PROJECT FINAL REPORT

Grant Agreement number: 312325

Project acronym: INFRAFRONTIER-I3

Project title: Development of mouse mutant resources for functional analyses of human diseases – Enhancing the translation of research into innovation

Funding Scheme: Combination of Collaborative Project and Coordination and Support Actions

Period covered: from January 2013 to December 2016

Name, title and organisation of the scientific representative of the project's coordinator:

Prof. Martin Hrabé de Angelis

Helmholtz Zentrum München

Deutsches Forschungszentrum für Gesundheit und Umwelt (GmbH)

Ingolstädter Landstraße 1

D-85764 Neuherberg

Tel. +49 89 3187-3502

Fax. +49 89 3187-3500

E-Mail: hrabe@helmholtz-muenchen.de

Project website address: www.infrafrontier.eu

Table of contents

1. Executive summary

The mouse shows great similarities in development, physiology and biochemistry to humans, which makes it a key model for research into human disease. The major challenges for mouse functional genomics in the 21st century are to:

· Develop a series of mutant alleles for every gene in the mouse genome
· Determine the phenotypic consequences of each mutation
· Identify mouse models for the complete disease spectrum in humans

To further develop and exploit the emerging mouse mutant resources, mouse models must be preserved and made available to the European biomedical research community. To this effect, the IINFRAFRONTIER-I3 project brought together the leading European Mouse Clinics, centers of excellence for systemic phenotyping of mouse mutants, and the European Mouse Mutant Archive (EMMA) network. The INFRAFRONTIER-I3 partners met the future challenges presented by phenotyping, archiving and disseminating mouse models in the ERA as follows:

· Resource development of the EMMA mouse repository by archiving of 1215 new mouse mutant lines supporting the development towards the 3rd largest mouse repository holding 6000 mouse mutants
· Provision of Trans-national Access to mouse production and 1st line phenotyping services delivering 54 new mouse models of human diseases for public access
· Free of charge access to a unique and specialized axenic service to derive, maintain and distribute germ-free mice underpinning the rapidly evolving field of microbiome research
· Provision of user friendly access to all INFRAFRONTIER resources and services via a new portal, extensive manual data curation of 2763 mouse mutant lines underpinning data quality, and cross referencing and integration with other mouse resources such as IMPC
· Improving user services by extensive technology development efforts leading to 3 novel phenotyping SOPs, six new cryopreservation protocols, six methods papers, cost reduction of services and a significant reduction in animal use
· Driving innovation by developing an entirely new Metabolic PhenoCage to measure a multitude of metabolically relevant parameters and with maximal flexibility to adjust to future scientific demands
· Engaging with the user community using a wide range of PR and outreach activities such as dedicated industry liaison and gnotobiology workshops
· Offering 16 state of the art cryopreservation and phenotyping training courses for 200 participants
· Benchmarking of INFRAFRONTIER services with other major mouse repositories and internally supporting a continuous improvement cycle

The comprehensive and unique physical and data resources developed by the INFRAFRONTIER-I3 project will support biomedical research in Europe by offering the opportunity to decipher molecular disease mechanisms and aiding the development of therapeutic strategies. Thus, the INFRAFRONTIER network will play a critical role in ensuring Europe maintains its leading role in the development of resources and knowledge of medically relevant mouse models by providing a user driven, sustainable platform offering access to unparalleled services and physical as well as data resources. Overall, the INFRAFRONTIER-I3 project contributed to the further development of the European Research Area (ERA), and supported and interacted with global initiatives such as the International Mouse Phenotyping Consortium (IMPC).

2. Project context and objectives

Research infrastructures and grand societal challenges

Research infrastructures (RI) are key pillars of the European Research Area (ERA) and provide a foundation for innovative research contributing to solutions for the grand societal challenges such as ageing societies and public health. To this effect Biological and Medical Sciences (BMS) RIs link basic research to medical application, drive innovation and thereby contribute to the development of a knowledge based economy and the objectives of the Europe 2020 strategy. The emerging BMS RI INFRAFRONTIER for phenotyping and archiving of model mammalian genomes brings together the leading European Mouse Clinics for systemic phenotyping of mouse mutants and mouse repositories of the European Mouse Mutant Archive (EMMA) network. Joint objective is the foundation of a sustainably funded pan-European RI ensuring access to mouse models for basic research of human health and disease and the translation of this knowledge into therapeutic approaches, personalized medicine and disease prevention strategies for the benefit of the European society.

The relevance of mouse as a model of human disease

The advent of next generation sequencing technology and genome-wide association studies has propelled medical genetics at an unprecedented pace. But despite these advances the function of at least half of the mammalian genome is poorly understood. However, understanding gene function and interaction, the impact of environmental factors and the basic biology of pathways and systems is a necessity to effectively treat symptoms and deleterious effects of human diseases. Thus, a systematic hypothesis generating program for the functional study of all mammalian genes is needed to usher in a new era of genomic medicine. The mouse model is one of the most important tools to understand mammalian gene function and ideally suited to undertake a comprehensive program for the phenotypic study of the function of all genes. The mouse model displays fundamental similarity to humans at the genetic level and similar physiology and anatomy. Furthermore, the mouse model provides 1) a wealth of information based on 100 years of extensive genetic study, 2) a comprehensive toolkit for the manipulation of the mouse genome and the generation of new disease models, and 3) mouse embryonic stem cells and the technology to manipulate them in vitro to modify genes at will. This allows to mimic human disease conditions and to transmit mutations into the germ line which revolutionized basic medical research. At the same time large scale chemical mutagenesis programs contributed hundreds of novel mouse models. Emerging resources such as the Collaborative Cross allow the genetic dissection of complex traits.

Large scale global mouse functional genomics initiatives

To ensure a systematic and cost effective approach, the European mouse community called for large scale mutagenesis and phenotyping approaches as well as archiving and distribution to ensure wide and open access of the resulting mouse resources (Auwerx et al., Nat Genet. 2004 Sep;36(9):925-7). As a result the European Conditional Mouse Mutagenesis Program (EUCOMM) joined forces with related global initiatives resulting in the International Knockout Mouse Consortium (IKMC, http://www.knockoutmouse.org). By 2012 the efforts of the IKMC members produced more than 14000 targeted knockouts in C57BL/6NTac ES cells, mostly conditional ready alleles accounting for more than half of the mammalian genome. Major advances of the European phenotyping programs EUMORPHIA and EUMODIC (www.eumodic.org) are the definition and harmonization of phenotyping protocols such as EMPReSSslim and the development of a dedicated database,

Europhenome (www.europhenome.org), capturing the phenotypic data produced by the EUMODIC large scale phenotyping effort. The consortium's findings and seminal work was recently published in Nature Genetics (Nat Genet. 2015 Sep;47(9):969-78). EUMODIC was the first step to creating a database of all mouse gene functional data, a vision now being realised by the International Mouse Phenotyping Consortium IMPC (www.mousephenotype.org). The INFRAFRONTIER mouse clinics contribute to this global endeavour with mouse model development, phenotyping, cryopreservation and distribution of mouse models via EMMA. A growing collection of at present 2500 IMPC mouse lines is accessible via the EMMA repository. The availability of the associated phenotype data provides fundamental new insights into the role of genes in basic physiological, developmental and cellular processes, and a better understanding of the underlying genetic pathways involved with disease, drives mammalian gene functional annotation, and fosters hypothesis-driven research. The IMPC has been included in the G7 Science Ministers report as a case study of how to achieve a globally distributed Research Infrastructure (https://www.bmbf.de/files/151109_G7_Broschere.pdf).

The INFRAFRONTIER-I3 project

The repositories of the European Mouse Mutant Archive (EMMA) network and the European Mouse Clinics have joined forces for the INFRAFRONTIER-I3 project. This combined effort is a response to the challenges and opportunities presented by the emerging mouse mutant resources for basic research into human health and disease and translation into therapeutics. The project builds on the ESFRI Infrastructure for Phenotyping and Archiving of Model Mammalian Genomes ("INFRAFRONTIER") and thus directly addressed and responded to the topic INFRA-2012-1.1.4. - Mouse archives and centers for phenotyping mouse models - of call 10 - FP7-INFRASTRUCTURES-2012-1.

The INFRAFRONTIER project and the emerging INFRAFRONTIER RI

The INFRAFRONTIER project was launched in 2008 as a preparatory phase project and brought together the leading European scientific institutions in the fields of high throughput phenotyping and archiving and distribution of mouse mutant resources as well as national ministries and funding bodies. Its main purpose was to initiate all necessary steps for the establishment of a sustainably funded pan-European research infrastructure for systemic phenotyping, archiving and distribution of mouse models of human disease.

To this effect a business plan was developed that laid down the organisational and financial requirements for the implementation of the INFRAFRONTIER legal entity, which will act as the pan-European coordination body for the INFRAFRONTIER research infrastructure. Open access to high-quality mouse models will be secured by the pan-European INFRAFRONTIER research infrastructure. It consists of the National INFRAFRONTIER Partners, which will provide capacities for transnational access, and a central coordination body. For this central unit a new INFRAFRONTIER legal entity will be created. The implementation of the INFRAFRONTIER research infrastructure started in 2011 with consultations on the business plan and the draft Articles of Association of the INFRAFRONTIER legal entity (figure 1).

Fig. 1: The INFRAFRONTIER preparatory phase project was funded by the EC to prepare the implementation of the INFRAFRONTIER research infrastructure consisting of the National INFRAFRONTIER Partners, and a central coordination unit, the INFRAFRONTIER Legal Entity.

A major milestone was then the foundation of the INFRAFRONTIER GmbH in 2013 by five partners of the INFRAFRONTIER network. These organisations are mandated by their national research ministries in Germany, France, Czech Republic, Greece, and Finland to participate in the activities of the INFRAFRONTIER GmbH. The INFRAFRONTIER legal entity joined the INFRAFRONTIER-I3 project as full partner in the final reporting period in 2016. A main goal of the founding partners and their ministries is to evolve the legal entity towards a European research infrastructure Consortium (ERIC), a dedicated Community legal instrument designed to facilitate the joint establishment and operation of research infrastructures of European interest. A formal application to obtain ERIC status was submitted to the EC in summer 2016.

The implementation of the INFRAFRONTIER research infrastructure supported by the foundation of a dedicated legal entity coordinating activities (INFRAFRONTIER GmbH, 2013), and the INFRAFRONTIER-I3 project supporting resource and service development (2013-2016) will advance biomedical research in Europe and also beyond. The major challenges for mouse functional genomics in the 21st century are to **1)** Develop a series of mutant alleles for every gene in the mouse genome, **2)** Determine the phenotypic consequences of each mutation, and **3)** Identify mouse models for the complete disease spectrum in humans. To further develop and exploit the emerging mouse mutant resource, mouse models must be preserved and made available to the European biomedical research community.

The INFRAFRONTIER-I3 partners aimed to meet the future challenges presented by phenotyping, archiving and disseminating mouse models in the ERA as follows:

• Contribute to resource development by archiving of 1215 new mouse mutant lines
• Provide Transnational Access (TA) to mouse production and 1st line phenotyping capacities
• Offer a specialized axenic service to produce, maintain and to distribute germ-free mice
• Provide user friendly accession to INFRAFRONTIER services, extensive manual data curation and cross referencing with other mouse databases
• Improve user services by developing novel phenotyping and cryopreservation SOPs and by refining innovative research instrumentation
• Engage with the user community using a wide range of PR activities, a dedicated user meeting and an industry liaison workshop
• Offer state of the art cryopreservation and phenotyping training courses
• Benchmark INFRAFRONTIER services with other major repositories

Expected project impact

The INFRAFRONTIER-I3 project was expected to contribute a large number of new mouse models to the EMMA repository. The projected growth and development of the EMMA repository in the coming years will be essential to support the transition to a sustainable research infrastructure. INFRAFRONTIER-I3 and complementary efforts led to the EMMA repository holding more than 6000 mouse mutant lines by the end of 2016. For many of these lines a comprehensive first line phenotypic screen was undertaken and the data are freely available. This comprehensive and unique physical and data resource will support biomedical research in Europe by offering the opportunity to decipher molecular disease mechanisms and aiding the development of therapeutic strategies. Thus, the INFRAFRONTIER network will play a critical role in ensuring Europe maintains its leading role in the development of resources and knowledge of medically relevant mouse models by providing a user driven, sustainable platform offering access to unparalleled services and physical as well as data resources.

It was further expected that INFRAFRONTIER-I3 activities will lead to significant advances in the operation and user services of the research infrastructure. Knowledge generated by the project will be widely disseminated by the website, presentations, scientific publications and SOPs and will contribute to standard development. Overall, the INFRAFRONTIER-I3 project will contribute to the further development of the European Research Area (ERA), and will support and interact with global initiatives such as the International Mouse Phenotyping Consortium (IMPC).

3. Description of main S&T results

3.1 WP2 - Communication and user interactions

The specific objectives of this work package were:

1) Foster a culture of co-operation between the partners of the INFRAFRONTIER-I3 project and in particular provide a means to effectively integrate new partners into the joint activities of INFRAFRONTIER

2) INFRAFRONTIER will undertake a number of external communication efforts to promote the activities and resources and services of the INFRAFRONTIER research Infrastructure (RI) among the general public and in particular the user community. The outreach efforts to attract potential new users who may benefit from the research infrastructure will comprise a variety of PR measures. External communication efforts will also involve coordination with other related European and global initiatives

3) Provide common procedures for Transnational Access (TA) management

4) Underpin the user focus of the INFRAFRONTIER RI

5) Support and reinforce the innovation activities of INFRAFRONTIER

6) Provide state of the art phenotyping and cryopreservation training courses

Progress towards objectives of work package tasks

Public relations and outreach activities to attract new users

Major aim of this task was to attract new users to the INFRAFRONTIER research Infrastructure using a number of outreach efforts. The main tool to disseminate INFRAFRONTIER resources and to promote services is the web portal at **www.INFRAFRONTIER.eu**. A redesigned web portal was launched in autumn 2013 and is now the major interface to the INFRAFRONTIER user community. The web portal provides background information on the organisation of INFRAFRONTIER, access to all resources and services, access to MTAs, genotyping protocols, cryopreservation SOPs, training course information, and literature references. The web site traffic is tightly monitored and at present an average of 6000 unique visitors per month are recorded. The INFRAFRONTIER portal relaunch has been described in detail in a dedicated publication in the Nucleic Acids Research database issue 2015 (Nucleic Acids Res. 2015 Jan;43 (Database issue):D1171-5.

INFRAFRONTIER services were also promoted regularly via Email lists such as the MGI-list, the ISTT transgenic list and an EMMA customer Email list. In particular the Trans-national Access calls and training courses were not only advertised via the INFRAFRONTIER web portal but foremost extensively via Email lists either targeting the EMMA customer base, the wider mouse community or national user groups. The **INFRAFRONTIER/EMMA customer base and Email list comprises a total of 7000 recipients** and is now more and more used to promote training courses of INFRAFRONTIER partners, open calls and newsletters. More recently and as a result of the **CORBEL project** (http://www.corbel-project.eu/home.html) we joined forces with other BMS research Infrastructures to disseminate information on project activities via Email and social media such as twitter.

INFRAFRONTIER-I3 scientists also very actively engaged and presented the consortium activities on an estimated 100 occasions at major **mammalian and human genetics conferences,** consortia meetings of the global IMPC phenotyping initiative and at related EU consortia such as IPAD-MD and CORBEL, and contributed presentations at training courses and workshops.

Furthermore, project outputs were disseminated by **scientific publications** in peer reviewed journals and as contributions to methods papers. A complete list of publications resulting from INFRAFRONTIER-I3 project activities are appended to this project report.

Industry liaison workshop

In an attempt to jointly present resources and services INFRAFRONTIER can offer to industry and to strengthen and develop new relations with partners from industry, a liaison workshop was planned as a major deliverable of the INFRAFRONTIER-I3 project. This is in line and supports the EU innovation policy. The effort to liaise with industry was jointly undertaken with the International Mouse Phenotyping Consortium (IMPC) as there is a common interest.

The INFRAFRONTIER-I3 industry liaison workshop was held in Barcelona on November 13th 2014 at the Majestic Hotel following the annual IMPC meeting. The workshop was attended by 70 participants from among INFRAFRONTIER and IMPC PIs, IMPC industry sponsors, BioPharma, research instrumentation developers, and CROs. A total of 25 companies were represented at the meeting. Specific objectives of the industry liaison meeting were to:

· Present INFRAFRONTIER / IMPC resources and services
· Present successful INFRAFRONTIER industry engagement models
· Promote industry access to EUCOMM resources
· Have presentations on the use of mouse models in industry
· Have a partnering session and exhibition

The industry liaison workshop is presented in detail on the INFRAFRONTIER portal where a meeting booklet and all presentations given at the meeting are available for download https://www.INFRAFRONTIER.eu/knowledgebase/workshops/INFRAFRONTIER-impc-industry-liaison-workshop. The industry liaison meeting was instrumental to establish new industry contracts and to solicit feedback from BioPharma on demands for animal model development.

