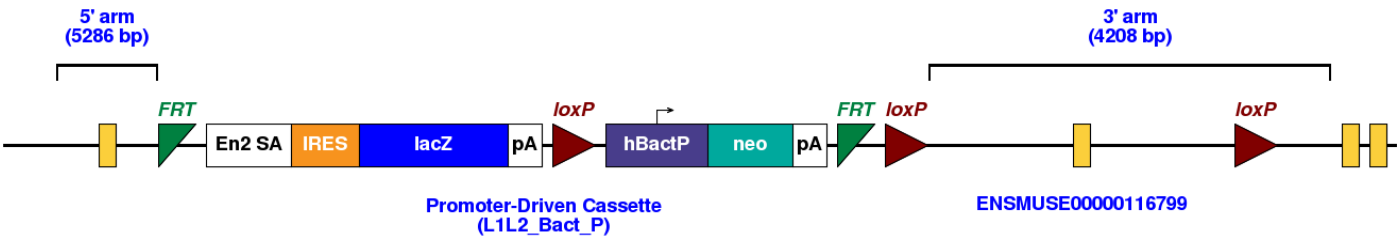
Knockout mouse lines presenting with welfare issues affecting their survival (abnormal survival [MP:0010769]) are processed through a bespoke sub-pipeline known as the "sick mouse procedure" (SMP) to maximise information collected on that mouse line. Matched wild-type controls are also processed to identify phenotypic abnormalities arising from the targeted allele.

Rala$^{tm1a(EUCOMM)Wtsi}$

V-ral simian leukemia viral oncogene homolog A (ras related)

Genetic Background: C57BL/6NTac; C57BL/6NTac; C57BL/6N-Atm1Brd/a

ES Cell Clone: EPD0532_3_H04



Affected genotypes

Homozygous (*Rala*$^{tm1a(EUCOMM)Wtsi}$).

Alternative breeding strategy

Following initial welfare observations, wild type x heterozygous mating strategy was employed to complete phenotyping work in standard pipeline using heterozygous mice only.

Heterozygous mice showed no significant phenotypic findings on the primary screen.

Welfare observations

Homozygous mice exhibit (observations recorded before mice reached P14):

- Domed head shape 6/17 (35%)
- Small body size 8/17 (47%)
- Trunk curling 6/17 and absence of righting reflex 7/17 (41%)
- Do not survive to weaning age (4 weeks) 17/17 (100%)

Homozygous Viability:

All genotyped mice from het x het intercross considered. When at least 28 mice are available, viability at p14 is calculated. *[>13% = Homozygous viable; >0% and <13% = Sub-viable; 0% = Lethal]*

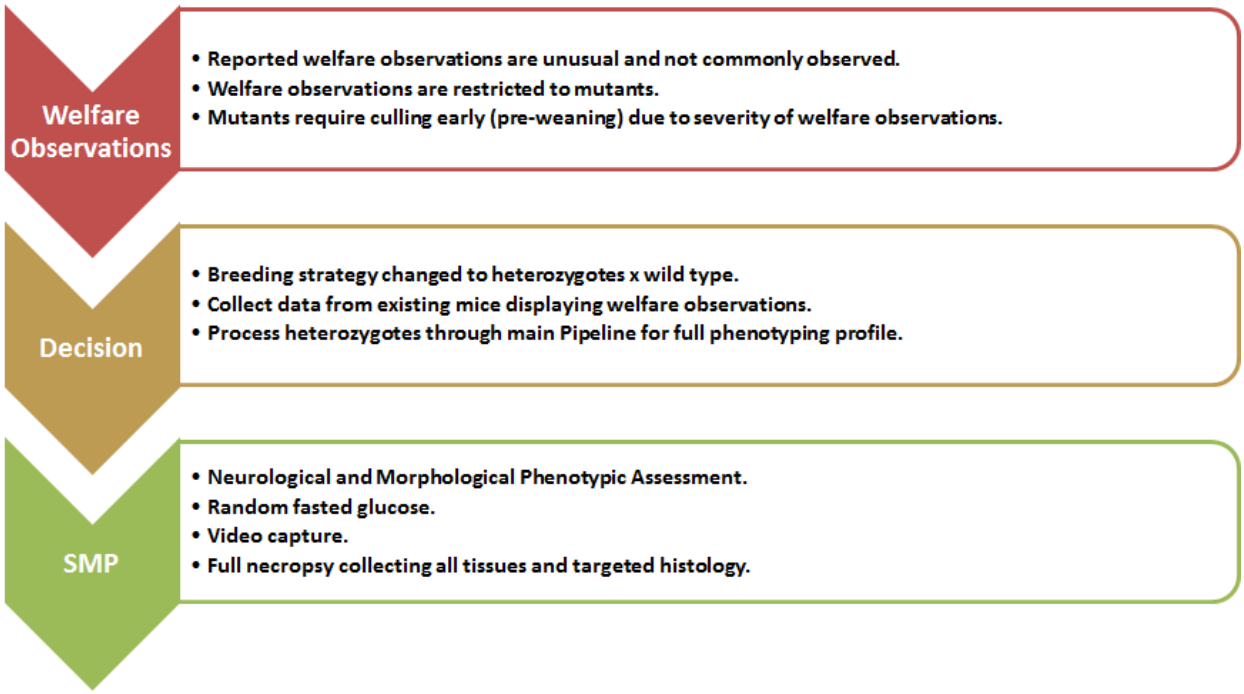
- **Sub-viable** : 3 Homs / 56 Total = 5.36 %

