Preface

The pace with which information about mammalian genomes has accumulated over the last few years is remarkable. Yet, despite this wealth of information, its immediate use in the diagnosis and therapy of human diseases is limited since only a small fraction of mutations causing congenital malformations or other human diseases has been identified.

Animal models are essential to understand genetics and pathogenesis of human diseases. The mouse is intensively used as a model system due to its similarity to humans in genome organization, development, biochemical pathways, and physiology. Mouse models have been key to the unravelling of several fundamental scientific findings important for understanding the molecular mechanisms underlying human diseases as well as the development and testing of drugs and therapies.

Specific advantages of the mouse as a model system include:

1. The genome is over 90% identical to the human genome.
2. It is possible to alter the genome in the mouse using gene-driven and phenotype-driven approaches and to produce models of human diseases, including genetic diseases.
3. Alteration of the mouse genome may also produce changes in the normal development and functioning of organs, systems, and behaviour, giving insight into the mechanisms behind their normal function and possible treatments for malfunction.
4. The mouse model is used for drug screening and testing of therapies, including gene delivery and gene therapy.

The bottleneck in the process of establishing suitable mouse models is quite often appropriate phenotyping. From my own experience as a postdoctoral fellow phenotypic analysis of „my” mouse mutants was focused on very specific organ systems and their function. Although this strategy was successful and unravelled several important functional aspects of genes, at the same time I was not able to detect additional phenotypic alterations in the very same mouse model. These additional alterations were caused by the pleiotropic effect of the genes of interest, and I simply missed these alterations since I either did not look for them or because of the lack of equipment and experience with specific methods of other research areas. Many investigators have encountered these similar observations.

Triggered by this experience and poised with the expertise in phenotype-driven forward genetics screens, the idea of the German Mouse Clinic was born.
Mouse Clinic at the GSF – National Research Center for Environment and Health in Munich is a unique platform for comprehensive standardized phenotyping of mouse lines. In all, 14 labs from different research areas work under “one roof” and measure over 240 parameters within every mouse line. Many new findings emerged from this platform. For almost all lines, and even well known mutant mouse lines, new phenotypes were identified; thereby corroborating the power and feasibility of standardized comprehensive phenotyping. The German Mouse Clinic has rather to be regarded as an impetus rather than a final product since mouse phenotyping is an ascending field leading to new observations. Furthermore, phenotyping will be considerably developed and revamped over the next coming years. The enormous progress in the field of in vivo imaging, for example, will change the accessibility of monitoring organ systems and cellular dynamics leading to a better understanding of homeostasis and disease. In addition new test systems are currently under development to gain insight into „gene – environmental“ interactions, another exciting field with great challenges for our center of environment and health research. A further challenge to foster the field of functional genomics is the proper archiving and dissemination of valuable biological material and datasets. The European Mouse Mutant Archive (EMMA) was created to serve as the main center for archiving and distribution of mouse models in Europe. EMMA is today a partnership of several laboratories and other institutions throughout Europe. The repository plays a crucial role in exploiting the tremendous conceivable benefits to human health presented by the current research in mammalian genetics. Over the last years we were able to develop EMMA into an international player, reflected by its important role in the Federation of International Mouse Resources (FIMRe). I hope this flyer is helpful in providing a brief overview to the interested reader and shows how interdisciplinary research in the field of life sciences will enable the next level of functional genomics.

Martin Hrabé de Angelis
Director GMC/EMMA

Neuherberg, July 2006
The German Mouse Clinic

Of Men and Mice

Why do we study mice? Diabetes, Alzheimer’s, hypertension and cancer – for many of these wide-spread diseases defective genes are at least partly accountable. If the respective genes would be identified and thereby gene function decoded, causes of diseases are easier to determine and new therapies can be developed. Mice are ideal model organisms for the investigation of gene functions. Not only do they reproduce fast, there is much knowledge about their genes available, whereby the mouse genome with its approx. 25,000 genes has been fully decoded. Apart from that, 95 per cent of the genome between humans and mice is identical, whereby many genes correspond and can cause the same diseases in mice and humans. There are mice which tremble like a Parkinson’s patient or have an elevated blood glucose level of diabetics. Therefore, fundamental research on mice allows important insights into the functions of the genes and their role in the development and course of human diseases.

The mouse models of the Mouse Clinic assist us: they carry defective genes causing the same diseases as in humans. For example, a mutant called “Beethoven” has been investigated, which – like the composer whose name it was given – loses its hearing with increasing age. This makes these and other mice valuable models for the corresponding human diseases.

