The main mission of the Biotechnology Programme Core Units is to provide expert technical and scientific support to CNIO Research Groups in a number of disciplines and technologies widely used in biomedical research, as well as to implement and develop state-of-the-art biotechnological tools and protocols. The Programme consists of 9 Core Units covering major areas in Biotechnology, namely Genomics, Proteomics, Monoclonal Antibodies, Histopathology, Flow Cytometry, Confocal Microscopy, Molecular Imaging and Mouse Genome Editing, as well as an Animal Facility. Although the Core Units are mainly focused on providing support and collaborating with the CNIO Research Groups, they also work with groups from other public research institutions as well as with private companies.

Faithful to its mission, a number of different technological innovations have been explored or implemented by the Core Units during 2019, often in collaboration with CNIO Groups. Among the new technologies introduced at the CNIO this year, it is particularly worth mentioning the Histopathology Unit’s implementation of the RNAScope system for the detection and visualisation of specific mRNAs by microscopy directly on tissue samples. Likewise, this year the Genomics Unit set up a new technology for single cell gene expression analysis (scRNAseq), based on a cell encapsulation system (Chromium 10X) acquired in late 2018. Moreover, the technological capabilities of the Units have been upgraded during 2019, with the acquisition of an Illumina NextSeq550 instrument for deep sequencing at the Genomics Unit, and a new ultrasound VisualSonics Vevo 3100 system at the Molecular Imaging Unit, among other equipment. Finally, the purchase process of a STED platform for super-resolution microscopy, co-funded with support from a call for scientific infrastructures from the Ministry of Science, Innovation and Universities (MCIU), has been initiated. The system will be deployed in 2020.

As an indication of our strong commitment to training, education and outreach, the Programme has been deeply involved in the organisation of courses, workshops, student visits, and meetings. We collaborated with the ‘CNIO & the City’ project, coordinated by the CNIO Institutional Image and Outreach to Society Office. The EuroMabNet network, chaired by the Head of the Monoclonal Antibodies Unit, held a workshop on the validation of antibodies, and specific training courses and workshops were organised by diverse Units (Confocal Microscopy, Flow Cytometry, Molecular Imaging, Animal Facility). Moreover, several members of our staff participated in various Masters courses and other training activities at the CNIO and elsewhere.

As usual, the Core Units were active in attracting funding from external sources through activities related to innovation, including contracts and agreements with private companies and public institutions based on the technologies mastered by several of our Core Units. Also, the royalties derived from the sales of the antibodies produced by the Monoclonal Antibodies Unit continue to represent a significant funding source for the CNIO, with an increase of 10% compared to the previous year; in addition, several new agreements have been signed with different companies to sell and distribute those antibodies.

Last but not least, 2019 was again a very productive year scientifically for the Programme. The contribution of the Units to the overall scientific performance of the CNIO is reflected in nearly 30 publications co-authored by members of the Units, many of them in top journals.

“The capability to maintain and upgrade state-of-the-art core facilities is one of the key elements behind the success of CNIO and its outstanding track record of scientific achievements.”
The Genomics Unit provides on-demand services in the genetics/genomics fields to CNIO researchers and the wider research community. Technologies are put in place with the capacity to interrogate genomes and their activities in a single process in homeostasis and disease at different levels of biological complexity. “The services in the genetics-genomics fields provided by the Genomics Unit contribute towards the understanding of molecular processes in homeostasis and disease at different levels of biological complexity.”

With its portfolio of services that survey different levels of biological complexity, the Genomics Unit contributes to the research projects of multiple CNIO groups. A wide genomic level is addressed by deep-sequencing technologies (NGS) and their applications. NGS permits a variety of different explorations, such as whole genome and whole exome tumour characterisation, transcriptomic analyses and location of chromatin interacting protein factors or RNA binding. This year we acquired and installed 2 pieces of equipment that enrich our capacity in this field: a Chromium Controller from 10x Genomics, suitable to perform single-cell genomics studies; and a sequencer (NextSeq, Illumina), a necessary element for the readout of NGS applications.

