D2.2 and D2.4: Combined C&D Group Publication on INFRAFRONTIER and IMPC Globally Available and Shared Resources

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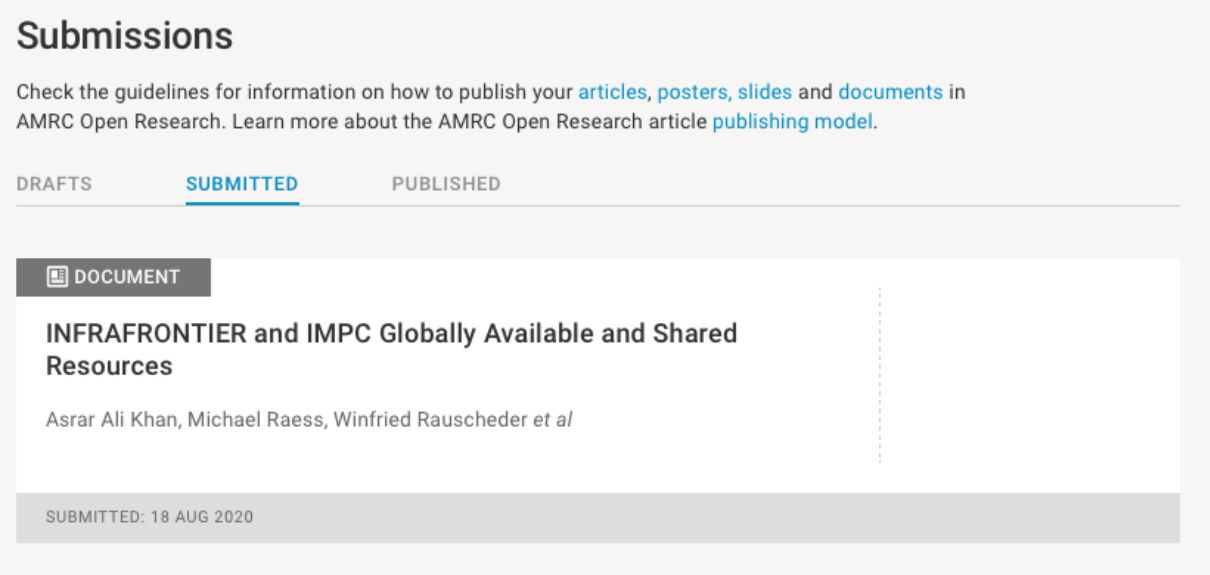

INFRAFRONTIER and IMPC Globally Available and Shared Resources

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INFRAFRONTIER / EMMA Services

European Mouse Mutant Archive (EMMA) is a non-profit repository for the collection, archiving (via cryopreservation) and distribution of relevant mutant mouse strains essential for basic biomedical research. It is the third largest mouse repository in the world with over 7500 strains archived. The laboratory mouse is the most important mammalian model for studying genetic and multi-factorial diseases in humans. The comprehensive physical and data resources of EMMA support basic biomedical and preclinical research, and the available research tools and mouse models of human diseases offer the opportunity to develop a better understanding of molecular disease mechanisms and help setting up the foundation for the development of diagnostic, prognostic and therapeutic strategies.

Some of the important INFRAFRONTIER / EMMA services include:

- EMMA database: The extensive online mouse strain catalogue (hosted on www.infrafrontier.eu) contains more than 7500 strains including major collections from large-scale projects like the International Knockout Mouse Consortium (IKMC) ES cell resources, Genome Editing Mice for Medicine (GEMM) programme and more.

- Archiving: Scientists who have developed medically relevant mutant mouse strains may submit their live models to EMMA for top level archiving - free of charge for all academic institutions.

- Distribution: Biomedical researchers worldwide who need mouse models for different disease areas can choose and order EMMA lines for their specific scientific needs.

- Scientific User Support: EMMA's scientific User Support team, with its scientifically trained staff, can offer different levels of support to all interested users. The team can advise users regarding multiple topics: e.g. archiving of their mutant strains at EMMA, requesting their strains of interest.

- Technology Development & Training: The INFRAFRONTIER Research Infrastructure and its partners offer a wide range of state-of-the-art training opportunities. These courses, offered by mouse clinics in different European countries, provide outstanding trainings in mouse phenotyping and specialised phenotyping courses. They also cover unique training opportunities in mouse model development as well as hands on cryopreservation courses offered by EMMA partners for many years. The list of courses is regularly updated (https://www.infrafrontier.eu/resources-and-services/infrafrontier-training-and-consulting-services).

- Protocols: A large collection of standardized protocols for cryopreservation and phenotyping available online to the scientific community on the INFRAFRONTIER portal (https://www.infrafrontier.eu/knowledgebase/protocols/cryopreservation-protocols).

