PROJECT FINAL REPORT

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Project title: Servicing the European Biomedical Research Community: Archiving and Dissemination of Mouse Models of Human Disease

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1. Final summary report

1.1 Executive summary

To exploit the emerging mouse mutant resources, mouse models must be preserved and made available to the European biomedical research community. Building on EMMA's previous achievements as the primary mouse repository in Europe, EMMAservice met the challenges presented by archiving and disseminating mouse models in the European Research Area as follows:

Focus of the EMMAservice project was WP4, offering a free of charge cryopreservation service. A total of 1224 mouse mutant lines were submitted to the EMMA repository out of which 1094 were fully archived and publicly available by the end of the project. Strain donations were made across Europe with major contributions from UK, France and Germany. Mouse mutant lines were donated by a total of 250 different depositors. A further focus of the EMMAservice research activities was technology development (WP5) which underpinned the EMMA cryopreservation service by refining existing EMMA sperm freezing technologies. Following discussions with collaborators in Japan, plus further development work in the laboratory we were able to establish a robust sperm freeze/recovery protocol that works extremely well on sperm harvested from inbred strains of mice. The efficacy of this technique has been demonstrated by several partners within the EMMA network and across the most common genetic backgrounds. This protocol is now presented on the EMMA website and available to the community. Furthermore, Laser IVF was firmly established within the EMMA consortium as a robust recovery technique and a protocol was uploaded onto the EMMA website. We have also made good progress with ICSI and were able to generate ICSI derived embryos using sperm and oocytes from several different strains of mice (CD-1, B6D2 and C3H). Finally, the transportation of unfrozen embryos will simplify the exchange of mouse stocks between laboratories. In keeping with this idea, we have demonstrated that frozen/thawed 2-cell embryos from several different strains (C3H, C57BL/6 & 129) will resume *in vitro* development after being held at 8°C for 72hrs. We now believe that we have defined the optimum conditions for transporting unfrozen embryos.

Free of charge access to EMMA mouse mutant resources was facilitated by the Transnational Access (TA) activity (WP3). A total of 15 TA calls were published on the EMMA website. In response to the calls a total of 311 genuine TA applications were submitted out of which 23 were rejected and 288 accepted. TA applications were submitted from 23 different countries with most applications being from Germany, France and the UK. In general, the EMMA distribution continues to grow. A total of 550 strain requests were received in 2012 adding to a total number of 2814 orders by the end of EMMAservice. The EMMA germ-free service (WP7) offered by the Gulbenkian Institute (Portugal) is a unique service not provided by other major repositories. The Gulbenkian fulfilled a total of 14 service requests by the end of EMMAservice.

WP6 coordinates the EMMA informatics activities such as database development and integration with other resources, web interface development and database curation. EMMAservice involved considerable development of the database schema and the database population mechanisms as well as extensive data curation. During the project a total of 1942 strains had their gene/allele and strain names/symbols manually reviewed and curated. New releases covered Spring internal interfaces, new searching and strain description pages and a new statistics/reporting package, which is key to control the progress of the EMMA archiving activities and to underpin further process improvements. Regarding data integration a BioMart search was set up that allows that EMMA data are accessible by multiple resources. Furthermore, links were set up to phenotype data of EMMA stocked mouse resources available at the Europhenome database or at the Sanger Mouse Portal. Finally, information available from MGI on human disease associations to mouse models were integrated into EMMA allowing the searching of EMMA mice by associated diseases.

Outreach efforts of WP2 focused on attracting new EMMA users by presenting the EMMA activities at 16 major mouse genetics meetings. Overall, EMMA PR activities covered a total of 115 presentations at scientific and science policy meetings and in addition publications and advertisements. The existing know-how and cryopreservation expertise was transferred to the community via a total of 16 training courses for 104 students and by a dedicated cryopreservation workshop.

The comprehensive and unique physical and data resources of EMMA will support biomedical research in Europe by offering the opportunity to decipher molecular disease mechanisms and aiding the development of therapeutic strategies. Thus, EMMA 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.

1.2 Project context and project objectives

EMMAservice project objectives

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:

1) Develop a series of mutant alleles for every gene in the mouse genome

2) Determine the phenotypic consequences of each mutation

3) Identify mouse models for the complete disease spectrum in humans

To exploit this emerging resource, mouse models must be preserved and made available to the European biomedical research community. Building on EMMA's previous achievements as the primary mouse repository in Europe, EMMAservice aimed to meet the future challenges presented by archiving and disseminating mouse models in the ERA as follows:

- Archiving of 1224 new mouse mutant lines in support of individual depositors and also of European mouse genetics programs
- Support of eligible customers with free of charge Transnational Access for up to 20% of requested EMMA mouse resources
- Technology development to underpin the archiving and distribution efforts by advancing current sperm freezing technology
- Training courses to promote the shipment of frozen germplasm rather than live mice
- EMMA informatics to support user services by setting new standards for user friendly accession of EMMA services, extensive data curation and cross referencing with other mouse database resources
- Widen outreach efforts to attract new users and to address specifically the translational research community

EMMAservice work packages and objectives

Project management **(WP1)** underpins all activities and specific objectives for the project were **1)** to ensure achievement of project results, **2)** to provide decision making and quality control, **3)** to support the implementation of changes in the activities and in the consortium if needed and **4)** to provide timely and efficient contractual, financial and administrative coordination of the project where needed.

A key objective of the EMMAservice networking activities **(WP2)** was to effectively integrate three new partners into EMMA leading to a significantly increased archiving and distribution capacity and to the representation of a new member state in the network. Furthermore, outreach efforts focused on attracting new EMMA users by presenting the EMMA activities at a total of 12 major mouse genetics meetings. A further project goal was to transfer the existing know-how to the community by organizing a total of 16 cryopreservation courses. Finally, a further aim was to solicit

comprehensive user feedback on EMMA services by the provision and analysis of user feedback forms.

Distribution of mouse mutant resources was supported in the EMMAservice project via the Transnational Access (TA) activity **(WP3).** The TA program provided free of charge access to a defined number of requested EMMA mouse resources. Deliverable for the project was the publication of 15 calls facilitating the provision of up to 330 access units.

Major objective and effort of EMMAservice was the archiving of mouse mutant lines that could be contributed from either the wider community or supported large European mouse production projects such as EUCOMM **(WP4).** Project deliverable was the archiving of 1224 new mouse mutant lines.

A further focus of the research activities was technology development **(WP5)** underpinning the EMMA cryopreservation service by the refinement of existing EMMA sperm freezing technologies. Focus of WP5 were the development and the publication of SOPs covering **1)** optimized sperm freezing technologies, **2)** improved laser IVF procedures **3)** improved ICSI capabilities at EMMA and **4)** refined conditions for transportation of unfrozen embryos.

WP6 coordinated the EMMA informatics activities such as database development and integration with other resources, web interface development and database curation. Key project objectives were **1)** maintenance and updating of the existing EMMA database including extensive curation of strain nomenclature, **2)** further development of the internal and external websites to improve the user-friendliness for customers and internal users and **3)** to facilitate integration of the EMMA database with other appropriate strain, genomic and phenotypic resources.

Another objective of the EMMAservice activities was to support interested customers with the transfer of up to 12 mouse mutant lines into germ-free conditions. Furthermore, customers could benefit from the availability of mice already made germ-free and available for distribution **(WP7).**

1.3 Work progress and achievements during the project

1.3.1 / WP1 Project management

The objective of WP1 was to set up an effective management framework for the EMMAservice project to ensure progress towards planned objectives and contractual commitments. The specific objectives were to:

- Ensure achievement of project results
- Provide decision making, quality control and conflict resolution mechanisms
- Support implementation of changes in the activities and in the consortium if needed
- Provide timely and efficient contractual, financial and administrative coordination of the project and amendments where necessary

Summary of progress towards WP1 objectives

Task 1: Project communication

Main objective of this task was to establish and maintain the **internal EMMAservice web pages** (technical implementation by WP6) to ensure the availability of all information concerning the project and its progress on the web server. To this effect a dedicated internal project webpage was set up that allows project participants access to and download of all relevant information such as the technical annex, annual reports and annual implementation plans, meeting minutes and presentations held at meetings.

Beyond this specific deliverable, communication within the network was mainly facilitated by **project and work package meetings** which are described under task 2 and in the work package 2 description of this report. In period 3 we further enhanced communication by **monthly teleconferences** for work packages 5 and 6.

Furthermore, to ensure that all partners of the EMMAservice project and in particular new partners of the EMMA network are familiar with all processes and responsibilities within the network, a comprehensive **EMMA Manual** has been compiled by the Project Office with support from the IT group. The manual describes in detail the external and internal websites and interfaces for data recording, the archiving and distribution workflows and requirements for data recording and in detail all automatic Email notifications sent to customers amongst other topics. This ensures that all processes are handled in a systematic way in a distributed infrastructure and underpins the project controlling.

Task 2: Project controlling

Project controlling was facilitated by **project meetings,** work package specific meetings and teleconferences where progress, problems, priorities and corrective actions were discussed in detail. Project controlling is foremost facilitated via the **EMMA database** which captures not only all strain related information that is displayed on the website but also information on the submission and evaluation process, information on archiving progress and order management. Based on captured data standardised reports can be prepared at any time and key stats are available live on the internal website. In the third reporting period Tableau software was purchased that aids visual analytics of data captured

in the database http://www.tableausoftware.com/. The software was used to prepare a set of standard reports covering the archiving and distribution activities of EMMA. Informatics and the Project Office will continue to develop standardized reports for the network partners. An example how tableau can be used is the visualization of requests, rather than presenting this data in excel files.

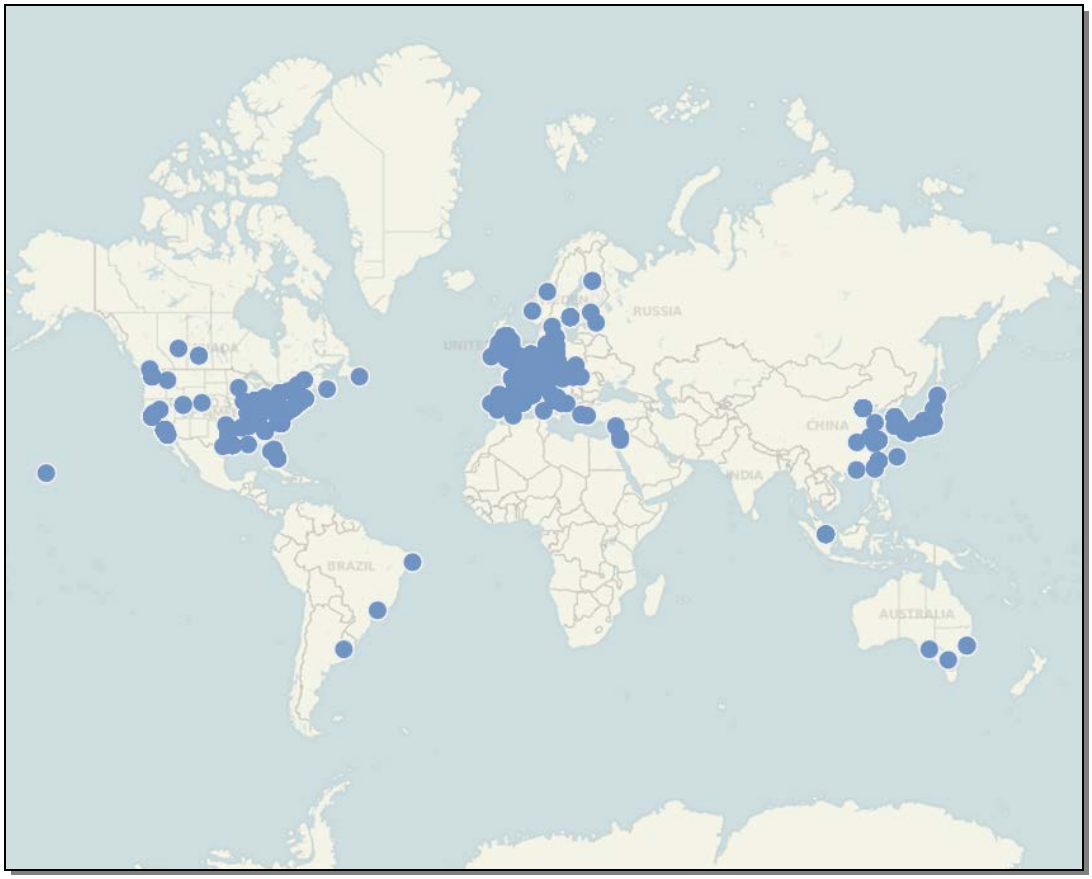

Fig. 1: Graphical display of EMMA requests using Tableau software

In addition, a software tool called JIRA is now being used routinely by the entire informatics team and the Project Office to track the numerous tasks of the informatics team, to prioritize tasks and to manage the assignment and monitor the completion of tasks (http://www.atlassian.com/en//software/jira/overview).

Task 3: Project reporting

This task was covered by the writing of three periodic and this final project report.

Task 4: Communication with EC and financial management

This task involved the transfer of funds from EC to the project participants. A key milestone of WP1 was the organisation of the midterm review meeting held at the Karolinska Institute in April 2011. The reviewer, Prof. Anton Berns, provided very valuable advice and recommendations covering mainly the monitoring of user satisfaction, a benchmarking of the EMMA services, and efforts to make EMMA more widely known to the user community. While activities to monitor user satisfaction and efforts to promote EMMA are underway, these activities will be further strengthened in the future. A

benchmarking of services was proposed as a deliverable in a grant application submitted in response to Call No 10 of the EC Capacities-Specific-Program published in July 2011. The submitted proposal has been positively evaluated and the benchmark analyses of EMMA services are now a contractual deliverable of the INFRAFRONTIER-I3 project which was launched in January 2013.

In addition to the specific tasks as described in the EMMAservice technical annex, the project management activities covered the appointment of board members of e.g. the Advisory Board or the Evaluation Committee. Furthermore, project management covered dissemination activities including the development of the project website and the further development of the EMMA network e.g. via the integration of new partners and the cooperation with other projects.

Advisory Board
For the EMMAservice project three Advisory Board members were appointed:

1) Prof. Ian Jackson, MRC-Human Genetics Unit, Edinburgh as a user representative
2) Dr. Belen Pintado, CNB-CSIC, Madrid as animal welfare expert
3) Prof. Maja Bucan, Pennsylvania State University, Pennsylvania as a mouse and human genetics expert

All three members of the Advisory Board are independent of any of the units operating the EMMA network.

Dissemination activities
A central aspect of the management activities are all EMMA dissemination activities. This covers a wide range of measures such as:

1) Presentation of EMMA at scientific and at policy meetings
2) Organisation of training courses
3) Production of flyers, brochures
4) Advertisements
5) Publications
6) Email lists and newsletters

A detailed description on these activities is provided in the work package 2 update of this report.