A Diagnostic Clinic for Mice

How does the Mouse Clinic work? The Mouse Clinic is a diagnostic clinic in which specialists in mouse genetics and clinicians work together. A standardised and largely non-invasive health check is carried out on the mouse mutants. Such a comprehensive characterisation is also called “phenotyping” by experts. On one single mouse more than 240 parameters can be determined in the first round of examination – the primary screen – resulting in a detailed picture. This provides the participating scientists with a considerable amount of information based on a minimum number of mice. This means that such a com-
prehensive analysis involves little time and cost as opposed to a comparative analysis in different laboratories. In order to conduct the large number of tests and to deal with the enormous logistics involved, numerous specialists work hand in hand at the Mouse Clinic. This is not just a figure of speech, but as a matter of fact, the mice are passed from one test module onto the next after a screen. This is why all laboratory modules and animal housing are under one roof. The spatial proximity does not only allow standardised measuring conditions and maintenance of animals, but also a high degree of interdisciplinary scientific exchange.

**GERMAN MOUSE CLINIC**

Structure of the Mouse Clinic consisting of 13 modules
The Modules

How can mice be thoroughly examined? In brief: comprehensive and standardized. In the screens medical diagnostic equipments are used much like the ones that are also found in „normal“ clinics: X-ray equipment, blood analyzers, ECG (electrocardiogram), and ultrasonic equipment, to mention just a few. They have partly been adapted for the size of mice. From behavioral tests to measuring metabolism and the analysis of gene expression, a total of 14 individual screens do not dismiss any important area. When interesting results are found, further analyses within
the secondary and tertiary screens provide the possibility of conducting more detailed tests. We are now going to show you the way mice are examined through our modules.

**Dysmorphology, Bones and Cartilages**
Prof. Dr. Martin Hrabé de Angelis, Dr. Helmut Fuchs
*GSF-Institute of Experimental Genetics*

Upon admission into this screen, the mice are weighted and examined for any malformations. Vital data, such as their date of birth, are recorded in our database. Later the mice return to this module in which the bones and cartilages are examined in detail. Together with bone density measurements, this helps diagnose diseases of the skeletal system, such as osteoporosis. In addition, a micro-computer tomography, developed especially for mice, gives us two- and three-dimensional image of the interior of the body.

**Behavior**
Prof. Dr. Wolfgang Wurst
*GSF-Institute of Developmental Genetics*

In the first two screens the mice are observed. Depending on how the mice behave on the hole board in the presence of their cage mates and respond to unknown or known objects, conclusions can be drawn as to their general activity, curiosity, emotionality, social behaviour and object memory. These behavioral tests help investigate the molecular and genetic foundations of psychiatric diseases, such as depression, posttraumatic stress syndrome or Alzheimer’s disease.

**Neurology**
Pd Dr. Thomas Klopstock
*Neurological Clinic of the LMU (Ludwig-Maximilians-University) Munich*

Our mouse neurologists test reflexes and record demeanour, activity, muscular tension, salivation and lacrimation. Using these simple tests, neurodegen-
erative and neuromuscular diseases of the nervous system, such as muscular dystrophies, multiple sclerosis or Parkinson’s can be diagnosed. In further tests EMG (electromyography) and EEG (electroencephalography) can be used to examine muscular and brain activity.

**Eye**

Prof. Dr. Joachim Graw and Dr. Jack Favor

*GSF-Institutes of Developmental and Human Genetics*

Eye screening tests the eyesight to detect any hereditary eye diseases, such as cataract and degeneration of the retina. A slit lamp helps detect turbidity and malformations of the cornea and the lens while the back part of the eyeball (fundus) is investigated by funduscopy. The eye size is measured by laser interference biometry (LIB). Additional tests include ERG (electroretinogram) which provides information on the function of the retina.

**Clinical Chemistry**

Prof. Dr. Eckhard Wolf

*Institute for Molecular Animal Breeding and Biotechnology of the LMU (Ludwig-Maximilians-University) Munich*

In this screen a blood sample is taken from the mice, which is portioned and distributed to the subsequent screens. Changed blood and urine values give us information on diseases of organ systems and metabolic disorders, such as hypercholesterol-
aemia or diabetes. The blood count tells us whether the mice suffer from chronic anaemia or leukaemia, and is carried out by an automatic blood analyzer which is similarly used in paediatric hospitals.

