IRTG Advanced Method Course (October 2016):

Genetic manipulation of mice

Genetically modified mice are commonly used for research in order to investigate the „in vivo“ function of a certain gene in a complex model organism or as animal models of human disease.

Basically, there are two technical approaches to produce genetically modified mice. First, injection of a foreign DNA (transgene) into the pronucleus of a fertilized mouse oocyte results in transgenic mice with random integrations of the transgene. This approach is usually used to add new information into the mouse genome (such as Cre-recombinase expression or marker gene expresssion) or to overexpress endogenous genes in wildtype or mutant forms. In the second approach, a targeted mutation of a certain gene of interest is made in embryonic stem (ES) cells with the help of a targeting vector that contains DNA sequences homologous to the target gene. Those ES cells, in which a homologous recombination occured and the endogeneous gene is replaced by a mutant form, are selected and then injected into mice blastocysts. This method is used to specifically manipulate a single gene, in most cases by destroying it leading to the generation of „knockout“ mice, although meanwhile more subtile genetic modifications, such as single amino acid substitutions or addition of tags, are introduced into the mouse genome via this method.

The lecture and the theroretical introduction of the course will give an overview of the differences between the two methods in terms of the manipulation of the early mouse embryo and vector design. The course will discuss the basic principles how to generate a targeting vector used for homologous recombination in ES cells and how to isolate and culture ES cells.

In the practical course the participants will follow and assist the standard procedure of ES cell injection into mouse blastocyts. This involves the isolation of early mouse embryos from the uterus of pregnant mice, culture and preparation of ES cells, injection of these ES cells into the blastocoel of isolated early embryos and their transfer into pseudopregnant foster mice.

Literature:

- Talts JF1, Brakebusch C, Fässler R. Integrin gene targeting. Methods Mol Biol. 1999;129:153-87.
- Nagy A, Gertsenstein M, Vintersten K, Behringer R. Manipulating the mouse embryo: a laboratory manual. Cold Spring Harbor Laboratory Press