Some of our contributions led to the following research reports being published in 2019, with the co-authorship of some of the Unit’s members: Fernández-Barral et al. report the presence of the vitamin D nuclear receptor (VDR) and stem cell markers (LGR5) in the human intestinal mucosa. Transcriptomics and ChIP-seq data support a direct effect of calcitriol, the active metabolite of vitamin D, in colon mucosa crypt stem cells. Vitamin D was found to display both pro-stemness and antiproliferative effects on the intestinal mucosa and is therefore proposed to contribute to the homeostasis of healthy intestine. The other report by Santos et al. describes organoids that recapitulate urothelial features in vitro. 90% of bladder cancer cases originate in the urothelium, and its growth and differentiation characteristics are poorly understood. The single-cell transcriptomics platform of 10x Genomics revealed cellular heterogeneity in different organoid culture conditions, and common and distinct cellular programmes under differentiation or proliferative responses. In addition, the study uncovered the involvement of Notch signalling in urothelial differentiation, which is consistent with the reported findings of mutated Notch pathway components in bladder tumours, and with the down-regulation of NOTCH1 transcript in vivo.

Vitamin D differentially regulates colon stem cells in patient-derived normal and tumour organoids. FEBS J. PMID: 31306552.

The services in the genetics-genomics fields provided by the Genomics Unit contribute towards the understanding of molecular processes in homeostasis and disease at different levels of biological complexity.
CRISPR/Cas based gene editing tools have revolutionised the way we approach genetic studies both in cells and in animals. The Unit has incorporated the CRISPR/Cas gene editing system for mouse germ line precise modification, replacing, in many cases, gene targeting in embryonic stem cells (ES cells) to generate knockout and knockin alleles with high efficiency. CRISPR reagents, introduced directly in mouse zygotes by pronuclear injection or electroporation, replace, in many cases, difficult and time-consuming ES cell culture and manipulation. The efficiency of knockout allele generation with CRISPR is often around 80-90% and bi-allelic knockout animals are frequently obtained. CRISPR-mediated homologous recombination directly in mouse embryos, using single stranded oligodeoxynucleotides as donor DNA for repair, leads to the efficient generation of point mutations or small tag insertions, thereby allowing precise and reliable genome edition. We have also developed strategies to increase the efficiency of CRISPR-mediated large (more than 3 Kb size) knockin integrations using, in this case, circular plasmids as donor DNA. As many as 40% of the pups born after zygote CRISPR microinjection carry targeted knockin inserts (FIGURE).

Zygote electroporation is a good alternative to microinjection for gene knockout generation. It is a much faster and easier process than embryo microinjection. Moreover, zygotes may also be obtained by in vitro fertilisation (IVF) and edited, on the same day, by CRISPR electroporation, increasing the chance of having a large number of fertilised mouse embryos. As many as 40 zygotes can be electroporated simultaneously in a single pulse and no embryo alteration (removing the zona pellucida) before electroporation is required. However, not all embryos are equally tolerant to the electroporation process. C57Bl6 zygotes exhibit a much lower viability than embryos of other genetic backgrounds, such as hybrid F1(B6, CBA) embryos, upon electroporation. We have also optimised protocols for electroporation of pure C57Bl6 embryos without compromising embryo viability.

**Research highlights**

CRISPR/Cas9 systems alone would be sufficiently well studied by using in vitro systems alone. The Mouse Genome Editing Unit is dedicated to the design, generation and cryopreservation of genetically modified mouse models of cancer, using state-of-the-art technology for the controlled modification of the mouse germ line. In collaboration with CNIO groups, we have created hundreds of genetically engineered mouse strains that are crucial for understanding the molecular basis of tumour development and for the preclinical validation of new and more efficient cancer therapies. The Unit currently maintains a collection of more than 1000 cryopreserved mouse strains from which the entire scientific community may benefit for the advancement of Science in many different research disciplines.