A major element of the dissemination activities is the development of the **EMMA project website**. This is an ongoing process and involves foremost provision of up to date strain related information and display of current strain availabilities. A major new activity started in the third reporting period is the complete re-launch of the EMMA website. This follows the approval of the INFRAFRONTIER-I3 project, which requires the provision of Transnational Access activities for mouse production and phenotyping via the INFRAFRONTIER website. Furthermore, following completion of the INFRAFRONTIER Preparatory Phase project, the INFRAFRONTIER Research Infrastructure is now moving forward to implementation. Thus, all EMMA and INFRAFRONTIER-I3 services will be made

available via www.infrafrontier.eu. Implementation of this major development is supported by a contractor with expertise in web design and the Drupal content management system. The EMMA URL at www.emmanet.org will be maintained and the INFRAFRONTIER website will be hosted at the EBI. The re-launch is scheduled for September 2013.

Development of the EMMA network

A central objective of the EMMA management activities is the continuous further development of the EMMA network encompassing the integration of new partners into EMMA, securing EMMA's involvement and cooperation with other projects and the diversification of the EMMA service portfolio. A continued and sustainable funding strategy of the EMMA repository is also being addressed by the EMMA management.

New EMMA partners

According to established policies (http://www.emmanet.org/rules_new_partners.php) a total of four new partners joined the EMMA network during the first project period representing Greece, Finland, Czech Republic and Austria as new member states. The integration of the new partners was facilitated by their active participation in all of the EMMAservice project meetings at their own expense. During the course of the second reporting period discussions were launched with additional prospective partners of EMMA, namely Israel represented by Prof. Fuad Iraqi of the Tel Aviv University (TAU) and The Netherlands represented by Dr. Jos Jonkers of the Netherlands Cancer Institute (NKI). The integration of TAU and NKI into the EMMA network was formalized in the third reporting period by signing of the EMMA cooperation agreement. Both TAU and NKI were visited by an EMMA visiting group to inspect the labs and facilities and to discuss further integration steps.

Co-operation with other projects / programs

The EMMA partners have established an extensive network and collaborations across the EU and worldwide, that are essential for the successful operation of the EMMA network.

1) EUMODIC, EUCOMM and EUCOMMtools, (http://www.knockoutmouse.org/about/eucomm): The EMMA network has for many years a successful collaboration with the EUCOMM project that extends now to the successor project EUCOMMtools. During the course of the EUCOMM project mice were produced from the ES cell resource as a quality control. All EUCOMM mice are publicly listed at the IKMC and at the EMMA websites and are distributed by partners of the EMMA network to users worldwide. The EUCOMM collection of mouse mutants is in high demand and accounts for 50% of all shipments from the EMMA network. Building on this successful collaboration the EMMA network will also support the EUCOMMtools project by archiving up to 250 lines of Cre-driver mice. These mice will be generated on a pure C57BL/6N background and their Cre expression patterns will be documented and annotated in day P7 and P56 mice. These mice will 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. EUCOMMtools was launched in 2011.

2) SYSGENET, (http://www.cost.eu/domains_actions/bmbs/Actions/BM0901): EMMA also initiated a collaboration with the COST action SYSGENET – the European systems genetics network for the study of complex traits linked to human diseases using mouse genetic reference populations (GRP). A close interaction will be ensured by Prof. Klaus Schughart (HZI) who is the co-ordinator of SYSGENET and partner in the INFRAFRONTIER project and by Prof. Fuad Iraqi who is a leading partner in SYSGENET and who is the Director of the new Israeli EMMA node. SYSGENET and EMMA collaborate to provide the EU research community with access to existing and future GRP resources such as the Collaborative Cross. This will create a basis for the European research community to make major scientific contributions in the field of complex genetics, systems biology and development of sophisticated experimental model systems for the better understanding of human diseases.

3) Biomedbridges: This is a consortium of 21 partners established in 2011 and aiming to implement a common e-infrastructure for the ESFRI BMS research infrastructures to allow interoperability of data and services. The project will deliver solutions for data harmonization, technical integration of services and secure access to data and specific use cases will be developed around these topics. EMMA / INFRAFRONTIER is represented in Biomedbridges by the Co-ordinator and the EBI (Co-ordinator of Biomedbridges) and leads the specific use case 'Phenobridge: crossing the species bridge between mouse and human'.

4) IMPC and InfraCoMP (http://www.mousephenotype.org/ and www.infrafrontier.eu): The aim of the EC funded InfraCoMP project is to coordinate the cooperation between the ESFRI project INFRAFRONTIER and the International Mouse Phenotyping Consortium (IMPC). The IMPC is 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 in order to create an encyclopaedia of mammalian gene function. Thus, whereas INFRAFRONTIER aims at building and operating a transnational European research infrastructure with all its organisational, 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. InfraCoMP addresses the need to develop an effective framework for aligning the objectives and resources of INFRAFRONTIER and IMPC to avoid duplication of efforts. This is aided by the fact that several of the research institutions represented in INFRAFRONTIER are also partners in the IMPC. InfraCoMP is coordinated by Prof. Hrabé de Angelis (HMGU), who is also the coordinator of INFRAFRONTIER. InfraCoMP will organise regular workshops that address the central issues that have to be resolved to ensure a cooperation of the two initiatives that minimises redundancy, maximises mutual benefit and leverages expertise and infrastructure capacity. Key topics addressed by InfraCoMP are **1)** Mouse Phenotyping, **2)** Mouse Production, Cryopreservation, and Distribution, **3)** Access to Phenotyping Data and **4)** Community Engagement. All mouse mutants produced by the European IMPC partners will be archived and distributed by the EMMA network.

1.3.2 / WP2 - Communication and user interactions

The specific objectives of this work package were:

1) To foster a culture of co-operation between the partners of the EMMA network
2) To effectively integrate new partners into the joint activities of EMMA
3) To promote the activities and services of EMMA among the general public and in particular the user community
4) To attract potential new users who may benefit from the EMMA research infrastructure
5) To coordinate activities with other related European and global initiatives
6) To disseminate the extensive state of the art knowledge of cryobiology available within EMMA

Summary of progress towards WP2 objectives

Task 1: Internal communication / networking meetings

The network internal communication was facilitated by several means such as project meetings of the entire network, work package specific meetings (will be discussed in the respective work package updates of this report) and via teleconferences as needed. This was further supported by network internal Emailing lists of the various management boards and by access to relevant documents such as meeting minutes and project contracts via the internal EMMA website.

The project meetings were held twice a year alternately at the EMMA core facility at the CNR-Monterotondo and at another EMMA node. The two day meetings covered parallel sessions of the Technical Working Group (TWG) and the Board of Participating Directors (BPD) or the Project Management Committee (PMC) as well as joint meetings of all management boards. Furthermore, meetings were attended by members of the Advisory Board and additional guests. Meetings covered presentations on work packages and the general development of EMMA as well as discussions of technical issues and discussions on the future development and the policies of EMMA. During the EMMAservice project a total of eight general project meetings were held.

In addition to the EMMAservice partners and representatives of the Advisory Board, all project meetings were also attended by the new EMMA partners representing Greece, Austria, the Czech Republic and Finland which were not partners in the EMMAservice project. Their participation in the meetings in addition to a number of other activities ensured a smooth integration of the new partners into the EMMA network. Meeting participation was covered at their own expenses. Furthermore, meetings were attended by external experts and guests. Prof Fuad Iraqi of the Tel Aviv University and Jos Jonkers of the Netherlands Cancer Institute both joined the 7th and 8th project meeting to discuss the participation of Israel and Netherlands in the EMMA network. The final project meeting had a technical focus on health monitoring. Thus, for the technical working group meeting two external experts, namely Prof Andre Bleich from Hannover and Dr Ricardo Feinstein from the Swedish Veterinary Association, were invited and joined the meeting. The participants, presentations, topics and discussions are all summarised in the meeting minutes which are available from the internal EMMA website. Furthermore, all presentations held at the meetings can be downloaded from the internal website or are provided by the Project Office.

Task 2: External communication / public relations and outreach activities to attract new users
Aim of this task was to attract new users to the EMMA infrastructure using a number of outreach efforts. Foremost, EMMA was extensively advertised and presented at major mammalian genetics conferences and meetings attended by clinicians and human geneticists. E.g. during the third reporting period EMMA was presented either by the EMMA Director (Prof. Hrabè de Angelis) or the Project Managers (Drs. Michael Hagn and Sabine Fessele) at the following scientific meetings:

1) International Mouse Genome Conference, St Pete's Beach, USA, October 2012, poster presentation, Dr. Michael Hagn, talk, Prof. Hrabé de Angelis
2) Mouse Molecular Genetics Meeting, Asilomar, USA, October 2nd – 6th, poster presentation, Dr. Sabine Fessele
3) Biocenter Finland Research Infrastructures Day, Tampere, Finland, August 2012, talk on EMMA and INFRAFRONTIER, Dr. Michael Hagn
4) ESOF European Science Open Forum, Dublin, Ireland, July 2012, talk on INFRAFRONTIER / EMMA, Prof. Martin Hrabè de Angelis

In addition, EMMA was represented and presented at numerous other meetings. Among these were project meetings of the EUCOMMtools projects and of the IMPC phenotyping initiative. Furthermore, EMMA was widely presented as part of the intensive promotion of the INFRAFRONTIER project. During the EMMAservice project Prof. Hrabé de Angelis and the Project Manager participated in about 115 national and international meetings and frequently presented on these occasions the objectives and resources and services of INFRAFRONTIER and EMMA. Furthermore, Dr. Raffaele Matteoni (CNR) and Dr. Martin Fray (MRC) contributed lectures on the occasion of a Wellcome Trust course on 'Managing Mouse Colonies: Best Practices, Genetics, Breeding and Welfare' held in Hinxton in June 2012. Dr Lluis Montoliu, Director of the Spanish EMMA node contributed an overview lecture of EMMA at the IUBMB & FEBS Meeting in Seville, Spain in September 2012.

EMMA related publications during course of EMMAservice project

1) Nucleic Acids Res. 2010 Jan; 38 (Database issue):D570-6. doi: 10.1093/nar/gkp799. Epub 2009 Sep 26. **EMMA--mouse mutant resources for the international scientific community**.
Wilkinson P, Sengerova J, Matteoni R, Chen CK, Soulat G, Ureta-Vidal A, Fessele S, Hagn M, Massimi M, Pickford K, Butler RH, Marschall S, Mallon AM, Pickard A, Raspa M, Scavizzi F, Fray M, Larrigaldie V, Leyritz J, Birney E, Tocchini-Valentini GP, Brown S, Herault Y, Montoliu L, de Angelis MH, Smedley D.

Abstract
The laboratory mouse is the premier animal model for studying human disease and thousands of mutants have been identified or produced, most recently through gene-specific mutagenesis approaches. High throughput strategies by the International Knockout Mouse Consortium (IKMC) are producing mutants for all protein coding genes. Generating a knock-out line involves huge monetary and time costs so capture of both the data describing each mutant alongside archiving of the line for distribution to future researchers is critical. The European Mouse Mutant Archive

(EMMA) is a leading international network infrastructure for archiving and worldwide provision of mouse mutant strains. It operates in collaboration with the other members of the Federation of International Mouse Resources (FIMRe), EMMA being the European component. Additionally EMMA is one of four repositories involved in the IKMC, and therefore the current figure of 1700 archived lines will rise markedly. The EMMA database gathers and curates extensive data on each line and presents it through a user-friendly website. A BioMart interface allows advanced searching including integrated querying with other resources e.g. Ensembl. Other resources are able to display EMMA data by accessing our Distributed Annotation System server. EMMA database access is publicly available at http://www.emmanet.org.

2) Mamm Genome. 2012 Oct;23 (9-10):559-71. doi: 10.1007/s00335-012-9420-4. Epub 2012 Sep 4. **Centralized mouse repositories.**
Donahue LR, **Hrabe de Angelis M, Hagn M**, Franklin C, Lloyd KC, Magnuson T, McKerlie C, Nakagata N, Obata Y, Read S, Wurst W, Hörlein A, Davisson MT.

Abstract
Because the mouse is used so widely for biomedical research and the number of mouse models being generated is increasing rapidly, centralized repositories are essential if the valuable mouse strains and models that have been developed are to be securely preserved and fully exploited. Ensuring the ongoing availability of these mouse strains preserves the investment made in creating and characterizing them and creates a global resource of enormous value. The establishment of centralized mouse repositories around the world for distributing and archiving these resources has provided critical access to and preservation of these strains. This article describes the common and specialized activities provided by major mouse repositories around the world

3) Mamm Genome. 2012 Oct;23 (9-10):572-9. doi: 10.1007/s00335-012-9423-1. Epub 2012 Aug 31. **Overview of new developments in and the future of cryopreservation in the laboratory mouse.**
Guan M, Marschall S, Raspa M, Pickard AR, Fray MD.

Abstract
The large-scale mutagenesis programs underway around the world are generating thousands of novel GA mouse strains that need to be securely archived. In parallel with advances in mutagenesis, the procedures used to cryopreserve mouse stocks are being continually refined in order to keep pace with demand. Moreover, the construction of extensive research infrastructures for systematic phenotyping is fuelling demand for these novel strains of mice and new approaches to the distribution of frozen and unfrozen embryos and gametes are being developed in order to reduce the dependency on the transportation of live mice. This article highlights some contemporary techniques used to archive, re-derive, and transport mouse strains around the world.

Email lists
EMMA services were also promoted regularly via email lists such as the MGI-list, the ISTT member list and an EMMA customer email list.

User feedback forms

A user feedback form was implemented in May 2010. During the course of the second reporting period a total of 87 export user feedback forms were returned and analysed. In the third period a further 88 feedback forms were returned adding up to a total of 175 analysed export feedback forms. Customers were asked to rate their views on how EMMA handled requests on a point scale 1-5 (1=Poor and 5=Very good) with regard to the following questions:

(a) Response to enquiries { }
(b) Time to provide materials { }
(c) Standard of communication { }
(d) Information and or protocols provided { }

The received feedback was overall very favourable. Average recorded values for all analysed feedback forms were for **a)** 4.78 **b)** 4.32 **c)** 4.72 **d)** 4.58. Most critical for customers is the time until the materials are shipped. This depends on several factors, also including the processing of MTA documents by customers or the involvement of third parties (e.g. Tet Systems) required for signing off MTA documents.

Similarly import user feedback forms were received and analysed during the course of the third period. Customers were asked to rate their views on how EMMA handled strain importations on a point scale 1-5 (1=Poor and 5=Very good) with regard to the following questions:

(e) Evaluation process { }
(f) Organisation of shipment { }
(g) Time to cryopreserve line { }
(h) Response to enquiries { }
(i) Standard of communication { }

The received feedback was also very favourable. Average recorded values for a total of 40 analysed import feedback forms were for **e)** 4.6 **f)** 4.7 **g)** 4.5 **h)** 4.7 and **i)** 4.7. The return of feedback forms is somewhat smaller as compared to export report forms, which is due to the submission of a significant number of strains produced from the EUCOMM ES cell resource via an automatic importer.

Task 3: Training courses

The annual EMMA cryopreservation courses were organised by CNR, MRC and the CNRS to teach basic and advanced techniques in cryopreservation for banking of mouse mutant strains and for re-derivation of mice from frozen stocks.

