# Two spontaneous mutant alleles, *rb* and $rb^{2J}$, of the *Epha4* gene on mouse Chromosome 1

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Mutation (allele) symbols: *rb* and $rb^{2J}$

Mutation (allele) names: rabbit and rabbit 2 Jackson

Gene symbol: *Epha4*

Strain of Origin: C57BL/6J

Current Strain names: C3Sn.B6-$Epha4^{rb}$/EiGrsrJ and C57BL/6J-$Epha4^{rb\text{-}2J}$/EiGrsrJ

Stock #s: 001502 for *rb*, 003129 for $rb^{2J}$ (jaxmice.jax.org)

Phenotype categories: neurological/behavioral: motor

capabilities/coordination/movement anomalies

**Origin and Description**

Both *rb* and $rb^{2J}$ alleles arose on the C57BL/6J background as spontaneous recessive mutations at The Jackson Laboratory in 1964 and 1997, respectively. The *rb* allele was transferred to the C3H/HeSnJ background and that congenic stock is designated C3Sn.B6-$Epha4^{rb}$/EiGrsrJ. Mutants for either allele are visibly identified by 2.5 – 3 weeks of age by a constant hopping gait of the hind limbs; the forelimbs may show a hopping gait or move normally. When picked up by the tail, mutants of either allele clasp their hindlimbs and show reduced ability to hold onto an edge; forelimbs show normal strength. More severely affected, $rb^{2J}/rb^{2J}$ mice also lean frequently to either side. *rb*/*rb* females breed well, producing 6-8 pups per litter. $rb^{2J}/rb^{2J}$ females may deliver 2-3 pups per litter but often exhibit poor nurturing. The *rb* mutation, currently at N37F1, is maintained by alternating generations of backcrossing female mutants to wildtype males of the C3H/HeSnJ strain and then intercrossing heterzygotes. The $rb^{2J}$ mutation currently at F200+18 is maintained by mating homozygous female mutants to heterozygous siblings. The C57BL/6J-$Epha4^{rb\text{-}2J}$/EiGrsrJ colony is supplemented by transplantation of mutant ovaries into histocompatible or immuno-deficient hosts.

DNA of both mutations has been cryopreserved in The Jackson Laboratory DNA Resource (stock numbers listed above). Embryos carrying the *rb* allele are cryopreserved as Stock #000938 (parents are B6J wildtype females x STOCK heterozygous males) and Stock # 001502 (parents are C3Sn.B6 congenic heterozygotes). Embryos carrying the

*rb*$^{2J}$ allele are cryopreserved as Stock # 003129 (parents are B6J wildtype females x heterozygous males).

**Genetic Analysis**

The *rb*$^{2J}$ allele is a remutation at the *rb* locus. (A C3Sn.B6-*rb*/*rb* female mated to a C57BL/6J +/*rb*$^{2J}$ male produced 4 visibly affected mice from 2 litters of 11 total progeny.) An early linkage cross located *rb* between the isoenzyme genes *ldh1* and *Pep3* on mouse Chromosome 1. To refine the position of *rb* on Chromosome 1, we used MIT microsatellite markers to type 75 F2 mutants from a B6-*rb*$^{2J}$ x CAST/Ei intercross. The results are given in centimorgans +/- standard error: D1Mit77—2.01 +/- 1.14—[*D1Mit181, D1Mit7, D1Mit46, D1Mit333, D1Mit132, rb-2J*] - 0.67 +/- 0.66—[*D1Mit216, D1Mit44*]. The order of markers was determined with Map Manager (Manly, 1993) and the linkage data are available from MGD accession number J: 87289). MGSC (2003) as shown by Ensembl (2003) agrees with our mapping order and places the cosegregant *D1Mit132* at map position 77.8 Mb near *Epha4* (Eph receptor A4) at 78.1 Mb. Because targeted and gene-trap mutations of the *Epha4* gene cause a hopping gait in mice, it seemed likely that *rb* and *rb*$^{2J}$ are mutations within the *Epha4* gene. The Tessier-Lavigne Laboratory at Stanford University kindly provided mice carrying a gene-trap mutation in *Epha4* for complementation testing. A *Epha4*$^{Gt(pGT1TM)38Wcs}$/+ female was mated to a *rb*/+ male. Of the 7 total progeny produced, 1 female and 2 males displayed a hopping gait with no leaning and were visibly indistinguishable from *rb*/*rb* mutants. Similarly, the reciprocal mating produced 3 mutants (2 females and 1 male) out of 24 total young. These results indicate that *rb* and *rb-2J* are mutations within the *Epha4* gene.

**Pathology**

Our standard pathology screen[1] revealed no anomalies in either *rb*/*rb* or *rb*$^{2J}$/*rb*$^{2J}$ adults. Auditory brainstem response testing revealed no significant hearing loss in *rb*/*rb* mice at 4 weeks of age or in *rb*$^{2J}$/*rb*$^{2J}$ mice at 4 - 6 months of age. *rb/rb* mutants on the congenic background do have retinal degeneration characteristic of most C3H strains. *rb*$^{2J}$/*rb*$^{2J}$ mutants have normal ERGs (electroretinograms), but may have a retinal histopathology awaiting verification. Fresh sperm specimens from two *rb*$^{2J}$/*rb*$^{2J}$ males at 4 months of age appeared normal by light microscopy.

**Acknowledgements**

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## References

MGD 2004, Mouse Genome Database, Mouse Genome Informatics Project, The Jackson Laboratory, Bar Harbor, ME. (URL: http://www.informatics.jax.org).

MGSC 16.30.1, Mouse Genome Sequencing Consortium 2003 (URL: http://www.ensembl.org/Mus_musculus/)

Manley KF (1993) A MacIntosh program for storage and analysis of experimental mapping data. Mamm Genome 4, 303-313.

**[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.