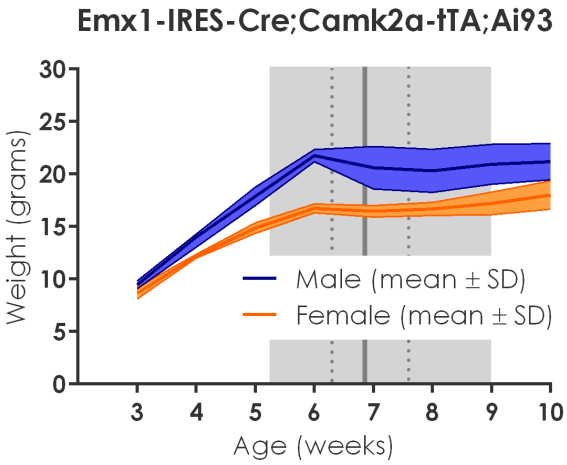
Native gene expression:

in situ hybridization of *Emx1* (NCBI Accession: XM_132640.3) in C57BL/6J
http://mouse.brain-map.org/experiment/show/100145374

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 weening (~p21):
Male: 8.53 ± 1.83
Female: 9.09 ± 1.66
Mean (±SD) Weight at surgery (~p40):
Male: 19.68 ± 3.24
Female: 17.00 ± 1.56
Coat Color: Black or Agouti
Mean (±SD) litter size: 4.14 ± 2.96 (5 breeding sets)
Post-weaning viability: 99.7%



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. Although the desirable genotype for experimental animals was heterozygous for all three transgenes, in some instances, animals homozygous for GCaMP6f were included. Actual genotypes of individual animals can be found the Genotyping whitepaper located in Documentation.

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:** No abnormal phenotype or behavior

References
Emx1-IRES-Cre
Source: https://www.mmrrc.org/catalog/sds.php?mmrrc_id=11830
Originating Lab: Kevin Jones
Gorski JA; Talley T; Qiu M; Puelles L; Rubenstein JL; Jones KR. 2002. Cortical excitatory neurons and glia, but not GABAergic neurons, are produced in the Emx1-expressing lineage. *J Neurosci* 22(15):6309-14.

Camk2a-tTA
Source: https://www.jax.org/strain/007004
Originating Lab: Mark Mayford
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.

Ai93(TITL-GCaMP6f)
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.

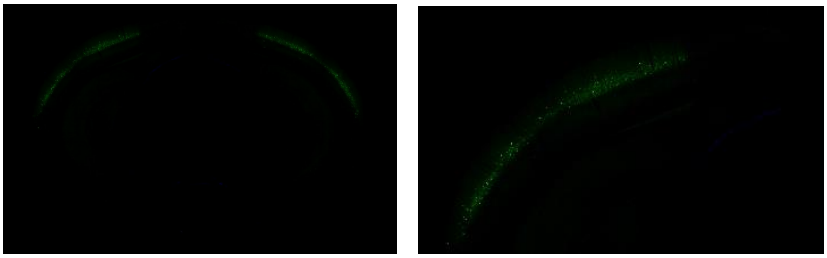
Nr5a1-Cre;Camk2a-tTA;Ai93(TITL-GCaMP6f)

Overview

Nr5a1-Cre;Camk2a-tTA;Ai93(TITL-GCaMP6f) 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 Nr5a1 promoter, resulting in mice that exhibit GCaMP6f in excitatory neurons in cortical layer 4 as well as in the ventromedial hypothalamus. Calcium influx associated with neural activity results in transient increases in fluorescence of CGaMP6-GFP.

Genetics
Transgene Expression

Native GCaMP6-GFP expression (green), 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 neurons that express CaMK2A.

Expressed Gene: cre, cre recombinase
Site of Expression: Expressed in cortical layer 4 as well as in restricted populations within the hypothalamus (ventromedial hypothalamus).

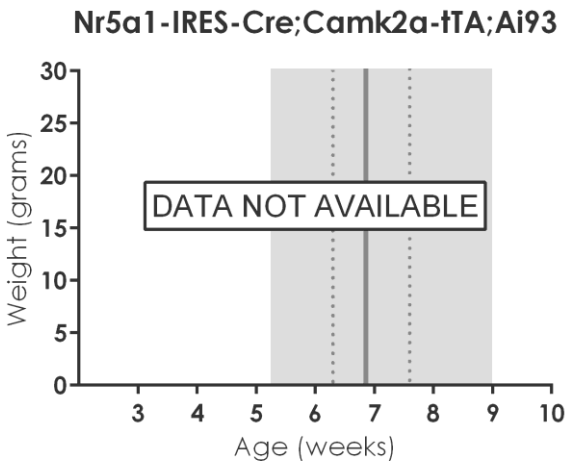
Native gene expression:

in situ hybridization of *Nr5a1* (NCBI Accession: NM_139051.2) in C57BL/6J
http://mouse.brain-map.org/experiment/show/734