Sick Mouse Procedure (SMP)

Initial welfare observations were reported when the first homozygotes were born during the colony breeding and expansion stage. Due to welfare considerations, these homozygotes required culling. Homozygotes were not considered sufficiently viable for primary phenotyping pipelines and so were not issued to the phenotyping pipelines at 4 weeks of age. New matings to generate homozygote mice were stopped.

Welfare observations in homozygotes described above progressed to moderate severity at 2 weeks of age upon which SMP (see schematic below) was initiated on the final litter of mice. 1 male and 1 female homozygote were processed alongside 1 male and 1 female heterozygote. Mice were genotyped by phenotype before processing and post mortem genotyping samples were taken to confirm a genotype. No further homozygotes were phenotyped due to the aforementioned alternative breeding strategy employed to reduce further welfare implications.

Schematic Outline of Bespoke SMP Pipeline



Phenotyping Heat Map

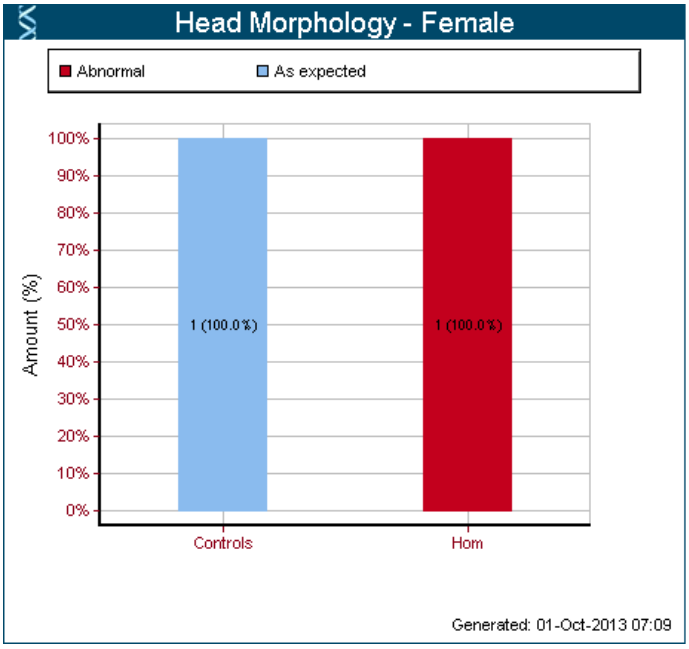
Colony Prefix	Allele Name	Genotype	Dysmorphology	Modified SHIRPA	Glucose Tolerance	Tissue Biobank
MEAH	Rala*tm1a(EUCOMM)Wtsi*	Homozygous				