Several methods for cryopreservation of mouse embryos, gametes and ovaries are presently available and no single method is adequate for all the various strains of mice being developed. Therefore, a variety of methods must be taught. The setup and performance of state of the art, comprehensive, theoretical and practical courses on cryopreservation of mouse embryos and

gametes is essential to disseminate the most advanced techniques on embryo handling, *in vitro* fertilisation, sample cryopreservation, thawing and culture, quality control and database management. The schedule and general plan of the Monterotondo courses was set up by CNR in collaboration with the Jackson Laboratory and the members of the course faculty. The courses were announced on the public EMMA web site (http://www.emmanet.org/about/courses.php), with links to the course online poster and the application form. In addition, the printed poster and flyer were mailed to various international scientific societies and groups active in the fields of biomedicine, cryobiology and animal science. The 4 main EMMA cryocourses were held at the EMMA core facility in Monterotondo. The courses were attended by an average of 10 participants from across Europe. The course format involved primarily a practical laboratory program in which students learned and practiced various techniques for the production, handling and cryopreservation of mouse cleavage-stage embryos, spermatozoa, and ovaries. Relevant principles and basic procedures of mouse colony breeding under conventional and SPF conditions were also discussed. The practical program included also techniques for sample recovery by thawing and culture and *in vitro* fertilisation of mouse oocytes by fresh or thawed sperm and quality control methods. In addition, the principles of cryobiology, long-term storage systems and cryogenic equipment, the use of specific inventory databases for storage and breeding programs, the public services for strain cryo-archiving, SPF recovery and distribution provided by the EMMA network and its partners, were presented and discussed.

The faculty members of the cryocourses were:
- Dr. Stanley P. Leibo, Department of Biological Sciences, University of New Orleans, Audubon Center for Research of Endangered Species (Course leader)
- Dr. Robert Taft, Jane Farley and Sian Clements, Reproductive Sciences, The Jackson Laboratory, Bar Harbor
- Dr. Marcello Raspa, Dr. Ferdinando Scavizzi and Dr. Raffaele Matteoni, Istituto di Biologia Cellulare CNR -EMMA, "A. Buzzati-Traverso" Campus, Monterotondo Scalo (Rome, Italy)
- Dr. Kent Lloyd, School of Veterinary Medicine, Mouse Mutant Regional Resource Center (MMRRC), University of California at Davis
- Dr. Susan Marschall, Helmholtz Zentrum München, German EMMA node
- Dr. Martin Fray, MRC Mammalian Genetics Unit – UK EMMA node, Harwell

In addition, to the joint effort of the main EMMA cryocourse, which is held by scientists of the Jackson Lab and EMMA, the MRC-MGU hosted eight additional cryocourses during the EMMAservice project which were exclusively organised by EMMA scientists. The four day courses were attended by an average of 6 students per course and covered practical sessions on embryo handling, embryo freezing and thawing, IVF and a demonstration of embryo transfer. The Harwell cryocourses were advertised on the EMMA website and in addition on the local MRC website at http://www.har.mrc.ac.uk/training/cryocourse/ unless they were booked in advance. Advance bookings are regularly made from interested candidates who were on shortlists from overbooked previous courses or by direct enquiries from interested students and scientists before the courses were announced on the EMMA website. Thus, an open access to interested candidates across Europe was always assured.

Finally, the French EMMA node in Orleans offered a new course format focussing on the handling of frozen material received from EMMA. The course covered sperm thawing, in vitro fertilization, embryo thawing and embryo transfer at different developmental stages. The 4 CNRS courses were advertised on the EMMA website at http://www.emmanet.org/courses.php.

Overall, in a total of 16 cryocourses EMMA trained during the EMMAservice project a total of 104 students in state of the art cryopreservation techniques.

Task 4: Cryopreservation Workshop

An EMMA cryopreservation technology-development workshop was held on 7-8th May 2012 at the CSIC (Consejo Superior de Investigaciones Científicas) main campus. It emerged as an initiative from the EMMA technology-development working group and was jointly organized by EMMA and the ISTT (International Society for Transgenic Technologies). At the workshop a group of leading experts in the cryopreservation field discussed in depth the latest technological advances in cryopreservation, including sperm and embryo cryopreservation, in vitro fertilization (IVF) methods and related techniques as ovary cryopreservation, laser-assisted and piezo-driven intracytoplasmic sperm injection (ICSI), transportation of frozen material and other technical and logistic challenges relevant to the operation of current mouse embryo/sperm archives.

Program and abstracts presented at this workshop

http://www.emmanet.org/pdfs/EMMA_cryopreservation_workshop_Madrid2012_website.pdf

Presentations given at the workshop

http://www.cnb.csic.es/~criocnb/emma/Madrid2012/

List of invited speakers and participants

http://www.cnb.csic.es/~criocnb/emma/Madrid2012/

Following the workshop the participating scientists agreed to publish their contributions in a dedicated book, which will provide an excellent and state-of-the-art summary of current technologies and approaches used by the leading experts in the cryopreservation field. The book is currently in preparation and will be published in 2013.

1.3.3 / WP3 Distribution of mouse mutant lines

The EMMAservice work package 3 covered the implementation of the mouse mutant distribution via EMMA as a **Transnational Access (TA) activity** to facilitate free-of-charge access for eligible customers to the EMMA mutant resources. During the EMMAservice project a total of 330 access units for either frozen embryos or live mice should be granted to EMMA customers. Focus of work package 3 during the first reporting period was the implementation of the Transnational Access activity which required significant changes on the public EMMA website, the internal website and data recording and promotion activities. Following implementation a total of 5 calls were published during the first reporting period and an additional 6 calls during the second reporting period. Another 4 calls were published during the third reporting period in 2012. The outcome of these 15 calls in total is summarised in this report. Before an update on the general development of the EMMA distribution statistics is given.

General update – EMMA strain distribution

As in previous years the EMMA distribution continued to grow. By December 2012 a total of 560 requests were received compared to a total of 473 for 2011. The total number of strain orders by the end of the EMMAservice project was 2814. Fig. 2 shows the distribution development during recent years.

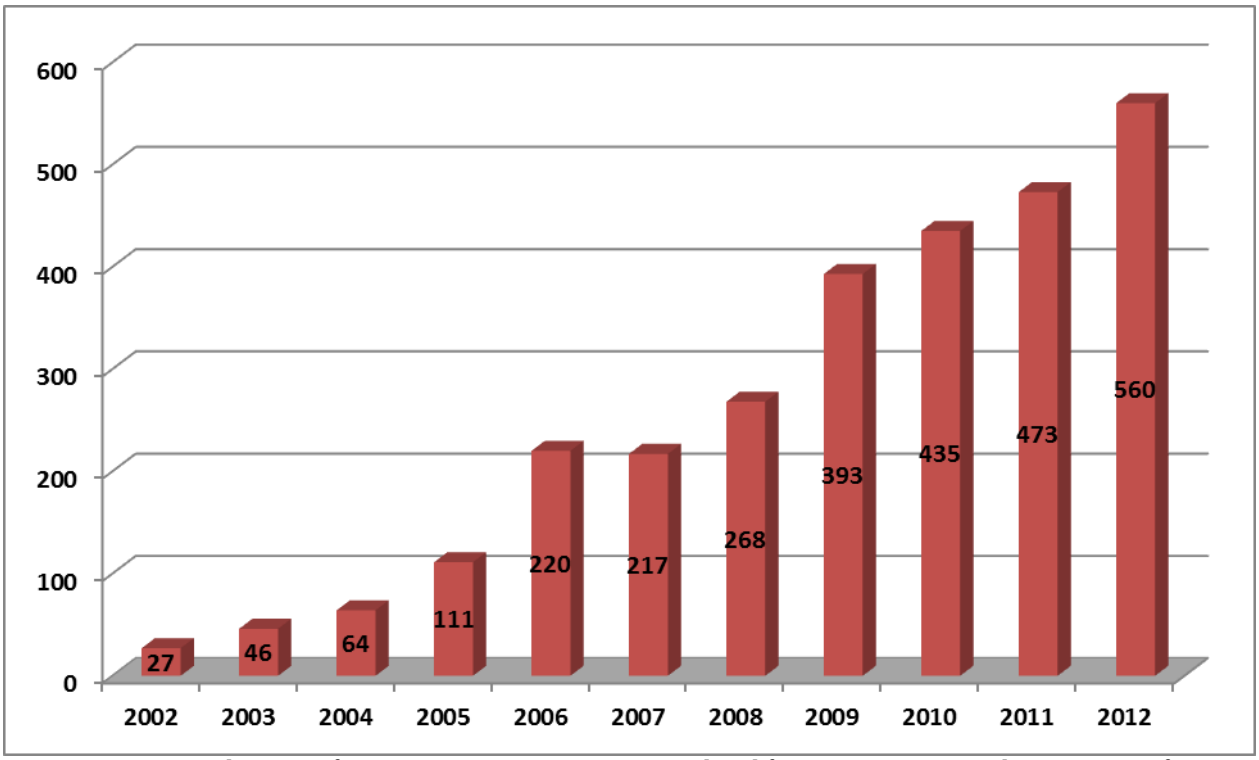

Fig. 2: Annual requests from EMMA repository. Note: Displayed figures are requests and registrations of interests. Cancelled orders are deducted

Summary of progress towards objectives

Promotion of the TA activity

To promote the option to access EMMA resources free of charge, a number of changes were implemented on the public EMMA website. Foremost, the process was described in a concise but comprehensive way on a dedicated webpage at http://www.emmanet.org/projects/ta-activity.php. There were in addition a number of links set up to this description on frequently used sites on the public EMMA web pages that made it virtually impossible for customers to miss the information. Furthermore, each strain description page and each online request form contained links that point to the TA info page. Finally, new calls were announced on the NEWS page at http://www.emmanet.org/news.php. Calls lasted three months and were run continuously. The TA activity was further promoted via email lists such as the transgenic or MGI lists and via a proprietary email list which contains all eligible EMMA customers recorded in the EMMA database. A concise synopsis of the TA service was circulated repeatedly via the EMMA Email lists.

Evaluation

All evaluators of the external EMMA Evaluation Committee were available for the TA evaluation process. The Committee is supported in the evaluation by the Project Managers. Due to the limited number of applications only two evaluators assessed the applications. Applications were generally of a solid quality and customers presented attractive research proposals for which the requested EMMA resources are needed. Because of the small number of requests it was so far not required to apply a strict ranking to the submitted applications. Rejections were largely based on formal grounds as customers requested more than the maximum two access units per call, project descriptions were not submitted or applications were retracted.

TA calls and outcome

During the EMMAservice project 15 TA calls were published on the EMMA website and promoted via the means described above. In total 339 TA applications were submitted to EMMA in response to published calls. Out of all received 339 applications the Evaluation Committee approved 308 applications and rejected 31 TA applications (9%). During evaluation and at later stages following the evaluation a total of 28 applications were cancelled by the applicants e.g. because proposed projects were not pursued or mice where sourced elsewhere. Thus, a total of 311 genuine applications were received out of which 288 were accepted and 23 were rejected by the Evaluation Committee. By far most of the customers opted for the application type A (255) which turns the TA application into a standard request upon rejection, whereas fewer customers (84) selected the option ‘TA only’ which leads to a termination of the request in case of a rejection. A total of 209 customers who were granted an access unit requested mice and 99 frozen embryos. TA applications were submitted from 23 different eligible countries with most applications from Germany France and the UK as shown on Fig. 3. Table 1 summarizes the call data.

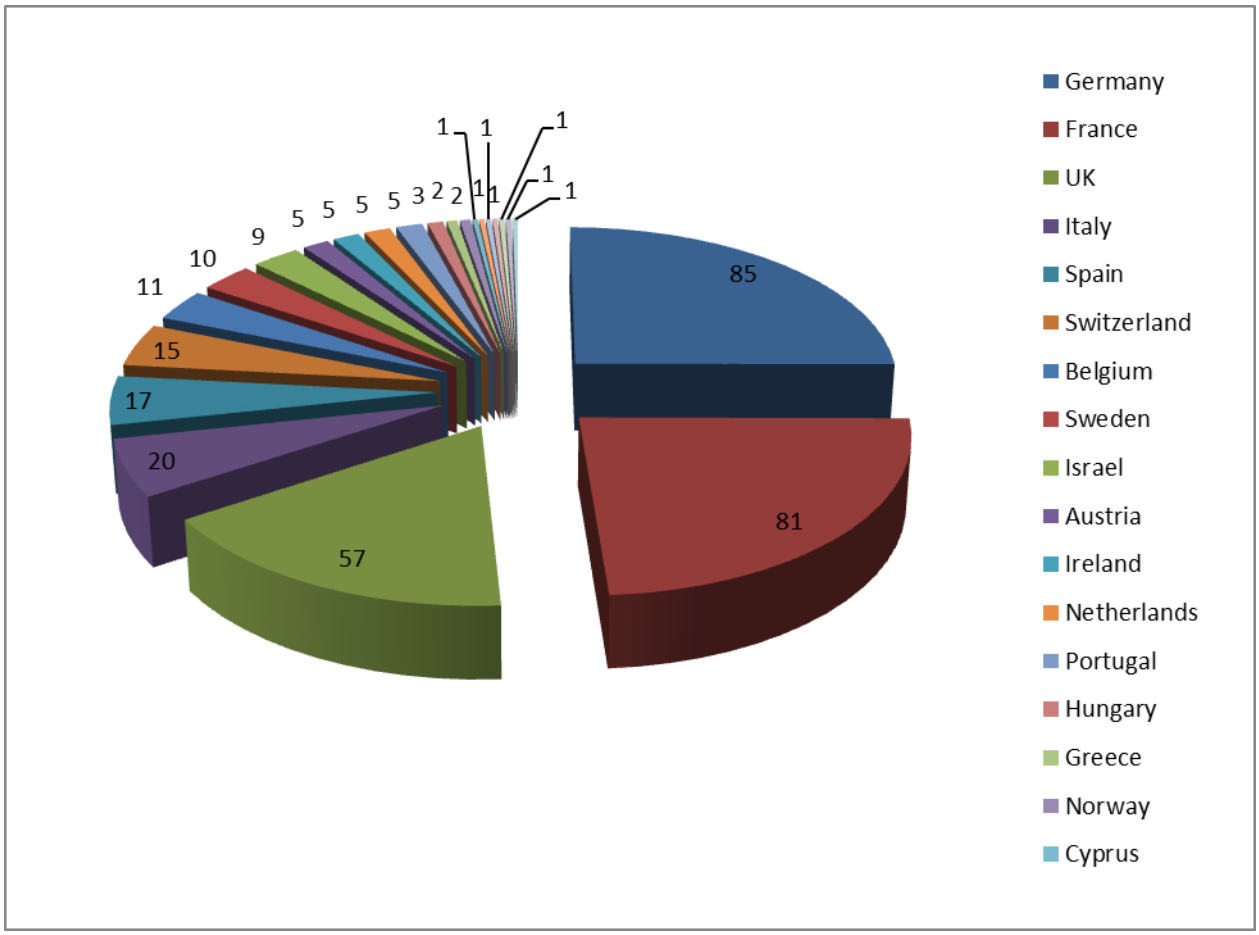

Fig. 3: Origin of applicants who were granted a WP3 TA access unit

	No of TA Applications 1st per	2nd per	3rd per	Rejected TA applications 1st per	2nd per	3rd per	Granted TA units 1st per	2nd per	3rd per	Total	Frozen embryos 1st per	2nd per	3rd per	Mice 1st per	2nd per	3rd per
CNR	**20**	**27**	**15**	**2**	**2**	**0**	**18**	**25**	**15**	**58**	**7**	**7**	**7**	**11**	**18**	**8**
CNRS	**34**	**49**	**20**	**5**	**5**	**1**	**29**	**44**	**19**	**92**	**9**	**8**	**4**	**20**	**36**	**15**
MRC	**14**	**28**	**16**	**4**	**2**	**0**	**10**	**28**	**14**	**52**	**4**	**8**	**2**	**6**	**20**	**12**
KI	**4**	**4**	**4**	**1**	**1**	**1**	**3**	**3**	**3**	**9**	**0**	**2**	**1**	**3**	**1**	**2**
HMGU	**14**	**24**	**14**	**2**	**1**	**0**	**12**	**23**	**14**	**49**	**5**	**7**	**5**	**7**	**16**	**9**
ICS	**4**	**11**	**14**	**0**	**0**	**2**	**4**	**11**	**12**	**27**	**0**	**6**	**8**	**4**	**5**	**4**
CNB	**6**	**10**	**7**	**1**	**0**	**1**	**5**	**10**	**6**	**21**	**2**	**5**	**2**	**3**	**5**	**4**
Total	**96**	**153**	**90**	**15**	**11**	**5**	**81**	**144**	**83**	**308**	**27**	**43**	**29**	**54**	**101**	**54**

