Cornell Heart Lung Blood Resource for Optogenetic Mouse Signaling
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CHROMus™ Strain Information Sheet

Strain Name:

Common Name: **HCN4-GCaMP8**

CHROMus™ designation and lines available: R24:B10:L3

Jackson Stock number: NA

Development: The green fluorescent calcium indicator GCaMP8 was inserted at the ATG site of the HCN4 gene in the BAC RP23-281H22 (CHORI) through homologous recombination. The resulting recombinant BAC was injected into the male pronucleus of fertilized oocytes which were then implanted into pseudo pregnant females. Resulting offspring were screened for the presence of the transgene (founders). Colonies were established from each founder and tested for expression.

Transgenic Numbers:

# pups born	# founders	# expressers
11	5	1

Description: The genetically encoded calcium indicator GCaMP8 is expressed under control of the HCN4 promoter, directing expression to the SA node and the AV node of the heart and the nervous system (brain and spinal cord). Expression is observed in the developing 13.5 dpc heart and in the brain and spinal cord. GCaMP8 responds to calcium levels in the cell. When calcium increases, a conformation change occurs resulting in an increase in fluorescence. When calcium decreases, fluorescence decreases. This mouse is useful for examining calcium signaling in the SA and AV nodes.

Phenotypic Data:

Native fluorescent images:

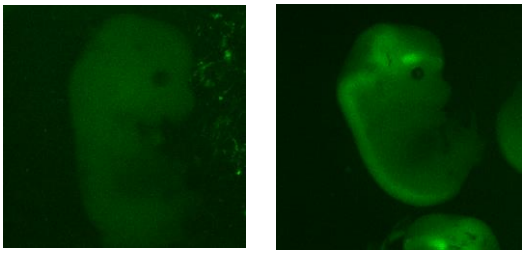

Figure 1: Native fluorescent images (GCaMP8) in 13.5 dpc embryo (left panel, genotypically negative, right panel genotypically positive) showing expression in the forebrain and spinal cord.

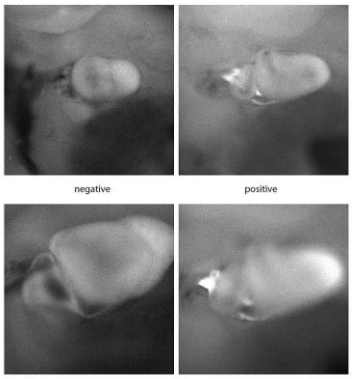

Figure 2: Native fluorescence (GCaMP8) in the heart of a 13.5 dpc embryo. Note the atrial signaling in the positive embryo.

Immunohisotchemistry:

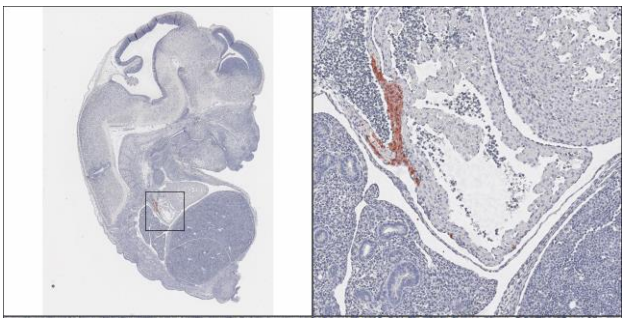

Figure 3: anti-gfp staining in the heart of a 13.5 dpc embryo. Whole embryo and heart showing staining in the SA node.

Also available online: video of calcium signaling in the 13 dpc heart. (http://chromus.vet.cornell.edu/hcn4gcamp8/)

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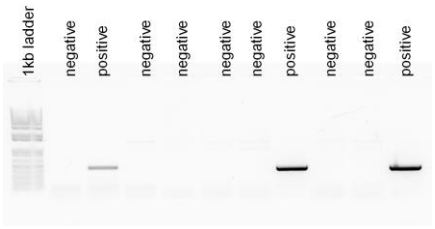
Genotyping Protocol:

Primer CalmF:	AAG GGC GAG GAG CTG TTC A
Primer CalmR:	CGA TCT GCT CTT CAG TCA GTT GGT
Expected size of product:	456 bp
Cycling conditions: 1. 94°C 3 minutes 2. Repeat 30 times a. 30 sec @ 94°C b. 30 sec @ 57°C c. 60 sec @ 72°C 3. 72°C 5 minutes 10°C hold	PCR results: 

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Acknowledgement: Please inform us of any publications resulting from the use of this mouse and use the following statement to acknowledge their development:

"The mouse strain HCN4-GCaMP8 was developed by CHROMus™; funded by NIH R24HL120847."