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 weening (~p21):
Male: Data not available
Female: Data not available
Mean (±SD) Weight at surgery (~p40):
Male: 22.3 ± 2.79
Female: 18.58 ± 1.14
Coat Color: Black or Agouti



Mean (±SD) litter size: 6.44 ± 2.83 (5 breeding sets)
Post-weaning viability: 97.8%

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. Although the desirable genotype for experimental animals was heterozygous for all three transgenes, in some instances, animals homozygous for GCaMP6f were included. Actual genotypes of individual animals can be found in the Genotyping whitepaper located in Documentation.

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:** No abnormal phenotype or behavior

References

Nr5a1-IRES-Cre
Source: https://www.mmrrc.org/catalog/sds.php?mmrrc_id=34276
Originating Lab: Bradford B. Lowell
Dhillon H; Zigman JM; Ye C; Lee CE; McGovern RA; Tang V; Kenny CD; Christiansen LM; White RD; Edelstein EA; Coppari R; Balthasar N; Cowley MA; Chua S Jr; Elmquist JK; Lowell BB. 2006. Leptin directly activates SF1 neurons in the VMH, and this action by leptin is required for normal body-weight homeostasis. Neuron 49(2):191-203.

Camk2a-tTA
Source: https://www.jax.org/strain/007004
Originating Lab: Mark Mayford
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.

Ai93(TITL-GCaMP6f)
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.

Rbp4-Cre_KL100;Camk2a-tTA;Ai93(TITL-GCaMP6f)

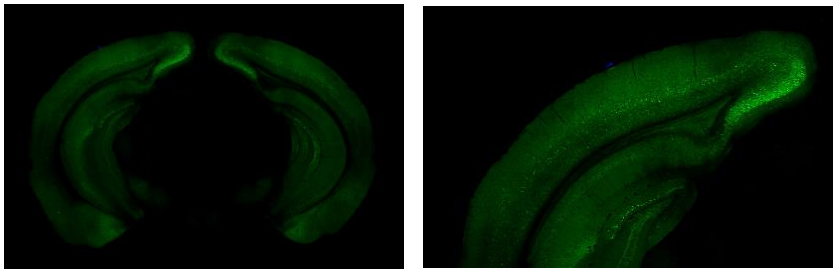
Overview

Rbp4-Cre_KL100;Camk2a-tTA;Ai93(TITL-GCaMP6f) 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 (green), 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 gene expression:

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

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

** Variable expression patterns were observed. All experimental animals in the dataset exhibited GCaMP6f expression in cortical layer 5.*

Phenotype

Growth and Development

Mean (±SD) Weight at weaning (~p21):

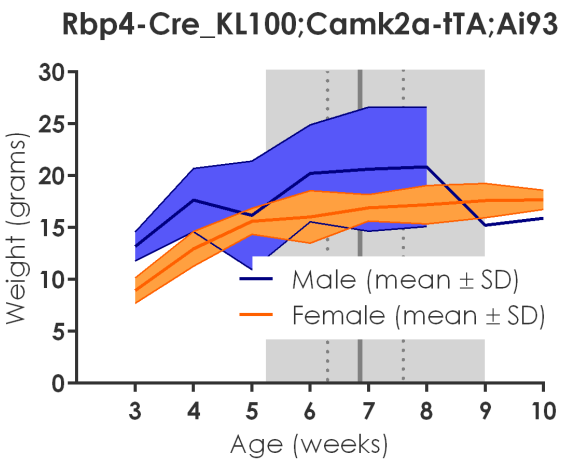
Male: 8.39 ± 1.56

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. Although the desirable genotype for experimental animals was heterozygous for all three transgenes, in some instances, animals homozygous for GCaMP6f were included. Actual genotypes of individual animals can be found in the Genotyping whitepaper located in Documentation.