Developing an INFRAFRONTIER communication concept

In order to professionalise outreach and communications within the INFRAFRONTIER research infrastructure we liaised with professional service providers and contracted Cyrano Kommunikation GmbH based in Münster, Germany http://www.cyrano.de/. Cyrano is already covering the topic ‘animal research’ by operating a dedicated platform on behalf of major German research organisations informing and promoting the wider public on the benefits of animal research (https://www.tierversuche-verstehen.de/). During the course of INFRAFRONTIER-I3, the INFRAFRONTIER GmbH as new partner led a dedicated task and worked with Cyrano on the development of a comprehensive communications concept. The development process involved the definition of communication objectives and the mapping of target groups for which core messages were developed for use in targeted promotion materials. As a first implementation measure an **INFRAFRONTIER newsletter** was circulated among all INFRAFRONTIER clients worldwide (7000 recipients) in December 2016.

Training courses

A major effort of the INFRAFRONTIER-I3 project was the organisation and running of training courses covering all aspects of mouse phenogenomics to pass on the unique expertise and skills among consortia members to the wider community. During the course of the INFRAFRONTIER-I3 project a total of **16 cryopreservation and phenotyping courses were held for about 200 students** from across the EU.

The annual **INFRAFRONTIER / EMMA cryopreservation** courses are organised by the CNR and MRC to teach basic and advanced techniques in cryopreservation for banking of mouse mutant strains and for re-derivation of mice from frozen stocks. Two additional courses were held by the University of Veterinary Medicine in Vienna. The regular Spring training course at MRC Harwell was held in 2016 in conjunction with the 13th ISTT conference in Prague as a satellite training course on March 16-18th. **Information on all INFRAFRONTIER cryopreservation training courses is available at:** https://www.INFRAFRONTIER.eu/resources-and-services/training-and-consulting-services/cryopreservation-training-courses

The **INFRAFRONTIER-I3 phenotyping training courses** covered highly specialised secondary phenotyping course formats and a broader mouse phenogenomics course.

The Autonomous University of Barcelona (UAB) organised trainings in:

- **Mouse osteology**: basis for X-ray and CT bone phenotyping (Nov 2013), 12 participants
- **Mouse blood and lymphatic vessel phenotyping** (Nov 2014), 12 participants

The Helmholtz Zentrum München (HMGU) operating the German Mouse Clinic offered training in:

- **Mouse metabolic phenotyping** (Oct 2016), 32 participants

Detailed course descriptions and access to course materials is accessible on the INFRAFRONTIER portal at: https://www.INFRAFRONTIER.eu/resources-and-services/training-and-consulting-services/phenotyping-training-courses

INFRAFRONTIER-I3 partners operating the French Phenomin network offered an international **training of excellence in mouse phenogenomics** providing engineers, PhDs and researchers with an innovative program focused on best practices in the use of the mouse model for biomedical research. Topics covered amongst others were standardized techniques, ethics and animal welfare, regulations, data analysis and resources. The course was held in June 2015 and attended by a total of 42 participants from nine different countries. A detailed description of the course and content is available at https://advanced-school.phenomin.eu. Because of the success of this comprehensive training course a second course is scheduled for June 2017 and the INFRAFRONTIER research infrastructure is planning to sustain the mouse phenogenomics course as a flagship training course format.

3.2 WP3 Innovation

The objective of this work package was to combine the typical indirect calorimetry set-up with features of metabolic cages allowing the sampling and quantification of urine and feces in parallel with gas exchange measurements. In addition, feeding and drinking behavior was monitored in detail along with activity measurement. This **innovation will refine the measurement of energy balance regulation in mutant mice and help to detect new animal models for human disorders**. A special focus was to provide a **Metabolic PhenoCage** (MPC) based on a balance between best possible animal welfare, technical complexity and commercially reasonable cost at a price which is accepted by the market.

We evaluated and documented the current state of technology for Metabolic PhenoCage systems as a first prerequisite for the establishment and operation of the refined MPC module. We identified three key topics for improvement of components: **1)** separation quality of feces and urine, **2)** functionality and animal welfare issues regarding water uptake, **3)** design and material of cage floor. These elements plus other functional units, e.g. food supply, have to be confirmed concerning their functionality before the overall design of the MPC, especially with regard to calorimetry, can be conceptualized and implemented.

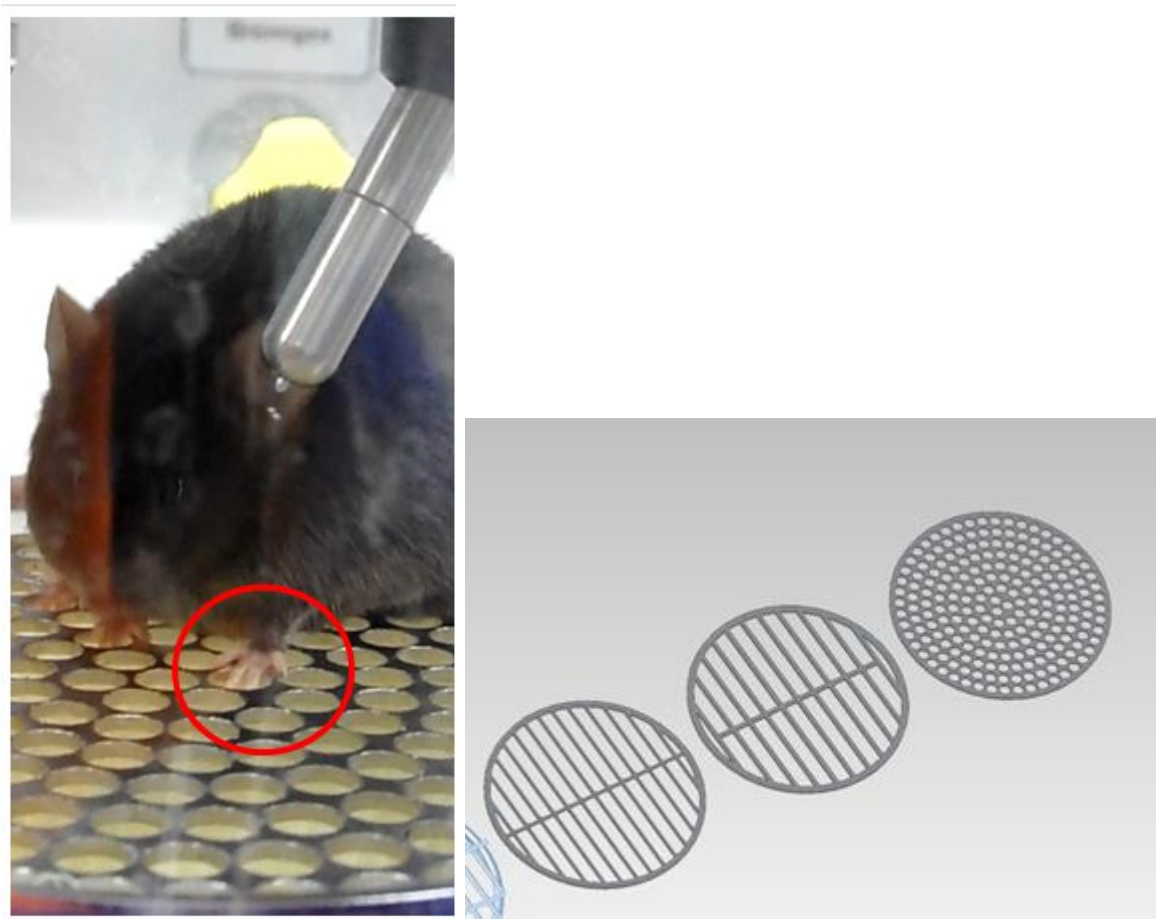

Figure 2: db/db mouse on cage grid / Different cage grid variants

Regarding **3)** the cage floor the bottom of the cage was re-designed under animal welfare aspects and different cage bottoms were developed and manufactured. Functionality tests with animals indicated that falling-through or run-off of feces and urine were optimized.

Overall, during the INFRAFRONTIER-I3 project **we developed an entirely new Metabolic PhenoCage to measure a multitude of metabolically relevant parameters and with maximal flexibility to adjust to future scientific demands**. Greatly improved animal welfare based changes adhering to current regulations and future anticipated changes were implemented. The latest technical advances in terms of materials and technologies were used to come up with a flexible production version at lower cost of ownership. The final version represents a complete new cage that meets and exceeds anything on the market today and can be used for a vast variety of possible experiments where metabolic monitoring in combination with urine and feces collection is needed under very controlled conditions in standard laboratory animals and including applications in germ-free animals. Further validation tests with laboratory animals are underway at our development partner institution as part of the ongoing scientific collaboration.

Figure 3: Overview Metabolic PhenoCage with weighing unit, freezer for urine and feces

3.3 WP4 Cryopreservation of mouse mutant lines

Objective of the INFRAFRONTIER-I3 work package 4 was to **support the European biomedical research community by archiving up to 1215 mouse mutant lines** during the course of the project. The mutants were cryopreserved as either embryos or sperm and the **ability to recover lines was ascertained by rigorous quality controls**. Archived lines were and are made available via the public EMMA strain list on the INFRAFRONTIER website at www.INFRAFRONTIER.eu and on the IMSR database at www.findmice.org. At least 608 archiving slots were allocated for strain donations from individual researchers, whereas the remaining archiving slots were allocated to support large scale mouse genetics programs.

Summary of progress towards work package objectives

The overall work package deliverable for 48 months covering all three reporting period was the submission and archiving of a total of 1215 mouse mutant lines to the INFRAFRONTIER/EMMA archive. **Overall, a total of 1871 mouse mutant lines were submitted during the course of the 4 years of the INFRAFRONTIER-I3 project exceeding the project deliverable by far.** As 37 archived lines contain more than one mutation and are counted twice as defined in the INFRAFRONTIER DoW, we report for the entire project a total of 1178 mouse mutant lines with an overall reporting count of 1215. All deposited lines are fully archived, quality controlled and accessible for the wider community via the INFRAFRONTIER website. The development of incoming strain donations of the EMMA repository is shown in figure 4.

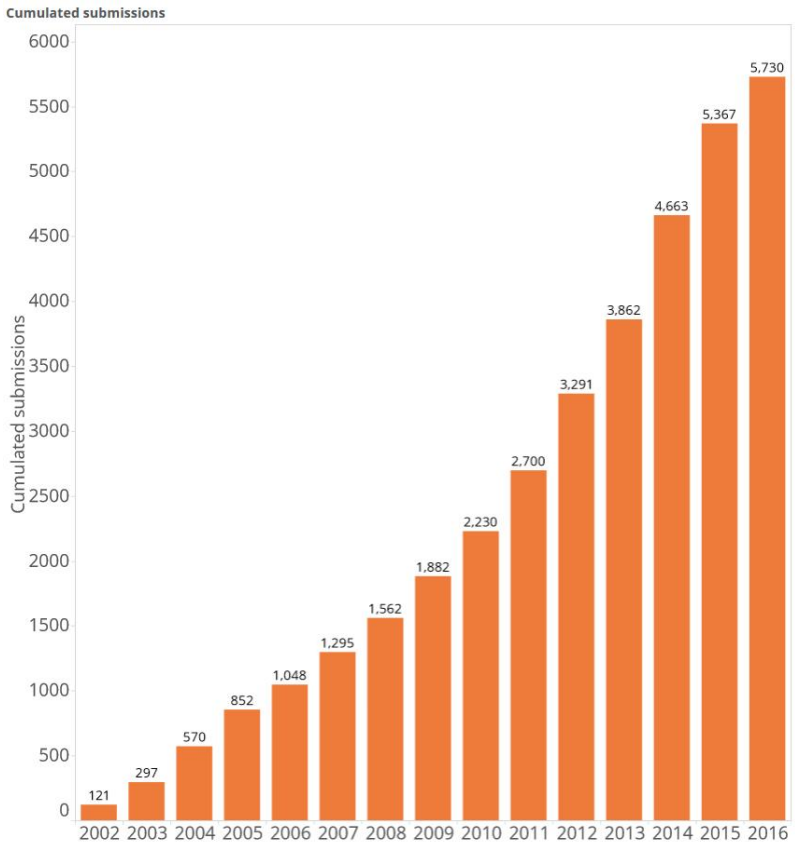

Figure 4: Number of submissions to the EMMA repository
(Cumulative figures until 2016)

The reported number of archived lines shows that the project deliverable has been completely fulfilled and 1215 mouse mutant lines were or will be fully archived and available to the biomedical research community at large. Beyond the INFRAFRONTIER-I3 project deliverable a total of 656 additional mouse mutant lines were submitted to the EMMA repository and archived by consortium partners using other funding sources e.g. national or institutional funding. **The processing of an additional 50% of lines clearly demonstrates the high demand of archiving services by the community that can be met by the INFRAFRONTIER consortium capacity**.

The INFRAFRONTIER-I3 project aimed to support foremost the wider community and also provided archiving slots to EU funded projects such as EUCOMMtools and to other large scale projects contributing to the International Mouse Phenotyping Consortium (IMPC). A total of 68% of the reported lines account for community contributed lines and the remainder of lines originate from large scale project efforts. Thus, also regarding contribution of both community and project contributed lines the overall project deliverable has been fulfilled.

Of the archived lines a total of 796 lines were archived as sperm, 115 as embryos and 267 as both formats. The **median archiving time (including a re-derivation control) for embryo freezing was 3.1 months, for sperm freezing 2.8 months**, respectively. Most submitted lines were targeted mutations, mainly KO mutations due to the contributions from large scale projects such as the International Mouse Phenotyping Consortium (IMPC). The second prominent class are transgenic lines followed by few chemically induced mutations, gene traps and spontaneous mutations.

Strain donations were made across Europe with major contributions from Germany, UK and France. See figure 5 for a graphic display of the origin of submissions. Mouse mutant lines were donated by a total of 320 different submitters or institutions of which 98% are not associated with the institutions operating the EMMA repository.

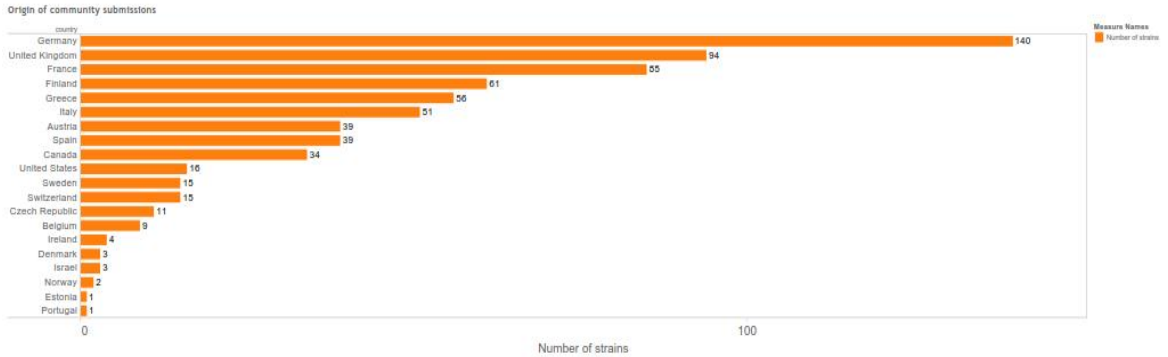

Figure 5: Origin of community submissions during the INFRAFRONTIER-I3 project

3.4 WP5 Technology development and implementation

WP5 Cryopreservation activity
Objectives:

- To enhance animal welfare by promoting the exchange of spermatozoa when transferring mouse stocks between institutions and thereby providing alternatives to live animal transportation.
- To allow the efficient exchange of mouse strains between institutions and at the same time minimise the cost to the end users.
- To reduce EMMA's dependence on surgical embryo transfer, thereby improving animal welfare.
- To minimise the costs and maximise the efficiency with which mouse stocks can be cryopreserved and subsequently recovered from the EMMA archive.

Summary of the progress towards the objectives
This work package has seen some excellent progress in the development of novel protocols for transporting germplasm in the absence of liquid nitrogen ($LN_{2)}$. These innovations have come about through the coordinated efforts of the INFRAFRONTIER partners, in addition to collaborations with colleagues in China and Japan. Protocols describing the rederivation of live mice from sperm transported in the absence of LN_2 were developed, validated and Standard Operating Procedures (SOPs) published on the INFRAFRONTIER portal. New protocols for shipment of sperm on dry ice are widely used among consortium members and to ship mouse resources to clients worldwide, and contribute to reducing live animal shipments and shipment cost for EMMA clients.

We have also been able to use Intracytoplasmic Sperm Injection (ICSI) to consistently produce pups from mice on an inbred or mixed background using either freshly harvested sperm or frozen/thawed sperm and a protocol has been released and published on the INFRAFRONTIER portal. Despite this success we decided to direct and use WP5 resources to developing an embryo/oocyte vitrification protocol because this has been seen as a more urgent need for the community. Robust vitrification protocols have been developed which give rise to high survival rates when used to freeze both embryos (~95% survival) and oocytes (~70% survival). What is more, the fertilisation rates for vitrified/warmed oocytes exceed 90% when freshly harvested sperm is used for *in vitro* fertilisation. Protocols and video demonstrations describing embryo vitrification have been presented on the INFRAFRONTIER website.