Immunology
Prof. Dr. Dirk H. Busch
Institute for Medical Microbiology, Immunology and Hygiene of the TU (Technical University) of Munich

The second part of the blood sample is in immunological test methods to examine the reactivity of the immune system. This allows the identification of mice susceptible to infectious, tumour and autoimmune diseases (e.g. Crohn’s disease, multiple sclerosis or rheumatoid arthritis).

Allergy
Prof. Dr. Markus Ollert
Department of Dermatology and Allergology of the TU (Technical University) of Munich

In the allergy screen mice which have a predisposition to allergic reactions are sought, and may serve as models for the respective diseases of the respiratory system (asthma) and the skin (atopic eczema).
Steroids
Prof. Dr. Jerzy Adamski
GSF-Institute of Experimental Genetics

In animal organisms the steroid hormones are important throughout life, and processes such as sexual development, bone metabolism or inflammations are decisively influenced by steroids. Altered steroid concentrations in the blood plasma provide information on mutations which may cause serious diseases, such as osteoporosis or Addison’s disease.

Nociception
Prof. Dr. Andreas Zimmer
Department of Molecular Neurobiology at the University of Bonn

The causes of acute and, in particular, chronic pain related to molecular and cellular biology have not been fully investigated. In addition pain perception depends on many psychological factors. In an injury-free hot plate test we look for mice with changed stimulus thresholds, i.e. mice showing a more sensitive or a more indifferent reaction to a stimulus.

Using high-throughput methods six different immunoglobulins can be identified in blood samples.
Cardiovascular
Dr. Boris Ivandic
University Hospital of Heidelberg

As in normal cardiological practice the blood pressure is measured and Doppler echocardiography and electrocardiography (ECG) are used in examinations to see whether the mice have a predisposition to cardiovascular disease. The resolution of the equipment has been increased so that even the heartbeat of embryos in the uterus can be recorded.

Gene Expression
Dr. Johannes Beckers
GSF-Institute of Experimental Genetics

In an organism the activities of the genes influence each other according to a highly complex set of rules. Although only one gene has been modified in the mouse lines, the delicate balance may be disturbed, so that one single mutation influences many other processes. The DNA

Lung Function
Prof. Dr. Holger Schulz
GSF-Institute of Inhalation Biology

Pulmonary diseases, such as chronic obstructive bronchitis or asthma, may well be considered wide-spread with three to five million patients just in Germany. Parameters like the respiratory rate, duration of inspiration and expiration or respiratory volume, provide information on disorders of the pulmonary function and their causes. Using a unique measuring unit miniaturised for mice, the same pulmonary function tests as for humans can be carried out.

Left picture:
Flow rate of the blood (A) in the pulmonary artery (B) of a mouse and the corresponding ECG (E).

Right picture:
At a higher resolution the contractions of the left ventricle (C) can be imaged. The left ventricle contracts and extends at a regular rhythm (D).
In the special lung function module the same tests as in humans can be carried out. For example, we measure the respiratory rate, the duration of inspiration and expiration and the respiratory volume.
chip technology allows the analysis of more than 20,000 genes in one single experiment, providing exact information on other processes which are affected.

*Energy Metabolism*
Prof. Dr. Gerhard Heldmaier,PD Dr. Martin Klingenspor
*Department of Biology at the University of Marburg*

Why is the natural balance between food intake and energy consumption disturbed in some patients, and how does obesity (adiposity) develop? Mouse models which become adipose during their natural development or whose obesity can be caused by high-calorie food, can help answer these questions. Using complex technical measuring methods mice are identified and their energy balance is characterised by determining their energy uptake and metabolized energy.

*Pathology*
Prof. Dr. Heinz Höfler,PD Dr. Leticia Quintanilla-Fend
*GSF-Institute of Pathology*

In the pathological screen the mice are fully characterised in terms of their morphology. The exact measurement and description of their organs as well as extensive microscopic tests allow us to define the disease so that it can be assigned to a human disease.

Left picture: Using so-called DNA chips the expression of individual genes can be examined in all tissues. For this purpose the comparative samples are labelled with fluorescent stains and hybridized to a probe with complementary DNA.

Right picture: Mice also follow a day-and-night rhythm, as is indicated by the activity and body temperature profile on the screen.
At the pathology module the histological sections are analysed by both human and veterinary medicine specialists and assigned to clinical pictures.
A detailed list of the available tests and parameters is found at http://www.mouseclinic.de.