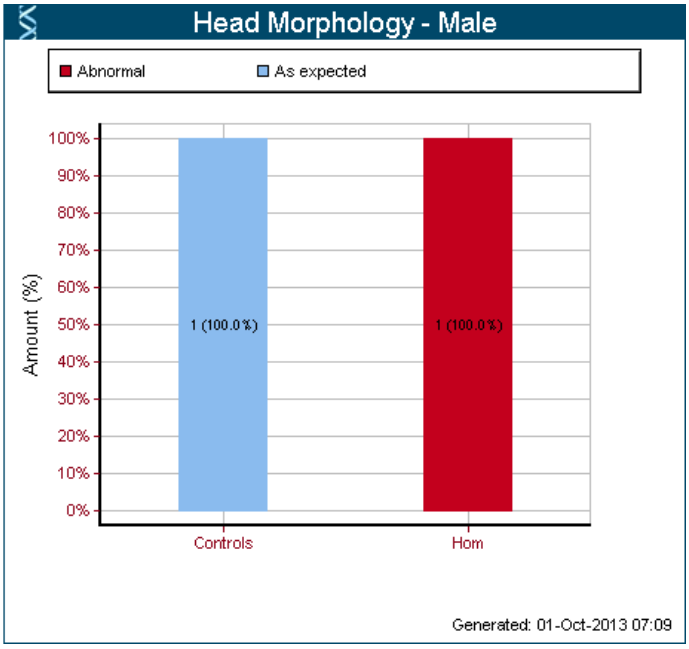
Significant / Interesting
Not significant
Resources available

Phenotyping data of interest (significant changes)

