Transgenic Facility
Operator and User Regulations

The Core Facility "Transgenic Facility" (TF) of the IZKF Aachen is a service platform for all users of the IZKF and of the medical faculty of the RWTH Aachen, as well as their collaboration partners to support their basic and applied science research projects.
The main services provided the TF include assistance with the breeding management, the consulting regarding projects aimed for the generation of novel genetically modified mouse lines, as well as the generation of those mouse lines itself, the management of the import and export of mice, and the hygienic clean-up of imported mice into the animal facility of the medical faculty. The high quality service provided by the TF is a prerequisite to align the governmental regulations regarding animal welfare with the requirements of our scientific costumers. To ensure the maintenance of this high standard, in parallel to the institute of laboratory animal science and its mouse holding facility, the Core Facility TF is certified according to the DIN-ISO 9001:2015

Our service spectrum includes:

1. Consulting for the generation of genetically modified mouse embryonic stem (ES) cells and mice
2. Genetic modification, culture and cryopreservation of murine ES cells
3. Generation of genetically modified mice
4. Rederivation of imported mouse lines (spf and non-spf)
5. Cryopreservation of mouse embryos, mouse sperms, and mouse oocytes (planned)
6. Revitalization from mouse sperms and embryos
7. In vitro fertilization (IVF)
8. Import and export management
9. Consulting for breeding management, genetics, and the 3R principle (refinement, replacement, reduction)
10. Organization of the animal databases "Tick@lab" and "Tierbase"
11. Organization of the animal capacity in our animal facility

In order to significantly shorten the governmental approval procedure for the generation of genetically modified animals, the TF in collaboration with the Institute for Laboratory Animal Science, holds already an animal license for the generation procedure itself. Thus, only a short project-specific extension license from the corresponding research group is required, for the drafting of which the TF will provide assistance too.

Contact information:

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On the University Hospital website (http://webap-uklac01.klinikum.rwth-aachen.de/content/folder/1019006) you will find all information and order forms concerning the Institute for Laboratory Animal Science and the TF. However, this website is only accessible for employees of the University Hospital Aachen. For requests from other collaborators, please contact us via Email.

Please note:

Users of an IZKF Core Facility are required to mention the Facility's support in the Acknowledgement of relevant publications. Please use the following wording for this purpose:

"This work was supported by the Transgenic Facility by a grant from the Interdisciplinary Centre for Clinical Research within the faculty of Medicine at the RWTH Aachen University"

or

„Diese Arbeit wurde unterstützt durch die Transgene Facility, gefördert mit Mitteln des Interdisziplinären Zentrums für Klinische Forschung in der Medizinischen Fakultät der RWTH Aachen."

In the following, some of our services are described in detail.
If you have any further questions, please do not hesitate to contact the TF.

Generation of transgenic mice via pronucleus injection

Transgenes can be integrated into the mouse genome by injection into the pronucleus of zygote stage embryos (fertilized eggs). These gene constructs can contain both species-foreign and -own genes under the control of either a foreign or an endogenous promoter. The exact site of integration and the number of inserted copies of the constructs are subject to chance. The transgenic embryos are then carried to term by foster mice (nurses). Some of the new-born mice have the transgene integrated into their germline. These fosters are the original animals of the transgenic breeding that follows.

Generation of genetically modified mice via injection of embryonic stem cells

Genetically modified murine embryonic stem cells, either externally purchased or generated by the TF, are injected into early mouse embryos (blastocysts). Given their pluripotency, these cells can contribute to all tissues of the adult mouse, including the formation of the germline, which allows a passaging of the modification to the next generation. Thus, mice with mutations in specific genes can be generated. This includes for example knock-outs, knock-ins, as well as the generation of conditional alleles.