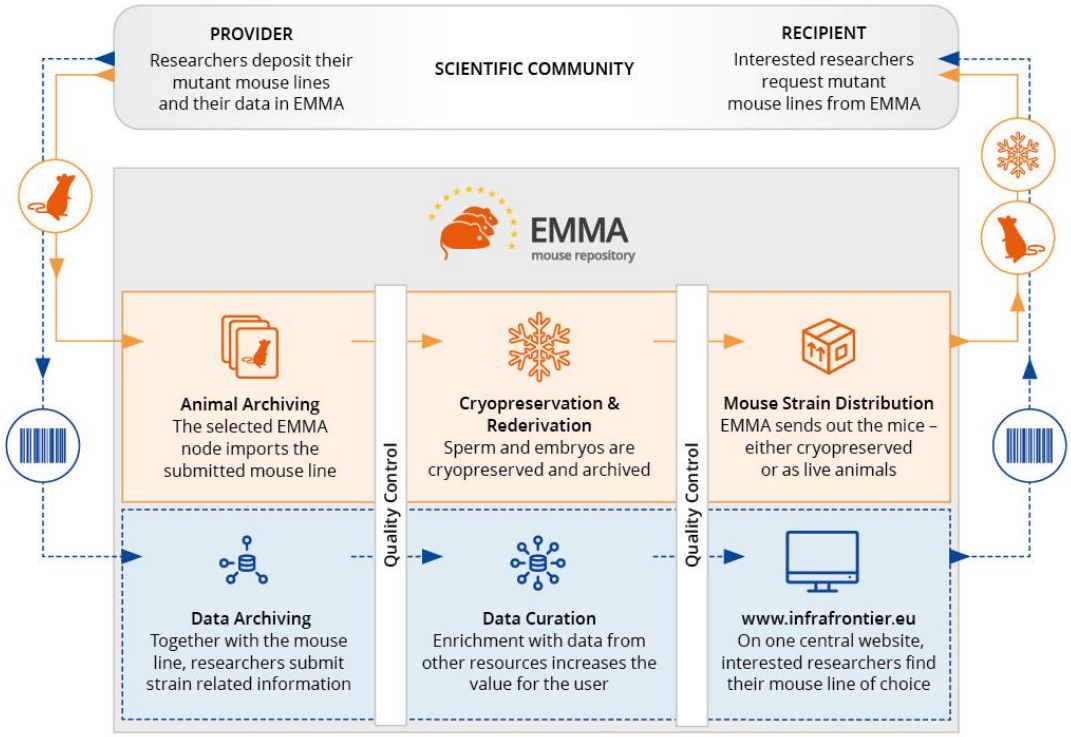

Figure 1: Scientific brokerage: EMMA archives mouse strains and data from the 'supply side' (providers) of the scientific community and offers them to the 'demand side' (recipients).

In essence, EMMA functions as a biological and digital scientific broker between the strain providers (supply side) and the strain recipients (demand side). On the biological side, this process starts with a selected EMMA node importing the submitted strain, archiving it by cryopreservation of sperms and/or embryos and distribution of the strain as cryopreserved materials or live animals. While on the digital front, the providers also submit strain-related information (like genotype, phenotype etc.). This data is enriched from other sources like MGI to add value to the end users and is made accessible to wider-scientific community at www.infrafrontier.eu. Quality control measures at each step ensure that these services hold up to high scientific standards.

The International Mouse Phenotyping Consortium (IMPC) Services

The International Mouse Phenotyping Consortium was founded in 2010 with the aim of generating a complete catalogue of mammalian gene function by systematically knocking out and phenotyping every mouse gene. With nearly a third of the mouse genome analysed by 2018, the IMPC website (www.mousephenotype.org) hosts 74 million data points and over 400,000 images.

The IMPC phenotyping centers use standardised allele production methods to knock out genes and a set of defined phenotyping tests to study the resulting phenotypes.

A variety of different and complementary gene targeting strategies are used to produce knockout alleles that involve deletion of a crucial exon common to all transcripts. Since its discovery, CRISPR/Cas9 has been widely adopted by the IMPC to generate knockouts.

The mouse phenotypes are characterized in 'phenotyping pipelines' formed from different phenotyping tests. These pipelines are used to acquire broad phenotype data at the embryonic and adult stages in a high-throughput manner. Multiple organ systems and disease states can be studied owing the broad range of phenotyping platforms.