**Publications**


**Overview**

Genetically modified mice represent one of the basic pillars that sustain cancer research at the CNIO. The term ‘cancer’ includes a variety of extremely complex diseases in which malignant cells communicate with different body systems, such as immune cells or blood vessels, that modulate tumour growth, expansion and invasion. Such complexity cannot be sufficiently well studied by using in vitro systems alone. The Mouse Genome Editing Unit is dedicated to the design, generation and cryopreservation of genetically modified mouse models of cancer, using state-of-the-art technology for the controlled modification of the mouse germ line. In collaboration with CNIO groups, we have created hundreds of genetically engineered mouse strains that are crucial for understanding the molecular basis of tumour development and for the preclinical validation of new and more efficient cancer therapies. The Unit currently maintains a collection of more than 1000 cryopreserved mouse strains from which the entire scientific community may benefit for the advancement of Science in many different research disciplines.
The production of monoclonal antibodies has had a profound impact on multiple branches of biomedical research, and has driven a fundamental shift in the analysis of biological problems. Monoclonal antibodies allow a better understanding of life processes, and can help in the discovery and elucidation of new pathways for the diagnosis, prevention and treatment of cancer.

The Monoclonal Antibodies Unit provides CNIO Research Groups with a la carte generation of mAbs. We are highly specialised in the production of mouse and rat monoclonal antibodies. The Unit also offers mAb characterisation and validation, medium-scale mAb production, as well as a service of Mycoplasma testing for the cell culture facility.

**Research activities**

In collaboration with P. Engel from the Universidad de Barcelona, we have produced and characterised several new mAbs against the leucocyte immunoglobulin-like receptor family (LILR, LIR, ILT, CD85). LILRs are widely expressed in haematopoietic-lineage cells and mediate activation or inhibition of the functions of various immune cells, primarily myeloid cells. It is becoming clear that LILRs, with their capacity to regulate immune responses and mediate protumour functions, represent a new class of receptors that can be targeted for the treatment of a variety of immunologic disorders and cancer.

Targeting one member of the LILR family with mAbs, however, is extremely complex due to the high homology shown among family members. The use of nonspecific antibodies might trigger the function of other members, which may complicate the interpretation of the biologic effects. The study of the functional role of LILRs in cancer is challenged by the lack of suitable mAbs able to specifically recognise each family member. For this reason, we developed and extensively validated novel mAbs specific for CD85A and CD85G that will help to study how LILRs regulate myeloid function and tumour progression, as well as to test the therapeutic efficacy of targeting LILRs for the treatment of malignant, autoimmune, and inflammatory diseases.

**EuroMAbNet**, a European consortium of experts in monoclonal antibody technology

Members include internationally distinguished academic laboratories that generate and validate mAbs. EuroMAbNet is strongly committed to improving the education and training of junior scientists in the field of antibody validation. We achieve this aim by organising annual Antibody Validation Workshops in different venues across Europe.

The final goal of EuroMAbNet is to strengthen European leadership in mAb technology, improve education in the field on an international level, and actively engage with industrial partners to ensure the optimum benefits from using mAb technology to improve human health.

**PUBLICATIONS**


**Research highlights**

During the last 19 years, the Monoclonal Antibodies Unit has generated a large number of mAbs, directed against more than 100 different antigens, mostly targeting molecules for which mAbs are not commercially available. Many of those mAbs have been licensed to external companies, generating royalties that represent an important source of revenue for the CNIO.

Each year, we prepare and update a detailed CNIO mAbs catalogue, which contains the datasheets of more than 100 thoroughly validated high-quality mAbs (accessible at http://www.cnio.es/ing/servicios/anticuerpos/default.aspx). This catalogue is offered to specialised companies that are looking for licensing opportunities.

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**Figure** Expression of CD85A mAb in diffuse large B cell lymphoma (DLBCL). Immunohistochemical approach. 1 cell: lymphoma (AITL), modular sciences; 2 cell: myeloid lymphoma (HD).
In 2019, we installed a new ultrasound system with better resolution to perform diagnosis and follow-up of tumours, as well as to phenotype different models and organs. The system improves the quality of ultrasound diagnosis by increasing the image resolution and signal-to-noise ratio (FIGURE).

The Molecular Imaging Unit continues to provide CNIO researchers with state-of-the-art molecular imaging equipment and human resources in order to guarantee the highest quality studies and to develop and update protocols and imaging techniques that optimise tumour visualisation in both the preclinical and clinical fields. The Unit also assesses and advises researchers on the best-suited imaging modality for their research projects.

