Colony Breeding Protocol

1. Purpose

To produce a cohort of mice for 1st tier phenotypic analysis for G2C programme.

2. Procedure

Establishing a colony:
Chimeras are produced by injecting modified E14 embryonic stem cells (derived from 129P2 mice) into C57Bl6 blastocysts. The first heterozygote(s) are produced in the F1 generation from chimera crossings with 129S5 wild-type mice. 6 back-crosses are set up in pairs or trios using F1 or Bx1 heterozygotes with 129S5 wild-types which are allowed to litter until at least 10 male and 10 female heterozygotes have been produced.

Cohort Production:
A cohort consists of 20 homozygotes & 20 wild-type mice produced in 3-4 months from setting up of intercrosses. Cohort production starts when 10-15 intercrosses (het x het) are set up as pairs and are allowed to litter until 20 homos & 20 wild-types have been produced. Ear clips are taken from pups for identification and genotyping purposes at 2-3 weeks old. Pups are weaned at 3-4 weeks. At weaning homozygote and matched wild-type animals are kept, heterozygotes are discarded. Where possible the weaned animals are housed under identical conditions with 2 homozygote and two wild-type animals per cage.

After the genotyping of first litters (~60 mice), if no homozygotes are produced, heterozygotes are kept at weaning and phenotypic analysis will be performed on heterozygote mice. If homozygotes are produced at significantly lower than mendelian ratio may use a minimal phenotyping strategy. Where individual pairs are not producing the ratios produced by other pairs in the same colony after 2-3 litters, these pairs are culled and replaced from heterozygote stocks.

Colony Maintenance:
1-2 F1 hets are each crossed with 1-2 129S5 wildtype mice and allowed to produce 3-4 litters. A Mendelian ratio of 1:1 (hets:WTs) is assumed. Crosses that have not produced any hets after 2 litters are replaced. Backcrosses are set up every 3 months using hets from the previous back-cross until at least back-cross 5 has been achieved. Eventually lines should all be frozen.

E17.5 Knockout Embryos Production:
Alongside cohort production, some homos are used to generate E17.5 knockouts. Homo:homo intercrosses are set up to mate overnight over four consecutive nights with the females checked for plugs each morning, with plugged permanently separated from the male. Plugged females are checked for signs of pregnancy at 14 days post-plugging and used for E17.5 cultures if pregnant. Where knock-outs are known to be homo lethal, het:het timed matings are set up.

3. Materials

The breeding of mice for use in scientific procedures in the U.K. is regulated by the Animals (Scientific Procedures) Act 1986 which requires a project licence granted by the Home Office. Individuals carrying out procedures require their own personal licence and all the animals are housed in a Home Office designated facility.

Colonies are maintained in a designated animal facility under barrier conditions Mice are housed in IVCs and fed on standard laboratory diet or Transwean if required for runts.

Ear clips are taken from pups for identification and genotyping purposes at 2-3 weeks old. Pups are weaned at 3-4 weeks.

Genomic DNA is extracted from ear clips using Wizard SV 96 Genomic DNA Purification Systems (A2371 - Promega) with manipulations performed on a Biomek FX station. Genotyping is performed by PCR.

4. Quality Control

The genotypes generated by each breeding is compared to the expected Mendelian ratios according to the genotypes of the parents, as described in the procedure for Cohort Production.

All progeny that survive are ear-clipped at 2 weeks old. Progeny that do not survive are collected from the breeding cages and frozen as soon as possible. Tissue is harvested from the remains, with genomic DNA extraction performed as for ear-clips.

All mice that are used in the phenotyping cohort are culled once phenotypic analysis is complete with tail tips being collected for genomic DNA extraction and confirmatory genotypic analysis.

5. Example Data

GNB-1

Intercrosses	Litters	Pups	Average	Homo	Het	WT	Start	Complete
11	28	132	4.7	20	78	28	25.10.07	20.2.08

6. Supporting information

7. Document history

This document created on 28 February 2008.