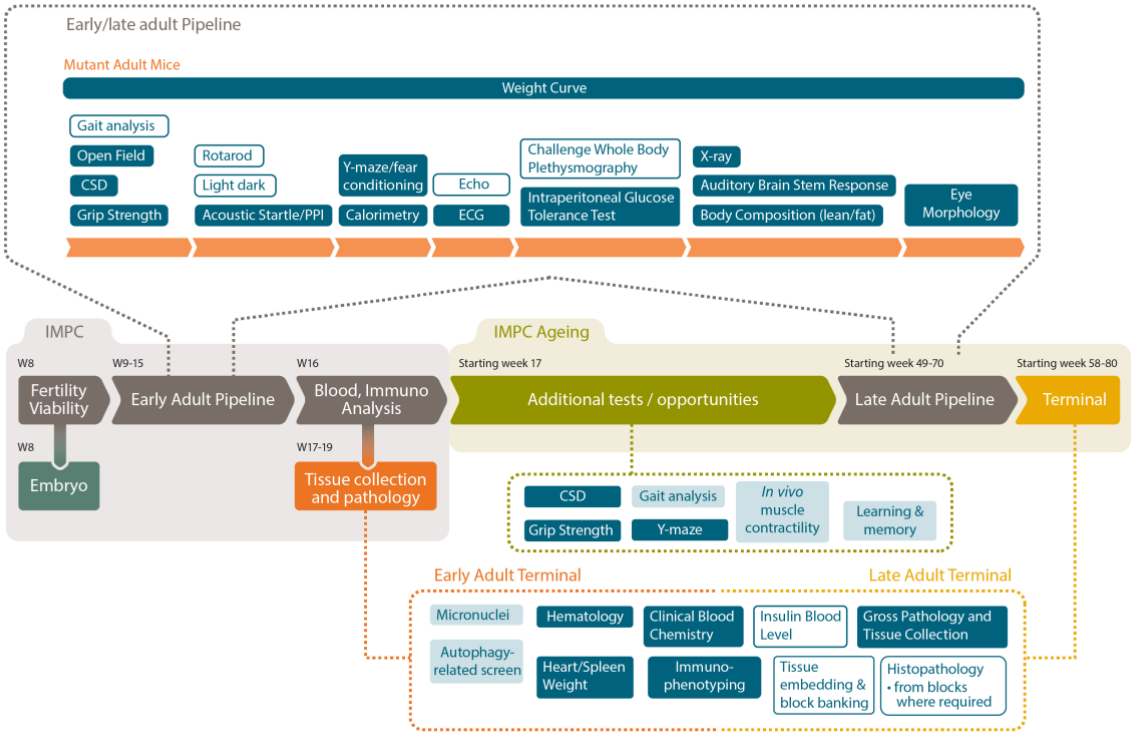

Figure 2: The IMPC phenotyping platform (https://www.mousephenotype.org/understand/the-data/)

Common INFRAFRONTIER and IMPC members and their shared services:

There are 9 institutes who are members of the INFRAFRONTIER RI and IMPC. These specialized institutes offer a wide range of shared services to the global research community and further the scientific goals of the respective research consortia. These institutes are also members of the IMPC Steering Committee.

Helmholtz Center Munich (HMGU), Germany

The Helmholtz Center Munich – German Research Center for Environmental Health (HMGU) is a member of the Helmholtz Association, dedicated exclusively to health-related scientific research. It is a part of the Helmholtz Association, the largest research organisation in Germany.

The central objective of HMGU's Institute of Experimental Genetics (IEG) is to explore the pathogenesis of human diseases. The IEG is a global leader in the systemic study of mouse models for human diseases and in the deciphering of involved genes.

As part of IEG, the German Mouse Clinic (GMC) is specialised on the comprehensive phenotyping of mutant mouse models evaluating numerous physiological pathways within a variety of therapeutic areas. In standardized pipelines, GMC contributes phenotyping data to the comprehensive databases of the **IMPC**. Additionally, it offers an open access platform of several phenotyping pipelines including the scientific interpretation of the data to clinical research teams from universities, scientific institutions and industrial partners.

Since 1997, IEG has built up its expertise in the development, cryopreservation and archiving of mutant mouse models. Today it provides an archive of more than 800 mutant mouse lines (sperm and embryos). IEG's Cryo Unit is operating as the German EMMA node: It ś actively archiving and distributing mouse strains, which have been developed in-house, as well as others submitted to EMMA by external research partners

List of shared services from HMGU:

- Mouse model generation
- EMMA archiving and distribution node
- IPAD-MD project coordinator
- Mouse phenotyping
- Mouse sperm and embryo cryopreservation
- In vitro fertilization (IVF)
- Revitalization of frozen mouse lines
- Rederivation of mouse lines
- Training in IVF, cryopreservation and rederivation techniques,
- Import and export service for INFRAFRONTIER and IMPC users

Czech Center for Phenogenomics (CCP), Czechia

The Czech Centre for Phenogenomics (CCP) is a large research infrastructure hosted at the Institute of Molecular Genetics CAS v.v.i. The CCP is unique in combining genetic engineering in mice and rats, comprehensive phenotyping including advanced imaging methods and metabolomics, SPF animal facility, cryopreservation and archiving, a PDX platform, and preclinical services – all in one central location at the BIOCEV campus. The concentration of specialized infrastructure and expertise provides a valuable resource for the biomedical and biotechnology research community.

The phenotyping module offers state-of-the-art tools for the physiological assessment of mice or rats with more than 700 parameters per model in basic phenotyping. The service can be expanded to disease-focused special screens or customized pipelines.

The transgenic and archiving module focuses on generation of mutant rodent models using cutting edge technologies such as zygote electroporation and microinjection of DNA and CRISPR/Cas targeting tools. Other services include microinjection of targeted ES cell lines to produce chimeric mice; mouse archiving; recovery of live mice from cryopreserved embryos and sperm, and also analysis of sperm viability.

