# A Spontaneous Mouse Strain with Cryptophthalmos

Authors: Michelle M. Curtain and Leah Rae Donahue

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Mutation Symbol: *ne*

Mutation Name: No Eyelid

Strain of Origin: STOCK Tg(CAG-Bgeo/GFP)21Lbe/J X CAST/Ei

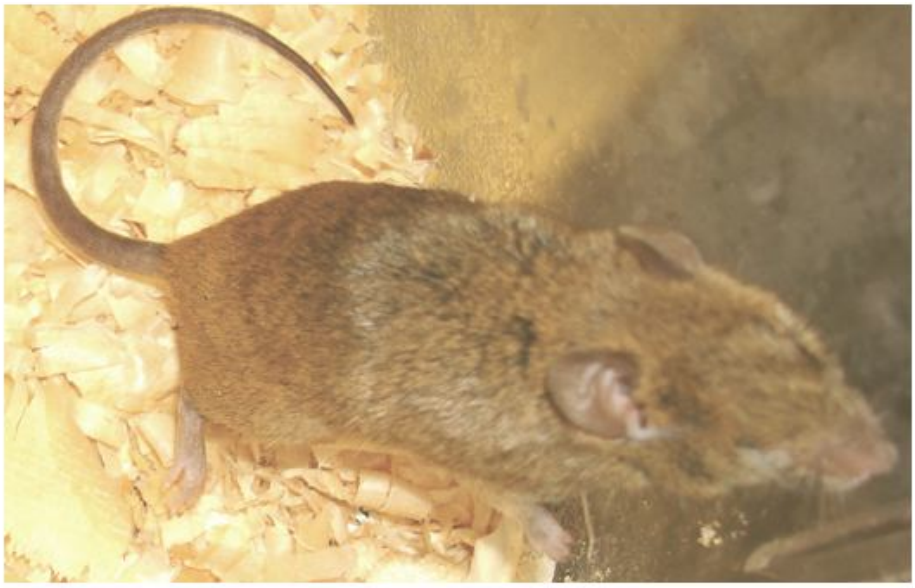
Current Strain Name: STOCK *ne*/J

Stock #006857 (jaxmice.jax.org)

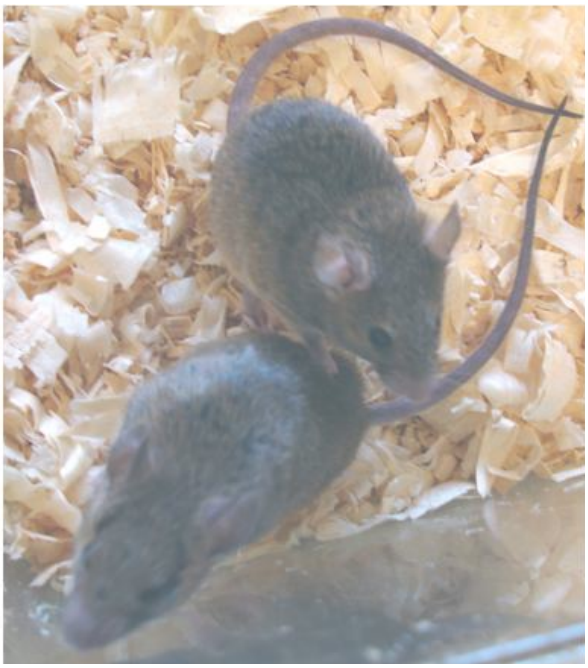
Phenotype Category: eye, skeleton, coat color, kidney

**Origin and Description**

No Eyelid (*ne*) was discovered in 2005 by Sandra Gray. It arose in the second generation of a research colony's mapping cross consisting of a STOCK Tg(CAG-Bgeo/GFP)21Lbe/J (Stock #3920) X CAST/Ei background. Our original mutant had a missing eyelid in that the eye was covered over by fur with no indication of eyelid formation. Similar phenotypes expressed were closed eyelids and small eyes. Other phenotypes that arose in the colony were fused and malformed digits, malformed ear pinnae, slight discoloration of the fur on the head, and missing kidneys; however these phenotypes are not fully penetrant. Malformed digits can appear in mice with and without eyelid abnormalities; discoloration of the fur and malformed ear pinnae sometimes appear in the eyelid mutants but not with regularity; the missing kidney phenotype has been observed only once. However due to the similarity between the *ne* phenotype and others reported for candidate gene *Frem2*, the inclusion of these not fully penetrant phenotypes should not be dismissed. The missing or closed eyelid is the most predominant feature of *ne* and what was used to map the mutation.