Table 1: TA applications during the first, second and third EMMAservice reporting period

In essence, an efficient TA system was set up in the first reporting period and was smoothly operating in the following periods. Slightly more TA applications (339) than the projected milestone of 330 were received. A total of 308 free of charge access units were then granted to EMMA customers corresponding to about 91% of the projected overall milestone for the EMMAservice project. Due to cancellations of TA applications at various stages a total of 288 granted TA applications were processed by EMMA and will be concluded in 2013 following the end of the project. To assess the impact of this work package and the possible achievements of users who benefited from the free of charge access to the EMMA resource, users were directed to a dedicated EC questionnaire for this purpose. Furthermore, all users who benefitted from this TA activity were asked to report on any publications that involved use of the requested EMMA mouse mutant resource.

Publications involving EMMA mouse mutant resources distributed via the EMMAservice TA

1) Requestor: Bert Brone, Request ID:1901
Glia 2013 Feb; 61(2):150-63. doi: 10.1002/glia.22421. Epub 2012 Sep 21.

Complex invasion pattern of the cerebral cortex bymicroglial cells during development of the mouse embryo.
Swinnen N, Smolders S, Avila A, Notelaers K, Paesen R, Ameloot M, Brône B, Legendre P, Rigo JM.
Hasselt University, BIOMED, Agoralaan (Gebouw C), Diepenbeek B-3590, Belgium

2) Requestor: Eric Lingueglia, Request ID:3457
Nature, 2012, 490: 552–555.
Black mamba venom peptides target Acid-Sensing Ion channels to abolish pain.
Diochot S, Baron A, Salinas M, Douguet D, Scarzello S, Dabert-Gay A, Debayle D, Friend V, Alloui A, Lazdunski M and Lingueglia E .

3) Requestor: Kleopas Kleopa, Request ID:1836
Markoullis K, Sargiannidou I, Gardner C, Hadjisavvas A, Reynolds R, Kleopa KA (2012)
Disruption of oligodendrocyte gap junctions in experimental autoimmune encephalomyelitis
Glia, 60:1053-66.

Vavlitou N, Sargiannidou I, Markoullis K, Kyriacou K, Scherer SS, Kleopa KA (2010)
Axonal pathology precedes demyelination in a mouse model of CMT1X neuropathy
J Neuropathol Exper Neurol, 69 (9): 945-958.

Sargiannidou I, Vavlitou N, Aristodemou S, Hadjisavvas A, Kyriacou K, Scherer SS, Kleopa KA (2009). Connexin32 mutations cause loss of function in Schwann cells and oligodendrocytes leading to PNS and CNS myelination defects.
J Neurosci, 29:4748-4761

1.3.4/ WP4 Cryopreservation of mouse mutant lines

Objective of the EMMAservice work package 4 was to support the European biomedical research community by archiving up to 1224 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. All archived lines will be made available via the public EMMA strain list on the EMMA website at www.emmanet.org and on the IMSR database at http://www.informatics.jax.org/imsr/index.jsp. At least 612 archiving slots were allocated for strain donations from individual researchers, whereas the remaining archiving slots were allocated to support EU funded mouse genetics programs. As the archiving of mutant lines carrying more than one mutation required extensive breeding and effort, the archiving of such lines was counted as the equivalent of two mutant lines.

Summary of progress towards work package objectives

Overall, at the project end we report the total submission of 1181 mouse mutant lines to the EMMA repository. As 43 lines contain more than one mutation and are counted twice as defined in the EMMAservice Description of Work, we report a total of 1224 mouse mutant lines thus reaching the overall project goal. Out of these lines a total of 1094 mouse mutant lines were fully archived at the project end and available via the EMMA website, whereas the remaining lines are still in the pipeline due to submission coming in close to the project end. The overall development of incoming strain donations of EMMA is shown in Fig. 4.

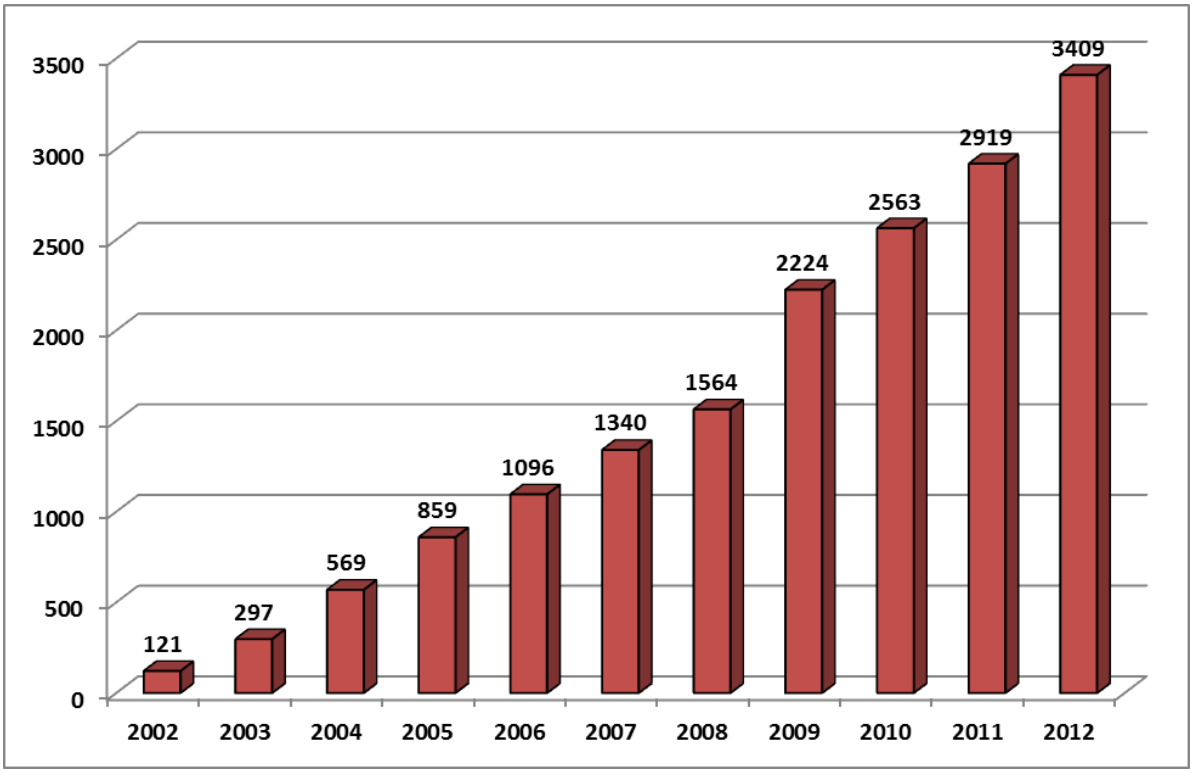

Fig. 4: Total number of submissions to the EMMA repository (cumulative figures till 2012)

The final status of the archiving and a breakdown of the cryopreservation effort per partner is summarised in table 2. Lines in progress at the project end will be fully archived and published on the EMMA website during the course of 2013.

Participant	In progress	Archived	Total Nr	48 months milestone
CNR	7	131	138	138
CNRS	12	168	180	180
MRC	9	171	180	180
KI	13	29	42	42
HMGU	33	147	180	180
ICS	16	128	144	144
SANG	0	180	180	180
CNB	40	140	180	180
Total	130	1094	1224	1224

Table 2: Archiving status of lines reported for the EMMAservice project

The reported number of lines corresponds to 100% of the project deliverable with regard to the number of deposited lines and to 90% with regard to archived lines. The EMMAservice project aimed to support foremost the wider community and also provided archiving slots to EU funded projects such as EUCOMM. The allocation of used archiving slots to both community-contributed and EU project-contributed mouse mutant lines and the project status of these lines is summarised in table 3. A total of 67% of the submitted lines accounted for community contributed lines and the remainder for lines originate from EU funded projects such as EUCOMM and EUMODIC.

	Community contributed lines		EU project contributed line		
Participant	In progress	Archived	In progress	Archived	Total
CNR	6	58	1	73	138
CNRS	12	159		9	180
MRC	9	96		75	180
KI	13	29			42
HMGU	31	84	2	63	180
ICS	3	7	13	121	144
SANG		180			180
CNB	30	102	10	38	180
Total	104	715	26	379	1224

Table 3: Breakdown of submitted mouse mutant lines into contributions from community and EU projects

Of the submitted lines 793 lines were archived as embryos and 398 were archived as sperm. Some are archived as both formats and thus the overall number of archived lines is 1191 (instead of 1181). The mean archiving time for embryo freezing was 3.8 months, for sperm freezing 3.5 months, respectively. Most submitted lines during the entire project were targeted mutations, mainly KO mutations due to the contributions from the EUCOMM mutagenesis project. This class accounts for 901 of the submitted lines, transgenic lines account for 176 lines, followed by chemically induced

mutations, gene traps and spontaneous mutations. Strain donations were made across Europe with major contributions from UK, France and Germany. See fig. 5 for a graphic display of the origin of submissions. Mouse mutant lines were donated by a total of 250 different submitters of which 96% are not associated with the institutions operating the EMMA repository.

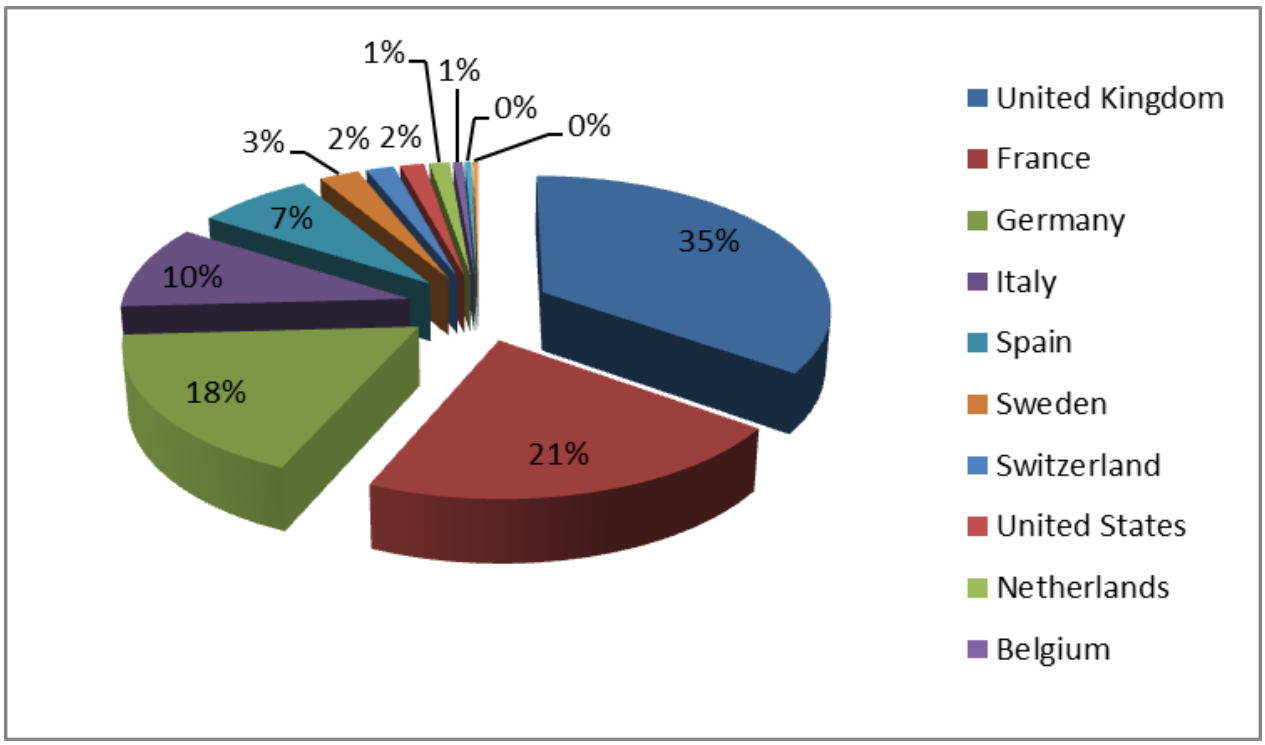

Fig. 5: Origin of submissions during the course of the EMMAservice project

While the overall number of submissions received by EMMA was sufficiently high to achieve the work package objectives, the origin of the submissions reveals some striking differences between the member countries of the EMMA network. By far most of the submissions were received from the UK, France and Germany which accounts for submissions from individual depositors and contributions from the EUCOMM mouse production centers. EUCOMM mouse production also accounts for the higher number of submissions from Italy compared to previous years. Also, a significant number of submissions were received from Spain which is most likely due to the extensive local PR efforts of the Spanish EMMA node at the CNB-CSIC. However, only few submissions were received from Scandinavia. Finally, a limited number of submissions were received from the U.S. and Canada, largely from researchers returning to Europe.