All developed **SOPs, flyers** and **video demonstrations** can be accessed at:
https://www.infrafrontier.eu/knowledgebase/protocols/cryopreservation-protocols

Overall, the **cryopreservation activities of WP5 were very successful and all deliverables were met**. **Six protocols** have been written and presented on the INFRAFRONTIER website, in addition to the **publication of six methods papers**, plus a further 5 articles in preparation on oocyte vitrification, frozen/thawed embryo shipments, shipping sperm on dry-ice, long term holding of sperm on dry ice, and trans-cervical embryo transfer. In addition to our dissemination activities via the SOPs on the INFRAFRONTIER

portal and publication of methods papers, new protocols and innovations are taught and demonstrated in the EMMA / **INFRAFRONTIER training courses** offered in WP2.

WP5 members had also taken the opportunity to **work with commercial partners** such as Air Sea Containers, to develop a cool box that can be used to maintain samples at refrigerated temperatures. These containers comprise a combination of cold packs and insulated materials and will keep the samples at 4-8°C for more than 72hrs. This allows sufficient time for transportation across Europe and between continents, if necessary. In addition, we have developed a cooling plate that can be used in conjunction with oocyte/embryo vitrification protocol. The cooling plate is now commercially available through Elim Springs Biotech Ltd.

WP5 Phenotyping activity

Objectives:

The overall objective was to carry out technology evaluation and piloting of new phenotyping assays that will:

1) Allow high-throughput analyses
2) Enhance user services for new types of data unmet by the classical assays or not yet covered in the International Mouse Phenotyping Consortium initiative (IMPC)
3) Reduce the cost of services
4) Reduce the number of animals used in research according to the 3R rules

Focus of activities were the development of novel protocols and SOPs for high-throughput micro-computed tomography (µCT) imaging of embryos and adults, and biomarker phenotyping to evaluate multiplex assays for profiling and discovery

Summary of progress towards objectives

From bench to bedside - high-throughput µCT imaging of embryos and adults

The goal of this task was the piloting of high-throughput imaging in order to identify relevant phenotypes in bone morphology, cardiovascular, muscular and respiratory systems in adult tissues as well as development defects during embryogenesis through 3-D image reconstruction. µCT is a preferred technology platform since it has very high resolution, is non-invasive and supports 3-D reconstruction. Conventionally, µCT has been limited in distinguishing soft tissues, which have inherently low contrast due to a narrow range of CT numbers. However, using contrast agents, it is now possible to achieve excellent soft tissue detail. A very successful collaboration between the Work Package 5 partners during the whole project duration supported excellent progress and led to the establishment of novel SOPs meeting all WP deliverables. A total of 3 novel imaging SOPs were developed during the course of the project and will be disseminated via dedicated databases for phenotyping SOPs such as IMPReSS (http://www.mousephenotype.org/impress).

Newly developed phenotyping SOPs cover:

· SOP I3\IMA-001-µCT EMBRYOS.pdf
  Using micron scale X-rays Computed Tomography (µCT) for E18.5 mouse fetuses phenotyping

· SOP I3\IMA-003 - µct Adult -bone.pdf

3D morphometric analysis of adult mouse bone microarchitecture by micron-scale X-rays Computed Tomography imaging: addressed to in vivo and ex vivo research

· SOP I3\IMA-002 - HREM.pdf

Using High Resolution Episcopic Microscopy (HREM) for mouse embryo phenotyping

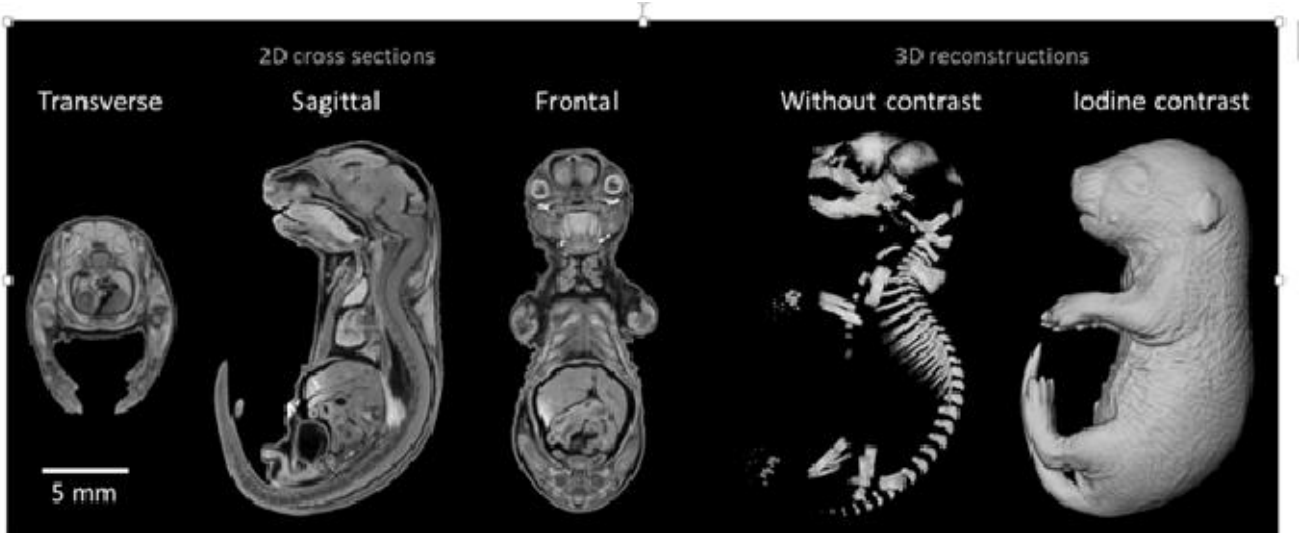

Figure 6: Imaging of E18.5 fetuses: 2D cross-sections in frontal, sagittal and transverse orientations and 3D reconstructions with and without iodine contrast.

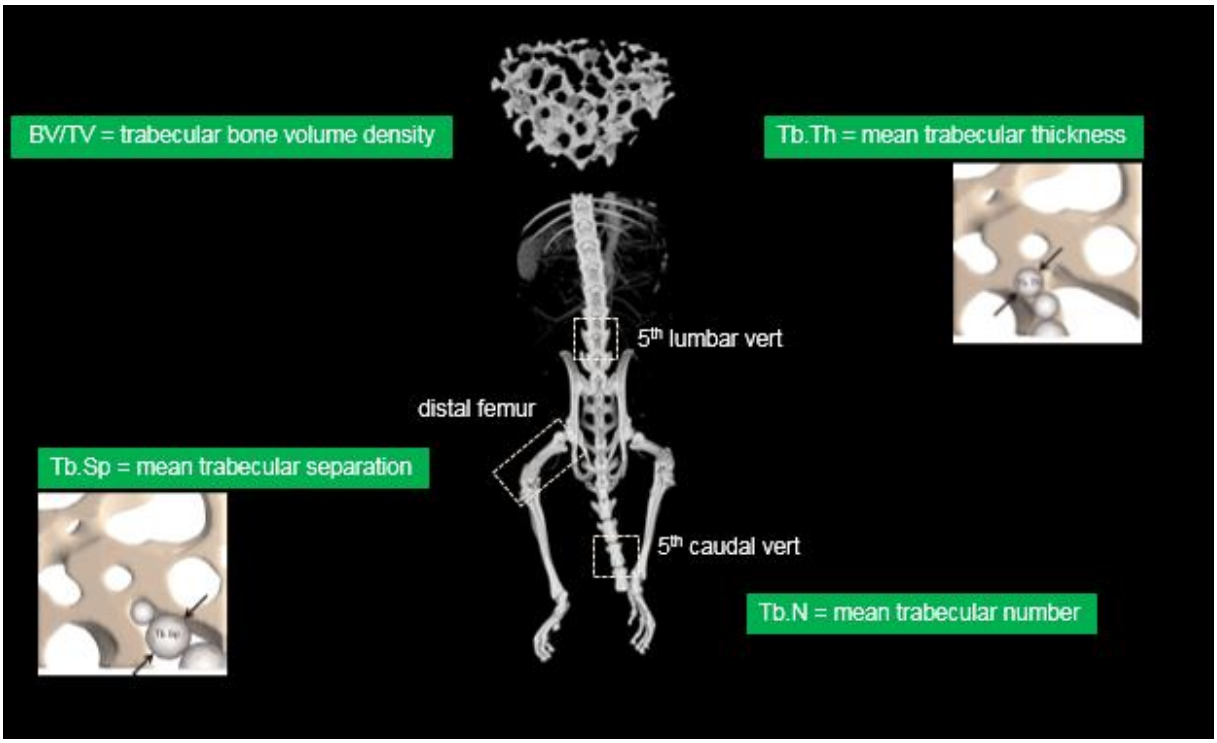

Figure 7: µCT bone phenotyping: Trabecular architecture

Biomarker phenotyping - Evaluate multiplex assays for profiling and discovery

Biomarkers can provide information on a range of biological processes and a variety of important physiological systems. They can be highly correlated with the onset of diseases ranging from diabetes to cancer. Analyzing biomarkers in serum and urine, or other biological fluids represents a treasure trove for phenotypic analysis and discovery of preclinical models for human disease. Our experience from EUMODIC and now the IMPC is that there is a real need for identifying the next generation of multiplex technology which will enable flexibility of assay design. This is important as the number of biomarkers is increasing rapidly and the costs for carrying out individual tests can be prohibitive when they are considered for incorporation into high-throughput screens. Multiplexing will also reduce the number of mice used in research since many tests will be performed on a single animal. Development of such assays will facilitate the comparison of mouse models with human disease conditions and so improve correlation between the phenotypes observed in mice and humans.

The **objectives** of this task were to evaluate multiplex assays for profiling biomarkers in adult mice to be able to deliver: **1)** a novel multiplex series with **2)** small sample volume requirements that would be **3)** amenable to automation, and **4)** would be suitable for biomarker detection in various fluids / tissues.

Summary of progress towards objectives

Following extensive discussions among the partners and within related networks a comprehensive short list of 15 biomarkers for assay development was compiled covering a range of physiological systems, including the skeletal, cardiac, renal, metabolic, and immune system. Based on this initial short list, we have conducted a **pilot cross validation study** with AYOXXA, a German biotech company providing its unique LUNARIS platform. LUNARIS™ is an innovative, patented beads-on-a-chip technology to analyze multiple biomarkers in low volume biological samples. LUNARIS BioChips bring the potential of microarray technology, successfully applied in genomics to the area of proteomics. The LUNARIS™ technology opens multiplexing opportunities to research areas all the way from basic research to clinical applications. A first **5-plex trial assay** incorporated cardiac troponin (cTnT), insulin, Interleukin 6 (IL-6), neutrophil gelatinase-associated lipocalin (NGAL) & osteoprotegerin (OPG). All centers have provided mouse plasma samples from relevant mutant lines and their control littermates. Plasma samples from mice fed with a standard chow diet (CD) and with high fat diet (HFD) were supplied by ICS for the validation of insulin measurement. Plasma samples from mice treated with lipopolysaccharide (LPS) were provided for the measurement of IL-6.

A pilot mouse 4-plex assay covering IL-6 / Insulin / OPG/ and TNFa was developed and successfully cross-validated against MSD and Luminex technologies. It was agreed with AYOXXA to aim for a joint publication to document the development effort. Towards this end and to generate additional data, AYOXXA is measuring in-house the samples of 10 IMPC mouse lines per mouse clinic on the pilot panel 4-plex, for primary screening, and the identified hits in inflammation phenotypes (IL-6 and TNFa) will then be analyzed on the secondary screening panel for inflammation, LUNARIS™ Mouse 12-Plex Cytokine Kit. The outcome of the multiplex screening will be analyzed in Spring 2017 and summarized in a joint methods paper.

3.5 WP6 EMMA informatics

WP6 is led by EMBL-EBI whereas CNR, MRC and INFRAFRONTIER contributed. All partners constitute the **INFRAFRONTIER informatics working group**. The objective of this work package was to coordinate and integrate informatics activities necessary to continue development of the database, curation of mutant mouse strains, improve user experience, and develop data analysis tools to control and improve INFRAFRONTIER services.

Summary of progress towards objectives

Manual curation of the INFRAFRONTIER-EMMA database

The **manual curation** of the database, according to the Guidelines and Rules established by the International Committee on Standardized Genetic Nomenclature for Mice, **is critical to ensure mutant model data accuracy and consistency** and their correct public and internal access, dissemination, analysis and reporting. **Accurate data is also a pre-requisite for automated cross-linking and integration with reference databases** such as ENSEMBL, MGI-MGD, IMSR and IMPC. The INFRAFRONTIER-EMMA database is built mainly from external user-entered data and, despite precautions being taken to ensure data consistency through use of drop-down menus, controlled vocabularies, ontologies, automated record updating, etc., there will always be a requirement for expert manual curation. This is for example necessary to remove or update incorrect, redundant and non-standard records, e.g. reference to incorrect transgene/gene/allele/background line records, multiple spellings of the same gene or allele symbols or background line, etc. in different external user submissions.

At the end of the INFRAFRONTIER-I3 project **2763 lines in total had their background, transgene / genes / alleles and strain names / symbols manually reviewed and curated**. The curated lines (2262 curated by CNR and 501 by MRC) had the following curation status: 1563 lines with nomenclature curated, assigned by INFRAFRONTIER-EMMA and approved by IMSR (Name status: IMSR_Approved), 796 lines with nomenclature curated, assigned by INFRAFRONTIER-EMMA and under review by IMSR (Name status: EMMA_Checked) and 404 lines with nomenclature curated, assigned by INFRAFRONTIER-EMMA, whose alleles / transgene are not yet annotated in MGI (Name status: EMMA_Preliminary). Overall, the objectives of this task have been fully met during the project and contributed to the overall data quality provided by the INFRAFRONTIER/EMMA repository.

Development of the public website for the external users

We **designed and implemented a new website (www.infrafrontier.eu)** as part of the merging of the EMMA services under the INFRAFRONTIER umbrella at the beginning of the INFRAFRONTIER-I3 project. This design was built upon user feedback with the aim of easing navigation by giving overviews of different aspects of the project coupled with the ability to obtain details on demand. We have worked on robustness of the web portal and creating an infrastructure that allows collaborators to contribute new features to the portal. To do this, we modularized the INFRAFRONTIER software from other mouse informatics projects at EBI and applied open source licenses. The software was then deposited in the public Github software repository and is being co-developed with collaborators.

Features of the revamped portal such as search and retrieval of strain information in the EMMA resource, access to key documents such as health reports and MTAs, and other features supporting user friendliness were comprehensively described in a dedicated publication of the Nucleic Acids Research database issue (INFRAFRONTIER Consortium /

INFRAFRONTIER - providing mutant mouse resources as research tools for the international scientific community. Nucleic Acids Res. 2015 Jan;43 (Database issue) : D1171-5. PubMed PMID: 25414328

Development of EMMA database schema and data

We have continuously improved and enhanced the EMMA database throughout the INFRAFRONTIER-I3 project. As most high-throughput production centers are involved in the International Mouse Phenotyping Consortium (IMPC) and the EC FP7 funded EUCOMMTools project, we have modified the EMMA database to receive data from import mechanisms built for the IMPC mouse production tracking resource “iMits”. By this manner we are able to gather data from thousands of mouse strains being produced by these projects. This also includes major modifications to the database to represent the CRISPR/Cas9 mechanisms being deployed that do not require an embryonic stem cell clone to develop a strain carrying a targeted mutation. As genome-editing technologies are quickly evolving, we have made our source code publicly available to allow other parties to modify the software for their needs. To support smaller groups not using the iMits tracker, we have also enhanced data submission by means of an online data submission form. This ‘wizard’ guides submitters through an 11-step process to record all the necessary data needed to deposit a strain. We gathered feedback from users and continuously improved the online submission form. Data integration was further refined by switching integration with MGI-Mouse Genome Database from the discontinued MGI BioMart to MGI’s new MouseMine data warehouse system.

Relocation of the INFRAFRONTIER IT infrastructure to INFRAFRONTIER

In order to support the long term sustainability of the critical INFRAFRONTIER / EMMA IT infrastructure, it was decided to relocate the database to the INFRAFRONTIER GmbH which coordinates all activities of the INFRAFRONTIER consortium and provides core funding for IT and operations. To achieve this EMBL-EBI worked on portability of the code base and made it available via the GitHub sharing platform (https://github.com/INFRAFRONTIER). The INFRAFRONTIER GmbH started by preparing the necessary virtual hardware infrastructure and then installed and tested all the components on different Ubuntu Linux machines. The main switch of the EMMA database and all user-facing applications happened seamlessly on 26 April 2016. The INFRAFRONTIER web portal represents one major part of the moved infrastructure. The web portal uses the content management system Drupal and has three separate instances for development, beta testing and production that are integrated by a managed deployment process. The web portal also includes the PHP/jQuery based public EMMA search and is integrated with a series of online forms that are implemented in Java. The Tableau Business Intelligence software was installed on a separate Windows server that also hosts the Active Directory system for user account management of Tableau and Drupal. All mail functionalities including automated confirmations and progress updates for customers, bulk mailing for PR measures and internal lists for communication within the consortium were also migrated.

