

**License Number: GZ: 311633/2014/9**

**Project Title: "Generation of genetically modified mice by breeding and keeping of these animals"**

**License Holder: Andreas Bichl**

**Number of Mice: 15,600 within 5 years**

**Severity classification according §3 TVG 2012: Moderate**

**License valid until: 2. June 2019**

**Project proposal and description:**

This project application covers breeding and keeping of genetically modified mice originating from breeding of genetically modified animals. Furthermore it also covers breeding and keeping of animals with spontaneous mutations. Although these animals are not subject to classic procedures, their breeding and keeping is deemed as use by the Animal Experiment Act of 2012 since it is at least possible that - due to genetic modifications (whether spontaneous or achieved through genetic procedures) - pain, suffering, anxiety or permanent damage occur primarily at the homozygous stage at such level which equates with or even goes beyond a cannula prick according to proper veterinary standards. It cannot be excluded that animals suffer from permanent minor pains or moderate impairment of their wellbeing or general condition.

The procedures applied to animals respectively use of the animals is limited to selection of appropriately suitable breeding partners and mating and/or subsequent keeping of the animals born. This selection procedure results in modifications of the genotype and/or phenotype of the filial generations. The creation of genetically modified animals follows the natural reproduction behaviour of the mice.

By mating the parent animals, the genomes of which have different mutations, which can either be homozygous, hemizygous or heterozygous, the attempt is undertaken to combine these mutations in the animals of the subsequent generations and then stabilize them in the genome. New genetically modified, recombinant inbred lines with mutations fixed in the homozygous stage are created through strict continued mating of litter mates. These animals mostly have mutations with a genetic background referred to as "mixed and undefined" to which various inbred lines have contributed to unpredictable degrees (e.g. C57BL/6, 129, CBA). To be able to analyse the effects of the new genotype on the phenotype in comparison with wild-type inbred lines it is therefore often necessary to reinsert the mutations through back-cross procedure into a standard background of a classically well-defined and researched inbred line (e.g. C57BL/6). This procedure involves mating of an animal from the subsequent generation with a representative of such background in which the mutation is to be inserted.

The genes involved may for example include the following; Pax5, Ebfl, Bhlhe41, Ikzf1, Ikzf3, Tcf3, Tcf4, Halorhodopsin (NpHR3), Channelrhodopsin (ChR2), Cre/Flp recombinase, green and red fluorescent protein, corticotropin releasing hormone, enkephalin, c-fos, CamK2, GAD, Smarca4, MII3, Brd2, Brd3, Gart, WAPAL,

CDCA5-Sororin, SMC3, PHF2, ESPLI-Separase, CTCF, REC8, Tet- inducible Caspase9, Dre Recombinase, DreERT, Rosa26, RANK(L), Hacer, NeuT, MMTV-NeuT, Ace2, Jnkl, Cardll, Trim28.

PCR based genotyping is used to identify the breed of the animals to be used. A biopsy specimen is taken from the tail tip using a scalpel or scissors prior to weaning of the young animals. The biopsy specimen maximum size of 5mm will not be exceeded (normally a biopsy specimen of 2 to 3mm is sufficient for animals of age < 14 days).

#### End-points

Since this project is focussed on evaluating the as yet unknown effects of mutation in genes and/or their functionality in the individual organic systems or the entire organism, the extent of any pathological changes can hardly be elaborated in great detail since these are indeed also the subject of this research project. We will therefore apply the general end-points also valid for animals which have not been subjected to any experimental procedures. Visibly sick animals will be handled according to clinical condition determined by veterinary inspection. As a guideline, occurrence of the following symptoms counts as an end-point:

- 1 Emaciation of animal, acra prominent. BCS 2-1 -> animal is immediately euthanized.
- 2 Loss of 20% of body weight -> animal is immediately euthanized.  
Loss of 10-15% of body weight within 3 days -> animal is immediately euthanized.
- 3 Irreversible inability to consume food and water -> animal is immediately euthanized.
- 4 Clearly ascertainable breathing problems, clearly impaired breathing, possibly accompanied by inhalation and exhalation wheezing, grinding of teeth, -> animal is immediately euthanized.
- 5 Heavy diarrhoea -> animal is immediately euthanized if accompanied by another symptom, otherwise after 4 days.
- 6 Clear apraxia, convulsions, cramps, paralyses, tremors, slowed or unresponsive reflexes, myasthenia or muscle rigidity, ambulation with curved spine, huddled posture, horrent fur, half closed eyes, dragging/swinging of hind quarters (animal is euthanized if there is no improvement four hours after the initial observation.)
- 7 Apathy, huddled posture, isolation from other animals, starkly reduced reaction to picking up, starkly reduced reaction to acoustic stimulation and contact, animal cannot be roused -> animal is euthanized if there is no improvement four hours after the initial observation.
- 8 Abnormal swellings or protrusions, stark asymmetry of body -> animal is immediately euthanized if symptoms in connection with one or more other symptoms. If the occurrence is not accompanied by other symptoms, further close observation of the animal is foreseen, should any additional symptoms occur the animal will be immediately euthanized.
- 9 Strong nasal or eye discharge -> animal is immediately euthanized if symptoms are accompanied by one or more other symptoms, otherwise four days after initial observation.

- 10 Bleeding from body orifices (light bleeding: animal is euthanized if the bleeding does not abate after 30 minutes. Heavy bleeding -> animal is euthanized if bleeding cannot be stopped immediately).
- 11 Severe skin lesions -> animal is immediately euthanized if accompanied by one or more other symptoms, or if attempted treatment fails with no prospect of healing, otherwise depending on clinical condition.
- 12 Strong aggressive reaction to picking up caused by pain, possibly accompanied by loud vocalisations, gnawing at parts of the body -> animal is euthanized if there is no improvement four hours after the initial observation.

#### Euthanasia methods

The deployed euthanasia methods follow the proven, mostly pain and stress free, standard procedures such as overdose of anaesthetics, or exposure to carbon dioxide with gradual filling of the container (will not be used for foetuses and newborn mice), or cervical dislocation, or decapitation (for foetuses and newborn mice); these procedures will only be applied by qualified, appropriately trained staff. Animal carcasses will be either examined or disposed off when apnoea and cardiac arrest occur or exsanguination has been performed.