In life phenotyping

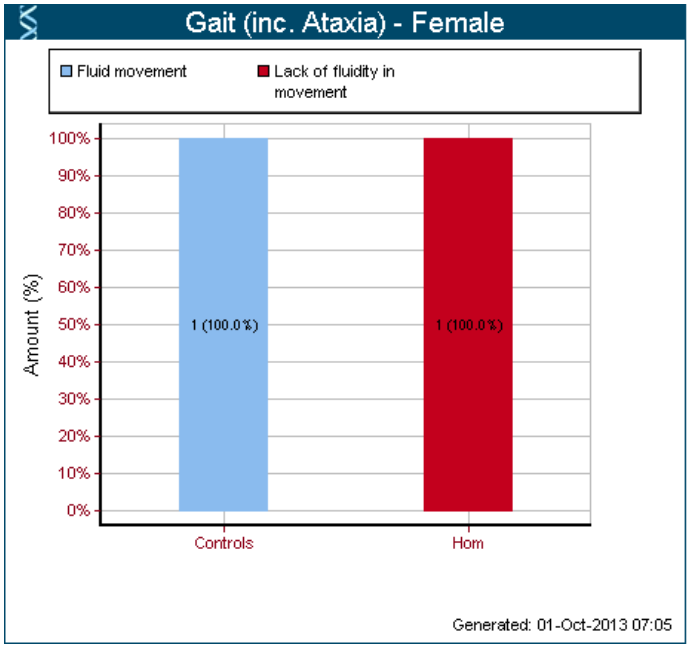
Dysmorphology

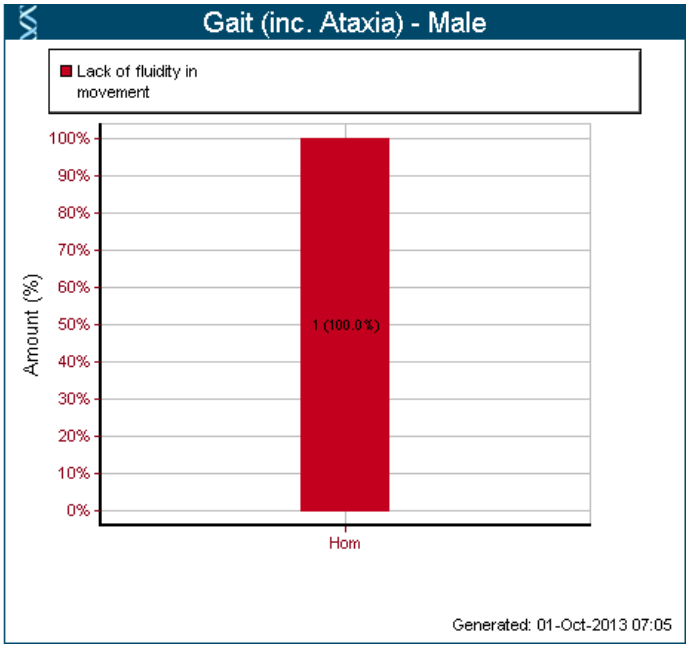




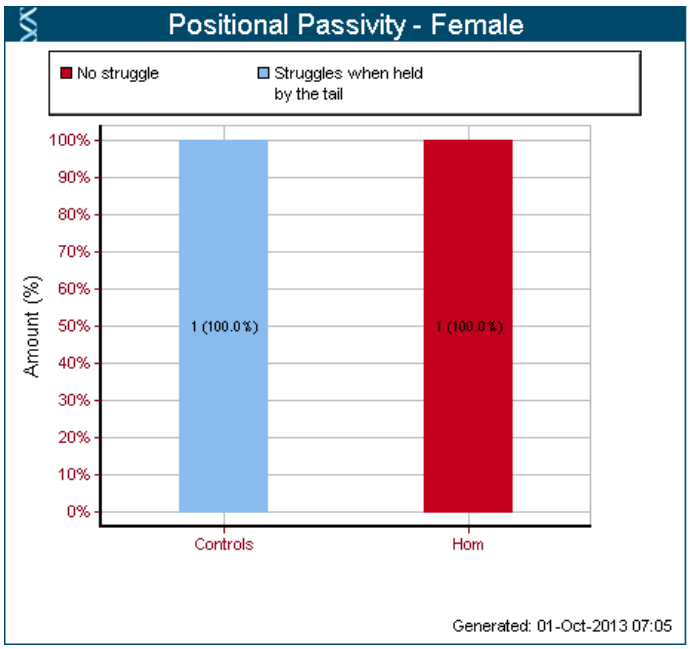
Males and Females – Abnormal head morphology [MP:0000432]