3.6 WP7 Axenic service - derivation and distribution of germ-free mice

The overall objective of this work package was to provide wild type and genetically modified animals raised in germ-free (GF) conditions to interested INFRAFRONTIER / EMMA customers. The activities concern the maintenance of GF isolators, the raising of GF foster mice, the maintenance of commonly requested strains as well as the importation, handling, introduction into GF isolators and the raising in GF conditions of specific requested strains. Germ-free mice support research into the complex interaction of host and microbiome.

Summary of progress towards objectives

Maintaining axenic live stocks

Frequently requested strains are the seeding colony (C3H), a control strain (C57Bl/6) or a popular research model (C57Bl/6 Rag2-/-). These commonly requested strains are maintained in breeding inside the isolators. Fast access to these lines is facilitated via standard service request forms, and a total of six such requests were processed during the course of the INFRAFRONTIER-I3 project.

Shipment of GF mice or tissues

GF mice can be shipped to the requester e.g. for requests to initiate an axenic colony. Alternatively, tissues and body fluids and also some cell subsets can be prepared by the technical staff and sent to the requester. Further manipulation on live animals, sophisticated extraction of biological material or immediate analysis of fresh tissues requires hosting of clients. The Gulbenkian Institute is a multidisciplinary research institute in the field of biomedical sciences with state of the art technical platforms, which conveniently enable visitor scientists to perform the necessary experiments on site. During the INFRAFRONTIER-I3 project a total of six clients were hosted by the axenic service provider at Gulbenkian, to follow up with further analyses of their newly derived germ-free mice on site.

Requester	Start date	Parental mice	End point	End date
Kollias	Dec 2010	Frozen embryos, Feb 2011	Analysis on site	Dec 2014
		From Live GF stocks		
Carding	Aug 2011	Live, not SPF, May 2012 Live SPF, Dec 2013	Live shipping	Nov 2014
Fallon	Oct 2011	Live, not SPF, Sep 2012	Analysis on site	April 2014
Hautefort	Apr 2013	In house SPF	Live shipping	Nov 2014
VFernandes	Apr 2014	From Live GF stocks	Tissues	May 2014
Eliopoulos	Jul 2014	From Live GF stocks	Tissues	Sept 2014
Lesnik	Feb 2015	In house SPF	Live shipping	Oct 2015
VargaWeisz	Apr 2015	Live, not SPF, Jan 2016	Analysis on site	Feb 2017*
Genestier	Aug 2015	Live, not SPF, March 2016	Live shipping	Feb 2017*
Wullaert	Oct 2015	Live, not SPF, Jan 2016	Live shipping	Feb 2017*
		From Live GF stocks		Feb 2017*
Silva-Santos	Aug 2016	From Live GF stocks	Analysis on site	Aug 2016
	Oct 2016	From Live GF stocks	Analysis on site	Oct 2016

Table 1: Overview of the requests accepted and processed during the course of INFRAFRONTIER-I3

Networking activities and outreach to promote gnotobiology

Networking is an essential component of WP7 e.g. to facilitate exchange of technological developments and quality control measures. Axenic and gnotobiology techniques were developed in the 1950s, and there is no clear common platform for either normalization or modernization of the approaches and techniques, although tools and materials obviously evolved tremendously. Finally, the number of studies relying at least in part on axenization and gnotobiology are rapidly increasing and several centers are considering the setting up of an in-house GF facility. This development indicates that a network of gnotobiology centers within Europe is highly desirable.

During the INFRAFRONTIER-I3 project the Gulbenkian Institute and partners KI and CNRS joined the ECGnoto initiative (http://www.ecgnoto.eu/), a novel bottom-up European consortium with no specific funding, dedicated exclusively to mouse axenization and gnotobiology (the science of controlled microbiota). In this frame, we have been developing an inventory of all GF facilities in Europe and their capacities as well as designing common good practices and shipment schemes.

As task of the INFRAFRONTIER-I3 project a specific workshop on axenic mice and gnotobiology covering both technological and scientific issues took place on the 26-27 March 2015 at the Gulbenkian Institute. This event brought together INFRAFRONTIER partners concerned by the matter as operators of a facility or users of axenic services as well as technical and scientific staff from the facilities identified during the inventory. The highly interactive workshop was attended by 12 renowned speakers of the field and about 70 participants. Additional sponsorship was obtained from isolator and isocaging system vendors.

Furthermore, the Gulbenkian Institute attended international meetings of the FELASA in Brussels, Belgium (June 2016) and the AALAS in Charlotte, North Carolina, USA (November 2016). At both meetings, Joana Bom contributed a lecture presenting the activities of this INFRAFRONTIER-I3 activity and the Gulbenkian axenic/gnotobiology facility and strategies.

3.7 WP8 Mouse model production (Trans-national Access)

Objective

To provide free of charge Trans-national Access for eligible users to 30 knockout mouse lines produced from ES cell clones that can be derived from the IKMC or from other sources, thereby supporting the development of mouse models of human diseases for functional genomics research.

Partners and infrastructure

The infrastructure required for running WP8 is provided by five independent INFRAFRONTIER nodes located in Vari-Athens (Greece), Madrid (Spain), Oulu (Finland), Prague (Czech Republic) and Vienna (Austria). All five nodes offer this service routinely to the researchers of their institutions and to external customers. They are equipped with up-to-date tools and have access to animal facilities where genetically modified animals are housed in specific units, their use is registered and reported to authorities and inspections are performed on a regular basis. They have proven capability to produce genetically-modified animals from embryonic stem (ES) cells. All five units have specialized personnel with the required expertise to produce genetically-modified animals using state-of-art technologies. All these five production units are associated with the corresponding archiving facilities at each center. This allows the cryopreservation of all new mouse lines created by the Trans-national Access service, thus allowing optimal integration of both production and archiving tasks and providing the new animal models to the requesting customer but also to the wider scientific community.

Progress towards objectives of work package tasks

Production of heterozygous mice carrying the inactivated gene allele from the corresponding gene-targeted ES cell clone(s) from IKMC or other sources

Details of the evaluation process and of the advertising of open TA calls are described in detail in the WP2 report. In brief, interested customers applied via specific online request forms accessible via the INFRAFRONTIER website for one of the 30 free access units qualifying for the production of heterozygous mice carrying the altered gene allele of choice. The **INFRAFRONTIER external Evaluation Committee** assessed all applications using defined criteria and recorded evaluations and the justification for their assessment in a dedicated reporting form. Each application was evaluated by two external committee members. Upon successful approval of TA applications the INFRAFRONTIER-I3 Project Office assigned the project to one of the WP8 participant nodes, who were then responsible for the production of the requested mouse knockout animal model following standard protocols. Making the mouse mutant lines that are produced with TA funding available to the wider community via the EMMA repository was a requirement, and ensured by signing strain deposition agreements with successful applicants.

INFRAFRONTIER-I3 Trans-national Access calls

During the first reporting period a total of 3 TA calls were published and fully executed resulting in the assignment of all available 30 mouse production TA slots to successful applicants. A first call was published in March 2013 followed by calls in December 2013 and May 2014. Overall, **a total of 130 project proposals from applicants across 17 eligible countries were submitted and evaluated**. The origin of all applicants is shown in Figure 8.

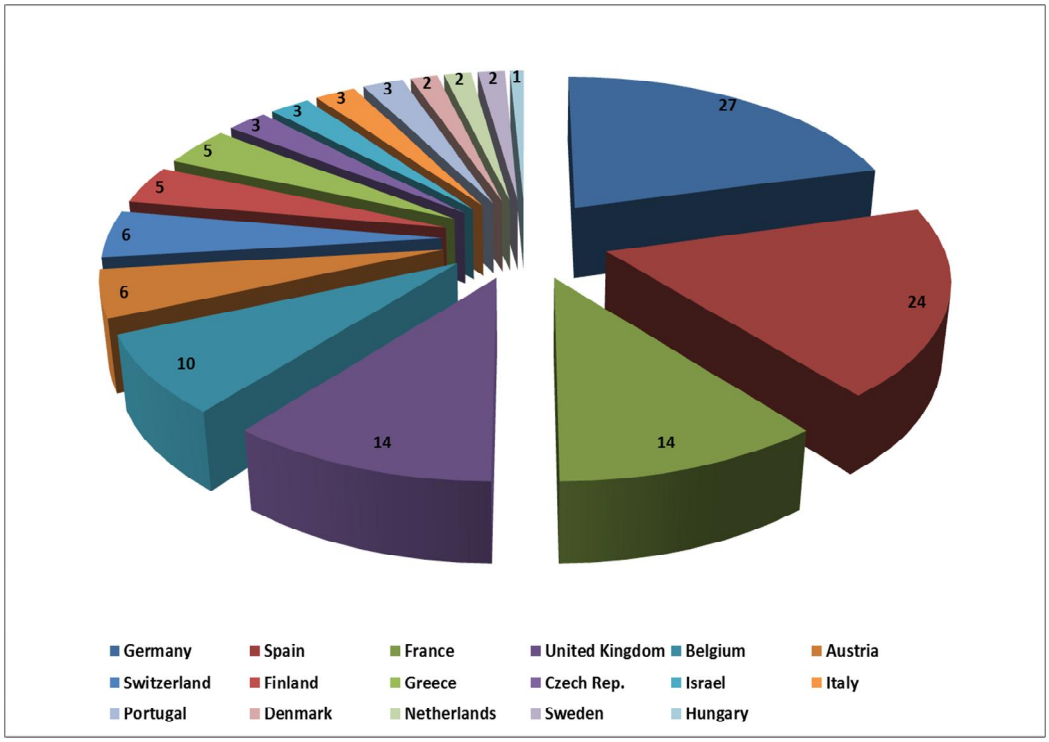

Figure 8: Origin of applications for mouse production TA calls

The origin of all successful applicants is shown in Figure 9. **A total of 30 / 130 applicants i.e. 23 % were awarded free of charge TA mouse production projects**.

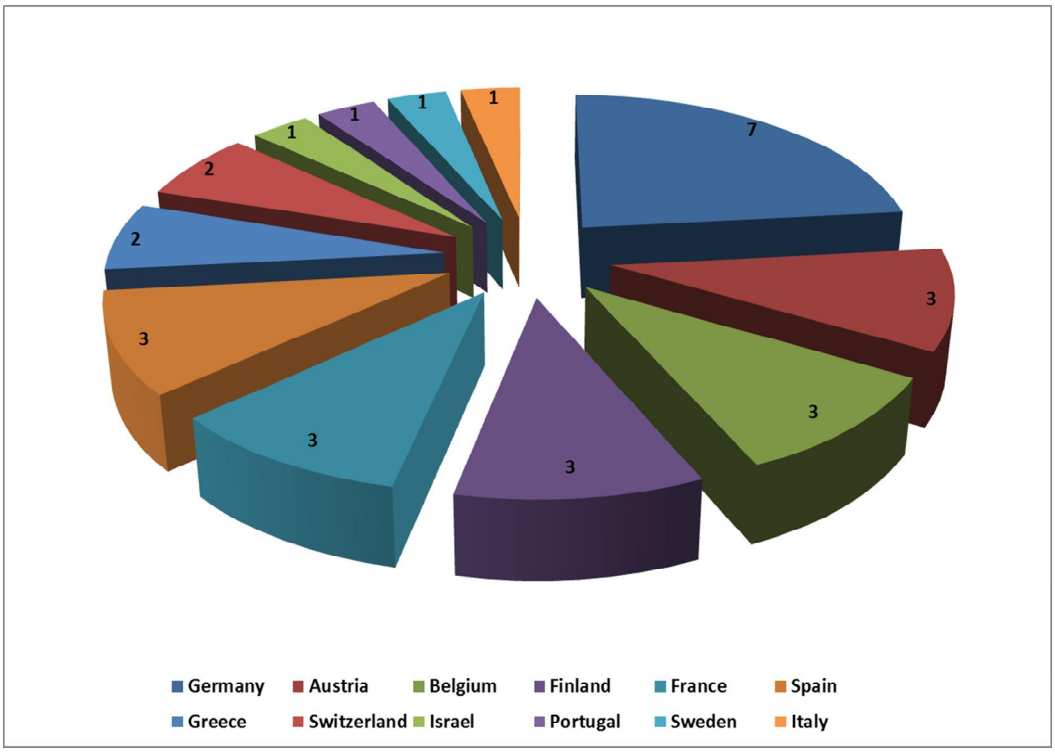

Figure 9: Origin of application for granted TA mouse production slots

An overview of all selected applicants and their projects is given in table 2, pages 26-27. Successful project proposals cover the production of new mouse models where no previous model exists, or the development of a conditional ready KO first allele that can be used in further steps to produce conditional lines to advance analyses of existing full KO lines. A **wide range of research areas was covered** as shown in table 2 such as **cancer research, motoneuron diseases, type 1 diabetes, metabolic diseases, liver diseases and mitochondrial diseases**, amongst others. The progress in actual mouse production is summarized in table 3. **Excellent progress has been made and in period 3 all running projects were finalized including the archiving in EMMA to facilitate sharing and long term availability of these valuable new mouse models of human diseases (**table 3, page 28). All newly developed mouse models of human diseases were shipped to selected applicants and are available to the wider biomedical research community.

Overall, a smoothly running and transparent process for promoting and evaluating Transnational Access calls for mouse production projects has been established. A good response from the wider European biomedical research community using mouse models resulted in the submission of a total of 130 project proposals. The most promising proposals, as assessed by an external Evaluation Committee, led to the development of 29 new mouse models of human diseases covering a wide range of key research areas such as cancer and diabetes, amongst others. The deliverable of WP8 has thus been completely fulfilled. In only one case mice could not be bred to germline and the project had to be terminated.

To fully exploit the resources that are being generated by this TA activity, all mouse mutant lines were archived by work package 4, distributed to selected applicants and are available under standard EMMA conditions to the research community at large via the INFRAFRONTIER/EMMA portal at www.infrafroontier.eu. Further value will be added to the mouse lines as several INFRAFRONTIER mouse clinics expressed interest to add the work package 8 lines to their phenotyping pipelines contributing to the International Mouse Phenotyping Consortium (IMPC).

Production centre	Call	Applicant	Gene of interest	Research area and project objective
Athens	**1**	**Carbone**	**Ehf**	Cancer research: Evaluate the role of the ETS transcription factor ESE3/EHF in prostate epithelial cell transformation and tumorigenesis
Fleming	**1**	**Aidinis**	**Lpar1**	Chronic inflammation: Role of lysophosphatidic acid (LPA) receptor 1 function in diseases like rheumatoid arthritis and idiopathic pulmonary fibrosis
	1	**Douni**	**Dnajc11**	Development of novel mouse model of autosomal recessive neuromuscular / mitochondrial disease with motor neuron pathology (complementary to ENU model)
	2	**Zick**	**Tm7sf3**	Type 1 diabetes: Study TM7SF3 - a Novel Promoter of Beta Cells Survival and Resistance to ER (Endoplasmic Reticulum) Stress
	3	**Sendtner**	**hNRNPR**	Motoneuron disease: Characterize the role of hnRNP R in the central nervous system with particular emphasis on the neuromuscular system
	3	**Weiskirchen**	**Plin5**	Liver diseases: Develop a cond KO mouse model to study the role of Perilipin 5 (PLIN5), also known as OXPAT, in hepatic steatosis
Prague	**1**	**Schneider Stock**	**Atf2**	Cancer research: Study role of the Atf2 transcription factor in the molecular and cellular mechanisms involved in inflammation and inflammation-associated colon cancer
IMG	**1**	**Gabellini**	**Frg1**	Myopathies: Study role of Frg1 in the molecular pathogenesis of facioscapulohumeral muscular dystrophy (FSHD)
	2	**van Veldhoven**	**Acox2**	Develop a novel mouse model of Acox2 encoding one of three acyl-CoA oxidases and study role in the peroxisomal beta-oxidation
	2	**Wachten**	**Gltp**	Develop a cond Gltp KO to unravel the mechanism of GlcCer-dependent signaling and to understand the molecular basis for the development of Gaucher disease
	3	**Hernando**	**Xpr1**	Develop a cond KO mouse model to study the role of XPR1 in phosphate metabolism and transport
	3	**van Veldhoven**	**Lonp2**	Develop a cond KO mouse model to study the function of LONP2 in mammalian peroxisomes
Oulu	**1**	**Lamkanfi**	**Caspase1**	Infectious dieases: Study role of caspase-1 (via a cond. Caspase1 specific KO) in infectious and non-infectious disease
Uni Oulu	**1**	**Welsh**	**Shb**	Use cond KO to study cell specificity of effects seen in KO line relating to reproduction, angiogenesis and hematopoiesis
	2	**Wickström**	**Xpo6**	Develop a cond KO model to study the role of Xpo6 in stem cell fate decisions
	2	**Battersby**	**2810006K23Rik**	Mitochondrial disorder: Develop a cond KO mouse model to faithfully replicate a human disease mutation affecting mitochondrial translation
	3	**Raivio**	**Ndnf**	Develop a cond KO model to study the role of Ndfn in Kallmann syndrome (congenital hypogonadotropic hypogonadism and anosmia/hyposmia)