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_KL100

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.jax.org/strain/007004

Originating Lab: Mark Mayford

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 (TITL-GCaMP6f)

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

Originating Lab: Allen Institute for Brain Science

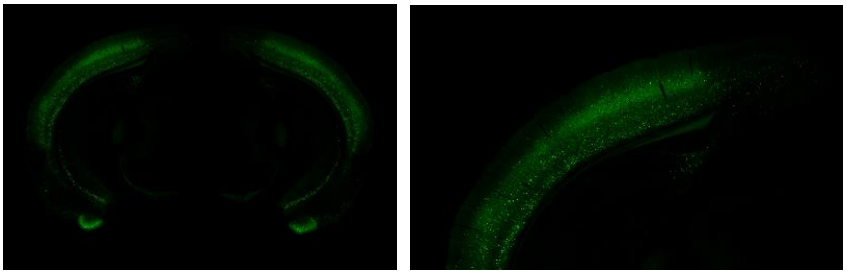
Madisen L; Zwingman TA; Sunkin SM; Oh SW; Zariwala HA; Gu H; Ng LL; Palmiter RD; Hawrylycz MJ; Jones AR; Lein ES; Zeng H. 2010. A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat Neurosci* 13(1):133-40.

Rorb-IRES2-Cre;Camk2a-tTA;Ai93(TITL-GCaMP6f)

Overview
Rorb-IRES2-Cre;Camk2a-tTA;Ai93(TITL-GCaMP6f) 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 (green), 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.

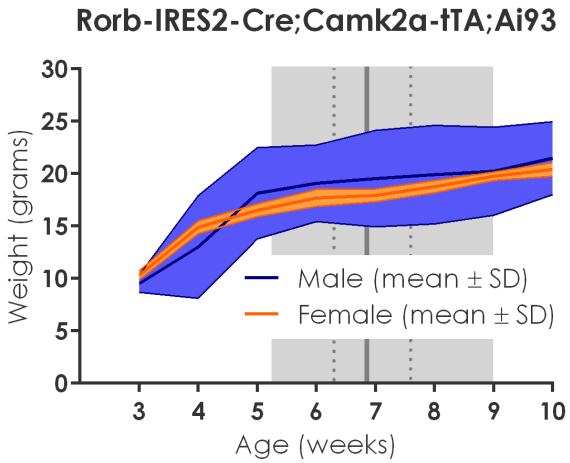
Native gene expression:

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

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. Although the desirable genotype for experimental animals was heterozygous for all three transgenes, in some instances, animals homozygous for GCaMP6f were included. Actual genotypes of individual animals can be found in the Genotyping whitepaper located in Documentation.

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.jax.org/strain/007004
Originating Lab: Mark Mayford
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(TITL-GCaMP6f)
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.

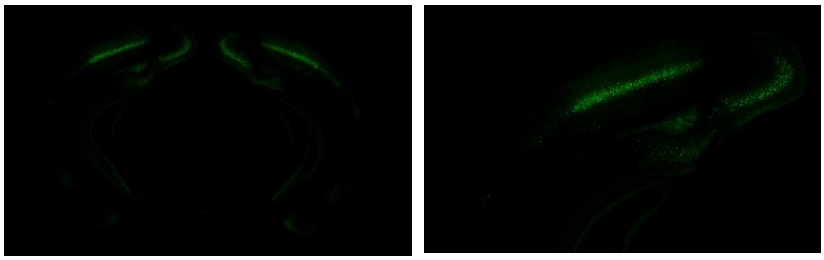
Scnn1a-Tg3-Cre;Camk2a-tTA;Ai93(TITL-GCaMP6f)

Overview

Scnn1a-Tg3-Cre;Camk2a-tTA;Ai93(TITL-GCaMP6f) 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 (green), 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 gene 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
	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):

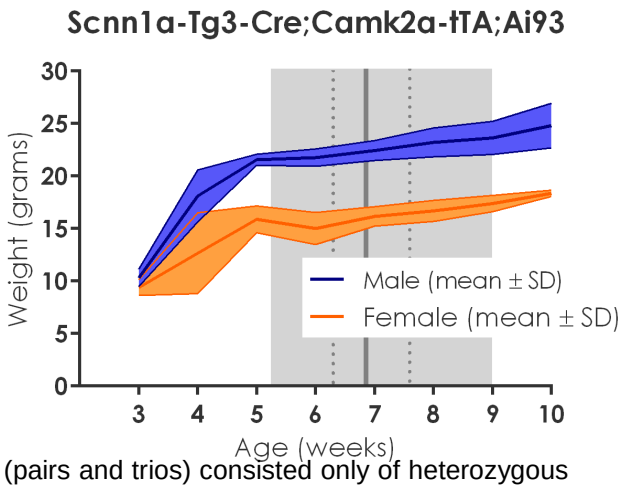
Male: 19.85 ± 2.29

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. Although the desirable genotype for experimental animals was heterozygous for all three transgenes, in some instances, animals homozygous for GCaMP6f were included. Actual genotypes of individual animals can be found in the Genotyping whitepaper located in Documentation.

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.jax.org/strain/007004

Originating Lab: Mark Mayford

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(TITL-GCaMP6f)

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.