CCP became fully operational in autumn 2016, and has since developed into one of the largest centers of its kind in Europe. Based on production numbers, requests and user feedback, CCP is a highly valued and productive member of INFRAFRONTIER and the **IMPC**. List of shared services from CCP:

- Mouse model generation
- EMMA archiving and distribution node
- IPAD-MD project partner
- Mouse phenotyping
- Mouse sperm and embryo cryopreservation
- IVF
- Revitalization of frozen mouse lines
- Rederivation of mouse lines
- Allele conversion services
- Training in IVF, cryopreservation and rederivation techniques,
- Import and export service for INFRAFRONTIER and IMPC users
- CRISPR/Cas and gene-editing courses and training for INFRAFRONTIER and IMPC users

MRC Harwell Institute, UK

The MRC Harwell campus is a centre of excellence in mouse genetics where the mouse is studied across a range of disciplines from early development through to aging. Harwell also specialises in mouse model generation, phenotyping, archiving and training.

Mouse model generation is offered through the Genome Editing Mice for Medicine (GEMM) programme. GEMM exploits Harwell's technical expertise and recent advances in genome editing to generate mouse lines that advance our knowledge or are of widespread use to the biomedical community.

Harwell generates/phenotypes 250 mouse lines each year for the **IMPC** and also offers its phenotyping expertise to investigators from other scientific institutions. The Harwell embryo bank was established in the mid-1970s and now acts as the UK's EMMA node offers public archiving, IVF and mouse import/ export services.

The new Advance training centre (https://www.har.mrc.ac.uk/training/) supports wet lab and theoretical learning in essential lab skills, in depth mouse genetics and genome editing. This training space is available to academic and industry partners.

List of shared services from MRC Harwell Inst.:

- Mouse model generation
- EMMA archiving and distribution node
- IPAD-MD project partner
- Mouse phenotyping
- Mouse sperm and embryo cryopreservation
- IVF
- Revitalization of frozen mouse lines
- Rederivation of mouse lines
- Allele conversion services
- Training in IVF, cryopreservation and rederivation techniques

Phenomin-ICS, France

The Mouse Clinical Institute (PHENOMIN-ICS) is a French research infrastructure of excellence for translational research and functional genomics operated by *INSERM* (National institute for biomedical research), CNRS (National center for scientific research) and the University of Strasbourg. It is part of the IGBMC platforms (Institute of Genetics and Molecular and Cellular Biology), and this close interaction strongly fuels its strong research and development program.

PHENOMIN-ICS provides state-of-the-art specialized services and consultancy to academic and industrial users generating approximately 70 genetically modified mice or rats per year and perform about 350 phenotyping projects per year. As part of the national research infrastructure CELPHEDIA (www. celphedia.eu), which focuses on all model animals used in scientific and biomedical research, PHENOMIN-ICS supports the French scientific community in the creation, care, phenotyping, distribution and archiving of animal models.

List of shared services from Phenomic-ICS:

- Mouse model generation
- EMMA archiving and distribution node
- IPAD-MD project partner
- Mouse phenotyping
- Mouse services: IVF, rederivation, axenization/gnotoxenization
- Technical and theoretical training (phenogenomics training schools)

Universitat Autònoma de Barcelona (UAB), Spain

The Universitat Autònoma de Barcelona (UAB) is one of the major public universities in Spain. The broad range of research activities includes Biotechnology, Biomedicine and Animal Health.

As part of the UAB, the Centre of Animal Biotechnology and Gene Therapy (CBATEG) is specialized on studying the pathophysiological causes of diabetes mellitus using transgenic animal models and in developing gene therapy approaches for this disease by *in vivo* genetic manipulation of tissues using adeno-associated (AAV) viral vectors. In recent years, CBATEG researchers also applied their know-how on gene transfer technologies to the development of gene therapies for severe inherited metabolic disorders such as Mucopoly- saccharidosis (MPS). Within the frame of the public-private partnership with the ESTEVE Pharmaceuticals Company, three gene therapy therapeutics have received the orphan drug designation by the European and North American authorities (EMA and FDA). The first clinical trial started in 2018, with a gene therapy treatment of MPSIIIA patients. Moreover, CBATEG researchers coordinate the ERASMUS+ Knowledge Alliance consortium PATHBIO, a project that will gather information, data and expertise to support teaching in mouse pathobiology and different aspects of mouse phenotyping. The CBATEG has also established a core of platforms that offer metabolic, morphological and pathological mouse phenotypic analyses to internal and external users.

List of shared services from UAB:

- IPAD-MD project partner
- Core platforms for metabolic, in vivo imaging, morphological and pathological mouse phenotypic analyses to internal and external users
- Training in mouse pathobiology – PATHBIO Summer Courses
- Contribute to phenogenomics training schools
- PATHBIO project Coordinator
- Production of adeno-associated viral (AAV) vectors for gene transfer and for the development of gene therapy approaches
- Mouse and rat model generation by CRISPR/Cas9 system
- Mouse and rat conventional transgenesis by pronuclear microinjection
- Mouse Blastocysts injection with recombinant clones of ESC (embryonic stem cells)
- Mouse sperm and embryo cryopreservation
- Rat embryo cryopreservation
- Revitalization of Mouse and Rat lines from frozen embryos
- Mouse IVF
- Health rederivation of mouse and rat lines

Consiglio Nazionale delle Ricerche (CNR), Italy

The Italian National Research Council is the largest multidisciplinary public research institution of Italy. It carries out advanced research in all sectors of knowledge and promotes its applications for the scientific and socio-economic development of Italy. CNR operates all over the Country, with over 90 institutes diffusing its competences and promoting cooperation with local and international institutions and companies.