Genetic modification of murine embryonic stem cells

The TF now also offer the genetic modification of murine embryonic stem cells. Our methodical repertoire thereby allows for the random integration of transgenes or modified bacterial artificial chromosomes (BACs), as well as the targeted manipulation of genes using state of the art-technology, such as CRISPR/Cas9 and homology directed repair. By this means it is possible to generate transgene-expressing cells, knock-outs, knock-ins, conditional alleles or more complex genetic modifications. The TF provides consulting to design these genetic modifications according to the researcher's specific requirements.

Cryopreservation and archiving

The TF offers the cryopreservation of murine embryos and sperms.

For the preservation of embryos, superovulated wild-type or genetically modified donor females are mated with the appropriate donor males. The resulting embryos are isolated and frozen in a medium containing propylene-glycol as a cryoprotective agent (CPA) using a slow freezing protocol. Thereby, the embryos are cooled down slowly to -32°C with a temperature decrease of -0.3 - -0.8°C/min and then kept in the gas phase of liquid nitrogen (-196°C) for long-term storage. The slow freezing process reduces the formation of ice crystals within the embryos which directly improves the survival rate upon thawing. An advantage of the

cryopreservation of embryos is the complete conservation of the given genomic state, enabling the direct rederivation of homozygosity and combinations of several modifications.

For the preservation of sperms, these are isolated from the cauda epididymis of donor males and frozen in a special cryoprotective medium. The sperm are first shortly incubated in the gas phase of liquid nitrogen and then transferred into liquid nitrogen. By this method we need only 1-3 males to freeze enough materials for a rederivation of the line. Thus, the cryopreservation is much faster compared to the preservation of embryos and the number of animals required is largely reduced. Sperms, on the other hand, have a haploid genome, so then restoration homozygosity or the simultaneous presence of multiple modifications usually requires further breeding after the rederivation.

The frozen mouse spermatozoa or mouse embryos, are divided into two storage tanks (capacity 300,000 embryos / tank) with liquid nitrogen and remain frozen in the gas phase at -196°C until further use.
Cryopreservation of mouse strains is an important part of the 3R principle (Replacement, Reduction, Refinement) and for animal welfare (here: reduction), as unused mouse strains are permanently and safely frozen and represent a reduction of animal numbers and husbandry costs for the user.

In addition, for requests from other institutes, frozen embryos or sperms can be shipped instead of live animals, which otherwise would only be allowed to be introduced into other animal husbandries after quarantine and embryo transfer. When cryopreserved mouse embryos are shipped, they need only be implanted in a sham-pregnant mouse and carried out by the wet nurse.

Hygienic clean-up of mouse lines

To ensure the SPF-status of the mouse holding rooms of the Institute of Laboratory Animal Science, imported living animals are only allowed to enter the quarantine room. To transfer a mouse line into any other holding room, an embryo transfer into a clean recipient foster mouse is required. The used preimplantation stage embryos are surrounded by the zona pellucida, a glycoprotein layer impermeable for most pathogens.
The the embryo transfer, superovulated donor females are mated with the corresponding donor males in our quarantine room. The embryos are then removed from the oviduct of the donor female and transplanted into an pseudo-pregnant foster mouse via oviduct embryo transfer. After weaning from the mother, the offspring can be genotyped and the wet nurse is examined for possible infections in our microbiology department according to the FELASA Hygiene Guidelines (Federation of European Laboratory Animal Science Associations).

***In vitro* fertilisation (IVF)**

An import of infected mice into the mouse holding of the Institute of Laboratory Animal Science is not possible. To introduce such lines, a hygienic clean-up via *in vitro* fertilization (IVF) is possible. Further, we offer IVF in cases of breeding difficulties, for example in fertility problems of mice due to e.g. genetic drift, inbreed depression or other genetic causes.
For the IVF, freshly isolated or frozen sperm (e.g. in cases if infectious mice) can be used. Sperm and oocytes from donor females are brought together in a microdrop culture and incubated for 4-6 h. The fertilized oocytes (zygotes) are incubated overnight and can be transferred into pseudo-pregnant foster mice as done for the standard hygienic clean-up.