In essence, the planned project milestones was achieved with regard to the total number of needed strain depositions and is nearly fulfilled with regard to completed archiving and publishing strain on the EMMA website. The latter is due to submissions arriving in the 2nd half of 2012 where either the mice are not yet shipped to EMMA or archiving is still in progress. All reported mouse mutant lines will be fully archived and published at www.emmanet.org. Most partners fully achieved their milestones for the project. Some difficulties were encountered for the Swedish partner due to the

limited number of strain submissions from Scandinavia. This has been addressed by adjusting the overall deliverable for KI and by re-allocating a total of 30 lines from KI to the CNR.

Another source of difficulties encountered in this research activity is the delayed shipment of mice from the contributors to the respective EMMA node where a particular strain is being archived. The average time between communicating acceptance of a line into EMMA and the arrival of mice at the assigned archiving centre is 3.5 months but occasionally it may take several months and up to a year until submitted mice are shipped to the EMMA partners. This is largely due to local logistics and beyond the control of EMMA.

Another persistent challenge is the cryopreservation of strains that are generally quite difficult to archive such as homozygous mutants, low fertility strains and double mutants. In such cases the breeding effort involved in the archiving process is far more extensive as in the case of fertile mutants that are archived as heterozygotes. The average archiving time of strains for freezing lines in form of embryos is about 4 months. However, for about 10% of the lines the archiving time is more than twice as high. The higher effort has been accounted for by counting double and multiple mutants with an equivalent of two mouse mutant lines.

1.3.5 / WP5 Technology development and implementation

WP5 objectives

- To reduce animal usage associated with EMMA's activities
- To refine existing technologies available within EMMA
- To reduce the costs and improve the efficiency with which mouse stocks can be cryopreserved and recovered from the archive
- To reduce the strain specificity of sperm cryopreservation
- To develop technologies that will facilitate *in vitro* fertilisation using sperm samples with normal motility profiles, as well as sperm samples with low cell counts and/or poor motility
- To improve the efficiency, reduce the costs and simplify the transportation of pre-implantation embryos

Summary of progress towards WP5 objectives

Task 1 - Optimise the procedure for freezing down mutants on genetic backgrounds that are difficult to recover from frozen/thawed spermatozoa e.g. C57BL/6, 129 & BALB/c

Sperm freezing represents an efficient mechanism for archiving mouse models. However, frozen / thawed C57BL/6, 129 and BALB/c sperm has historically yielded poor results in IVF recovery experiments. There is a pressing need to overcome these problems because **a)** C57BL/6 has proved to be the strain of choice for the large scale mutagenesis programs and **b)** the archiving centres need to be able to efficiently freeze down the large numbers of strains being submitted by the community, and sperm freezing has the potential to meet this demand.

The HMGU, CNRS and MRC compared the efficacy of the in-house sperm freezing protocols with recently published sperm freezing protocols and / or post thawing sperm treatments. These methods include:

- The addition of 477 µM mono-thioglycerol added to 18% raffinose 3% skimmed milk (Ostermeier, 2008)
- Addition of 100mM L-Glutamine to the cryoprotective agents used to freeze sperm (Takeo, 2011a)
- The addition of 1.0mM methyl β cyclodextrin added to post thaw media (Takeo, 2007)
- Sperm selection and addition of methyl β cyclodextrin, plus enrichment of pre-incubation and IVF medium (Taguma, 2009)
- The addition of reduced glutathione to the IVF media (Takeo, 2011b)

We have previously reported that the addition of 477µM mono-thioglycerol to the standard cryo-protective agent (18% raffinose, 3% skimmed milk) can improve the average fertilisation rate of frozen / thawed C3H and C57BL/6J sperm. However, there is considerable variation in the fertilization rates obtained across all the genetic backgrounds tested so far.

Initially we were concerned that we were seeing the same degree of variability with other sperm freezing techniques. Nevertheless we decided to use the 477µM mono-thioglycerol data as a bench mark for further development of alternative protocols. In particular, we focused on the potential benefits of adding the cholesterol sequestering agent, methyl-β-cyclodextrin to post-thaw sperm incubation drops.

The results obtained using this approach were extremely encouraging. In fact we saw a profound increase in IVF fertilisation rates, and an associated reduction in the variability between IVF sessions when using frozen / thawed sperm harvested from C57BL/6N, C57BL/6J, BALB/c and C3H/HeH males. Although we only saw a modest increase in fertilization rates when using 129 frozen / thawed sperm, see Table 4.

Table 4: The comparison of MTG and MBCD+GSH protocols on the fertilisation rate of the frozen/thawed sperm sample from C3H/HeH, 129, C57BL/6J, C57BL/6N Tac and BALB/c background.

Methods	**C3H/HeH (±sem)**	**129 SvEv (±sem)**	**BALB/c (±sem)**	**C57BL/6N (±sem)**	**C57BL/6J (±sem)**
MTG	72.12 (5.5)	10.23 (4.4)	50.89 (13.5)	37.82 (4.7)	23.5 (4.1)
MBCD+GSH	85.92 (5.3)	15.85 (2.7)	93.61 (2.9)	78.98 (9.5)	70.4 (12.2)

In these experiments sperm was harvested from 3 separate males and subjected to two different freeze / thaw protocols.

a) MTG treatment – half of the sperm recovered from each male was frozen down using a cryoprotective agent (CPA) supplemented with 477µM mono-thioglycerol. After freeze/thawing the sperm was taken through the MRC's standard IVF protocol using regular human tubal fluid (HTF) IVF medium.

b) L-glutamine treatment – the other half of the sperm recovered from each male was frozen down using CPA supplemented with 100mg/ml L-glutamine. After freeze/thawing the sperm was taken through a modified IVF procedure which incorporated 0.75 mM methyl-β-cyclodextrin in a sperm pre-incubation drop. After 30 minutes in the pre-incubation drop the sperm was transferred into a fertilisation drop containing 1mM reduced glutathione, plus a high Ca++ HTF (75.5mg/100ml).

Each of these modifications to the IVF recovery protocol was able to enhance the fertilisation rates we achieve using frozen / thawed sperm independently of the other reagents. Table 5 shows the effect of raising the Ca++ concentration in the HTF medium from the standard 0.25mg/100ml to 0.75mg/100ml. Similarly, table 6 shows the effect of supplementing the IVF fertilisation drops with 1.0mg/ml reduced glutathione.

Subsequent analysis during the third reporting period demonstrated that the addition of 0.75mM methyl-β-cyclodextrin to the sperm pre-incubation drop was associated with the loss of the zona pellucida from a significant proportion of the fertilised oocytes. This represents an infection risk during embryo transfer. Fortunately we have been able to demonstrate that reducing the concentration of the concentration of methyl-β-cyclodextrin down to 0.25mM practically eliminates this problem.

Table 5: The effect of adding 1.0mg/ml reduced glutathione to the IVF fertilisation drops using frozen/thawed sperm pre-incubated in MBCD

Group	Genetic background of sperm	Genetic background of oocyte	Fertilisation rate (%)
MBCD	C57BL/6J	C57BL/6J	20.59
MBCD+GSH			74.29
MBCD	C57BL/6NTac	C57BL/6NTac	60.82
MBCD+GSH			70.06
MBCD	C57BL/6NTac	C57BL/6NTac	31.31
MBCD+GSH			38.96
MBCD	C57BL/6NTac	C57BL/6NTac	14.56
MBCD+GSH			15.46

Table 6: showing the effect of adding 1.0mg/ml reduced glutathione to the IVF fertilisation drops using frozen/thawed sperm preincubated in MBCD

Genetic background of oocyte and sperm	Group	Fertilisation rate (%)
C57BL/6J	MBCD	20.59
	MBCD+GSH	74.29
C57BL/6NTac	MBCD	60.82
	MBCD+GSH	70.06
C57BL/6NTac	MBCD	31.31
	MBCD+GSH	38.96
C57BL/6NTac	MBCD	14.56
	MBCD+GSH	15.46

Using this modified IVF protocol we were able to consistently achieve substantial increases of fertilization rates across a wide range of sperm samples harvested from mutant mice on a C57BL/6N background. What is more, we were able to adapt this IVF procedure for the use with freshly harvested sperm and more importantly sperm frozen down using older protocols. This novel 'rescue' protocol is an extremely valuable tool because it allows archive managers to take advantage of these improvements in IVF technology when recovering legacy samples from their archives.

These refinements to the combined sperm handling/IVF procedure have improved the reliability of IVF and reduced mouse usage and costs. For example, the average number of females used / IVF at the MRC has been reduced from 17.9 / IVF in 2011 to 11.8 / IVF in 2012 i.e. a reduction of 34% in mouse usage and associated costs. Three relevant protocols have been uploaded onto the EMMA website and are freely available to the scientific community. These are:

a) A sperm freeze/thaw and IVF recovery protocol
b) An IVF protocol using freshly harvested sperm
c) A 'rescue' IVF protocol for use with frozen sperm samples from legacy archives

Task 2 - Optimise the technique of laser assisted *in vitro* fertilization

A robust laser assisted IVF procedure will significantly improve fertilisation rates when used in conjunction with sub-optimal sperm samples. These could be frozen / thawed samples or freshly harvested sperm collected from stocks such as those with a 129 background which traditionally show poor fertilisation rates even in conventional IVF systems. The CNRS, HMGU and MRC are all now equipped with laser drills attached to inverted microscopes and have worked towards refining this procedure. Initially our results showed a consistent increase in fertilisation rates often reaching >10 times the fertilisation rates achieved with conventional IVF. These benefits are most frequently observed when the sperm sample is very poor. What is more, elevated fertilisation rates have been consistently observed with C3H, C57BL/6J and 129 sperm.

Later we noted that the increase in fertilisation associated with laser IVF occurred in conjunction with a reduction in the percentage of transplanted embryos that develop into live born pups. Even though this reduces the efficiency of laser IVF it does not negate the value of the procedure. The reason for this reduced birth rate is being investigated but no firm conclusions have been made so far. However, we have established that laser IVF is not triggering parthenogenic embryonic development. *In vitro* studies conducted within WP5 comparing the development of oocytes following laser drilling in the presence or absence of frozen/thawed sperm indicate that laser drilling does not induce parthenogenic embryo development. We have also investigated the possibility that breaching the zona pellucida so comprehensively with the laser allows more than one spermatozoa to penetrate the ooctye and induce polyspermy. By examining the oocytes between 4 and 6hrs post-fertilisation and counting the number of visible pro-nuclei we have come to the conclusion that polyspermic fertilisation is not responsible for the reduced implantation rate associated with laser IVF. The most likely explanation still appears to be that the duration of the laser pulse used to penetrate the zona pellucida adversely affects the developmental potential of the embryos following formation of the zygote. This phenomenon has been observed by the CNRS when the duration of the laser pulse was progressively increased from 100µs to 400µs.

In order to meet our commitments to the EMMAservice grant we have prepared a detailed protocol and made it available to the scientific community via the EMMA website. Although we feel that laser-IVF may have been largely superseded by improvements to conventional techniques (as detailed for task 1), we believe the laser-IVF protocol is still a useful addition to our tool kit of assisted reproduction techniques.

Task 3 - Establish an intra-cytoplasmic sperm injection (ICSI) capability within EMMA

The development of a reliable ICSI (alternatively referred to as micro-insemination) procedure within the EMMA consortium would underpin the on-going sperm freezing activities. Not least because EMMA would be able to guarantee the recovery of valuable mouse models, even when required to handle sperm samples containing low counts, non-motile sperm or even sperm frozen in the absence of cryo-protectants. The MRC, CNR and HMGU have initiated active ICSI development programmes in their laboratories and made substantial progress during the third reporting period.

To support this task a 3 day course on ICSI was commissioned at the EMBL-Monterotondo. This course was the first one of its kind in Europe and was specifically designed for EMMA and organised by Dr. Pedro Moreira. The course was held between the 19th & 21st July 2010 and was attended by delegates from eight of the EMMA partners (MRC, CNB, ICS, HMGU, CNRS, CNR, Fleming and IMG). During the ICSI course the participants were shown improved methods for preparing sperm, plus improved oocyte injection procedures which can be easily implemented by the EMMA laboratories. Building on the lessons learnt at the ICSI course, the MRC and CNR embarked on a series of *in vitro* experiments designed on improving the overall survival rates of oocytes, post-sperm injection. It was hoped that a reduction in the percentage of oocytes that lyse after sperm injection could be used as a metric for indicating improved implantation potential following surgical embryo transfer. With practice we have seen a steady reduction in the number of

oocytes that lyse after sperm injection. This clearly demonstrates the training benefits of the EMBL ICSI training course.

In Dec 2011, the MRC employed Dr Jie Zhu who has had previous experience of mouse nuclear cloning. The MRC have produced 9 live born pups from 4 litters (see table 7) of ICSI derived embryos and are continuing to develop the programme.

Table 7: Summary of ICSI development programme at the MRC using CD-1 oocytes

No. ICSI sessions	No. Oocytes Injected	No. Survived (%)	No. 2 cell (%)	No. Embryo transferred	No. Pups (%)
68	4141	1339 (32.34)	606 (45.76)	203	9 (4.43)

In addition the CNR have been extremely successful in developing their ICSI programme. Using freshly harvested or frozen thawed B6D2 sperm the CNR have achieved birth rates in excess of 17%. The birth rates using sperm frozen in the absence of any cryoprotectant, nominally referred to as ‘dead’ are <4%. However, 50-55% of the embryos develop to the blastocyst stage in culture, compared with 70-80% of embryos developing to the blastocysts stage when frozen and/or freshly harvested sperm is used. This lower birth rate indicates that the sperm is severely compromised probably because the DNA is damaged when sperm is frozen in the absence of cryoprotectants. Understanding this mechanism behind these poor implantation rates will be the focus of future study. The main aim behind these studies will be to develop a reliable protocol for enhancing sperm quality and integrity of sperm DNA. This will underpin the development of the ICSI procedure and help make ICSI more accessible to the community. In particular, we wish to develop the capacity to use ICSI to recover live born pups from male germ cells recovered from partially degraded sperm samples and/or gonadal tissues frozen in conventional freezers. Overall, we have developed a reliable ICSI protocol and can confidently say that the EMMA consortium is now competent to perform ICSI at two nodes (MRC and CNR).

Task 4 - Establish the optimum conditions for transportation of unfrozen pre-implantation embryos across international boundaries

The transportation of frozen embryos offers many advantages over the shipment of live mice but it is still relatively expensive and relies on the recipient being able to handle the frozen embryos competently. Access to a reliable and carefully defined protocol for transporting unfrozen (frozen/thawed) embryos across international boundaries would facilitate the dissemination of stocks held in the EMMA archive, reduce shipping costs and simplify the re-derivation procedure for the client receiving the embryos. The experiments conducted to date continue to focus on the use of M2 medium which is an easily accessible HEPES buffered embryo culture media. Embryos cultured in this media have been used to determine the optimum embryo holding temperature and duration. The results so far continue to indicate that embryos survive better at a holding temperature

of 8°C vs. 4°C or room temperature, and that the embryos will develop successfully up to the blastocyst stage *in vitro,* even after being held at 8°C for 72 hours.