As a result of a collaboration with the CNIO Breast Cancer Clinical Research Unit, we contributed to establishing the clinical usefulness of 18F-FDG PET as a tool to assess patients’ tumour responses in clinical trials.

The Molecular Imaging Unit continued to work on 2 main projects. Granted in collaboration with the CIEMAT group, one focuses on developing and labelling nanobodies produced by camelids based on the ImmunoPET strategy; this strategy combines the high specificity and selectivity of the antibodies with the high sensitivity and quantitative capabilities of PET. We also continued our participation in the RENIM Network. Our project focuses mostly on developing nanoparticles for optical and multimodality (optical-MRI or PET-MRI) imaging to detect primary tumours and distant metastasis. The results of this research will directly benefit CNIO scientists, who will be able to use and test these new imaging tools.

OVERVIEW

Molecular imaging is defined as the in vivo measurement of biological processes at the cellular and molecular levels. These techniques can visualise pathophysiological processes noninvasively in real time, with the potential for serial monitoring, and provide information about specific molecular alterations underlying the disease status of individual subjects. By complementing conventional ‘anatomical or physiological’ imaging, molecular imaging enables early detection of disease, disease staging, and quantitative assessment of therapeutic response.

“Molecular imaging with tumour-specific probes acts as a virtual biopsy, providing biological characterisation in a non-invasive way.”

• PUBLICATIONS

• AWARDS & RECOGNITION
  • Project evaluator of the Junta de Andalucía and Generalitat Valenciana. Investigación, Desarrollo e Innovación Biomédica y en Ciencias de la Salud 2019.
FLOW CYTOMETRY
CORE UNIT

Lola Martínez
Core Unit Head

Technicians
Renan Antonioli (until March) (TS)*,
Julia García (TS)*, Sara García (since November) (TS)**

November (TS) *, Tania López (until April) (TS)**

*Titulado Superior (Advanced Degree)
**Plan de Empleo Joven (Youth Employment Plan - Graduate)

OVERVIEW

Flow Cytometry is an indispensable tool in the oncology field. It allows multiparametric analysis for the identification, quantification and isolation of defined subpopulations of cells, based on the levels of expression of fluorescent markers and their relation to each other.

Our aim is to provide CNIO groups with technical and scientific advice regarding the use of cytometric technologies, collaborating with them in the design, acquisition, data analysis and interpretation.

We have 4 analysers and 3 high-speed cell sorters, with different configurations of lasers and detectors, to cater to all our users’ needs. We also have an automated magnetic bead separation system (AutoMACS), 2 automated cell counters and a tissue homogeniser (GentleMACS). Analysers are user-operated upon appropriate training, and cell sorters are operated by the Unit staff. Our sorters can separate up to 4- or 6-defined populations simultaneously, as well as perform single cell cloning. We can accept human samples to sort according to Biosafety regulations.

RESEARCH HIGHLIGHTS

We provide state-of-the-art equipment and software packages in flow cytometry and collaborate with CNIO investigators in setting up and optimising flow cytometry techniques of their interest. Some of the applications developed and validated at our Unit are:

- Cell proliferation studies (CFSE, Cell Trace Violet, BrdU or EdU, DNA content, etc.)
- Apoptosis studies (Annexin V, Mitochondrial Membrane Potential, Caspase 3, etc.)
- Multicolour immunophenotyping panels (B and T cell development, Tregs, Inflammation, etc.)
- Functional assays (side population detection, Ca++ flux, intracellular pH, etc.)
- Cytometric bead arrays to measure several cytokines from cell extracts and plasma
- Platelets studies
- Extracellular vesicles detection (microvesicles and exosomes)
- Single cell sorting for omics analysis.

We further optimised our multicolour flow cytometry panels to characterise immune response in various samples from haematopoietic tissues, pancreas, skin, liver, lung, brain, as well as different tumour types. Single cell deposition into 96 or 384 PCR plates to perform single omics techniques is now part of our routine portfolio. We perform 4-way sorting based on DNA content on live stained samples and are advancing to separate even further to isolate 6 different fractions of DNA content. Additionally, we are also pushing the power of our analytical tools by moving towards high dimensional analysis, performing ‘unsupervised’ clustering analysis on our multiparametric panel assays.