	3	**Tynnismaa**	**Grpel2**	Mitochondrial diseases and neurodegeneration: Develop a cond KO mouse model to study the role of GRPEL2 in mammalian mitochondria
Vienna	**1**	**Villunger**	**Pdcd5**	Cancer research: Investigate the role of PDCD5 in autophagy and as a modulator of p53
VUW	**1**	**Leveillard**	**Nr4a1**	Develop a cond KO mouse model to study the role of Nr4a1 in retinitis pigmentosa the most common form of inherited retinal degeneration
	2	**Buchner**	**Syap1**	Develop a cond KO model to study a possible role of SYAP1 in motoneuron disease
	2	**Kindler**	**Mkrn1**	Develop a cond KO mouse model to study the role of Makorin in behavior, learning and memory
	3	**Fink**	**Ing3**	Ing3 has been shown to be down regulated in various cancer types and is therefore designated as a candidate tumour suppressor gene
	3	**Kratky**	**Lipa**	Develop a cond KO mouse model to study the role of lysosomal acid lipase (Lal) in the regulation of lipid and energy metabolism in cells and tissues
Madrid	**1**	**Pujol**	**Il10**	Develop a faithful mouse model for the lethal in childhood, cerebral inflammatory and demyelinating form of X-linked adrenoleukodystrophy (X-ALD)
CNB-CSIC	**1**	**Mendez**	**Cpeb3**	Develop a cond KO model to understand the role of CPEB3 in learning, renal cell differentiation and cancer
	2	**Bähler**	**Myo19**	Myo19-deficient mice represent a model to study the importance of actin-based movements of mitochondria. Mouse model may contribute to efforts of finding ways to delay or treat degenerative diseases
	2	**Almeida**	**Mex3a**	Develop a cond KO mouse model to study the role of Mex3a in homeostasis (maintaining a balance between stem cell self-renewal and differentiation) and in carcinogenesis
	3	**Charlet Berguerand**	**Smcr8**	Neurodegenerative diseases: Develop a cond Smcr8 mouse line as MODEL OF AMYOTROPHIC LATERAL SCLEROSIS & FRONTOTEMPORAL DEMENTIA
	3	**Medina**	**Arid1b**	Develop a novel mouse model for ARID1B (Arid1b can be altered in several human pathologies including cancer, Coffin-Siris syndrome and intellectual disability)

Table 2: Allocated mouse production TA slots and research area of proposed projects

	Call	Applicant	Gene of interest	Customer contacted	ESC clones ordered	ESC clones arrived	ESC clones injected	Chimeras born	Hets produced	Hets shipped	Archived in EMMA
Athens	1	Carbone	Ehf								
Fleming	1	Aidinis	Lpar1								
	1	Douni	Dnajc11						cancelled		
	2	Zick	Tm7sf3								
	3	Sendtner	hNRNPR								
	3	Weiskirchen	Plin5								
Prague	1	Schneider Stock	Atf2								
IMG	1	Gabellini	Frg1								
	2	van Veldhoven	Acox2			Talens used	Talens in prod	Talens tested	Talens pups born		
	2	Wachten	Gltp								
	3	Hernando	Xpr1								
	3	van Veldhoven	Lonp2								
Oulu	1	Lamkanfi	Caspase1								
Uni Oulu	1	Welsh	Shb								
	2	Wickström	Xpo6								
	2	Battersby	2810006K23Rik								
	3	Raivio	Ndnf								
	3	Tynnismaa	Grpel2								
Vienna	1	Villunger	Pdcd5								
VUW	1	Leveillard	Nr4a1								
	2	Buchner	Syap1								
	2	Kindler	Mkrn1								
	3	Andrukhova	Fgf23								
	3	Kratky	Lipa								
Madrid	1	Pujol	Il10								
CNB-CSIC	1	Mendez	Cpeb3								
	2	Bähler	Myo19								
	2	Almeida	Mex3a								
	3	Charlet Berguerand	Smcr8								
	3	Medina	Arid1b								
				30	30	29 (1)	29 (1)	29 (1)	29	29	29
				Customer contacted	ESC clones ordered	ESC clones arrived	ESC clones injected	Chimeras born	Hets produced	Hets shipped	Archived in EMMA

Table 3: Progress of allocated mouse production TA projects / Dec 2016

3.8 WP9 Mouse systemic phenotyping (Trans-national Access)

Objective

The aim of WP9 was the provision of free of charge access units for a comprehensive 1st line phenotyping in the participating mouse clinics. A standard 1st line phenotyping pipeline was used that builds on the pipeline developed by the International Mouse Phenotyping Consortium (IMPC, http://www.mousephenotype.org/impress). The primary phenotyping pipeline is designed to cover a wide range of biological functions and takes into account the timing and order of tests, cohort size, and statistical issues associated with the use of the controls. As a standard, a minimum of 7 homozygous mutant mice per genotype and per gender are analyzed. Mutant lines that display homozygous lethality are phenotyped as heterozygotes.

Partners and infrastructure

The WP9 partners are independently operating four **European Mouse Clinics** and have an outstanding track record of scientific achievement and successful collaborative work as demonstrated in the EC funded **EUMORPHIA** and **EUMODIC** projects. The participating centers are HMGU operating the German Mouse Clinic, the MRC Harwell, CERBM-GIE (Institute Clinique de la Souris) and The Wellcome Trust Sanger Institute running the Mouse Genetics Project (Sanger MGP). The WP9 partners offer eligible customers access to the unique resources and expertise provided by the world leading mouse clinics. Each center is fully equipped with state of the art research instrumentation to facilitate **comprehensive phenotypic analyses covering a wide range of physiological functions ranging from cardiovascular, neuro-behaviour and metabolism expertise to detailed pathology assessments.** Routinely, all phenotyping activities are performed in a high-throughput fashion, as a service to support internal programs or for the wider scientific community. For WP9 the partners offer access to this unique infrastructure. The free of charge access unit offered covers the production of a cohort and a comprehensive first line phenotyping.

Progress towards objectives of WP

1st line phenotyping analysis of 22 mouse mutant lines

Details of the evaluation process and of the advertising of open TA calls are described in detail in the WP2 report. In brief, interested customers applied via specific online request forms accessible via the INFRAFRONTIER website for one of the 22 free access units qualifying for the phenotyping of a cohort of mutant and control mice. The **INFRAFRONTIER external Evaluation Committee** assessed all applications using defined criteria and recorded evaluations and the justification for their assessment in a dedicated reporting form. Each application was evaluated by two external Committee members. Upon successful approval of TA applications the INFRAFRONTIER-I3 Project Office assigned the projects to one of the WP9 mouse clinics in consultation with the centers and taking into account applicant's preferences. The cohort production and phenotyping was then carried out as described in detail in the Description of Work. In case of Sanger the service provided covered the production and phenotyping of a mouse mutant line from the available in-house ES cell library. As a first step, the mouse clinics contacted the successful applicants to initiate the project by organizing the importation into the respective INFRAFRONTIER mouse clinic where cohorts were bred, phenotyped and archived. Making mouse mutant lines and associated phenotype data available to the wider community is a requirement of the INFRAFRONTIER-I3 project.

INFRAFRONTIER-I3 phenotyping Trans-national Access calls

During the first reporting period a total of 2 TA calls were published and fully executed resulting in the assignment of 25 mouse phenotyping TA projects to successful applicants. Sanger has taken on three additional projects to account for possible dropouts during mouse production. A first call was published in May 2013 followed by a further call in April - May 2014. Overall, **a total of 76 project proposals from applicants across 14 eligible countries were submitted and evaluated**. The origin of all applicants is shown in Figure 10.

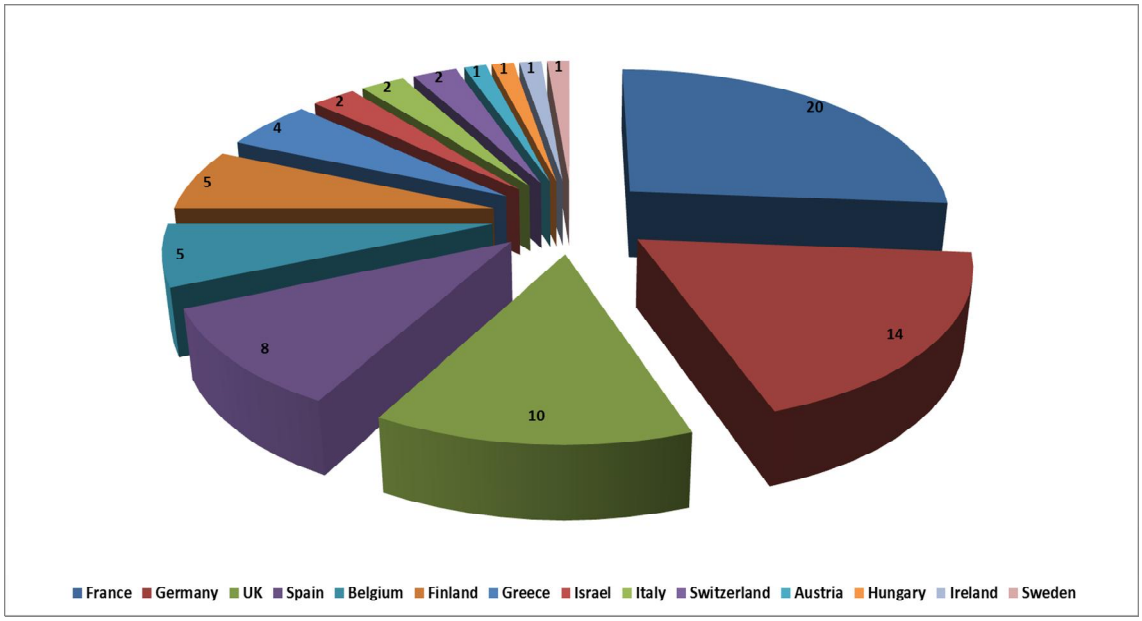

Figure 10: Origin of applications for mouse phenotyping TA calls

The origin of all successful applicants is shown in Figure 11. **A total of 25 / 76 applicants i.e. 33 % were successful**.

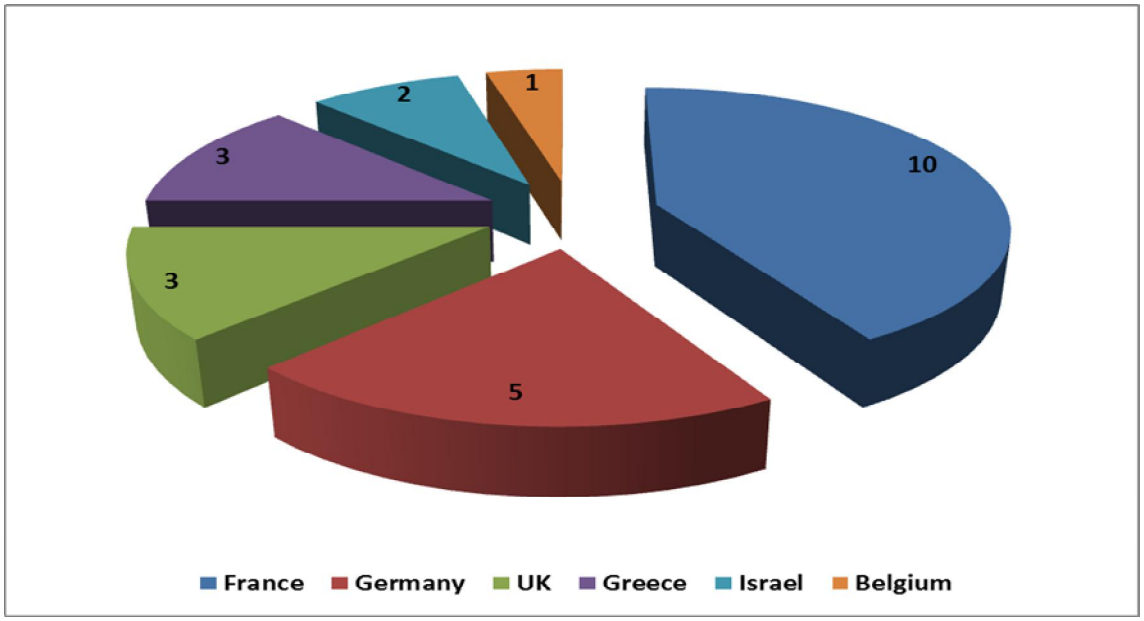

Figure 11: Origin of applications for granted mouse phenotyping TA slots

An overview of all selected applicants and their projects is given in table 4, pages 32-33. Successful project proposals cover the production and phenotyping of new mouse models where no previous model exists, or the development of a conditional ready KO first allele that can be used in further steps to produce conditional lines to advance analyses of existing full KO lines. Other projects characterize e.g. triple KO lines, models for Down-Syndrome with large duplications or conditional KO lines. It should be emphasized that these are all types of projects that are not covered by large scale initiatives like the International Mouse Phenotyping Consortium (IMPC) and that the INFRAFRONTIER-I3 project is providing a unique phenotyping service to the wider community. A wide range of phenotypes / research areas is covered by the accepted projects as shown in table 4. Examples are mice with **phenotypes indicating metabolic and / or growth deregulation and may be related to obesity, a KO mouse model able to suppress toxicity observed in existing polyQ mouse models, a new Spinocerebellar ataxia 7 (SCA7) mouse model, Down Syndrome models, and a G-protein coupled receptor GPR85 KO line and potential schizophrenia model**, amongst others.

Excellent progress has been made in period 3 and all projects required to fulfill the WP9 deliverable of phenotyping 22 mouse mutant lines were finalized. By the end of period 3 a total of 24 phenotyping projects were completed. The Sanger Institute generated 10 new mouse models of which 9 are completely phenotyped thus exceeding their project deliverable of 7 lines. The progress and final status of WP9 is shown in table 5, page 34.

Overall, as for the mouse production TA a smooth running process for promoting and evaluating mouse phenotyping TA calls has been established. Following a good community response a total of 25 project proposals were allocated that led to the development and characterisation of novel mouse models of human disease as well as to the in depth characterisation of existing mouse mutants. Importantly, the **projects run by the GMC, ICS and the Mary Lyon Centre cover a broad range of mutations and genetic backgrounds going far beyond the activities of large scale initiatives like the IMPC that rely on standardised processes**. In this respect this is a special service providing a unique infrastructure and technical expertise to individual investigators.

The resources and data generated by this work package will be exploited by making all data and mouse lines publicly available via EMMA / INFRAFRONTIER and the IMPC database. Furthermore, the in depth phenotype analysis by the mouse clinics led already to **joint publications with the applicants**. The project of David Elliott (Khdrbs3 T-STAR, TA provided by MRC) has resulted in a publication in Cell Reports

Cell Rep. 2016 Dec 20;17(12):3269-3280. doi: 10.1016/j.celrep.2016.12.002. Ehrmann I et al., A SLM2 Feedback Pathway Controls Cortical Network Activity and Mouse Behavior.

A further publication has been submitted to Scientific Reports of the NATURE publishing group. Two more publications are currently being prepared and some TA phenotyping projects led to follow-up studies to generate more extensive data towards scientific publications.