In the early stages of this project we reported that *in vitro* development was not reflected in the embryos ability to establish a pregnancy following embryo transfer. This poor implantation rate was seen in embryos transferred immediately after cold storage or after *in vitro* culture to confirm that the embryos have survived cold storage. Since that last report we have spent considerable amount of time investigating the cause behind inconsistent developmental potential of embryos following cold storage and either poor implantation rates. We now believe the rate at which the embryos are cooled before being placed in the low temperature chamber profoundly affects embryo viability. By the end of the second reporting period we had data indicating that embryos cooled slowly by placing them is an insulated flask before transfer to the low temperature holding chamber improves embryos survival rates. Subsequent studies have shown that culturing the embryos in KSOM+AA for 3 hours before being put in the low temperature holding chamber greatly improves embryo survival (see table 8).

Table 8: In vitro development of frozen/thawed C57BL/6 embryos frozen at the 2-cell stage. Immediately after thawing the embryos were cultured in KSOM+AA for 3hrs and then placed in M2 medium and held at either room temperature or 8˚C for 24, 48 or 72hrs

Groups	Number of embryos thawed	Normal of embryos recovered (%)	Normal development (morula) after 2 days (%)	Normal development (blastocysts) after 3 days (%)
Control	33	33(100)	32(97.0)	28(84.8)
Room Temp for 24hrs	33	33(100)	30(90.9)	27(81.8)
8°C for 24hrs	31	31(100)	31(100)	27(87.1)
Room Temp for 48hrs	33	33(100)	9(27.3)	4(12.1)
8°C for 48hrs	33	33(100)	29(87.9)	27(81.8)
Room Temp for 72hrs	34	33(97.0)	0	0
8°C for 72hrs	32	32(100)	31(96.9)	29(90.6)

*RT = room temperature (22˚C)

We have continued to test the *in vivo* viability of embryos following low temperature holding. In these experiments 36 embryos are being divided between two recipient females after holding the embryos at 8ºC for 0 hours, 24 hours, 48 hours & 72 hours. In addition, we have developed a simple an inexpensive transport chamber that allows the EMMA nodes to send unfrozen embryos between partner laboratories at a defined holding temperature. This work was aided with the use of data logger to monitor chamber temperature during transportation. Using this transport box unfrozen embryos have been exchanged between the MRC and the HMGU, ICS, CNRS and CNR. Table 9 shows the *in vitro* development rates of a cohort of embryos, alongside the success rates following the implantation of a second cohort of embryos into recipient females.

Table 9: Birth rate of frozen/thawed C57BL/6NTac embryos frozen at the 2-cell stage. Immediately after thawing the embryos were cultured in KSOM+AA for 3hrs and then placed in M2 medium and dispatched in a cold package to ICS, HMGU, CNR and CNRS. The 2-cell embryos were then transferred to the oviducts of 0.5 day pseudopregnant foster mothers.

Institute	No. embryos tested	No. Normal Embryos found	No. embryos transferred	No. off springs	Birth Rate (%)
ICS (24h)	50	48	48	19	39.58
HMGU (24h)	50	50	50	11	22.00
CNR (48h)	50	50	40	4	10.00
CNRS (48h)	50	50	45	0	0.00

In summary, we have developed a robust protocol for transporting unfrozen embryos using conventional courier services. The embryos can be transported for a duration of up to 72hrs which would be sufficient to reach any laboratory in Europe, North America or Asia under normal circumstances. The copy of this protocol has been presented on the EMMA website and is available for use by the scientific community.

Overall, the WP5 program has met its deliverables and developed a comprehensive tool kit of procedures available to EMMA and the wider scientific community. Access to these new tools will enable EMMA to deliver a more efficient and cost effective cryopreservation and dissemination service to the community. Protocols for the efficient recovery of live mice from frozen thawed/sperm, laser IVF and the transportation of unfrozen embryos have all been presented on the EMMA website. Publication of an overview of new developments in cryobiology, published in Mammalian Genome (Guan et al 2012, Volume 23, Issue 9, Page 572-579) as well as access to comprehensive protocols on the EMMA website will help propagate the advances made by WP5 across the wider scientific community.

1.3.6 / WP6 EMMA informatics

The objective of this work package was to coordinate any informatics activity required by the EMMA project including interactions with other activities such as the EC FP7 EUCOMMtools project, the International Mouse Phenotyping Consortium (IMPC), the InfraCoMP coordination action and the INFRAFRONTIER project. The majority of the work involves maintaining and updating the existing EMMA databases and web interfaces that external and internal users use to query the data, deposit and order mouse lines. As well as developing the EMMA database, this work package also aims to integrate the data with other appropriate strain, genomic and phenotypic resources to raise the profile, usage and usefulness of the EMMA resources.

Summary of progress towards WP6 objectives

Milestones: EMMA IT network meetings

The EMMA informatics team interacts constantly with the EMMA Project Office and the Technical Working Group (TWG) to drive the development of the database schema and interfaces to suit the requirements of the EMMA partners as well as of external users. This interaction involves informal day-to-day contact as well as formal planning meetings at the biannual EMMA meetings and dedicated biannual EMMA IT meetings. During the course of the project a total of eight IT meetings were held.

Task 1: Development of the EMMA database schema and data

The primary goal of the data curation work was to define and provide gene / allele and strain names / symbols, according to the complex rules that define the 'International Standards of Genetic Nomenclature for Mice'. This is necessary for correct cross-reference with MGI / MGD and IMSR databases and IKMC and related project databases (EUCOMM, EUCOMMTOOLS, IMPC, etc.). Appropriate, up-to-date curation of genetic data and strain nomenclature is also essential for efficient access and use of EMMA database resources by requesting scientists, who can readily identify, compare and study mutated gene and allele sequences, with their linked bibliographic references. This applies also to the increasing number of currently mapped and sequenced alleles of ENU- and other chemically- or radiation-induced mutants, which were originally identified on the basis of phenotype analysis. The identification and assignment of correct gene and allele names / symbols / IDs is also necessary for allowing immediate user access to cross-linked human and mammalian resources of related genomic / phenomic / pathology data (ENSEMBL, Mammalian Phenotype Ontology, OMIM, etc.).

Two full-time curators, one at CNR-IBC and one at MRC-MGU (MRC funded, not charged to EMMAservice contract), take care of the manual curation of the EMMA database. The curators closely interact with the other EMMA IT workgroup members at EMBL-EBI, CNR-IBC and MRC-MGU, the members of the EMMA Project Office and the other members of the EMMA TWG.

At the end of the EMMAservice project 1942 lines in total (out of a total of 4272 deposited lines) had their mutated genes / alleles and strain names / symbols manually reviewed and curated (1526 lines were curated by CNR-IBC and 416 by MRC-MGU). These include 1060 lines with nomenclature curated, assigned by EMMA and approved by IMSR (IMSR_Approved), 494 lines with nomenclature curated, assigned by EMMA and under review by IMSR (EMMA_Checked) and 388 lines with nomenclature curated, assigned by EMMA, whose alleles / transgene are not yet annotated in MGI / MGD (EMMA_Preliminary).

Furthermore EMMA-DB curators actively participated in the definition, application and reviewing of programmatic procedures, implemented by the EMBL-EBI group, for the automated assignment of correct background, gene / allele and strain nomenclature for large scale mutant production programmes (IKMC EUCOMM/EUMODIC/KOMP, Wellcome Trust Knockout Mouse Resource and Sanger Mouse Genetics Programme, etc.), currently comprising more than 1800 strains that are archived and distributed by EMMA.

The EMMA-DB curators also collaborated on the definition, application and updating of programmatic procedures for:

- Weekly automated comparison of EMMA-DB gene/allele records with corresponding MGI-MGD data and automated completion/update of EMMA-DB records, if necessary
- Daily automated comparison of EMMA-DB bibliography records with corresponding PubMed records and automated completion/update of EMMA-DB records, if necessary
- Weekly automated upload of EMMA-DB strain / background / gene / allele / bibliography records to the IMSR database.

Main priorities for the manual curation of alleles / genes and strain data included:
(i) To review and curate nomenclature of targeted mutants (knock-outs, knock-ins, LoxP- or FRT-flanked conditionals, etc.), Cre- or Flp- expressing strains, Tet-controlled transgenic strains, that are frequently requested and distributed and whose allele/gene data are already annotated at MGI / MGD.
(ii) To assign correct allele / gene / strain nomenclature to other published or unpublished lines, whose allele / gene data are NOT already annotated at MGI / MGD; in particular, this includes defining and submitting to MGI / MGD new allele / gene names for transgenic strains, chemically / radiation-induced and spontaneous mutants, etc.
(iii) To review and curate data of newly submitted strains immediately at the point of submission. This greatly increases the overall efficiency of the curation effort, enabling effective interaction with submitter, for rapid identification and insertion / revision of missing or incorrectly submitted data, updating of bibliographic references, etc. It also allows correct identification and listing of lines carrying or expressing >1 targeted mutation / transgene, as they are increasingly produced and used for appropriate modelling of complex phenotypes and multi-factorial pathogenic traits.

The EMMA data curation team has also continued its collaborative efforts with the Jackson Laboratory and IMSR to ensure mouse strains within EMMA are properly represented and can be integrated with their resources. Representatives of the EMMA data curation group also collaborated with the IT groups of IMPC and IKMC (EUCOMM, EUCOMMtools and KOMP) projects and participated in the meetings of the InfraCoMP coordination action and of the INFRAFRONTIER project.

Overall, the objectives of task 1 have been met during the EMMAservice project, with considerable developments of the database schema and database population mechanisms as well as the curation of more than 1900 mouse mutant lines of the EMMA repository.

Task 2: Improved user interactions
We have continuously expanded the completely revamped EMMA internal site for the partners by adding several new features. The Jira Bug Tracker tool allows EMMAservice partners to create, modify, track and prioritize issues concerning all aspects of the EMMA IT infrastructure from new feature requests to data curation support. Since its inception the tool has advanced database development by facilitating communication between developers at EBI and EMMAservice partners

throughout Europe. Over 200 issues were reported during the 3rd reporting period with the majority addressed in the same time frame.

Another feature we have added is the dynamic generation of submission and request forms in a PDF format. These files are permanently stored in the EMMA database, creating a snapshot of the original submission that can be easily retrieved by EMMA partners. New PDFs can be dynamically generated when curators change the information associated with a submitted mouse strain. While we met the deliverable of re-implementing existing interfaces in the first and second reporting period, we have continued to improve the interfaces by migrating to the latest Spring / Hibernate libraries and using Tableau to generate reporting interfaces. Tableau is superior to the previously used ChartDirector software in several aspects including the ability to create intuitive administrative dashboards and to generate geographical reports.

We also improved the user-friendliness of EMMA resources to outside users. The dynamically generated PDF files described above are now sent to submitters allowing for review in an easy to read format. We further improved usability by the use of Silverback recording software to document how volunteers use the online form on the EMMA website. From our usability studies, we have modified our plan to track user data in cookies to instead directly store submitter contact and shipping information in the database. Users of submission forms will be prompted to use the contact and shipping details stored in the EMMA database after entering in an email address. This will reduce the time needed by users to fill forms while also reducing errors. We have also developed an efficient online submission form that will ease the entry of information into the EMMA database. The submission form and storing of user details has been implemented and is in the final stages of quality assurance testing. A further key achievement was the development of a comprehensive search strategy for users of the EMMA resource. While at the beginning of EMMAservice the search for mouse mutant strains was gene based, now users have the option to also search for phenotypes and associated human diseases.

Task 3: Integration with other resources

Integration of the EMMA resource with other data is a critical component of our activities. Considerable data has emerged from the International Knockout Mouse Consortium (IKMC: EUCOMM, KOMP, TIGM, NorCOMM); phenotyping efforts such as the Sanger Mouse Genetics Program (MGP) and the International Mouse Phenotyping Consortium (IMPC); and existing mouse resources such as the Mouse Genome Database (MGD) from the MGI group of Jackson Laboratory and the International Mouse Strain Resource (IMSR). We have continued collaborative efforts with these groups to ensure robust data exchange between these resources and EMMA.

In the final reporting period, a tracking resource known as iMits has been extensively developed as part of the IMPC project (www.mousephenotype.org). Built upon the i-DCC informatics platform responsible for tracking EUCOMM ES cell production, iMits tracks the production of knockout mouse strains for the IMPC project. As European partners in the IMPC will produce hundreds of knockout mouse strains in the next four years, it is crucial that an automated pipeline is built to import relevant strain information into EMMA. We have modified our automated annotation

pipeline for the i-DCC platform to capture information from the more complex iMits platform. In addition, we collaborated with the IMPC resource to add links to EMMA to allow users to order mouse strains that have a phenotype of interest.

All deliverables associated with task 3 were achieved. We will continue to ensure that such data is integrated with EMMA to improve the usefulness of the resource to the whole scientific community, either by bringing the data into the EMMA database itself or through the ability of the BioMart interface to perform distributed and integrated querying. The BioMart interface was also implemented during the EMMAservice project.

1.3.7 / WP7 Axenic service – Production 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 EMMA customers. The activities concerned the maintenance of GF isolators, the raising of GF fosters, 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.

Summary of progress towards WP7 objectives

Task 1: Increase the visibility of the EMMA germ-free service

The EMMA web site offers a unique opportunity to promote the study of mouse mutants raised in germ-free conditions. We developed a page that provides general information, selected literature references and links to specialized web sites, illustrating the recent contribution of gnotobiology to the advancement of biomedical science. These data are available on the EMMA web site at http://www.emmanet.org/axenic/intro.php.

Task 2: Transfer of mouse lines into germ-free conditions

GF isolator maintenance

For this work package six GF isolators were reserved: One hosts the seeding colonies, another hosts the common strains (including that of the seeding colony), two serve to raise the novel transfers, and one has a special design to perform the foetus transfer. The sixth isolator is undergoing a rotating annual maintenance. Each isolator undergoes a full reset every year, during which all filters and gloves are changed, potential leaks tested and cleaning and double sterilization by fumigation is performed. This procedure ends by several stringent sterility tests and takes all together about 2 months, whether the isolator has been heavily used or not. Hence, during the last 48 months a total of 24 rounds of such maintenance sessions were performed. Introduction of food, water and other material into the GF isolators is performed through steal metal cylinders that can stand long sterilization cycles and can be hooked to the isolators by a specially designed door that does not allow environmental air exchange either to the cylinder or to the isolator. These are under intensive use and any wearing-out can jeopardize the sterility of the isolator. Hence, we not only check them carefully, changing joints regularly but also replace them when at risk, which correspond to the equipment budget used in this reporting period. Sterility inside each isolator is confirmed every other month upon culture of multiple swabs in 4 different media and in aerobic and anaerobic conditions.