**Who Can Use the Mouse Clinic?**

Basically any scientist who is interested can have his mice examined. The requirements to hygiene and quality of the lines are high, whereby a total of 60 animals of the same age per line must be provided. There is much interest, and enquiries are received from all over Europe, the US and Japan. When there is a shortage in capacity, a scientific committee from the National Genome Research Network (NGFN) decides which mice to include.

**Participating Partners**

Most of the funding is provided by the National Genome Research Network (NGFN).

The Mouse Clinic Consortium:

- GSF – National Research Center for Environment and Health, Neuherberg
- Helmholtz Centre for Infection Research (Research Centre for Biotechnology, GBF), Brunswick
- Ludwig-Maximilians-University Munich (LMU)
- Philipps University Marburg
- Technical University of Munich (TU)
- University of Bonn
- University Hospital of Heidelberg
EMMA
The European Mouse Mutant Archive

The Objectives of the European Mouse Mutant Archive

There are many thousand scientifically relevant mouse lines available worldwide whose maintenance is very costly. Breeding involves a lot of work and there is always the risk that a valuable line is lost due to an infectious disease. The method of cryopreservation, i.e. freezing in liquid nitrogen, allows the storage of mutant mouse lines in the form of frozen sperm and embryos. The mouse lines can be revitalised and further investigated at any point in time. Cryopreservation requires much technical know-how and is, therefore, not established at all.

<table>
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<tr>
<th>Partners</th>
<th>Tasks</th>
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<tbody>
<tr>
<td>Consiglio Nazionale delle Ricerche, Istituto di Biologia Cellulare (CNR-IBC) in Monterotondo</td>
<td>EMMA central facility, organisation of cryocourses, embryo cryopreservation</td>
</tr>
<tr>
<td>Institute of Experimental Genetics (IEG) GSF – National Research Center for Environment and Health in Neuherberg</td>
<td>EMMA Director, overall project coordination, sperm and embryo cryopreservation</td>
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<tr>
<td>European Bioinformatics Institute (EMBL-EBI) in Hinxton</td>
<td>Establishment and maintenance of the EMMA database</td>
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<tr>
<td>CNRS Centre de Distribution, de Typage et d’Archivage animal (CNRS-CDTA) in Orléans</td>
<td>Embryo cryopreservation</td>
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<tr>
<td>MRC Mammalian Genetics Unit (MRC-MGU) in Harwell</td>
<td>Sperm and embryo cryopreservation</td>
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<tr>
<td>Karolinska Institute (KI) in Stockholm</td>
<td>Embryo cryopreservation and production of germ-free mice</td>
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<tr>
<td>Fondação Calouste Gulbenkian, Instituto Gulbenkian de Ciencia (FCG-IGC) in Oeiras</td>
<td>Production of germ-free mice</td>
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research institutions. In order to allow scientists to preserve their lines and make it easily available to interested researchers the European Mouse Mutant Archive (EMMA) was established. EMMA is a project initiated by scientists for scientists and its purpose is to support the world’s scientific community. Mutant mouse lines are cryopreserved and maintained under the highest standards and the archive makes mutant mouse lines available to interested scientists worldwide. EMMA thereby contributes to a reduction of the number of experimental animals. In addition to these core services EMMA offers additional services such as the production of germ-free (axenic) mice. Such strains are important tools to study certain human disease conditions. The dissemination of knowledge is also an important part of the project. Know-how in cryopreservation techniques is passed on in two annual cryocourses.

The EMMA Consortium

A project of this dimension cannot be carried out by one institution on its own. Therefore, the...
The sperm is collected from the epididymis before freezing.

Tasks are distributed over seven institutes in six different European countries. The institutes involved in the project are among the most renowned in their countries and have extensive experience in mouse genetics, mutagenesis and in the cryopreservation of mutant mouse lines. The EMMA network is a founding member of FIMRe, the Federation of International Mouse Resources (www.fimre.org). FIMRe is 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, ES cell lines and live breeding stock to the research community.

What is the Role of the GSF?

The GSF-Institute of Experimental Genetics (IEG), headed by Prof. Martín Hrabé de Angelis, has several tasks in this project: Prof. Hrabé de Angelis is the EMMA
director and chairman of the Board of Directors of the EMMA consortium. Furthermore, he is in charge of the overall coordination of the project and supported in this role by two project managers who are running the EMMA project office at the IEG. Within FIMRe, Prof. Hrabé de Angelis acts as vice chair of the FIMRe Board of Directors and represents Europe in this board.