Figure Upgrade of CNIO’s LSR Fortessa. We have optimised an 18-marker immunophenotyping panel in human blood samples and we are characterising different T and Myeloid subsets and developing a 20-marker one in murine tissues.
The Confocal Microscopy Unit is equipped with 3 laser scanning confocal systems (Leica SPS) that incorporate UV and multiphoton excitation, as well as a white light laser and hybrid detection, and 2 wide-field systems (a Deltavision 4D deconvolution station and a Leica DMIRE6000 system, equipped with microinjection and microfluidics control). All the microscopes are automated and equipped with incubators for live cell imaging.

In addition, the Unit has implemented high throughput technologies applied to confocal microscopy using 2 different systems:

- An Opera (Perkin Elmer) High Content Screening (HCS) system, which allows HCS experiments to be run on fixed and live cells in multiwell plates, and enables the monitoring of cell dynamics (translocation, cell division, etc.) through the use of fluorescence.
- A Matrix Screening Application integrated into the SP5 confocal systems, enabling high throughput feeding of the instrument, not only in multiwell plates but also in tissue sections.

These advances enable us to increase the level of information obtained from a sample as well as to carry out the automated screening of cell behaviour under different treatments.

The Confocal Microscopy Unit continues to dedicate significant effort towards developing and implementing High Content Screening technology at the CNIO. In 2019, we further developed new advanced Machine Learning solutions oriented towards data mining and classification, thus allowing us to manage multiple object features applied to, for example, HCS multi-parametric treatment activity classification. Our activity in this field has been recognised and has led to the publication of several articles in journals as well as scientific book chapters.

"The Confocal Microscopy Unit is fully committed to disseminating advanced microscopy methodologies that are useful for cancer research and society at large; we have organised courses, talks and visits, always with the aim of increasing our understanding of cell biology and that leads to cancer."
Proteins act as the molecular effectors of cells and catalyse virtually all biological processes. In this regard, proteomics aims to characterise the complete repertoire of proteins to better understand how cells function at the molecular level. Global analysis of proteins is challenging, owing to their high complexity (>12,000 genes transcriptionally active in mammalian cells) and high dynamic range (9 orders of magnitude between high- and low-expressed proteins).

Furthermore, proteins are post-translationally modified (e.g. phosphorylation) and interact with each other to form complexes; both processes are highly divergent in time and interact with each other to form complexes. To tackle these analytical challenges, proteomics uses mass spectrometry-based proteomics techniques to explore proteome structure and function. In the last decade, mass spectrometry-based proteomics has emerged as the most powerful technique to answer biological questions relevant to our understanding of cancer biology. State-of-the-art MS technology coupled to quantitative multiplexing approaches enable profiling the entire proteome across multiple biological conditions (11 in a single experiment).

In collaboration with the Hereditary Endocrine Cancer Group, this technology was applied to identify potential targets of the miRNA hsa-miR-139-5p, which has been associated with thyroid cancer through its involvement in alternative splicing. Furthermore, exosomes and extracellular vesicles have gained great interest in the medical community as possible sources of biomarkers in non-invasive liquid biopsies. In this regard, in collaboration with the Microenvironment & Metastasis Group, we have explored the protein content of extracellular vesicles extracted from lymphatic drainage as surrogate markers of melanoma progression. We found that seroma-derived exosomes are enriched in proteins mimicking melanoma progression. Along the same lines, in collaboration with the Genes, Development and Disease Group, we applied this strategy to perform proteomic analysis of exosomes in a mouse lung fibrosis model and found that they are enriched in collagen-related proteins secreted by macrophages. Finally, the Unit continues to implement new technologies to the catalogue of available services. Throughout 2019, we optimised protocols for the global analysis of protein methylation, including mono- and tri-methylation of lysines as well as mono- and di-methylated proteins from cell samples. Currently, all forms of methylation can be purified through highly specific monoclonal antibodies.

In 2019, the Proteomics Unit continued working on the application of proteomic approaches to answer biological questions relevant to our understanding of cancer biology. State-of-the-art MS technology coupled to quantitative multiplexing approaches enabled profiling the entire proteome across multiple biological conditions (11 in a single experiment).