Specific strain requests

For any strains to be introduced into GF conditions, customers submit a request to EMMA that is evaluated by an external expert. Upon approval by EMMA, arrangements are set between the requester and the GF service at IGC to import the mouse strain of interest. According to the health status of the strain it will first be rendered SPF by embryo transfer. To this effect, animals received in a quarantine area are first bred to guarantee maintenance of the colony. Then, according to the number of females provided by the requester or to the nature of the genetic modification (maintained heterozygote or homozygote) natural mating or IVF is chosen to produce 2 cell embryos that are implanted into SPF foster mothers. Once sufficient SPF mice are available, they are set in a timed-pregnancy breeding. The pregnant females are sacrificed one day before natural birth and submitted to caesarean sections. The full uterus is clamped before surgery and introduced into the GF isolator through a bath of a strong antiseptic. The foetuses are then adopted by a GF foster mother that had delivered her own progeny less than 3 days before. The time laps between the approval of a request and its conclusion varies from 12 to 36 months according to i) the requester's ability to send animals in a timely manner, ii) the number of animals or the quality of frozen embryos provided, and iii) the strain breeding capacity. Foster mothers at time of adoption and pups at 1 month and 2 months of age are confirmed GF by faeces as well as mouth and skin swab cultures in 4 different media in aerobic and anaerobic conditions.

Task 3: Keeping live stocks (maintenance of commonly used GF strains)

Commonly requested strains were defined from our past experience with the EMMAinf project. It concerned C3H, the seeding and foster colony; C57BL/6, often used as controls for various mutants and for which we had several tissue or body fluid requests, and C57BL/6 Rag2-/-, a line requested several times by independent users. The C57BL/6 Rag2-/- strain bred poorly and finally stopped breeding. We decided that a better strategy for this line is to regularly introduce foetuses according to the demands. Hence we maintained a robust colony in SPF ready for transfer in GF. We actually did not receive new requests for this strain, but several interests were expressed. We had evidence that GF NMRI mice would be better fosters. However, with more extensive practice we realized that these animals are much bigger than C3H, and although good mothers, they require much more care and frequent cage changes inside the isolator. Hence, we discontinued this strategy and established new breeding schemes for C3H that ensure that foster mothers are not the bottleneck of the whole work package. Colonies are confirmed GF every other month by faeces as well as mouth and skin swab cultures in 4 different media in aerobic and anaerobic conditions.

Task 4: Shipment of GF mice or tissues

Most of the GF animal analyses are conducted on site by visiting scientists from the requesting laboratory. Occasionally tissues and body fluid are requested. These are prepared on site by our technical staff and sent by express mail. This was the case for a subset of dendritic cells from WT mice raised in germ-free conditions and their SPF controls (Travis 2011) and for WT sera (Treise, 2012). The former request benefitted from the help of immunologists and the cell sorting facility at the Instituto Gulbenkian de Ciencia. Four investigators requested live animals as part of the EMMAservice project. We have previously satisfyingly tested the quality of a transport company

that uses a van equipped with a mini-isolator operating on the same principle as ours. Our test concerned a transfer from Orleans (France) to Oeiras (Portugal). The same company will transport live animals from Oeiras Portugal to UK (Carding, Wakenshaw).

Interaction with other EMMA partners

The seeding colony of GF mice is the C3H strain and was imported from the EMMA partner CNRS-CDTA in Orleans, France. It serves as foster mother to newly introduced foetuses (see below). This colony is occasionally consolidated with new breeders produced at the CDTA, which was the case during the first reporting period.

Requests received and processed during the EMMAservice project

An overview of the requests handled during the EMMAservice period and their status is presented in table 10. Further details are presented below.

Requester	Nbs	Submitted	Mice received	Concluded	Deliverables
Travis 09	2	Jan 2009	Apr 2009	Jun 2011	2
Stockinger	2	Sept 2009	December 2009	Feb 2011	2
Loh	1	Mar 2010	NONE	*Retracted*	0
Travis 10	1	Aug 2010	From live GF stock	Sep 2010	1
Kollias	1	Dec 2010	Frozen embryos	*2013*	
Lino	3	May 2011	In house, large numbers	Sep 2011	3
Soares	2	Jun 2011	In house, non SPF	Nov 2012	2
Carding	1	Aug 2011	Live, non SPF, Feb 2012	*2013*	
Wakenshaw	1	Aug 2011	Being ordered from Jackson	Oct 2012	1
Fallon	1	Oct 2011	Not available	*Postponed*	
V-Fernandes	1	Mar 2012	From Live GF stocks	Mar 2012	1
Treise	2	April 2012	From Live GF stocks	Apr 2012	2

Table 10: Requests and deliverables for month 48 of the EMMAservice project

Dr. Mark Travis (2009), Faculty of Life Sciences, University of Manchester, UK. (2 requests)

The project concerned the comparative analysis of C57BL/6 WT and C57BL/6 beta8 integrin conditional knockout driven by the CD11c-Cre transgene raised in GF and in SPF conditions. This double request accounts for 2 deliverables. Breeding pairs were indicated as male beta8 flox/flox, CD11c-Cre x female beta8 flox/flox. The animals provided were not SPF. They have been received in our quarantine, bred locally to secure the line, and were re-derived to our SPF facility after natural mating. The progeny was genotyped and set in breeding. Young adult males and females produced in SPF in sufficient numbers allowed a first set of foetus transfer to GF foster mothers in September 2010. A second set of GF animals was produced in February 2011. All animals were fully analysed by June 2011. The remaining animals in SPF were used for embryo and sperm freezing, which could enter the EMMA archive if the owner agrees. **These two requests are concluded.**

Dr. Gitta Stockinger, MRC National Institute for Medical Research, London, UK (2 requests)
Interleukin-17-Cre RosaGFP, progeny of males: Interleukin-17-Cre KI heterozygotes x Females Rosa GFP. Half of the progeny bears the genotype of interest, thus this request corresponds to 2 project deliverables. The requester sent us 3 males (not SPF) that were hosted in our quarantine and bred with C57BL/6 to secure the colony, and was then further used for embryo transfer to our SPF zone. The progeny was genotyped and male Interleukin-17-Cre KI heterozygotes were raised to adult age. These were mated with females from our own SPF colony of ROSA-GFP. Foetus transfer to GF foster mother took place in November 2010 and the animals were analysed in February 2011. **These two requests are concluded.**

Dr. Mark Travis (2010), Faculty of Life Sciences, U. Manchester UK
This request concerned a specific subset of dendritic cells resident in the mesenteric lymph nodes and in gut epithelium. Cells from GF and SPF C3H animals were prepared, purified by cell sorting, lyzed for RNA preparation and the samples sent by express mail to the requester. **This request is concluded**

Dr. George Kollias, Biomedical Sciences Research Center Alexander Fleming, Institute of Immunology, Greece
The request concerns TNFdeltaARE mice that are bred and studied as heterozygotes. The breeding scheme proposed is TNF-deltaARE/+ male with wild type females. Frozen embryos were received and thawed. Only one animal out of 10 recovered carried the mutation. This male did not breed. By 5 months of age, when showing the expected signs of inflammatory disease, an IVF with WT oocytes was attempted to recover the line. 13 pups were obtained among which 5 carried the mutated allele. These were set in breeding and their progeny will be used as donors of foetuses for transfer in GF isolators early in 2013.

Dr. Andreia Lino, Instituto Gulbenkian de Ciência, Portugal, (3 requests)
The request submitted in May 2011 concerned the transfer of a homozygote double knockout AIDμS on a B6 background as well as B6 and C3H WT controls. As the animals were on site, bred as homozygotes for both alleles, provided in good numbers and were healthy, the request was immediately processed and 8 pups were raised for 2 months in GF condition. Animals were analysed on site, and control B6 and C3H WT mice raised in GF were provided from our stock. **Altogether these three requests were concluded by September 2011**. These requests should serve also to illustrate that when parents and progenies are healthy, and the genotype straightforward, EMMA requests could be processed and concluded in less than 4 months.

Miguel Soares, Instituto Gulbenkian de Ciência, Portugal (2 requests)
The request concerns homozygote GalT-/- mice. These animals were available at the IGC in a non-SPF area of the facility. They have been successfully rederived by embryo transfer to our SFP zone. Maturation of this progeny provided the parents for foetus transfer in GF isolators. The requester asked us to postpone the transfer to 6 months to match colonies in GF and SPF. Foetus transfer to GF isolators was successfully performed in October 2012. Control WT animals were provided from our stock of GF mice. **These two requests are concluded.**

Simon Carding, Institute of Food Research, Department Integrated Biology of the Gastrointestinal Tract, Norwich UK
This service requests concerns the transfer of mice homozygote for a null mutation in the TCRdelta gene to germ free conditions to support the set-up of their own GF facility. Animals were received in two batches, in February 2012 and May 2012 and received in our quarantine for embryo transfer in SPF conditions. Embryo transfer was performed in June and 7 pups were born (6 males, 1 female). A single breeding pair served to rebuild the colony in SPF. Foetus transfer to GF isolators is planned for early 2013.

Louise Wakenshaw, Institute of Food Research, Department Integrated biology of the Gastrointestinal tract programme, Norwich UK
This request concerns the transfer to GF conditions of mice homozygote for a null mutation in the CCR5 gene. The evaluation process of the proposal required additional information, which delayed for a few months the agreement. SPF animals will be purchased from Jackson, shipped directly to our SPF facility and received in May 2012. These animals bred very well and foetus were successfully transferred to and adopted (17 pups) in GF isolators in October 2012. **This request is concluded.**

Henrique Veiga Fernandes, **Molecular Medicine Institute, Lisbon, Portugal**
The request concerned GF newborn WT animals. It was immediately satisfied with pups from the C3H stock colony. **This request is concluded.**

Irina Treise, Helmholtz Zentrum Munchen, Germany
The request was for serum from 10 C3H and 10 B6 WT young adults born and raised in GF conditions. It was immediately satisfied from our stock colonies. **These two requests are concluded.**

Overall, since the start of EMMAservice we received 18 formal requests of which 14 are concluded and 2 are predicted to be concluded in 2013. The expected deliverable of 12 service requests was fulfilled during the EMMAservice project.

1.4 The impact of the EMMAservice project

1.4.1 Strategic impact of EMMAservice

EMMAservice shaped the European Research Area (ERA)

The EMMAservice 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. Overall, 10 partners from 7 different countries constituted the EMMAservice consortium. This included three new partners joining EMMA for the EMMAservice project, namely the CNB-CSIC from Madrid representing Spain as a new EMMA member, and the ICS and the Sanger Institute contributing outstanding resources and expertise to the network. The new EMMA project partners contributed to an increasing archiving and distribution capacity and beyond the EMMAservice project also provided new resources into the EMMA repository. Furthermore, during the course of the EMMAservice project more partners from Greece, Finland, Czech Republic, The Netherlands and Israel joined EMMA (but not the EMMAservice project) to further strengthen the network. Thus, EMMA will further expand into a network of national archiving nodes with mandates from the member states and is open for new member states to join. The structure and organisation of EMMA will allow an effective management, ensure the highest quality standards and cost efficiency, a centralisation on national levels, the required capacity and the appropriate coverage and outreach to the local user communities throughout the European Research Area. Overall, the EMMA consortium assembles the leading experts in Europe for archiving of mouse models and offers the needed capacities to fully exploit the opportunities presented by the emerging mouse mutant resources which cannot be provided on a national level but only by a concerted European approach.

EMMAservice supported FP7 Cooperation Specific Programs

The EMMAservice project significantly contributed to other EC funded research projects and EU and global initiatives in mouse functional genomics and also greatly benefits from these collaborations. Specifically, EMMA successfully collaborated with the EC funded EUCOMM and EUMODIC projects by archiving 500 mouse mutant lines that were produced by these projects from conditional IKMC resources. Furthermore, phenotyping by EUMODIC added significant value to these mouse mutant lines. This unique EUCOMM mouse mutant resource is highly demanded and accounts for more than half of mouse mutant lines shipped via EMMA. Archiving and distribution via EMMA facilitates the full exploitation of the conditional IKMC mouse resources by the wider biomedical research community.

EMMAservice supported and interacted with global initiatives such as the Federation of International Mouse Resources (FIMRe)

Towards a global sharing of available mouse resources the EMMA partners have previously build collaborations within FIMRe (Federation of International Mouse Resources) a network of 17 major resource centres from four continents that was founded in April 2005. FIMRe's overall goal is to ensure availability, assure quality, promote sharing and preservation of genetically defined mice, and disseminate information, affiliated resources and expertise in their use to the global biomedical research community. Specifically, all EMMA mouse mutant resources are also displayed at the International Mouse Strain Resource (IMSR, www.findmice.org), a searchable online database of

mouse strains and stocks available worldwide. Most FIMRe partners do display their available mouse mutant resources in the joint IMSR database which is widely used by the mouse community.

1.4.2 EMMAservice advanced the operation and user services of the EMMA network

The EMMA network is committed to continuously improve its operation and user focus and services. The EMMAservice project implemented a variety of measures that improved operational efficiency and the quality of user services.

- Invitation of a user representative to the EMMAservice Advisory Board (WP2, Networking)

To reinforce the user focus of the EMMA network we invited Prof. Ian Jackson a renowned representative of the mouse genetics community, who is not affiliated with any of the participating centres, to join the EMMAservice Advisory Board as a user representative. Prof Jackson very actively participated in all EMMAservice project meetings and provided invaluable advice.

- Implementation of user feedback forms (WP2, Networking)

A more user friendly online feedback form for all user services will be implemented. This should further increase the return rate compared to the current option to provide feedback to the Project Office via dedicated forms send by Email.

- Improving EMMA resources and services

1) EMMAservice provided a free of charge archiving service adding a further 1224 mouse mutant lines to the EMMA archive (WP4). 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 production of a single knockout line can easily exceed a cost of 50000 EUR and may need up to 18 months. The addition of 1224 mouse mutant lines to the EMMA repository 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 EUCOMM. Mice produced from the EUCOMM resource are among the most widely used research tools from the EMMA repository.

2) Free of charge access to EMMA mouse mutant resources was facilitated by the Transnational Access (TA) activity (WP3). A total of 15 TA calls were published on the EMMA website. In response to the calls a total of 311 genuine TA applications were submitted out of which 23 were rejected and 288 accepted. Shipped EMMA mouse mutant resources underpinned the selected applicant's research and will eventually contribute to scientific applications. By the end of the EMMAservice project first publications resulting from the EMMAservice TA were reported. The TA service also supported a number of researchers with limited financial resources who otherwise were not able to carry out their proposed projects.

Similarly, the EMMA germ-free service (WP7) offered by the Gulbenkian Institute (Portugal) is a unique and free of charge service not provided by other major repositories. The Gulbenkian fulfilled a total of 14 service requests by the end of EMMAservice. This service also underpinned research efforts of approved proposals (following an evaluation of received applications by an external expert), and may now also contribute to joint scientific publications of applicants and Gulbenkian.

3) A further focus of the EMMAservice research activities was technology development (WP5) which supported the EMMA cryopreservation service by refining existing EMMA sperm freezing technologies. The development work in the laboratory established a robust sperm freeze/recovery protocol that works extremely well on sperm harvested from inbred strains of mice across the most common genetic backgrounds. This protocol is now presented on the EMMA website and is publicly available to the wider community. Furthermore, Laser IVF was firmly established within the EMMA consortium as a robust recovery technique and a protocol was uploaded onto the EMMA website. All SOPs provided on the EMMA website are frequently downloaded as tracked by Google analytics software. Progress was also made with ICSI (Intracytoplasmic sperm injection) and we were able to generate ICSI derived embryos using sperm and oocytes from several different strains of mice (CD-1, B6D2 and C3H). Finally, we have demonstrated that frozen/thawed 2-cell embryos from several different strains (C3H, C57BL/6 & 129) will resume *in vitro* development after being held at 8°C for 72hrs. We now believe that we have defined the optimum conditions for transporting unfrozen embryos. The transportation of unfrozen embryos will simplify the exchange of mouse stocks between laboratories.

