SMP: Mammalian Models

Project: European Mouse Mutant Archive (EMMA)

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Introduction

The European Mouse Mutant Archive (EMMA) is the main center for the archiving and distribution of mutant mouse strains essential for biomedical research in Europe. The laboratory mouse is the most important mammalian model for studying genetic and multi-factorial diseases in man.
Based on its excellent genetics and long history in biomedical research numerous mouse mutants were identified. In the last two decades, technological improvements provided the ability to produce mouse mutants for virtually any gene that has been cloned by gene specific approaches (knock-outs, knock-ins, conditional mutagenesis), complemented by large scale, genome-wide ENU mutagenesis and gene trap approaches. Since it is impossible to retain all of these mutant mouse strains, it is essential that all mutants that have been created are held in a repository from which mutant mice can readily be made available to interested investigators. If this is not done, then many valuable mutant animals will be lost because of the constraints of space and finance that affect the individual laboratories and industry *(1, 2).* To meet these needs in Europe the European Mouse Mutant Archive was established and implemented. Six partners and the European Bioinformatics Institute (EBI) joined the EMMA consortium with the GSF Institute of Experimental Genetics being the German representative. In addition to its financial support from the EU the German partner is funded by the national NGFN research program. Main objectives of the German partner within this program are 1) to increase the central archiving unit at the GSF, 2) provide access to archived mutant mouse lines for the scientific community, 3) improvement of databases containing the genetic and phenotypic properties of the archived mice. Ensuring a rapid access to interesting mouse models of human diseases is a key factor for functional genomics research.

Project Status

The EMMA network is a partnership of several laboratories and other institutions throughout Europe *(3).* The current membership includes the CNR Istituto di Biologia Cellulare in Monterotondo, Italy, the CNRS Centre de Distribution de Typage et d'Archivage animale in Orleans, France, the MRC Mammalian Genetics Unit in Harwell, UK, the Karolinska Institutet in Stockholm, Sweden, the Instituto Gulbenkian de Ciência in Oeiras, Portugal, the GSF Institute of Experimental Genetics in Munich, Germany and the EMBL European Bioinformatics Institute in Hinxton, UK, being responsible for the development of the EMMA resource database. Every partner is a major player in mouse genetics in their countries. The German node of EMMA is the GSF Institute of Experimental Genetics being responsible for the administrative coordination of EMMA. The GSF possesses the largest animal facility in Germany and has a long standing expertise in mouse genetics, mutagenesis and cryopreservation. In addition, EMMA is a founding member of FIMRE (Federation of International Mouse Resources, www.fimre.org) a collaborating group of mouse repository and resource centers worldwide whose collective goal is to archive and provide strains of mice as cryopreserved embryos, gametes, embryonic stem (ES) cell lines and live breeding stock to the research community. The EMMA director, Prof. Hrabe de Angelis acts as vice chair of the FIMRE Board of Directors representing Europe.

The Management structure of the EMMA consortium was set up to ensure an effective operation of such a large and international enterprise and consists of several components. EMMA is headed by the director who leads the Board of Participating Directors (BPD), the decision making level within the consortium. The BPD discusses and decides on recommendations forwarded by the Advisory Board and the Board of Activity Group Leaders (AGL). The AGL board develops and agrees on EMMA Standard Operation Protocols (SOP) for quality control, archiving and distribution. The AGL Board is headed by the Project Coordinator who supports the EMMA director. To guarantee an effective communication within the consortium an internal webpage was set up. This resource database constitutes a fundamental tool for the management of scientific, technical and logistical activities carried out by each EMMA partner. Furthermore, the EMMA resource database provides up to date information about the archiving status of mice and describes the genetic and phenotypic properties of all the mutant strains that EMMA stocks. The available strain list is displayed on the public EMMA website (www.emmanet.org). This website is the link to the scientific community and facilitates the submission of mice to EMMA and requests of mice from EMMA. In addition, information on the SOPs used by EMMA are provided on the public website.

Procedures

These SOPs regarding freezing procedures, health status and animal handling were developed to control the quality of the EMMA processes. The first control step after a mouse is taken into an EMMA facility is the genotypic and phenotypic verification. This is followed by a test freezing of either sperm or embryos, depending on the genetic background and a revitalisation test. The ability to reconstitute a stock from frozen material will be ascertained at the time the strain is being frozen. Therefore, embryos derived from frozen sperm by *in vitro* fertilisation or thawed frozen embryos are transferred into pseudopregnant recipients, recovered as live-born and raised to maturity (4).