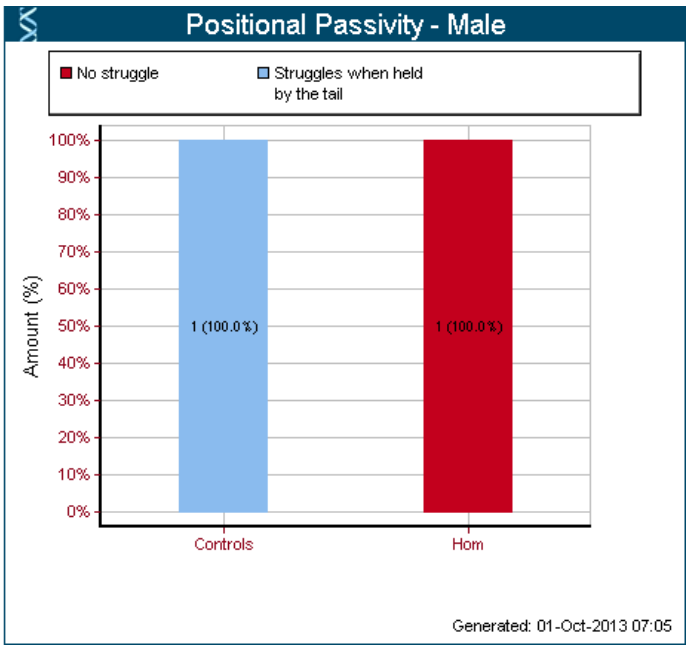
Modified SHIRPA



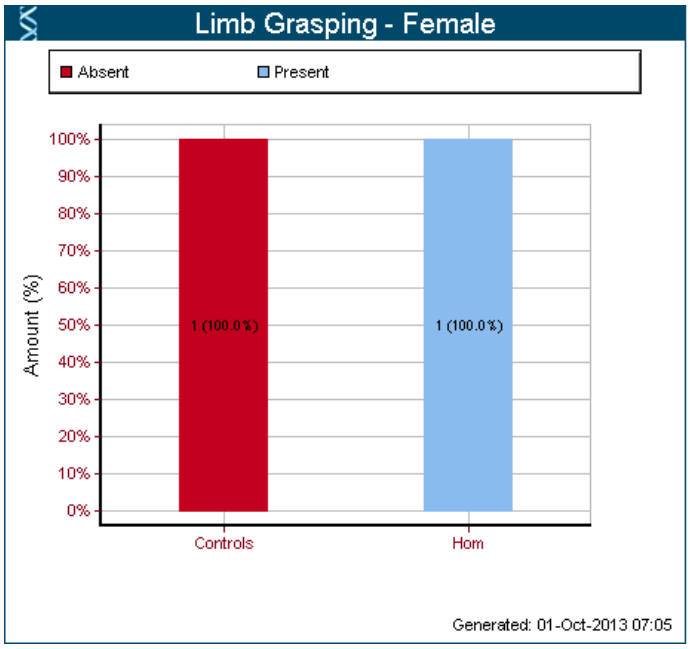


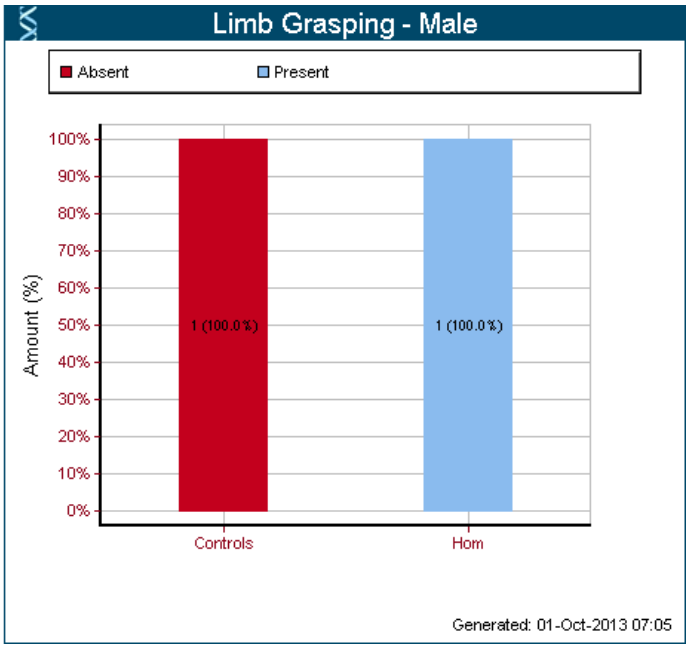
Males and Females – Abnormal gait [MP:0001406]