4) WP6 coordinated the EMMA informatics activities such as database development and integration with other resources, web interface development and database curation. EMMAservice involved considerable development of the database schema and the database population mechanisms as well as extensive data curation. During the project a total of 1942 strains had their gene/allele and strain names/symbols manually reviewed and curated. Up-to-date curation of genetic data and strain nomenclature is essential for efficient access and use of EMMA database resources by requesting scientists, who can readily identify, compare and study mutated gene and allele sequences, with their linked bibliographic references. New software releases covered Spring internal interfaces, new searching and strain description pages and a new statistics/reporting package, which is key to control the progress of the EMMA archiving activities and to underpin further process improvements. Data integration was facilitated via a BioMart search that allows that EMMA data are accessible by multiple resources. Furthermore, links were set up to phenotype data of EMMA stocked mouse resources available at the Europhenome database or at the Sanger Mouse Portal. Finally, information available from MGI on human disease associations to mouse models were integrated into EMMA allowing the searching of EMMA mice by associated diseases.

1.4.3 The EMMAservice project increased the potential for innovation and will strengthen the connection with human health

The EMMAservice project provided foremost access to a free of charge cryopreservation service. All 1224 mouse mutant lines submitted for the archiving service potentially constitute valuable mouse models of human diseases. Beyond the EMMAservice 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 EMMA can be used to address basic and fundamental scientific questions about in vivo gene function and may further our understanding of disease genetics. The number of human genetic studies (e.g. GWAS) has increased phenomenally over the last five years and a great opportunity now exists to validate possible disease candidates and pathways in human using mouse models. 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 EMMA 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 EMMA 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.

1.5 The project public website and contact details

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The European Mouse Mutant Archive (EMMA)

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10: Consejo Superior de Investigaciones Scientificas, Centro Nacional de Biotecnología (CNB-CSIC), Madrid, Spain

2 Use and dissemination of foreground

2.1 Dissemination and exploitation of project results

Major EMMAservice project results for exploitation were delivered by work package 4 (archiving) and work package 5 (technology development). The EMMAservice cryopreservation activities and archiving efforts led to a repository holding nearly up to 3500 mouse mutant lines by the end of the project. This entire physical resource and the associated data will be available for exploitation by the global biomedical research community. The technology development activities led to novel SOPs for cryo-technologies that are publicly available for use by the wider scientific community.

Dissemination and exploitation of the EMMA services and resources

1) Public relations: The EMMA network is generally undertaking a wide range of PR and outreach activities to attract new users to the research infrastructure and in addition aims to raise the awareness of the wider scientific community and general public for the objectives of EMMA. The general dissemination activities used by EMMA cover a variety of complementary measures such as:

- Talks or poster presentations foremost of the Co-ordinator and the Project Manager but also of the Management Board in general to promote the EMMA resources and services at conferences. This covers major mammalian genetics and human genetics conferences but also other EU consortia meetings, conferences on Research Infrastructures, policy workshops, FELASA meetings, ESF meetings on Functional Genomics amongst others. The Board of Directors also represents EMMA at numerous national conferences. During the course of EMMAservice the EMMA network activities were promoted by a total of 115 presentations at various meetings
- Hand-outs of an EMMA poster describing EMMA services and resources are distributed at conferences
- Placement of advertisements in scientific journals and in magazines covering general EU policy and research policy
- The EMMA resources and services are promoted among members of the International Society for Transgenic Technologies (ISTT) which closely collaborates with EMMA

Access to the EMMA services and resources

Access to the resources and services offered by EMMA is facilitated via the EMMA website and in future via the INFRAFRONTIER website at www.infrafrontier.eu. From 2013 onwards, following the implementation of the INFRAFRONTIER Research Infrastructure, EMMA is an integral part of INFRAFRONTIER and all resources and services can be accessed via the revamped INFRAFRONTIER website which will be launched in September 2013. This website provides general information about the EMMA network and its objectives, further it describes all procedures for using the EMMA services and provides online service request forms and a list with all publicly available strains and the associated strain related data. The available EMMA strains are also displayed at the International Mouse Strain Resource (IMSR), a searchable online database of mouse strains and stocks available

worldwide. EMMA requests a charge of 2400 EUR per strain when it supplies live mice or 1100 EUR if frozen materials are provided to customers.

Access to data resources

The EMMA strain description pages provide links to other mouse databases and informatics tools e.g. to the prominent Mouse Genome Informatics (MGI) website. A BioMart data management system has been deployed on the EMMA resource to provide an advanced query interface for users. This system also allows joint querying of any public BioMart. The EMMA BioMart is part of a collection of mouse BioMarts including the IKMC BioMart and the EuroPhenome phenotyping and EurExpress expression resources. Cross referencing with further database resources will be widened in future with a focus on the integration with databases capturing phenotype data generated by projects under the umbrella of the International Mouse Phenotyping Consortium (IMPC).

Users of the EMMA resources and services and expected impact

The comprehensive physical and data resources of EMMA 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, may facilitate the identification of potential new drug targets, and provide the foundation for the development of diagnostic, prognostic and therapeutic strategies.

Dissemination and exploitation of the EMMAservice work package on technology development and implementation (WP5)

The outcomes of the EMMAservice technology development efforts will be exploited in a variety of ways which are:

- Publications in peer reviewed scientific journals
- EMMA network internal application of developed SOPs to improve user services
- Displaying SOPs on the EMMA website

Users of the SOPs developed by EMMAservice and expected impact

Potential users of the SOPs are the archive managers of the EMMA repositories and foremost EMMA customers who will greatly benefit from easier resource shipments without liquid nitrogen. This innovative approach will simplify the procedures for exchanging mouse strains. Users will also benefit from advanced sperm freezing SOPs. Promoting the exchange of sperm will benefit EMMA and the scientific community as whole by, a) reducing the costs associated with archiving mice, b) increasing the efficiency of the cryopreservation process and c) minimising the welfare issues associated with live animal transportation. SOPs are frequently downloaded from the EMMA website as tracked using Google analytics software.

Training courses and cryopreservation workshop - Dissemination of knowledge

The extensive practical experience in cryopreservation technology available in the EMMA network is disseminated by hosting a series of EMMA cryocourses. The EMMAservice partners CNR, MRC and CNRS have organised 16 training courses which were attended by 104 participants. The courses cover mainly cryobiology but also more general topics such as handling of experimental animals. During EMMAservice the practical training courses were complemented by a dedicated Cryopreservation workshop with contributions from the leading experts in the field.

Users of the EMMA training courses and expected impact

Participants of the courses are scientists and technicians e.g. working in transgenic units. The training by EMMA in state of the art cryopreservation technologies supports the 3R principles and may lead to an overall reduction of mice that are kept on shelf and promotes the usage of frozen samples as the preferred format for shipments. The output of the cryopreservation workshop that was organised by EMMA is an abstract book and presentations of the contributing experts that are available for download from the EMMA website. This will be complemented by a dedicated book with contributions from workshop participants that is scheduled to be published in 2013.

2.2 Contribution to standards

The EMMAservice project contributed to the development of standards by constantly reviewing and updating protocols and SOPs that are used for cryopreservation and distribution of mouse resources. These standards will be disseminated by the following means:

- Publications in peer reviewed scientific journals
- Displaying SOPs on the public EMMA and INFRAFRONTIER websites
- Teaching SOPs developed in training courses

2.3 Contribution to policy development

A key EU policy development is the Innovation Union initiative which highlights the increasing relevance of research infrastructures to enable world class basic research and innovation. The Innovation Union policy stresses the need of pooling resources across Europe to build and operate research infrastructures due to their high cost and complexity. The EMMAservice project is fully in line with the Innovation Union policy and supports its implementation. The EMMAservice project integrated three new partners into the EMMA network. Furthermore, new partners from Greece, Finland, Czech Republic, the Netherlands and Israel also joined the network during the course of the project. Overall, the EMMAservice project assembled the leading experts in Europe for the cryopreservation of mouse models of human disease and offers the needed capacities to fully exploit the opportunities presented by the emerging mouse mutant resources which cannot be provided on a national level but only by a concerted European approach. The enlarged partnership of the EMMA network results in a more balanced distribution of mouse repositories across Europe and is thus contributing to Cohesion Policy and shaping of the ERA.

List of scientific publications										
No	**Title**	**Main author**	**Title of the periodical or the series**	**Number, date or frequency**	**Publisher**	**Place of publication**	**Year of publication**	**Relevant pages**	**Permanent identifiers if available**	**Is/Will open access provided to this publication?**
1	EMMA – mouse mutant resources for the international scientific community	Phil Wilkinson	Nucleic Acids Research / database issue Jan 2010	*Nucl. Acids Res.* (2010) 38 (suppl 1): D570-D576.	Oxford Journals	Oxford	2010 Epub September 26, 2009	570-576	PMID: 19783817	Y
2	Centralized mouse repositories	LR Donahue	Mammalian Genome, Special Issue on Mouse Genomics Programs and Resources	Mamm Genome. 2012 Oct;23(9-10):559-71	Springer	New York	2012 Epub September 4, 2012	559-571	PMID: 22945696	Y
3	Overview of new developments in and the future of cryopreservation in the laboratory mouse	Mo Guan	Mammalian Genome, Special Issue on Mouse Genomics Programs and Resources	Mamm Genome. 2012 Oct;23(9-10):572-579	Springer	New York	2012 Epub August 31, 2012	572-579	PMID: 22936001	Y

Note: These are publication authored by EMMAservice network partners. Publications resulting from WP3 i.e. from recipients of free of charge Transnational Access units are not listed here. Publications of recipients of TA units where the provided resources were used are detailed on page 21 of this report.

List of dissemination activities								
No	**Type of activities**	**Main leader**	**Title**	**Date / period**	**Place**	**Type of audience**	**Size of audience**	**Countries addressed**
1	Cryocourses - MRC	MRC	Cryocourses	January and September each year of project Total of 8 training courses	MRC Harwell	Technicians, scientists	For each course about 6 participants	Participants from across EU
2	Cryocourses - CNR	CNR	Cryocourses	November each year of project Total of 4 training courses	CNR Monterotondo	Technicians, scientists	For each course about 10 participants	Participants from across EU
3	Cryocourses - CNRS	CNRS	Cryocourses	January 2010, October 2010, March 2011, March 2012 Total of 4 training courses	CNRS Orleans	Technicians, scientists	For each course about 4 participants	Participants from across EU
4	Workshop	CNB-CSIC	Cryopreservation Workshop	May 7th-8th 2012	Madrid CSIC headquarter	Scientists, technicians	60	Participants of workshop from across the world

5	Oral presentation	HMGU	NorIMM Symposium	June 2009	Rovaniemi, Finland	Scientists	100	Mainly Skandinavian area
6	Poster presentation	HMGU	Mouse Molecular Genetics Meeting	September 2009	Hinxton, UK	Scientists	250	Mouse geneticists from across the world
7	Oral presentation	HMGU	MUGEN meeting	October 2009	Athens, Greece	Scientists	100	Immunologists mainly from EU
8	Poster presentation	HMGU	International Mouse Genome Conference	November 2009	La Jolla, USA	Scientists	250	Mouse geneticists from across the world
9	Poster presentation	HMGU	2nd annual NGFN meeting	November 2009	Berlin, Germany	Scientists	400	Human geneticists from across Germany
10	Oral presentation	HMGU	9th Transgenic Technology Meeting	March 2010	Berlin, Germany	Scientists	400	Transgenic technologists from across the world
11	Poster presentation	HMGU	International Mouse Genome Conference	October 2010	Crete, Greece	Scientists	250	Mouse geneticists from across the world
12	Poster presentation	HMGU	3rd annual NGFN meeting	November 2010	Berlin, Germany	Scientists	400	Human geneticists from across Germany
13	Oral presentation	HMGU	Cryo-Workshop	March 2011	University Hospital Hamburg	Scientists, technicians	50	Mouse community from local University Hospital
14	Oral presentation	HMGU	International Mouse Genome Conference	June 2011	Washington DC, USA	Scientists	400	Mouse geneticists and complex trait community from across the world
15	Poster presentation	CSIC, HMGU	10th Transgenic Technology Meeting	October 2011	St. Pete's Beach, Florida, USA	Scientists	350	Transgenic technologists from across the world
16	Oral presentation	HMGU	ESOF European Science Open Forum	July 2012	Dublin, Ireland	Scientists, policy makers	100	Scientists, policy makers, infrastructure operators from across world

17	Oral presentation	HMGU	Biocenter Finland Research Infrastructure Day	August 2012	Tampere, Finland	Scientists, students	100	Largely Finnish scientists
18	Poster presentation	HMGU	International Mouse Genome Conference	October 2012	St. Pete's Beach, Florida, USA	Scientists	250	Mouse geneticists from across the world
19	Poster presentation	HMGU	Mouse Molecular Genetics Meeting	October 2012	Asilomar, USA	Scientists	250	Mouse geneticists from across the world
20	Advertisements	HMGU	2 page advert describing EMMA in Public Service Review / Issues 17 and 18	2009	PSCA publisher, Newcastle, UK	Politicians, science policy makers	15000	Politicians and policy makers across EU
21	Email lists	CNB-CSIC	Promoting EMMA services via Email lists e.g. transgenicos@listas.cnb.csis.es List used to circulate posters and letters	2009 / 2010	Spain	Mouse geneticists across	300	Spanish mouse community

Notes: Beyond the presentations funded by EMMAservice about 100 additional presentations were given by the EMMA Director and other members of the Board of participating Directors as well as from scientists of the Technical Working group to various audiences covering both scientists and policy makers. Distribution activities via the EMMA website are described on pages 46 / 47 of this report. Publications are listed on page 49.

Type of exploitable foreground	Description of exploitable foreground	Confidential YES / NO	Foreseen embargo date	Exploitable product or measure	Sector of application	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
Cryopreserved mouse mutant lines in EMMA repository	Cryopreserved mouse mutant lines	No (However, distribution of some lines may be restricted by a max grace period of 2 years granted to depositors of mouse mutant lines	No	EMMA distributes live mice or frozen stock for basic or applied research to recipient scientist	Biomedical research	Depending on research of user	EMMA has no rights in deposited lines. Distribution may be subject to MTAs	Depositor of mouse mutant lines. EMMA acts as broker
SOPs developed by WP5(technology development)	SOPs	No	No	Application of SOPs for in house use by EMMA partners, in EMMA cryocourses or by any interested scientist e.g for local cryopreservation via sperm freezing	Biomedical research	Biomedical research	No	All EMMA partners