Mouse clinic	Call	Applicant	Gene of interest	Project objective
HMGU / GMC	**1**	**Kollias**	**TNFRSF1A**	1st line phenotyping of transgenic line expressing human TNFR1 under the CollagenVI promoter. Mice are characterised by increased body size and mass. Phenotype indicates metabolic and / or growth deregulation and may be related to obesity
	1	**Reiner**	**Zdhhc2**	1st line phenotyping of Zdhhc2 mice. Zdhhc2 is an acyl transferase which is palmitylating Ndel1 in the developing brain
	1	**Voigt**	**TRMT2A**	Phenotyping of TRMT2A (tRNA Uracil5 methyltransferase) KO mouse to develop a mouse model able to suppress toxicity observed in existing polyQ mouse models
	2	**Zerial**	**Appl1/Appl2**	1st line phenotyping of APPL1KO/APPL2KO double-knockout mice. Mutants display hyperleptinemia, hypertriglyceridemia and hyperinsulinemia but no changes in glucose levels. Model to study metabolic dysfunctions (e.g. diabetes)
	2	**Kontoyiannis**	**HnrnpA3**	1st line phenotyping of hnRNPA3 mutant mice. hnrnpA3 (heterogeneous nuclear ribonucleoprotein A3) may be a key post-transcriptional organizer for stress-related post transcriptional regulation
CERBM-GIE / ICS	**1**	**Brault**	**Stch-App 9.2 Mb duplication**	Phenotyping of Ts2Yah mouse model that was developed to search for dosage-sensitive genes involved in DS. Ts2Yah mice carry a tandem duplication of the Stch-App interval on mouse chromosome 16. The model displays impaired locomotion that gets worse with age associated with increased muscle force
	1	**Trottier**	**Ataxin-7**	Comprehensive first line phenotyping of a new Spinocerebellar ataxia 7 (SCA7) mouse model / SCA7100Q/5Q . SCA7 is a neurodegenerative disease characterized by progressive ataxia and blindness
	1	**Blasig**	**Claudin-12**	First line phenotyping of Claudin 12 mutant mice. Claudin 12 (Cldn12) is a Tight Junction protein with unknown function up to now
	1	**Boucher**	**ShcA**	Characterize in vivo the role of ShcA in energy balance and weight management and to determine whether ShcA is indeed an interesting candidate to protect against diet-induced obesity and its comorbidities
	1	**Kieffer**	**GPR85**	1st line phenotyping of G-protein coupled receptor GPR85 KO mice. GPR85 function is poorly understood, it has been proposed to play a role in the development of schizophrenia
MRC / MLC	**1**	**Elliott**	**Khdrbs3 T-STAR**	Comprehensive phenotyping of T-STAR KO mice to develop a model for neurological disorders
	1	**Tybulewicz**	**Ts(lipi-Znf295)1Tyb**	1st line phenotyping of Ts1Tyb mice, a mouse model of Down Syndrome. s(Lipi-Znf295)1Tyb (Ts1Tyb) has a duplication of the entire 22.9Mb region of Mmu16 increasing the dosage of about 233 genes from 2 to 3 copies
	1	**Stockinger**	**CYP1A1 CYP1A2 CYP1B1**	1st line phenotyping of a triple knockout for the three cytochrome P450 enzymes. It is particularly the potential link between perturbations of metabolic pathways and immune functions that is of interest to study in this mouse
	2	**Goridis**	**Phox2b**	1st line phenotyping of Phox2blox/lox;Lbx1cre/+ mutants providing for the first time critical information on the function of dB2 interneurons as a whole. Phox2b is a transcription factor essential for proper development of the autonomic reflexes controlling cardiovascular, respiratory and digestive functions

	2	**Leveillard**	**BSG1 isoform**	First line phenotyping of Bsg1 KO mice. The Bsg gene is expressed specifically by photoreceptors (PRs) as an RdCVF interacting protein. The Bsg KO mouse shows a progressive degeneration of PRs
Sanger	**1**	**Eisenbach**	**Ifitm6**	Production and phenotyping of Ifitm6 mice to investigate the roles of IFITM6 in inflammation and cancer of the gastro-intestinal tract
	1	**van Veldhoven**	**Hao2**	Production and phenotyping of a novel Hao2 KO model. Hao2 encodes a FMN dependent oxidase associated with renal peroxisomes
	1	**Schob**	**Lsm14b**	Production and 1st line phenotyping of mice lacking Lsm14b / RAP55B, a protein involved in primary biliary cirrhosis
	2	**Laporte**	**Mtmr1**	Production and 1st line phenotyping of Mtmr1 KO mice. Mtmr1 expression is altered in myotonic dystrophy and MTMR1 homologs are implicated in several neuromuscular diseases
	2	**Kindler**	**Begain**	Production and 1st line phenotyping of Begain knockout mice, a putative animal model for human mental diseases
	2	**Duchesne**	**Cep250**	Assess the phenotype associated with a knockout of the mouse gene encoding Cep250. This animal model could be of particular relevance for extending studies on this spectrum of human disease that encompass autosomal recessive microcephaly and Seckel-like syndrome
	2	**Aidinis**	**Sh3pxd2a**	Production and first line phenotyping of Sh3pxd2a mice. The conditional KO mouse line will be further used in examining the role of SH3PXD2A in lung pathophysiology
	2	**Bahram**	**MYH3**	Production and 1st line phenotyping of Myh3 KO mice to confirm the pathological role of MYH3 in Spondylocarpotarsal synostosis syndrome (bone disorder) and to better understand the pathophysiology of this disease
	2	**Ghyselink**	**8030411F24Rik**	Production and 1st line phenotyping of 8030411F24Rik (Cystatin14) KO mice to investigate the physiological function(s) of 8030411F24Rik in vivo and to assess whether the gene is regulated by retinoic acid
	2	**Martinez-Chantar**	**DCUN1d3**	Production and first line phenotyping of DCNL1 (Defective in cullin Neddylation) KO mice to broaden knowledge into the real dimensions of the occurring NEDDylation posttranslational modifications and their role in the regulation of liver physiology

Table 4: Allocated mouse phenotyping TA slots and research area of proposed projects

Call	Applicant	Gene of interest	Customer contacted	MI in progress	Germ line transmission	Importation / cohort breeding	Phenotyping started	Phenotyping completed
1	Kollias	TNFRSF1A		not applicable	not applicable			
1	Reiner	Zdhhc2		not applicable	not applicable			
1	Voigt	TRMT2A		not applicable	not applicable			
2	Zerial	Appl1/Appl2		not applicable	not applicable			
2	Kontoyiannis	HnrnpA3		not applicable	not applicable			
1	Brault	Stch-App 9.2 Mb duplication		not applicable	not applicable			
1	Trottier	Ataxin-7		not applicable	not applicable			
1	Blasig	Claudin-12		not applicable	not applicable			
1	Boucher	ShcA		not applicable	not applicable			
1	Kieffer	Penk		not applicable	not applicable			
1	Elliott	Khdrbs3 T-STAR		not applicable	not applicable			
1	Tybulewicz	Ts(lipi-Znf295)1Tyb		not applicable	not applicable			
1	Stockinger	CYP1A1 CYP1A2 CYP1B1		not applicable	not applicable			
2	Goridis	Phox2b		not applicable	not applicable			
2	Leveillard	BSG1 isoform		not applicable	not applicable			
1	Eisenbach	Ifitm6						
1	van Veldhoven	Hao2						
1	Schob	Lsm14b						
2	Laporte	Mtmr1						
2	Kindler	Begain						
2	Duchesne	Cep250						
2	Aidinis	Sh3pxd2a						
2	Bahram	MYH3						
2	Ghyselink	8030411F24Rik						
2	Martinez-Chantar	DCUN1d3		Crispr/Cas				
			25	10	10	25	24	24
			Customer contacted	MI in progress	Germ line transmission	Importation / cohort breeding	Phenotyping started	Phenotyping completed

Table 5: Progress of allocated mouse phenotyping TA projects / Dec 2016

3.9 WP1 Project management

The objective of WP1 was to set up an effective management framework for the INFRAFRONTIER-I3 project, to ensure progress towards its planned objectives and contractual commitments. The specific work package objectives were:

- Project communication and integration of all project activities
- Project controlling, benchmarking
- Project reporting
- Dissemination and exploitation of project results
- Communication with EC and financial management

Beyond the standard project management activities a focus of WP1 activities was on the definition of **key performance indicators and data analysis tools to support project and research infrastructure performance controlling** and on **benchmarking studies** and in particular for the EMMA repository and related international mouse archives.

As a new analysis tool **Tableau software** was implemented and integrated into the INFRAFRONTIER IT architecture by the informatics team and the Project Office in the first reporting period. As part of the database relocation task also Tableau software and associated licenses were relocated from EMBL-EBI to the INFRAFRONTIER GmbH and integrated into the local IT architecture. Tableau software (http://www.tableausoftware.com/) aids the visual analytics of data captured in the INFRAFRONTIER / EMMA database.

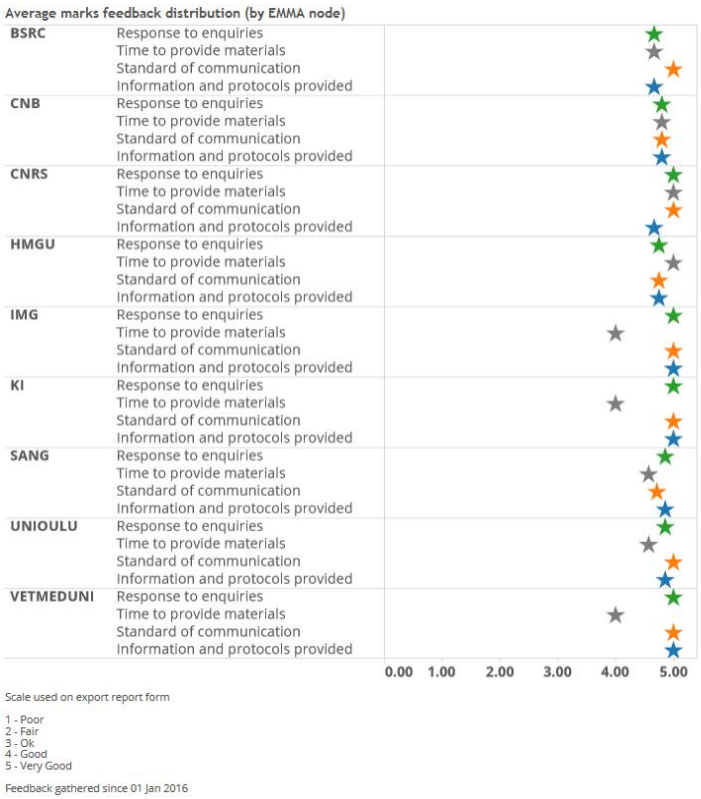

Fig 12: Analysis and visualisation of user feedback using Tableau software

The Tableau software integrates with the EMMA database and is continuously being used by the Project Office to prepare a comprehensive set of live standard reports covering the archiving and distribution activities of EMMA. A focus in period 3 was on the implementation of online user feedback forms and the integration with Tableau software for data visualisation and analysis (see figure 12). This simplifies the user feedback analysis which was managed in previous periods via Emailing feedback forms and analyses in Excel. At present a total of 30 instant data reports are available and accessible for all project partners, and data are regularly used for controlling and reporting purposes.

Benchmarking of INFRAFRONTIER services

A major deliverable of INFRAFRONTIER-I3 was a **benchmark analysis that will cover both an internal benchmarking as well as a comparative analysis using performance data of other major mouse repositories**. Benchmarking is a systematic process for identifying and implementing best or better practices. A benchmark study can be implemented as a performance benchmarking whereby performance levels of organizations for a specific process are compared, or as a best practice benchmarking to assess processes rather than associated performance levels. For a performance benchmark study **key performance indicators (KPIs)** that are used to gauge performance must be defined. KPIs are (i) a way to measure progress towards the benchmark goals, (ii) reflect organizational goals and (iii) must be quantifiable. Benchmark data are then the foundation for a **Continuous Improvement Plan (CIP).**

As a first step KPIs were defined as basis for the benchmark study. Comparative data that exist and are publically available are **1)** the available mouse mutant lines listed at the International Mouse Strain Resource (IMSR) database. The IMSR (http://www.findmice.org)/ is a searchable online database of mouse strains, stocks, and mutant ES cell lines available worldwide, including inbred, mutant, and genetically engineered strains. The goal of the IMSR is to assist the international scientific community in locating and obtaining mouse resources for research. The data content found in the IMSR is as supplied by strain repository holders. As a further indicator we use **2)** the referrals from the IMSR to repository websites that are provided by the IMSR operators. Further KPIs that are recorded but require the cooperation and provision of data by other repositories are **3)** web access statistics, **4)** the number of strain deposits per year, **5)** the number of orders and shipments per year, **6)** the number of infrastructure users per year, **7)** turnaround times for archiving and distribution services (for distribution the turnaround times for re-derivation of mice from frozen stock, the shipment of frozen material and the shipment of mice kept on shelf are recorded).

Following the implementation of the Tableau data analysis and reporting software, reports for the above mentioned KPIs 4 to 7 were prepared and are now available at any time with current data. Web access stats were recorded by the EBI and now by INFRAFRONTIER and monthly reports were provided to the INFRAFRONTIER Project Office. IMSR data are publicly available or provided by IMSR upon request. Additional performance monitoring data that are recorded via Tableau are electronic user feedback forms.

A report with INFRAFRONTIER 2013 data and 2014 data for the first half of the year was compiled and sent to other major repositories which expressed their interest to participate in this benchmarking study. Comparative data were provided by RIKEN BRC and the Australian Phenomics Network. During the second reporting period benchmark data were collated for 2014 from all participating repositories. The data for 2015 and 2016 are now being compiled and

compared with RIKEN BRC only. The RIKEN repository operation is of a similar scale and ideally suited for performance comparisons. It should be noted that EMMA compares very favourably to other repositories when frozen versus live shipments are compared, which are twice as high for EMMA as for other repositories. During the course of the project the number of frozen shipments increased from 40% to 49% by the end of 2016.

As stated above, existing benchmark data that can be obtained from IMSR are the number of available mouse mutant lines in the IMSR database. EMMA records in IMSR are automatically updated on a weekly basis. Figure 13 shows the data as recorded in Dec 2016.

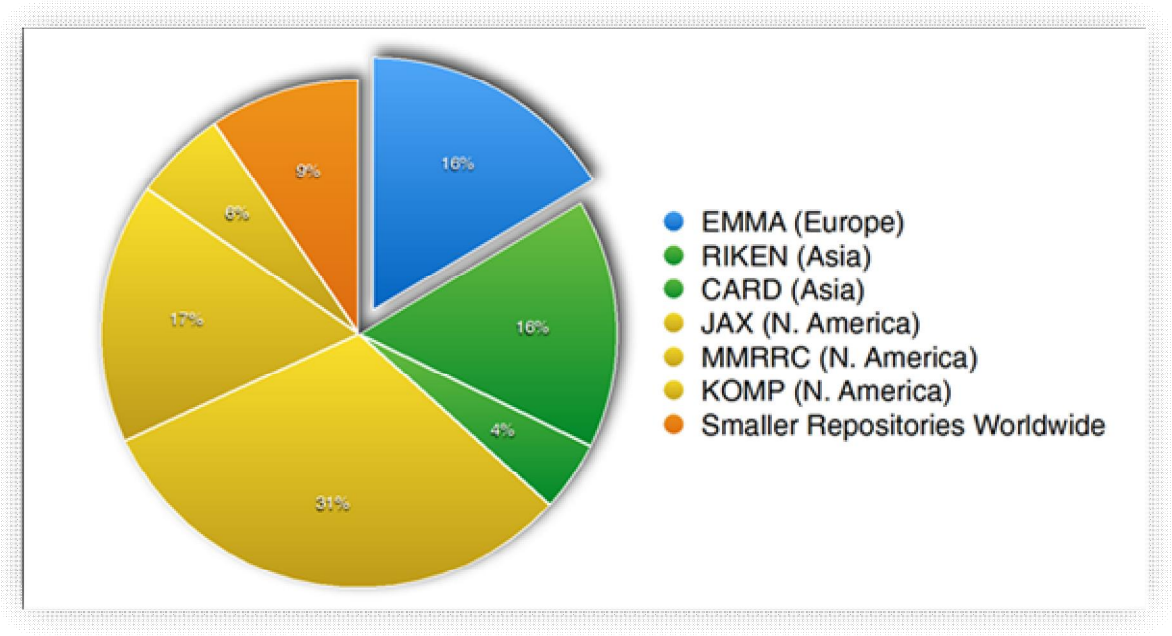

Figure 13: Repository contributions to IMSR data (Dec 2016)

The IMSR data show that **EMMA is firmly established together with RIKEN BRC as the 3rd largest mouse repository worldwide** with a stock holding that is close to that of the US Mutant Mouse Regional Resource Centre (MMRRC).

The recorded performance data allow also an internal benchmark analysis. Figure 14 shows the service times of individual EMMA partners for the distribution of mouse resources in 2016. Recorded are the times **1)** from order receipt until the MTA has been processed, **2)** from order receipt until payment information has been provided, **3)** from order receipt until all required paperwork such as import permits are in place and **4)** from order receipt until actual shipment. The average time across all centres from order receipt until shipment of frozen stock is now at 2 months and decreased from 3.2 months in 2014. The observed differences among centres, offer the opportunity to assess local processes in detail to identify best practices. This will facilitate a continuous improvement of the overall performance across the network.