CNR is a founding partner of the European Mouse Mutant Archive (EMMA) and INFRAFRONTIER networks and has established their Italian node and the new Mouse Clinic at the "A. Buzzati-Traverso" Campus, Monterotondo (Rome).

Since June 2019 these infrastructures are part of the new CNR's Institute of Biochemistry and Cell Biology (IBBC). They are integrated components of INFRAFRONTIER and **IMPC** for generation, phenotyping, cryopreservation and dissemination of mutant models of human diseases, with their bioinformatics resources. Advanced imaging modalities are also major research areas, in collaboration with IBBC's multi-sited cellular/ biological node of EuroBioImaging.

Complete infrastructures, equipment and services are readily available to international users for mouse mutant model production, phenotypic and advanced imaging analysis, cryopreservation and distribution, genotypic/phenotypic data curation and disease model annotation, as well as related training activities.

List of shared services from CNR:

- Mouse model generation
- EMMA archiving and distribution node
- Mouse model data curation
- IPAD-MD project partner
- Mouse phenotyping
- Mouse sperm and embryo cryopreservation
- IVF
- Revitalization of frozen mouse lines
- SPF Rederivation of mouse lines
- Training in IVF, cryopreservation and rederivation techniques

European Bioinformatics Institute (EMBL-EBI)

EMBL-EBI manages large-scale biological databases, which are available to clients via 24/7 web services. The demand for rapid access to all publicly available biological databases is constantly growing, as is the volume of biological information held within the databases. To support these needs, EMBL-EBI manages an extensive high-performance computer and data-storage infrastructure. It hosts some of the world's most important collections of biological data and has been supporting the informatics needs of INFRAFRONTIER / EMMA for over 15 years as well as **IMPC**.

EMBL-EBI is located in South Cambridgeshire on the Wellcome Genome Campus, which also hosts the Wellcome Sanger Institute, making the campus one of the world's largest concentrations of expertise in genomics and bioinformatics. EMBL-EBI comprises two purpose-built, state-of-the-art buildings for bioinformatics services, research, training, industry collaboration and European coordination. The buildings house dedicated office workspaces, meeting rooms, a lecture theatre, two training rooms and multiple video-conferencing suites. They are a part of the European Molecular Biology Laboratory (EMBL), an international, innovative and interdisciplinary research organisation funded by 26-member

states and two associate member states. The Laboratory has six units: the main Laboratory in Heidelberg, and Outstations in Hinxton (the EBI), Grenoble, Hamburg, Monterotondo near Rome and Barcelona.

List of shared services from EMBL-EBI:

- IMPC database and web portal including FAIR-sharing data and sustainability
- Informatics and statistical support

The Center for Phenogenomics (TCP), Canada

TCP is owned and operated by The Hospital for Sick Children and Mount Sinai Hospital. It is a unique resource that provides critical tools (mouse and rat models) and services for high calibre biomedical research. TCP was designated a national research facility by the Canada Foundation for Innovation in 2014, and a Genomics Technology Platform by Genome Canada in 2015.

TCP's facility is uniquely programmed to design, produce, manage, analyse, and dis- tribute mouse models to enable discovery, translational studies, and preclinical bioavailability, safety, and effect evaluation for therapeutic discovery.

Via projects like IPAD-MD, TCP has established cooperative and productive collaboration with all other INFRAFRONTIER members.

TCP is a founding member of the International Mouse Phenotyping Consortium (IMPC) and is designated and supported as a National Research Facility (one of 13) by the Canada Foundation for Innovation (www.innovation.ca/MSI). TCP is also funded by and a Node (one of 9) of Genome Canada's Genomic Innovation Network (www.genomecanada.ca/GIN). TCP is an integral networking partner in several INFRAFRONTIER EC-grants but does not offer any services directly.

List of shared services from TCP:

- Mouse & rat model generation
- Mouse breeding, procurement, & in vivo mouse studies
- Rederivation of mouse lines
- ES cell derivation
- Clinical Phenotyping
- Imaging
- Infection & Inflammation models
- Pathology
- Cryopreservation & Recovery

National Centre for Biotechnology (CNB-CSIC), Spain

The National Centre for Biotechnology (CNB) forms part of the Spanish National Research Council (Consejo Superior de Investigaciones Científicas, CSIC) the largest public research institution in Spain with 120 research institutes in all areas of knowledge. The CNB was inaugurated in 1992 with the vision to spearhead biotechnology research in Spain. Since its inception, the center has strived for scientific diversity, bringing together the expertise of molecular biologists, biochemists, geneticists, physicists and engineers to create a unique multidisciplinary research environment. The CNB is one of the largest research centers of the CSIC, with 70 research groups working in five departments. The CNB encompasses an animal facility with a capacity for 25,000 mice, including BSL2 area.