At the IEG the focus of cryopreservation techniques is on sperm preservation. Methods have been established which can be applied to successfully revitalise mutant mouse lines not only from frozen embryos, historically the older method, but also from frozen sperm. At the IEG both methods are applied.

Animal housing at the GSF facilitates work according to highest standards. The sperm of the mutant mouse lines is frozen in cryo-straws.
quality standards. To ensure these, various control processes have been implemented and standard operating procedures (SOPs) have been developed and established throughout EMMA. They control all processes from the freezing process, health control and handling of mice to the transport of live animals as well as frozen material.

From Mice to Ice and Back

When animals arrive at an EMMA facility, they undergo a detailed morphological characterisation. Apart from that, a genetic fingerprint is taken. This so-called phenotyping and genotyping respectively guarantees the specificity of the frozen mouse line. This is followed by a test run to determine the suitability of the mouse line for cryopreservation. For this purpose sperm is frozen in liquid nitrogen and then thawed again, oocytes are fertilised with this sperm in vitro and the resulting embryos are transferred into foster mice. If mutant offspring is born following this procedure, the revitalisation has been successful and the mouse line can be fully archived. During this entire procedure stringent health control is performed based on the FELASA recommendations (Federation of European Laboratory Animal Science Associations). All mice provided by EMMA are classified as SPF (specific pathogen free) according to the FELASA guidelines.

The EMMA Archive

Since its inception the number of mutant mouse strains submitted to EMMA and the number of requests has steadily increased. To date nearly 1000 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 and strains with skeletal malformations among many others. Also the number of requests made to EMMA is steadily increasing. To date more than 400 requests were submitted to EMMA. Several strains representing interesting disease models and valuable Cre-expressing lines are highly demanded and are kept on shelf as live stocks facilitating a fast delivery to the customers.
Who Can Use EMMA?

EMMA can be used free of charge by all scientists who agree that their mouse line be made available to other scientists for research purposes. The ownership of the mutant mouse line stays with the producer. Applications for archiving and requests for mutant mouse strains are submitted through the EMMA website (http://www.emmanet.org). A public description of current EMMA activities and procedures is also available from the EMMA website. After evaluation and approval by EMMA’s scientific committee, depositors are asked to send mice of breeding age to one of the EMMA partners for embryo or sperm cryopreservation. Requested strains may be sent as frozen samples or re-derived upon request. Highly demanded lines are kept on shelf to facilitate a fast delivery to customers and breeding pairs can generally be provided with little or no delay.

Why Should You Use EMMA?

- Archiving of mutant mouse lines free of charge
- No loss of intellectual property rights
- EMMA SOPs ensure consistently high standards
- EMMA distributes mutant mouse lines worldwide
- Mutant mice obtain SPF-status (FELASA)
- Contribution to the scientific community

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Research for the Health of Man and the Environment

The GSF contributes to the foundation of Future Medicine and Health Care as well as Ecosystems, which are of critical importance for health. Our focus is on chronic degenerative diseases like lung diseases, allergies, cancer, and cardiovascular diseases that are influenced to a large extent by environmental conditions, personal risk factors, and life style. Therefore, in order to generate a knowledge base, we analyse interactions between genetic disposition, biological systems, and environmental factors. This will lead to the development of new personalised approaches in prevention, diagnostics, and causal therapy – the future direction of medicine. Our strategy is based on translational approaches that bridge basic research with clinical application to provide an immediate benefit to the patient.

The research projects of the GSF are focussed on four complementary areas:

• Environmental Factors and Health,
• Mechanisms of Health and Disease,
• Infection and Immunity,
• and Ecosystems and Health.

Networking across disciplines facilitates exchange of knowledge and value creation. In addition, GSF scientists ensure that the most recent research results can flow into guidelines and new legislation by participating in national and international advisory commissions. As the national centre of competence, the GSF carries out important tasks within the field of Radiation Research and Radiation Protection. The GSF is highly committed to scientific and technical excellence in its institutes and departments, as well as the promotion of young scientists.

The GSF is a research institution of the Federal Government and the State of Bavaria within the Helmholtz Association of German Research Centres with approximately 1700 associates in 24 institutes and departments.

The main research site is located in the north of Munich. Several groups are closely linked to the universities and hospitals.

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