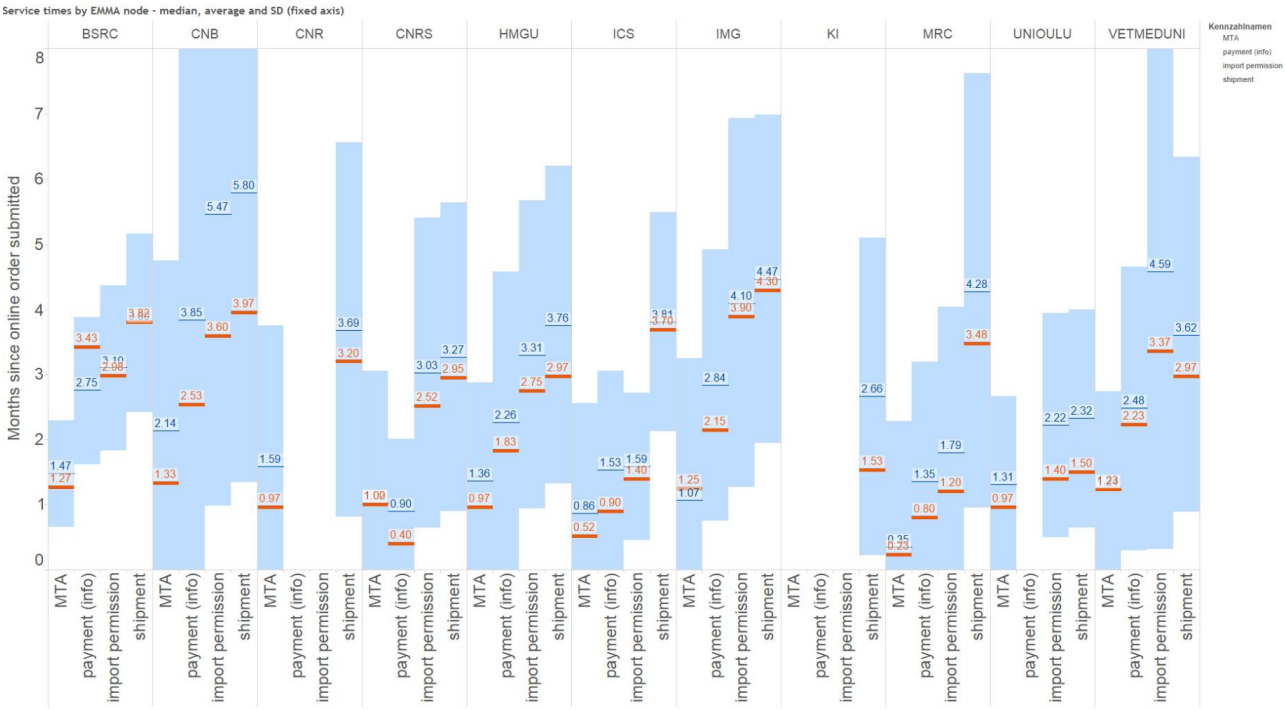

Figure 14: Service times for processing requests across EMMA partners
Shipments of mouse resources in 2016

4. Potential impact, dissemination activities and exploitation of results

The key **expected impacts** of the INFRAFRONTIER-I3 project as listed in the 2012 FP7 Capacities Work Programme were as follows:

1) Structuring impact on the ERA and on the way research infrastructures operate, evolve and interact with similar facilities and with their users
2) Operators of infrastructures will develop synergies and complementary capabilities in such a way as to offer an improved access to researchers and to develop their innovation potential
3) A more coordinated approach between infrastructure operators, users and public authorities will enable to optimize the development, use and sustainable operation of the identified research infrastructures.
4) A closer interaction between a large number of researchers active in and around a number of infrastructures will facilitate cross-disciplinary fertilizations and a wider sharing of knowledge and technologies across fields and between academia and industry.
5) Integrating Activities should also contribute to increase the potential for innovation of the related research infrastructures

The executed INFRAFRONTIER-I3 project did directly and comprehensively address in many ways all the impacts as described in the 2012 work program.

4.1. INFRAFRONTIER-I3 did contribute to shaping the European Research Area

The INFRAFRONTIER-I3 project had a significant impact on structuring the European Research Area (ERA) by bringing together the leading European mouse repositories under the umbrella of the EMMA network and existing European mouse clinics as well as the Czech Centre for Phenogenomics (CCP) as a new emerging mouse clinic. Overall, 23 partners from 16 different countries constituted the INFRAFRONTIER-I3 consortium, encompassing a total of 16 EMMA nodes representing 14 European countries including three new member states, namely Belgium, Netherlands and Israel. In particular the Dutch EMMA node was incorporated into the INFRAFRONTIER/EMMA service portfolio by providing access to a unique collection of ES cell models for rapid target gene validation in complex cancer mouse models.

Furthermore, the four European mouse clinics at the Helmholtz Centre Munich, the ICS in Strasbourg, MRC Harwell and the Sanger Institute, which have undertaken the pioneering phenotyping of 500 mouse mutant lines during the course of the EUMODIC project, contributed their unique infrastructure and expertise to the INFRAFRONTIER-I3 project. In addition to the mouse clinics already in full operation, three partners were implementing new mouse clinics, namely the Monterotondo Mouse Clinic at CNR, the Czech Centre for Phenogenomics (CCP) and INSERM / CIPHE providing highly specialised expertise in immunophenotyping (INSERM / CIPHE), and greatly benefitted from engaging in the INFRAFRONTIER-I3 project and the exchange of best practices. All participating mouse clinics also contribute to the International Mouse Phenotyping Consortium (IMPC, www.mousephenotype.org) underpinning their visibility on a global scale.

.

Access to mouse production expertise and capacities was provided by the INFRAFRONTIER-I3 partners CNB-CSIC (Madrid), Fleming (Athens), University of Oulu, the IMG Prague and the VUW in Vienna. The highly specialised axenic service was provided by the Portuguese partner with support from CNRS (Orleans), MRC (Harwell) and the CNR (Monterotondo) in networking activities. The European Bioinformatics Institute (EBI) was the lead partner for the INFRAFRONTIER-I3 informatics activities and supported by the CNR (Monterotondo) focusing on data curation, partners at MRC Harwell and the Sanger Institute providing user input into the informatics group, and the Project Office at HMGU contributing project management requirements. Finally, the INFRAFRONTIER GmbH, coordinating activities of the INFRAFRONTIER research infrastructure, joined as additional project partner during the 3rd reporting period and has taken over the lead in IT developments.

Finally, addressing and underpinning the EU Innovation Union policy the INFRAFRONTIER-I3 project also included a highly innovative German SME, TSE Systems GmbH, one of the leading manufacturers of high-quality research instruments in the world. Engagement in the project supported the development of a new and complementary project funded by the German Research Ministry (BMBF) involving TSE, the Czech Centre for Phenogenomics and the Helmholtz Centre Munich. What is more, WP5 technology development efforts involved a highly innovative German SME, namely AYOXXA Biosystems GmbH for the joint development of multiplex protein analysis assays.

4.2 INFRAFRONTIER-I3 supported FP7 Capacities and Cooperation Specific Programs as well as H2020 projects

The INFRAFRONTIER-I3 project significantly contributed to and impacted other EC funded research projects and EU and global initiatives in mouse functional genomics and also greatly benefited from these collaborations. Specifically, building on the successful collaboration with EUCOMM the EMMA network supported also the **EUCOMMTools** project by archiving up to 250 lines of Cre driver mice on a pure C57BL/6N background. These mice form a matched Cre driver resource for C57BL/6N mice produced from conditional IKMC resources. It is anticipated that this unique resource will be highly demanded, as it allows capitalizing on and fully exploiting the conditional IKMC resources.

The ERA also greatly benefits from a close collaboration of the **Biomedical Sciences research infrastructures,** which collectively provide support to the wider European biomedical research community along the entire value chain ranging from target identification and validation to preclinical models, translational and clinical research. An overarching activity needed to facilitate and optimise the collaboration and interaction among the BMS RIs is bioinformatics and the interoperability of data and services. To develop and implement a common e-infrastructure among the BMS research infrastructures the **Biomedbridges** consortium consisting of 21 partners formed in 2011. INFRAFRONTIER was represented in Biomedbridges by the co-ordinator and the EBI and led a specific use case 'Phenobridge: crossing the species bridge between mouse and human' aiming to identify and develop a set of annotations, necessary terminologies, and mappings between terminologies for human and mouse models of diabetes and obesity. INFRAFRONTIER is also involved in a follow up project of the BMS infrastructures, **CORBEL** - Coordinated Research Infrastructures Building Enduring Life-Science Services (2015-2019, coordinated by ELIXIR). CORBEL will establish a collaborative framework of shared services between the ESFRI Biological and Medical

Research Infrastructures that transform the European research community from discovery of basic biological mechanisms to applied medical translation – through the provision of a unified interface, aligned services and coordinated user access to a range of advanced technology platforms. INFRAFRONTIER contributed systemic phenotyping services offered as Trans-national Access in the INFRAFRONTIER-I3 project as a service offer to the first joint public call of the CORBEL project.

4.3 INFRAFRONTIER-I3 supported and interacted with global initiatives such as the International Mouse Phenotyping Consortium (IMPC)

The INFRAFRONTIER partners have established an extensive network and collaborations across the EU and worldwide, that are essential for the successful operation of the network. A key INFRAFRONTIER partner is the IMPC, a global project to carry out systemic phenotyping of mouse lines for each of the approximately 20.000 protein-coding genes in the mammalian genome to create an encyclopedia of mammalian gene function. Thus, whereas INFRAFRONTIER aims at building and operating a transnational European research infrastructure with all its organizational, legal and administrative consequences, the IMPC is a research project, which merely by its scale requires the participating facilities, both within Europe and globally, to upgrade their infrastructure. At present 9 INFRAFRONTIER partners contribute to this global phenotyping effort. The IMPC and INFRAFRONTIER data are tightly integrated, facilitating easy access of phenotype data if clients navigate the INFRAFRONTIER / EMMA portal and fast access of mice if clients search the IMPC portal for phenotype data. By the end of 2016 INFRAFRONTIER/EMMA had a collection of 2684 IMPC mice available for distribution. This collection is widely used by the research community and contributes about 50% to all mouse resource shipments from the EMMA repository.

4.4 INFRAFRONTIER-I3 advanced the operation and user services of the emerging INFRAFRONTIER research infrastructure (RI)

The INFRAFRONTIER RI is committed to continuously improve its operation and user focus and services. The INFRAFRONTIER-I3 project implemented a variety of measures that significantly improved operational efficiencies and the diversity and quality of user services. Specifically, these measures cover:

· **Benchmark analyses and performance controlling (WP1, Management)**

The INFRAFRONTIER Project Office and the informatics team implemented Tableau Business Intelligence software facilitating data analyses and instant reports on INFRAFRONTIER key performance indicators. A comprehensive set of reports covering service times and archiving and distribution capacities of the EMMA repository and of individual nodes were implemented and inform a continuous improvement cycle to advance operations. Furthermore, data reports provided a foundation for an internal but also an external benchmarking exercise into which major other repositories such as RIKEN-BRC (Japan) actively engaged.

· **Invitation of a user representative to the INFRAFRONTIER-I3 Advisory Board (WP2, Networking)**

To reinforce the user focus of the INFRAFRONTIER-I3 project and research infrastructure we invited Prof Ian Jackson a renowned mouse geneticist of the MRC Human Genetics Unit in Edinburgh as a representative of the mouse genetics community. This builds on a successful practice followed in the INFRAFRONTIER preparatory phase project and in the EMMAservice project.

· **Implementation of an online user feedback form (WP2, Networking)**

A more user friendly online feedback form for all user services was implemented during the course of the INFRAFRONTIER-I3 project, replacing previous forms that were sent to clients via Email. The user feedback forms are linked to Tableau software facilitating instant provision of user feedback reports and analyses.

· **Organizing an industry liaison workshop (WP2, Networking)**

To facilitate engagement with industry stakeholders we organised a dedicated meeting for 80 participants including INFRAFRONTIER and IMPC PIs, funders, and representatives of 30 companies. Represented companies included BioPharma, CROs and research instrumentation developers amongst others. The meeting facilitated direct input of BioPharma service needs e.g. emphasizing use of rat models and humanised mouse models in preclinical research. This was taken into account in the development of pilot services in further INFRAFRONTIER grant applications and service developments. The industry liaison meeting further supported the initiation of contacts that were strengthened at follow-up industry engagement meetings supported by complementary INFRAFRONTIER projects, and also led to two joined grant applications with industry partners.

· **Improving quality and diversity of INFRAFRONTIER resources and services**

The INFRAFRONTIER-I3 project had a significant **scientific impact** by offering access to state of the art archiving, mouse production and phenotyping services leading to the development of novel resources available to support basic and applied biomedical research. Specifically, improvement of resources and services covers:

o **Providing a free of charge archiving service adding a further 1215 mouse mutant lines to the EMMA archive (WP4)**. A further 656 mouse lines were archived by EMMA partners during the course of the INFRAFRONTIER-I3 project using complementary national funding sources. This clearly documents the demand for the INFRAFRONTIER/EMMA archiving service and the research infrastructure capacity that has been developed by the consortium. The deposition of mouse lines into the EMMA repository ensures that lines are cryopreserved under the highest quality standards. **Archiving benefits the depositors** by conserving the financial, animal house and labour resources which would otherwise be needed to maintain the breeding colonies. In addition, cryopreservation also ensures the availability of these valuable resources to future generations of research scientists and thus **maximizes the return of the public investment** made into generating these resources. This obviates the need to reproduce these resources at a high cost and investment in time. **The addition of a total of 1871 mouse mutant lines to the EMMA repository also significantly contributed to the quality of the available resource for the benefit of the wider community.** This can be

attributed to the strain donations from individual researchers as well as to the collaboration with large scale projects such as EUCOMMtools and the IMPC.

- **Incorporation of the Netherlands Cancer Institute GEMM-ESC archive into INFRAFRONTIER (WP2).** The INFRAFRONTIER-I3 project facilitated the distribution of a unique archive of embryonic stem cells derived from validated genetically engineered mouse models of cancer. The INFRAFONTIER partner Netherlands Cancer Institute is developing this ES cell collection and INFRAFRONTIER informatics was involved in providing an online resource to allow users to obtain information and order these ESC clones. The INFRAFRONTIER webpage gives access to extensive documentation about these lines by providing allele maps, protocols and links to publications describing the resource. The NKI GEMM-ESC can now be accessed to **support rapid target gene validation in complex cancer mouse models.**

- **Trans-national Access to mouse production and phenotyping capacities (WP8 and WP9).** This unique and free of charge service offered by INFRAFRONTIER-I3 facilitated use of the world class high-throughput phenotyping infrastructure and expertise offered by the participating mouse clinics. **This bottom up access for individual researchers on a European scale offers a service quality that goes beyond the more standardised approach of high-throughput top down projects such as EUMODIC or IMPC.** Specifically, flexibility for the phenotyping service was provided with regard to the nature of the mutant, the genetic background and the exact design of the phenotyping pipeline. There was a high demand for offered TA services as documented by a total of 206 proposals in response to 5 public calls. This led to 54 projects to develop and / or characterise novel mouse models of human disease covering a wide range of research areas such as metabolism and diabetes, neurodegenerative diseases and cancer. **Scientific excellence was demonstrated by high profile publications e.g. in CELL reports resulting from offered services**.

- **Technology development (WP5).** A key objective of the technology development efforts undertaken by INFRAFRONTIER-I3 was the improvement of existing user services as well as developing new service offers. To this effect the **cryo-group** optimised the procedures for transporting sperm in the absence of liquid nitrogen. This will significantly ease shipments between laboratories and also reduce the associated cost. Quality of services was furthermore improved by expanding the ICSI capabilities to virtually guarantee the recovery of mouse mutant lines from frozen gonadal tissue. Overall, the technology development efforts have been very successful and led to new SOPs that are implemented into the EMMA operation and are accessible for the community on the INFRAFRONTIER portal supported by training videos. Beyond the planned activities new development projects on robust embryo/oocyte vitrification were initiated which will have a significant impact on EMMA operation and external users. All technology development efforts had tremendous impact on cost, reduction of animal use and throughput of archiving projects.

  The technology development efforts of the **phenotyping group** focused on the development of new phenotyping protocols. This is in response to extensive surveys undertaken by IMPC where the community asked for novel phenotyping tests to be included in the pipelines. Focus for new phenotyping tests were **biomarkers** which

measure a variety of important physiological systems and can be highly correlated with onset of disease ranging from diabetes to cancer. Analyzing biomarkers in serum and urine, or other biological fluids represent a treasure trove for phenotypic analysis and discovery of preclinical models for human disease. There is a real need for identifying the next generation of multiplex technology which will enable flexibility of assay design covering different therapeutic areas and at lowered cost. Multiplexing will also reduce the number of mice used in research since many tests will be performed on a single animal. Development of such assays will also guarantee a better comparison of the models with a stronger predictivity to human disease conditions. In **collaboration with the German biotech company AYOXXA a pilot multiplex assay on their proprietary Lunaris™ platform was tested and validated** and a joint publication describing the study is underway. In addition, focus was on the **development of imaging protocols for high-throughput applications**. Molecular imaging allows non-invasive assessment of biological processes in living subjects. It is well recognized that applying imaging modalities to small animal preclinical models can increase our understanding of pathophysiological processes and can boost the development of new imaging probes and drugs. Especially in the field of genetically engineered mice, imaging brings key information thanks to its ability to measure the *in vivo* anatomic and functional phenotypes of the models. Extensive testing and validation of imaging modalities supported the **development of three novel SOPs on µCT imaging** of E18.5 mouse fetuses, High Resolution Episcopic Microscopy HREM for embryo phenotyping, and µCT for analysis of adult mouse bone microarchitecture. All newly established SOPs are available for implementation by all consortium partners and beyond e.g. in the global IMPC network.