Since 2007, the CNB joined the EMMA consortium with the support of the Spanish scientific community thanks to the existing and active Mouse Sperm and Embryo Cryopreservation Facility that provides both EMMA and private mouse banking services for researchers. To date 440 EMMA mouse lines and more than 500 private mouse lines have been cryopreserved and many distributed world-wide. In addition, under the leadership of Dr. Lluis Montoliu, who pioneered the use of CRISPR tools for the generation of genome-edited mouse models in Spain, the Spanish EMMA node has contributed with the generation of numerous new mouse models of interest in biomedicine.

List of shared services from CNB:

- Mouse model generation
- EMMA archiving and distribution node
- IPAD-MD project partner
- Promotion of INFRAFRONTIER model development platform
- Invited speaker and instructor (Dr. Lluis Montoliu) in CRISPR Courses editions 2019, 2018, 2017, 2016, co-organized by CCP.
- Mouse sperm and embryo Cryopreservation
- IVF
- Revitalization of frozen mouse lines
- Rederivation of mouse lines

Globally available shared mouse resource for archiving and distribution from INFRAFRONTIER / EMMA and IMPC

The Knock-out Mouse Consortium (IKMC) was developed after the completion of the mouse genome sequence to systematically generate mutant ES cells for every gene in the mouse genome (more than 20,000 genes). The IMPC builds on the efforts of IKMC in developing these knockout mice and carries out high-throughput phenotyping of each line to functionally characterise every gene in the mouse genome. These strains are cryopreserved in various mouse repositories and are readily available to the global research community. A majority of these strains are archived and distributed via INFRAFRONTIER / EMMA nodes.

Nearly half of the strains archived in EMMA are IMPC strains with an average of about 390 submissions per year during 2015 – 2019 (figure 3).

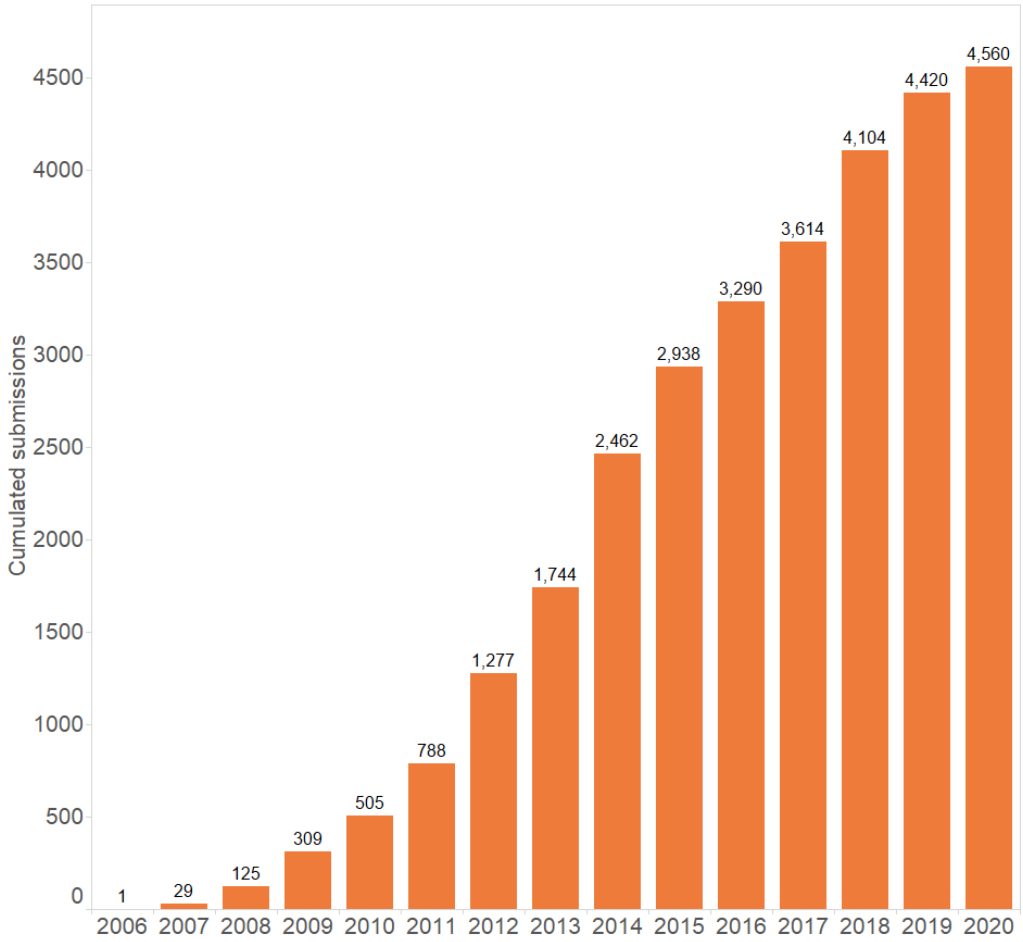

Figure 3: Cumulative number of IKMC / IMPC submissions per year to the EMMA public strain list

In total, there are 4514 IKMC / IMPC strains on the public EMMA strain list with 4469 alleles and 3587 genes (figure 4). The majority of these strains are Tm1a – knockout-first allele and CRISPR-generated strains. The latter are expected to increase significantly in the upcoming years owing to the widespread use of CRIPR-Cas9 in the generation of mouse models.