Nine-month-old male *ne*/*ne*. The eye phenotype here is a missing eyelid.



A breeder pair at ten-months of age. The *ne*/*ne* female has closed eyes and is shown on left of the photos. The *ne*/+ male is shown on the right of the photo.

The colony is maintained by mating a +/*ne* by a *ne*/*ne* or the reciprocal. Male and female mutants are fertile but have small litters. A homozygote mated to a heterozygote, based on an average of ten litters, had 4.5 pups per litter. Ten litters of heterozygote x heterozygote matings averaged 5.7 pups per litter.

**Genetic Analysis**

No Eyelid is a recessive mutation determined by mating a *ne/ne* to a CAST/Ei inbred strain (Stock # 000928) then intercrossing F1 carriers. There were no mutants in the F1 mice (0/20) and about 11% (8/71) of F2 mice were affected.

To determine a genetic map position, we mated a *ne/ne* to an A/J inbred (Stock #000646) and another *ne/ne* to a BALB/cBy inbred (Stock #000650). From each cross, sibling carriers were mated and mutants appeared in the F2 generation. Spleen and tail tips were collected from each set of mutants and stored at -80 degrees C. DNA was extracted using standard phenol extraction methods. Polymerase chain reaction was done with MIT or Research Genetics primer pairs. Twenty-three homozygotes were born out of 157 total F2 progeny from the two crosses. The mutation is located between markers *D3Mit65* and *D3Mit241*. *D3Mit65* is at 23.3 cM (according to MGI) or 50.4 Mb (according to Ensembl), and there was one recombination out of twenty meiosis or 5% recombination. There were two out of 18 for 11% recombination at *D3Mit241* at 33 cM (MGI) or 66.3 Mb (Ensembl). There was no recombination (0/20) at *D3Mit120* at 28 cM (MGI).

A candidate gene is *Frem2* located at 53 Mb (Ensembl) or 29.9 cM (MGI). The *Frem2* reported alleles have cryptophthalmos, microphthalmia, malformed ear pinnae, syndactyly, abnormal coat color, renal defects and heart defects. Our *ne* strain exhibits the eye, ear pinnae, coat color, renal and digit phenotypes with variable penetrance. The heart is normal in *ne/ne* mice up to two months of age; we have not looked at these organs in older mice.

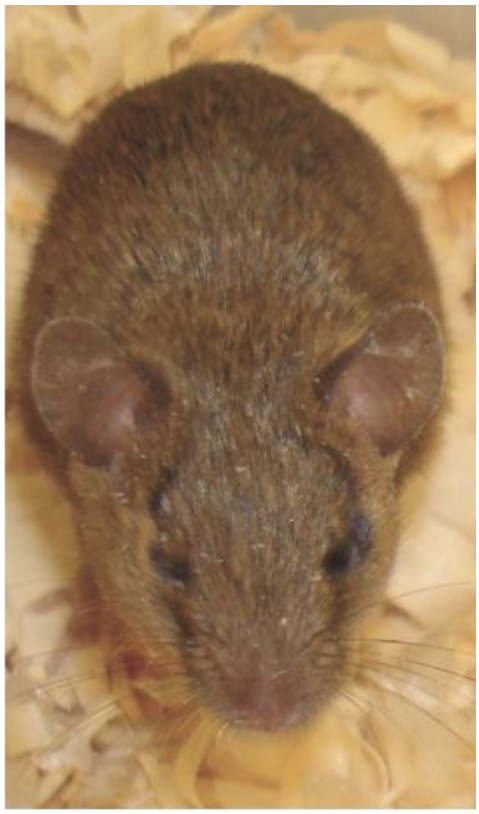
**Pathology**

Using our standard pathology screen[1], a seven-week-old homozygote had a disorganized

eye. The eye of a 26-day-old homozygote revealed the inner nuclear layer had dark cells that were either dying cells or had failed to develop. A three-week-old *ne/ne* female had no right kidney and both eyes had thin retinas. Another eight-week-old female homozygote had degeneration of the Organ of Corti at the tip of the cochlear. She also had subpial Rosenthal fibers and hypertrophic astrocytes in the ventral medulla and cervical spinal cord.