- **INFRAFRONTIER informatics (WP6).** All informatics activities underpin user services by ensuring data quality and maximizing integration with other relevant data resources. The extensive expertise of the **curation group ensured accurate nomenclature and quality of data** associated with strain submissions to EMMA. By the end of the project a total of **2763 lines had their background, transgene, genes, alleles and strain names manually reviewed and curated**. The use and accessibility of the EMMA resources was further enhanced by the integration with other appropriate strain, genomic and phenotypic resources such as ENSEMBL, and the International Mouse Phenotyping Consortium (IMPC) portal, human disease databases including Online Mendelian Inheritance in Man (OMIM), the Mouse Genome Database (MGD) and the International Mouse Strain Resource (IMSR) at the Jackson laboratory.

4.5 The INFRAFRONTIER-I3 project increased the potential for innovation

Economic impact of resources and services offered via INFRAFRONTIER-I3

The INFRAFRONTIER-I3 project provided access to mouse production and phenotyping services which may contribute to the development and characterization of new mouse models of human disease. Furthermore, all submitted mouse mutant lines for the archiving service potentially constitute valuable mouse models. Beyond the INFRAFRONTIER-I3 project, all mouse mutant resources available from EMMA will be distributed on a cost recovery basis to support basic and applied research. In **basic biomedical research** the identification of the genetic bases for human disease is a fundamental goal and the investigation of gene function through mouse mutants and phenotyping is a central element

in achieving this goal. **The disease models available from INFRAFRONTIER can be used to address basic and fundamental scientific questions about in *vivo* gene function and may further our understanding of disease genetics**. Overall, the mouse is widely regarded as the best model system for developing an understanding for human biology. In **BioPharma** mouse models are used for addressing more applied questions ranging from the **identification and validation of novel drug targets to the analysis of drug action and side effects and safety and efficacy testing of potential drugs**. Drug companies exploit phenotype results from mouse models at multiple key decision points during pre-clinical research, including target and compound selection, not just to validate which to go forward with, but also for avoiding unwanted target liabilities that could lead to failures later on in the clinic. Furthermore, genetically engineered mouse models are successfully used for testing treatment regimes in co-clinical trials in mouse and humans contributing to the rational design of clinical trials. The expectation from systematic functional annotation of the genome will be for the emerging mouse models to play an even more important role as the tool of choice to accelerate drug development. The comprehensive physical and data resource of INFRAFRONTIER will be available to support basic biomedical and preclinical translational (bench to bedside) research in Europe and globally. The available research tools and mouse models of human disease offer the opportunity to develop a better understanding of molecular disease mechanisms and may facilitate the identification of potential new drug targets thereby providing the foundation for the development of diagnostic, prognostic and therapeutic strategies. Thus, the **INFRAFRONTIER resources and services contribute to tackle grand challenges such as aging societies and public health and have a potentially huge societal and economic impact**. The potential economic impact of mouse models of human disease has been described in a seminal review by Zambrowicz and Sands (NATURE REVIEWS / DRUG DISCOVERY, Volume 2, January 2003) which analysed the 100 top selling pharmaceutical drugs in 2001. This analysis is a **compelling case for the power of gene knockout technology to describe the action of blockbuster drugs**. The analysed top 100 drugs modulate 43 host targets of which 34 were knocked out. A total of 29 of the resulting knockout phenotypes have been informative in terms of illuminating gene function and pharmaceutical utility and providing in most cases a direct correlation between KO phenotype and the therapeutic effect of the drug.

4.6 Dissemination and exploitation of project results

Major INFRAFRONTIER-I3 project results for exploitation were delivered by work package 3 (innovation), work package 4 (archiving) and work package 5 (technology development). In addition, WP8 and WP9 led to complementary resources and data for exploitation via INFRAFRONTIER or the users benefitting from the Trans-national Access. The innovation work package led to the development of a superior Metabolic PhenoCage system for use in phenotyping programs by mouse clinics and specialized phenotyping centres and is close to market entry. The INFRAFRONTIER-I3 cryopreservation activities and archiving efforts supported the development of the EMMA repository to the 3rd largest mouse repository worldwide holding now 6000 mouse mutant lines. This entire physical resource and the associated data are available for distribution and exploitation by the global biomedical research community. The INFRAFRONTIER-I3 technology development activities facilitated the development of novel SOPs for cryo-technologies and high-throughput phenotyping technologies that are made public and accessible for use by the wider scientific community.

4.6.1 Dissemination and exploitation of the INFRAFRONTIER services and resources

Public relations: The INFRAFRONTIER research infrastructure is undertaking a wide range of PR and outreach activities to attract new users and aims to raise the awareness of the wider scientific community, industry and general public for the objectives of INFRAFRONTIER. The general dissemination activities used by INFRAFRONTIER and the specific measures that were used to promote the INFRAFRONTIER-I3 project and all related research infrastructure activities covered a variety of complementary measures such as:

- **About 100 talks or poster presentations** foremost of the Coordinator and the Project Manager but also of the Management Board in general to promote the INFRAFRONTIER resources and services at conferences. This covered major mammalian genetics and human genetics conferences, EU consortia and IMPC meetings, conferences on research infrastructures, policy workshops, and FELASA meetings amongst others. The Board of Directors also represented INFRAFRONTIER at numerous national conferences.
- **INFRAFRONTIER flyers, brochures and USB sticks** with relevant information about the INFRAFRONTIER infrastructure and resources and services were distributed at conferences and workshops
- An INFRAFRONTIER **newsletter** was circulated via comprehensive customer mailing lists covering 7000 recipients
- **Trans-national Access calls** were extensively advertised via INFRAFRONTIER customer mailing lists, national contact lists, and community Email lists such as Mouse Genome Informatics (MGI)
- The INFRAFRONTIER services were promoted among members of the **International Society for Transgenic Technologies (ISTT)** via the ISTT mailing list and at the ISTT society conferences
- Complementary PR measures used in the INFRAFRONTIER-I3 project were the organisation of a **gnotobiology workshop** and an **industry liaison workshop**

Access to the INFRAFRONTIER services and resources

At the beginning of the INFRAFRONTIER-I3 project we redesigned and implemented a new website as part of the merging of the EMMA services under the INFRAFRONTIER umbrella, which can be seen at www.infrafrontier.eu. This design was built upon user feedback with the aim of easing navigation by giving overviews of different aspects of the project coupled with the ability to obtain details on demand. The search and retrieval of strain information in the EMMA resource is available by a highlighted section on the home page as well as an integrated search box. The EMMA search offers sub-lists for e.g. strains with certain mutation types, strains that can be used as research tools or strains available to industry users via genOway.

In addition to mouse strains, INFRAFRONTIER aids in the distribution of a unique archive of embryonic stem cells (ESCs) derived from validated genetically engineered mouse models of cancer. The INFRAFONTIER partner Netherlands Cancer Institute (NKI) is developing this resource and INFRAFRONTIER informatics was involved in providing an online resource to allow users to obtain information and order these ESC clones. The INFRAFRONTIER webpage gives access to extensive documentation about these lines by providing allele maps, protocols and links to publications describing the resource.

Finally, Trans-national Access (TA) to model development and phenotyping services and TA call documents were also provided to INFRAFRONTIER users on the portal. Training courses were announced on the INFRAFRONTIER portal and SOPs resulting from project activities are available for download. Monitoring of web access shows that strain searches and access to SOPs and protocols are the most widely used resources on the INFRAFRONTIER portal. A **detailed overview of all newly developed features of the web portal has been comprehensively described in the Nucleic Acids Research Journal dedicated Database Issue** in 2015.

Access to data resources, data integration and exchange methodologies

To support use and to add value to INFRAFRONTIER mouse resources we have continuously developed data integration and exchange methodologies with major mouse resources. To achieve this, we took a multi-pronged approach to support both high-throughput production centers and smaller groups of the INFRAFRONTIER consortium. As most high-throughput production centers are involved in the International Mouse Phenotyping Consortium (IMPC) and the EC FP7 funded EUCOMMTools project, we have modified the EMMA database to receive data from import mechanisms built for the IMPC mouse production tracking resource “iMits”. By this manner we were able to gather data from thousands of mouse strains being produced by these projects to facilitate their long term distribution via INFRAFRONTIER/EMMA. Further modifications to the iMits database allowed the incorporation of genome-edited mouse models from the iMits mouse production tracking database into INFRAFRONTIER/EMMA. As genome-editing technologies are quickly evolving, this is a critical step to provide access to the strongly growing collection of genome-edited mouse models. To support smaller groups not using the iMits tracker, we have also enhanced data submission by means of an online data submission form. A ‘wizard’ guides submitters smoothly through an 11-step process to record all the necessary data needed to deposit a strain. Data integration was further enhanced in the 3rd reporting period with the Mouse Genome Informatics (MGI) new MouseMine data warehouse system.

Users of the INFRAFRONTIER resources and services and expected impact

The comprehensive physical and data resources as well as the Trans-national Access services of INFRAFRONTIER supported basic biomedical and preclinical translational research in Europe and globally. The **available research tools and mouse models of human disease offer the opportunity to develop a better understanding of molecular disease mechanisms, may facilitate the identification of potential new drug targets, and provide the foundation for the development of diagnostic, prognostic and therapeutic strategies**. Many partners of the INFRAFRONTIER-I3 project have established user networks for their mouse programs. This proactive strategy is establishing consortia of scientists, including basic, clinical and industrial researchers, who can collaborate together around accessing mouse infrastructure and resources. Such active engagement will result in future collaborative research, both within and between EU countries, with the potential to increase industry participation.

4.6.2 Dissemination and exploitation of the INFRAFRONTIER-I3 innovation activities

Promoting the INFRAFRONTIER resources and services to the BioPharma industry via an industry liaison workshop

The INFRAFRONTIER research infrastructure and the INFRAFRONTIER-I3 project may benefit the BioPharma industries by providing access to its current and its pipeline of new

mouse mutant and data resources, which may add value to internal research and development programs. Industry access to infrastructures providing the resources that emerge from major new initiatives like the IMPC where 20,000 knockouts are being produced are of the highest priority with regard to driving the innovation process.

To support industry engagement the INFRAFRONTIER-I3 industry liaison workshop was held in Barcelona on November 13th at the Majestic Hotel following the annual IMPC meeting. The workshop was attended by 70 participants from among INFRAFRONTIER and IMPC PIs, IMPC industry sponsors, BioPharma, research instrumentation developers, and CROs. A total of 25 companies were represented at the meeting. Specific objectives of the industry liaison meeting were to:

- Present INFRAFRONTIER / IMPC resources and services
- Present successful INFRAFRONTIER industry engagement models
- Promote industry access to EUCOMM resources
- Have presentations on the use of mouse models in industry
- Have a partnering session and exhibition

The industry workshop is presented in detail on the INFRAFRONTIER portal where a meeting booklet and all presentations given at the meeting are available for download at: https://www.infrafrontier.eu/knowledgebase/workshops/infrafrontier-impc-industry-liaison-workshop.
The industry liaison meeting was instrumental to establish new industry contracts and to solicit feedback from BioPharma on demands for animal model development.

Building on the first industry liaison workshop and on the established contacts INFRAFRONTIER has taken advantage of the IPAD-MD project and organised an IPAD Industry & Innovation Worskhop, on June 28th to 29th 2016 in Munich, Germany. In future it is planned to sustain and further develop industry contacts and engagement via regular INFRAFRONTIER Annual Stakeholder meetings that will be funded by the H2020 funded INFRAFRONTIER2020 project from 2017 onwards.

Exploitation of the INFRAFRONTIER-I3 work package on innovation (WP3)
The innovation work package is a paradigm for partnering with industry. Focus was on the refinement of a Metabolic PhenoCage system currently in development at TSE systems. This joint approach and user driven product development provides added value for all parties, eventually leading to a superior product based on detailed user specifications with a more competitive market position. Overall, **during the INFRAFRONTIER-I3 project we jointly developed an entirely new Metabolic PhenoCage to measure a multitude of metabolically relevant parameters and with maximal flexibility to adjust to future scientific demands. Greatly improved animal welfare based changes adhering to current regulations and future anticipated changes were implemented**. The latest technical advances in terms of materials and technologies were used to come up with a flexible production version at lower cost of ownership. The final version represents a complete new cage that meets and exceeds anything on the market today and can be used for a vast variety of possible experiments where metabolic monitoring in combination with urine and feces collection is needed under very controlled conditions in standard laboratory animals and including applications in germ-free animals. **TSE Systems will now take the resulting project output further through the established in-house product development process and sell it on its established markets in Europe, USA and Asia**. As metabolic

research is an important concern for the public health sector as a whole, and is currently gaining in importance as a result of the worldwide obesity epidemic, the potential clients cover the whole spectrum of biomedical researchers in academia and industry.

Dissemination and exploitation of the INFRAFRONTIER-I3 work package on technology development and implementation (WP5)

Overall, the technology development efforts supported by the INFRAFRONTIER-I3 project (WP5) were very successful. A total of **six new protocols** related to cryopreservation technologies and **three new phenotyping SOPs** were developed and are now presented on the INFRAFRONTIER website for download. Access of users to the available SOPs is monitored and confirms a wide use of the protocols provided by INFRAFRONTIER. Development outputs were further disseminated by writing **six methods papers** plus a further 5 articles in preparation on various topics such as oocyte vitrification, frozen/thawed embryo shipments, shipping sperm on dry-ice, long term holding of sperm on dry ice, and transcervical embryo transfer. Beyond these dissemination activities new protocols and innovations are taught and demonstrated in the INFRAFRONTIER / EMMA training courses offered in INFRAFRONTIER-I3 WP2. All developed SOPs and videos can be accessed at: https://www.infrafrontier.eu/knowledgebase/protocols/cryopreservation-protocols

WP5 members had also taken the opportunity to work with commercial partners such as Air Sea Containers, to develop a cool box that can be used to maintain samples at refrigerated temperatures. These containers comprise a combination of cold packs and insulated materials and will keep the samples at 4-8°C for more than 72hrs. This allows sufficient time for transportation across Europe and between continents, if necessary. In addition, we have developed a cooling plate that can be used in conjunction with oocyte/embryo vitrification protocol. The cooling plate is now commercially available through Elim Springs Biotech Ltd.

4.6.3 Training courses - Dissemination of knowledge

The unique practical experience in cryopreservation technology and in systemic and secondary phenotyping available in the INFRAFRONTIER consortium was extensively disseminated by hosting a series of training courses and workshops. Overall, a total of **12 cryocourses covering mainly cryobiology but also more general topics such as handling of experimental animals were organised for nearly 100 students**. One cryocourse was held as a satellite event in conjunction with the International Society for Transgenic Technologies (ISTT) conference in Prague March 2016. This strongly supported visibility of INFRAFRONTIER training activities. Feedback on training courses was solicited and informed the agenda of follow-up courses. User feedback was also assessed six months after the courses to assess implementation of taught protocols. While INFRAFRONTIER partners run cryo-courses since 1999 phenotyping courses were implemented as a new training course format for the INFRAFRONTIER-I3 project. Overall, **4 phenotyping courses were attended by 100 students across Europe**. The comprehensive Phenogenomics course pioneered by the Phenomin network will be sustained beyond the INFRAFRONTIER-I3 project as a flagship training course format. Participants of the courses were scientists, students, and technicians e.g. working in transgenic units and phenotyping facilities. The training by INFRAFRONTIER in state of the art technologies and best practices supports the 3R principles and may lead to an overall reduction of mice that are used for biomedical research.

5. Project public website and contact details

Project acronym: INFRAFRONTIER-I3
Funding scheme (FP7): Combination of CP & CSA
EU financial contribution: €9.9 million
Total budget: €12.4 million
EU project officer: Keji-Alex Adunmo
Duration: 48 months
Start date: 1 January 2013
Completion date: 31 December 2016
Partners:
Medical Research Council - MRC Harwell, UK
Consiglio Nazionale delle Ricerche - Monterotondo, IT
Centre Européen de Recherche en Biologie et Médecine - Groupement d'Intérêt Economique, FR
Genome Research Limited, UK
Biomedical Sciences Research Centre Alexander Fleming, GR
Karolinska Institute, SWE
Fundação Calouste Gulbenkian, Instituto Gulbenkian de Ciencia,PT
Centre National de la Recherche Scientifique, FR
Universitat Autonoma de Barcelona, ES
Consejo Superior de Investigaciones Cientificas, ES
University of Oulu, FI
European Molecular Biology Laboratory - European Bioinformatics Institute, DE
Helmholtz Centre for Infection Research, DE
University of Copenhagen, DK
Institute of Molecular Genetics of the ASCR, v.v.i, CZ
University of Veterinary Medicine Vienna - Biomodels Austria, AT
Toronto Centre for Phenogenomics, CAN
Institut National de la Sante et de la Recherche Medicale, FR
Tel Aviv University, IL
Netherlands Cancer Institute, NL
TSE Systems GmbH, DE
Katholieke Universiteit Leuven, BE
INFRAFRONTIER GmbH, DE
Coordinator: Martin Hrabé de Angelis, Helmholtz Zentrum München - München (DE), hrabe@helmholtz-muenchen.de

Project webpage: www.INFRAFRONTIER.eu