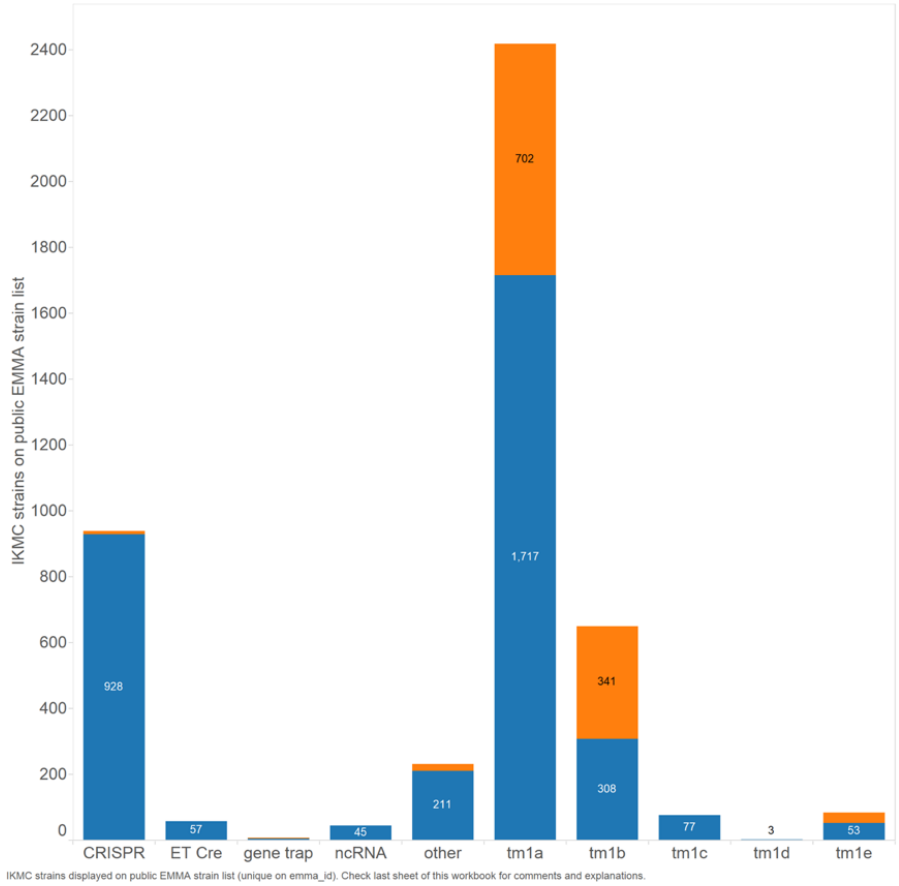

Figure 4: Different types of IKMC / IMPC strains on the EMMA public strain list. Orange segments denote strains with available IMPC phenotyping data and blue are strains without IMPC phenotyping data.

Currently, INFRAFRONTIER / EMMA is the largest distributor of IKMC / IMPC mouse strains with about an average of 350 shipments per year from 2015 – 2019 (figure 5). These numbers denote completed shipments from EMMA nodes excluding strain requests that in process or third-party transfers.