A clinical eye exam revealed a greater range of abnormalities in the eyes of mutants. In a four-month-old male mouse, both eyes had dermoid. A five-and-a-half month old mouse had a bloody white cornea. A two-and-a-half month old male had one eye with a retinal spot and one eye with a broken cornea. Heterozygotes had normal eyes. Homozygotes from the mapping cross also revealed severe infection in the eyes with blood and cataracts; unaffected siblings were normal.

Hearing was assessed by auditory brainstem response (ABR) on four homozygotes and four unaffected mice all around two months old. Three of the homozygotes did not survive the test, perhaps in response to the anesthesia, and the fourth mutant had normal hearing as did the unaffected mice.



This is a *ne*/*ne* female at ten months of age with one small eye and the other eye closed shut. Also note the coat color anomaly on the face.

**Discussion**

We have not proven or disproven our strain to be an allele of *Frem2*, and we do not know if the digit, coat color, kidney and ear pinna phenotypes are part of the *ne* mutation. But if *ne* is another allele of the *Frem2* gene, these phenotypes may be part of the mutation. Also, the variability of the *ne* eye pathology and phenotype may be explained by the myelencephalic blebs reported in some *Frem2* strains during embryogenesis. We have not looked at embryos young enough to determine if *ne* also has these blebs or blisters; however, we looked at an E 17.5 litter where one embryo had an asymmetrical face in that

one side was narrower than the other. Another from the same litter had one eye open at an age when eyelids should be covering the eye. These two examples may be due to myelencephalic blebs reported to occur earlier in development from E 11.5 to E 13.

## Acknowledgements

We would like to thank Chantal Longo-Guess for ABR analysis, Norm Hawes for clinical eye evaluation, Coleen Kane for preparation of tissues for histological assessment, and Dr. Roderick Bronson for pathological evaluation.

## Protocols

### [1]Standard Histology Protocol used in the Mouse Mutant Resource

For fixation of tissues, mice were deeply anesthetized with tribromoethanol (avertin) until they no longer displayed a withdrawal reflex in the hind limbs and then perfused intracardially with Bouin's fixative following a flush of the vasculature with saline solution. After soaking in Bouin's for one week to demineralize bones, tissues were dissected. Six segments of spine with axial muscles and spinal cord in situ, representing cervical, thoracic and lumbar spinal segments, were dissected. The brain was removed and sliced into 6 cross sectional pieces at the levels of olfactory lobes, frontal cortex, striatum, thalamus, midbrain, rostral and caudal medulla with cerebellum. Midsagittal slices of hind leg through the knees were prepared. Slices of basal skull through the pituitary and inner ears were taken. Both eyes, salivary glands and submandibular lymph node, trachea plus thyroid and sometimes parathyroid were removed and cassetted. A longitudinal slice of skin from the back was removed. The thymus, slices of lung, and a longitudinal slice of heart were cassetted. Similarly slices of liver through gall bladder, kidney with adrenal attached, pancreas and spleen were prepared. The stomach was sliced longitudinally to include both squamous and glandular portions. Loops of small intestine from 3 levels and slices of large intestine and cecum were removed, as were slices of urinary bladder. The whole uterus, with ovaries attached, was taken. In males testes were sliced longitudinally. The accessory male organs including seminal vesicles, coagulating gland and prostate were removed en block. Altogether in most cases all tissue fit into a total of 10 cassettes. The cassettes were processed in an automatic tissue processor to dehydrate tissues which were then embedded in paraffin. Six micron sections were cut and stained with hematoxylin and eosin (H&E). Sections of brain and spinal cord in vertebral bones also were stained with luxol fast blue (LFB) for myelin and cresylecht violet (CV) for cellular detail.

### [2]Auditory-Evoked Brainstem Response (ABR) Thresholds

ABR thresholds in mice are determined using a semi-automated computer system (Intelligent Hearing Systems, Miami, Florida). Subdermal needle electrodes are inserted at the vertex and ventrolaterally to both ears of anesthetized mice. Specific auditory stimuli from 10-100 dB SPL are delivered binaurally through plastic tubes from high frequency transducers. ABR thresholds are obtained, in an acoustic chamber, for clicks and for 8, 16, and 32 kHz pure-tone pips. ABR thresholds of all mice and strains tested are entered in spreadsheet files for storage, easy access, and for the production of periodic progress reports. Click-evoked ABR waveforms, obtained at threshold (T) and at T+10, T+20 and T+30 dB or each mouse, are also stored for future reference. Mice of the CBA/CaJ strain are tested periodically as references for normal hearing, and for monitoring the reliability of the equipment and testing procedures.