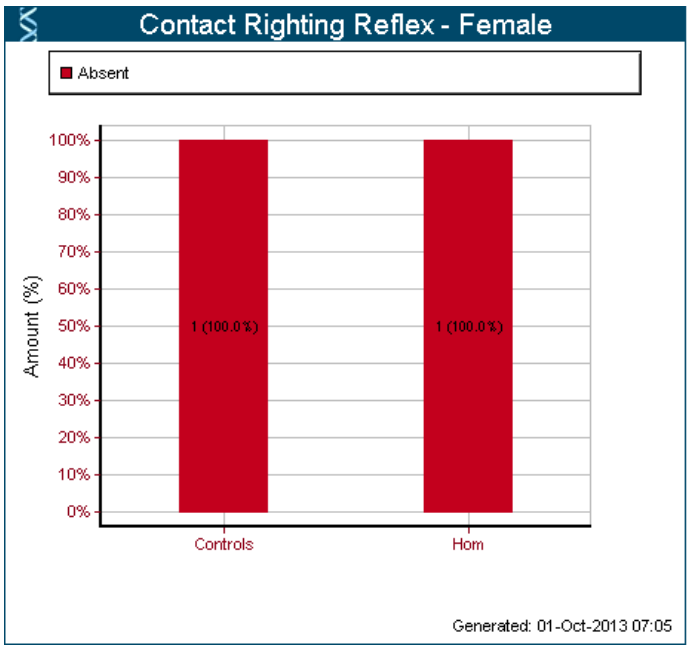


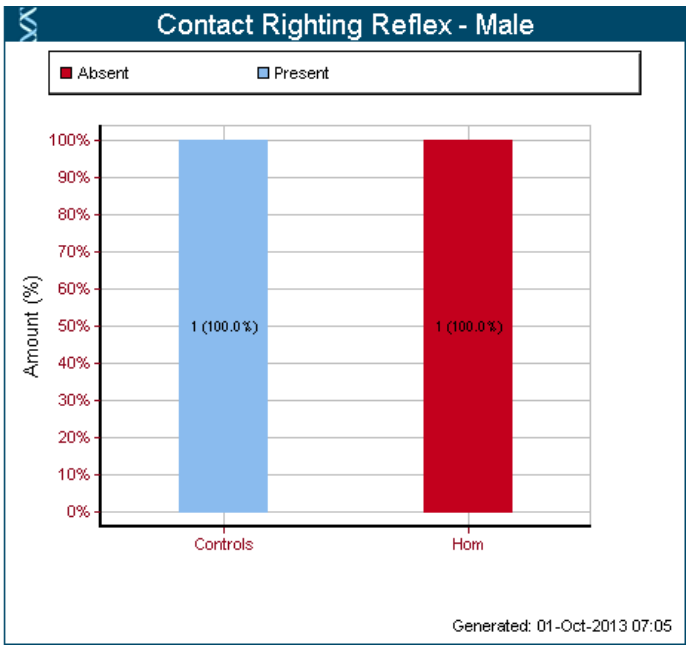
Males and Females – Abnormal Behaviour [MP:0004924]





Males and Females –Limb Grasping [MP:0001513]





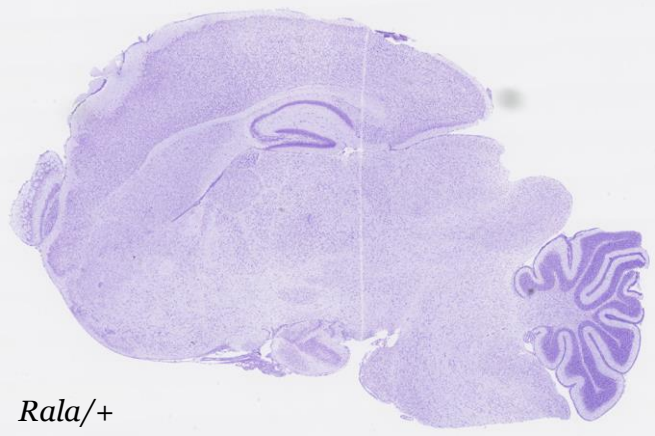
Males and Females – Impaired Righting Response [MP:0001523]

Dysmorphology Images

Videos displaying abnormal gait are available for this line.

Necropsy observations

Macroscopy observation of 2 homs showed abnormal brain shape. Hydrocephaly MP:0001891, Small Cerebellum MP:0000852.



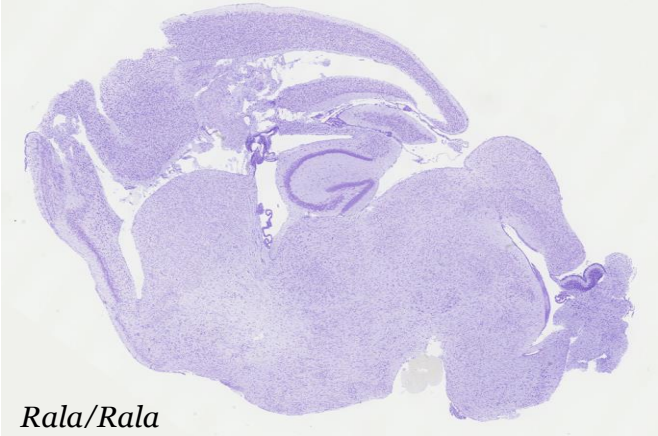


Figure 1. Sagittal section of the brain (5 μm - Nissl's staining) showing hydrocephalus and a small cerebellum in homozygous mice.