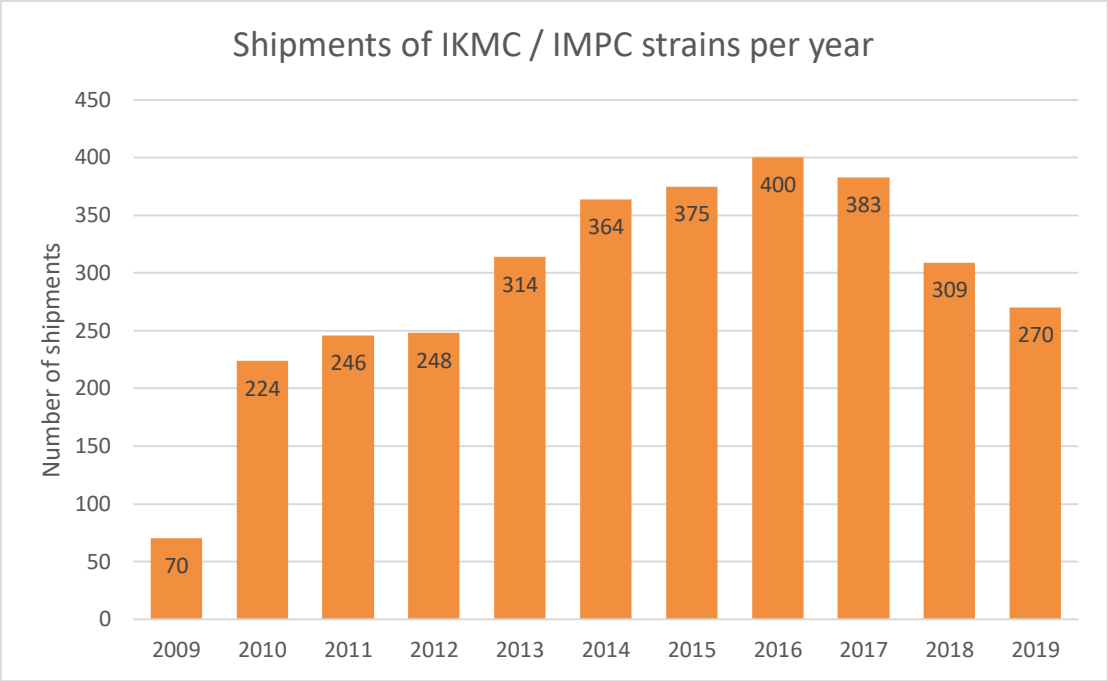

Figure 5: Number of shipments for IKMC / IMPC mouse strains from the EMMA public strain list.

North America is a major shipment destination of the IKMC / IMPC strains with a total of 1355 strains shipped (2009 - 2020), closely trailing Europe with 1416 strains. This positions INFRAFRONTIER / EMMA as a globally available and shared resource among the life science researchers across the world (figure 6).

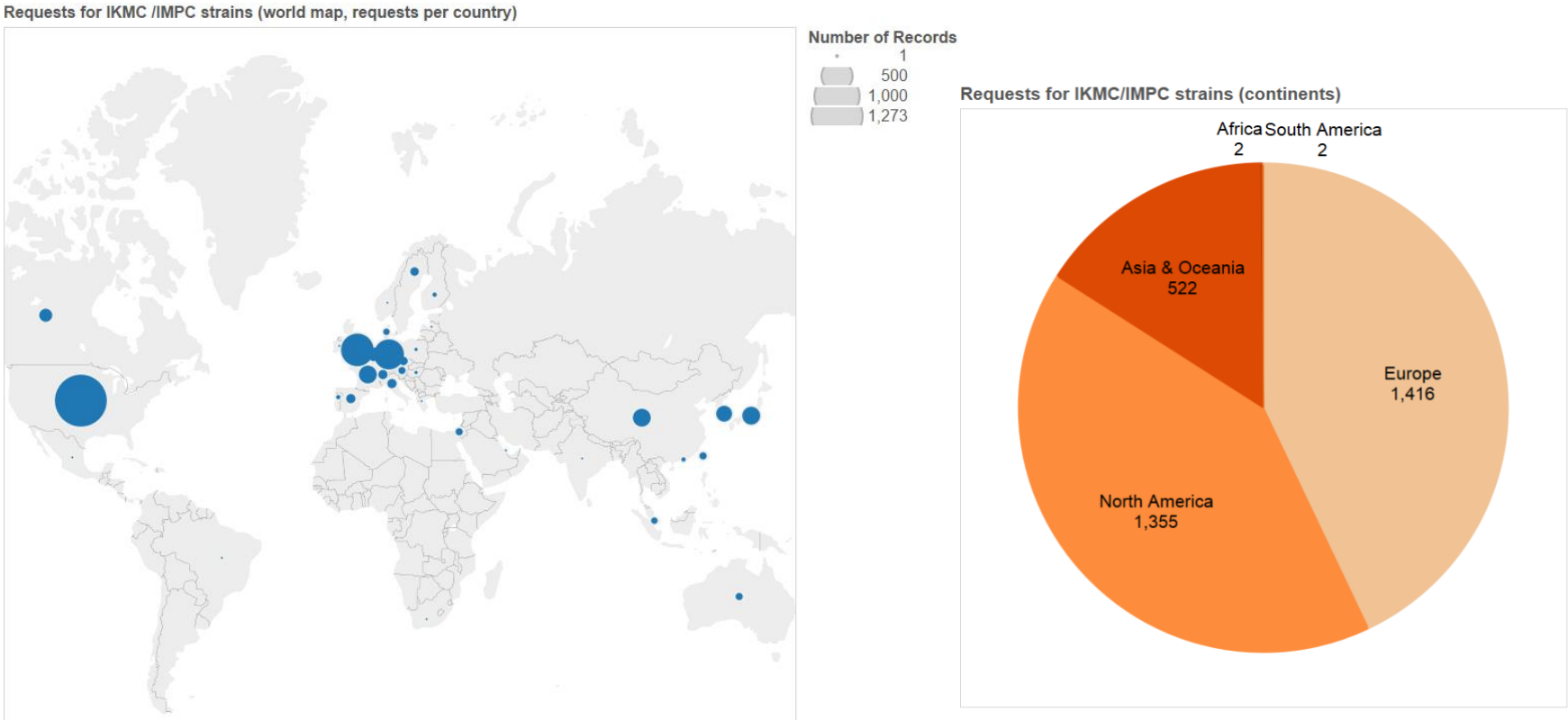

Figure 6: IKMC / IMPC strain shipments (from 2009 – 2020) quantitatively depicted on the world map (left) as requests per country and as a pie chart per continent (right).