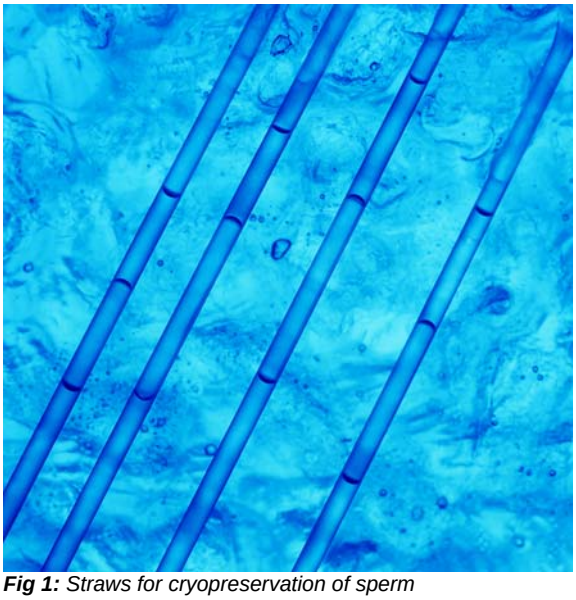

Fig 1: *Straws for cryopreservation of sperm*

Health monitoring is performed on all in- and outgoing mouse lines. All mice supplied are subjected to obtain a specific-pathogen-free (SPF) status according to FELASA guidelines. After the freezing and rederivation steps requested mice will be supplied to customers with SPF status and a health certificate according to FELASA rules. The most frequently requested strains are maintained as small colonies of breeding animals, which can be obtained in small numbers by customers.

Results

Since its inception the number of mutant mouse strains submitted to EMMA and the number of requests has steadily increased. To date more than 700 mutant strains have been submitted to EMMA. Among the mutants archived by EMMA are targeted mutant strains including numerous Cre-expressing lines and knock-outs, transgenic lines and ENU-induced mutants. Mutant phenotypes of archived strains cover neurological defects, hearing defects, strains with skeletal malformations and a strain serving as model for erythroleukemia among many others. Also the number of requests made to EMMA is steadily increasing. To date more than 250 requests were submitted to EMMA covering 107 different mutant strains. Several strains representing interesting disease models and valuable Cre-expressing lines are highly demanded. These strains are kept on shelf as live stocks facilitating a fast delivery of these strains to the customers. Interestingly most requests in 2005 were made from researchers in the US closely followed by requests from German investigators.

Fig 2: *Liquid nitrogen tanks for cryopreservation*

Networking within NGFN is essential to achieve the ambitious goals of this interdisciplinary consortium. EMMA is collaborating with numerous German partners from outside and within the NGFN network. Mice submitted to EMMA from NGFN collaborators include eg. an adhesion molecule on glia knockout (AMOG, beta 2 subunit of murine Na,K-ATPase) provided by Prof. Schachner (Hamburg). These mice exhibit motor incoordination at 15 d of age, subsequently tremor and paralysis of extremities and die at 17–18 d after birth. The mutant phenotype might be primarily related to reduced pump activity, with neural degeneration as a possible consequence of osmotic imbalance. In addition, Prof. Bernd Arnold (Heidelberg) submitted a transgenic line to EMMA that contains the Tie2CreERT2 transgene facilitating a temporal Cre-mediated recombination exclusively in endothelial cells. Furthermore numerous ENU-induced and gene-trap mutants were submitted to EMMA from collaborators within the NGFN network.

Value for research community

The mouse is the key model organism to study mammalian gene function mainly due to its excellent genetics and a plethora of available mutants modelling human diseases. These mutants offer the opportunity to decipher molecular disease mechanisms and provide in some instances a basis for the development of diagnostic, prognostic and therapeutic strategies. Therefore it is essential that these valuable resources are retained in a central repository from which they can be readily made available to interested investigators Thus EMMA plays a crucial role in exploiting the potential benefits to human health presented by the research in mammalian genetics.

Outlook

The number of mutant mouse lines submitted to EMMA as well as the number of requests for mutants from EMMA is likely to increase significantly in the near future. This is due to the growing name recognition of EMMA and an increase in the acceptance of EMMA services by researchers. In addition, large scale and systematic efforts worldwide to perform saturation mutagenesis of the mouse genome using gene targeting and gene trapping approaches will increase the number of mouse mutants. The EUCOMM network has been established to contribute to this ambitious goal. EMMA as a EUCOMM partner is expected to archive up to 320 mutant mouse lines generated within this research network. Projects such as EUCOMM, with the objective of generating mutations in ES cells, indicate that it is likely that ES cells will be archived and distributed by resource centers in the future besides cryopreservation of mutants as either frozen sperm or embryos. In addition to the archiving and distribution of mutant mouse lines EMMA offers a service to generate germ-free mice. By offering this service EMMA responds to the growing demand and interest of researchers for mice converted to a gnotobiotic condition. Within FIMRE the EMMA germ-free service was identified as a speciality of EMMA. As a founding member of FIMRE, a collaborating group of mouse repository and resource centers, EMMA will continue its efforts to contribute to the further development of this network. The long term goal and vision of the FIMRE network is to secure availability, to assure quality and to promote sharing of mutant mice and to disseminate information and resources to the global biomedical research community with EMMA being the European representative.

Lit.: ***1.*** *Abbott, A. Full house, Nature, Vol. 417, 20 June 2002, 785 -86.* ***2.*** *Marschall S., Hrabé de Angelis M. Cryopreservation of mouse spermatozoa - double your mouse space. Trends in Genetics, 15, 128 - 131(1999).* ***3.*** *Zeretzke, S. Das Europäische Maus Mutanten Archiv. Biospektrum 5 / 2003, 9. Jahrgang, 628 – 630.* ***4.*** *Marschall S, Hrabe de Angelis M. In vitro fertilization / cryopreservation. Methods Mol Biol.2003; 209:35 – 50.*