

Mouse Mutant Core Facility

Genetically modified (GM) mice play a vital role in both fundamental and applied biomedical research. Examples of their use include the modelling of disease processes, the study of individual genes, and the testing of novel drugs and treatments on disease models.

A wide range of modifications can now be made to the mouse genome, including the introduction of simple expression cassettes, targeted deletions and insertions, conditional sequences, point mutations and other more complex modifications. These mutations are produced using a variety of techniques, most of which involve the manipulation of pre-implantation stage embryos or mouse embryonic stem (ES) cells.

The purpose of the Mouse Mutant Core Facility is to generate genetically modified mouse models for use in the study of development and disease. The facility has been in existence since January 2007 and is staffed by scientists with extensive experience in cell culture, embryo manipulation and molecular biology.

The facility has two separate laboratories. Our main lab is a 60-sq m space containing a small self-contained tissue culture lab and a fully equipped lab for carrying out molecular biology work. In addition, we have a dedicated microinjection laboratory within the Animal Research Center (SEA) that houses two independent microinjection stations, as well as other equipment required for embryo manipulation and mouse surgery.

We have generated several lines of GM mice during the course of 2009, both transgenic and gene-targeted. Recently, various improvements and modifications have been made to the established protocols for the generation of GM mice. We have been assessing some of these modifications in an attempt to improve efficiencies.

We continue to develop and adapt existing molecular biology technologies used in both the generation of transgenic and gene-targeting vectors, and in screening for the resultant genetic modifications. The main aim of this is to assist research groups in the production of recombinant DNA molecules required for the production of GM mice. Much work has been carried out in the last 12 months to improve and simplify cloning protocols, particularly recombineering protocols.

Services for IRB Barcelona researchers **Experimental design**

The facility provides consultation on all aspects of the design of gene-targeting and transgenic DNA vectors. These designs usually begin with an examination of the gene structure, derived from data generated by the relevant research group, or from databases (such as Ensemble or Vega) or a combination of both. We then propose an appropriate strategy and a suitable cloning protocol.

We can also give advice on screening strategies for transgenic and gene targeted mice and cells.

Vector construction

DNA vector design and construction is crucial for the success of the transgenic and gene-targeting process. We have a full-time molecular biologist who provides support for IRB Barcelona researchers. This member of staff designs targeting vectors and cloning plans, designs screening strategies, and develops and maintains a set of molecular and cellular tools and protocols. All of this work is designed to make the creation of recombinant DNA molecules and the screening for mutations both easier and faster.

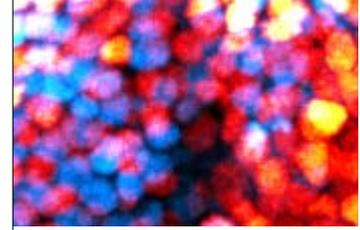
Generation of gene-targeted and transgenic mice

In recent years, publically funded initiatives aimed at creating ready-made ES cell mutants, such as the European Conditional Mouse Mutagenesis Programme (EUCOMM) and the International Gene Trap Consortium (IGTC), have generated mutations in thousands of genes. We use these resources wherever possible, and in the last year we have been involved in several gene-targeting projects using ready-made ES clones or ready-made gene-targeting vectors.

However, many types of genes and genetic modifications are not covered by the aforementioned consortiums, thus these types of projects require the design and building of gene/mutation-specific vectors in collaboration with the research groups.

Mouse embryonic stem cell culture

The facility has a dedicated tissue culture laboratory devoted to the culture and manipulation of mouse ES cells and mouse embryonic fibroblasts. We offer a complete gene-targeting serv-



ice, from the transfection of ES cells with gene-targeting vectors, drug selection of transfected cells, picking, and expansion of drug resistant clones, to the archiving of duplicate clones. After correctly targeted cell clones have been identified, potential positives are expanded and further analysed before being microinjected into pre-implantation stage mouse embryos.

Microinjection

The facility has two dedicated microinjection stations equipped with state-of-the-art micromanipulators. Two specialist technicians carry out microinjection and associated microsurgery techniques, in addition to overseeing breeding strategies for lines generated or maintained by the facility.

During 2009, we have assessed some recent developments in the protocols used for the generation of GM mice. These include the use of improved media for embryo culture, microinjection techniques into early stage embryos, and the transfer of embryos into foster mice using non-surgical methods.

Scientific output

Publications

Samuel MS, Munro J, Bryson S, Forrow S, Stevenson D and Olson MF. Tissue selective expression of conditionally-regulated ROCK by gene targeting to a defined locus. *Genesis*, 47(7), 440-46 (2009)