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Pathology is the branch of science devoted to the study of the structural, biochemical and functional changes in cells, tissues and organs underlying disease. The Histopathology Unit offers support and expertise throughout a full range of services covering from paraffin embedding and tissue microarrays, to other institutions, including hospitals, research centres and private companies.

The implementation of the *in situ* hybridisation technique RNAScope and multiplexed immunohistochemistry staining, to enable the detection of several protein markers and mRNAs on the same tissue section, is an example of the Unit’s commitment to innovation to facilitate the progress of research projects at CNIO.\(^1\)

*Pathway visualization of those markers.*

Training and outreach activities are also a critical component of the Unit’s activity. This includes our participation in modules of *Formación Profesional* for pathology technicians, mentoring of high school students during short-term stays at the Unit, conducting guided visits to the laboratories for students and other audiences, as well as offering practice sessions on the different technologies run by the Unit in Masters and other courses, among other activities.\(^2\)

In line with the activity carried out over the last few years, the Unit has maintained the portfolio of services demanded by its users in accordance with the needs of their projects. Thus, about 30,000 paraffin blocks of tissue samples were generated, and ca. 25,000 techniques performed, including histological and IHC techniques, *in situ* chromogenic hybridisation, tissue microarrays, slide scanning, etc. Also, during this time we introduced new IHC markers useful for the study of tumour development, as well as new chromogenic substrates for the visualisation of those markers.

During 2019, the Unit implemented RNAScope technology for *in situ* hybridisation, using the Ventana–Roche automatic platform for IHC stains. This new technique allows the efficient detection of specific mRNAs directly on sections from formalin-fixed paraffin-embedded (FFPE) tissues, thus providing a spatial dimension to gene expression analysis. The applications of this new technology are expected to be manifold, *e.g.*, as an alternative to IHC whenever it is difficult to find specific antibodies that work well on FFPE tissues, or to validate results from other technologies, among others. Also, sometimes it is feasible to combine this technique with IHC, allowing for double stains to detect both mRNA and protein, or any other marker of interest.

The high quality of the techniques run by the Unit continues to be endorsed by External Quality Assessment Schemes. Thus, our histochemical techniques were evaluated by UK NEQAS. On the other hand, NordiQC and SEAP has evaluated a subset of our IHC techniques under different modules, including general markers, breast cancer markers and PD-L1; these all obtained very high scores.

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**Core Unit Head**

Vacant

**Staff Scientist**

Eduardo José Calatrava

**Technicians**

Nuria Cabrera, Maria Gómez, Patricia Gonzalez, Sabino

**Overview**

“Pathology is the branch of science devoted to the study of the structural, biochemical and functional changes in cells, tissues and organs underlying disease. The Histopathology Unit offers support and expertise throughout a full range of services covering from paraffin embedding and tissue microarrays, to other institutions, including hospitals, research centres and private companies. The implementation of the *in situ* hybridisation technique RNAScope and multiplexed immunohistochemistry staining, to enable the detection of several protein markers and mRNAs on the same tissue section, is an example of the Unit’s commitment to innovation to facilitate the progress of research projects at CNIO.”

**Publications**

The CNIO has a state-of-the-art Animal Facility, managed by Vivetecnia Management & Services. The Animal Facility’s primary responsibility is the supply, husbandry and quality control of laboratory animals used by the Research Programmes in their experimental protocols. The strict compliance to national, EU and international recommendations regarding the use and care of animals in research is of paramount importance to the CNIO.

The high standards achieved by the CNIO with regards to the use and care of animals for experimentation have been recognised by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International. AAALAC accreditation, considered one of the top international recognitions in this field, was first obtained in October 2016, and was renewed in 2019 for a new 3-year period. AAALAC International is a private non-profit organisation that promotes the humane treatment of animals in science through voluntary accreditation and assessment programmes.

“Mouse models are essential tools in cancer research. The Animal Facility offers to CNIO researchers all the capabilities needed in this area, in compliance with the highest standards of animal care and welfare.”

The CNIO Animal Facility was established to assist researchers in the development and analysis of in vivo models. We are currently collaborating with as many as 27 Research Groups, Sections and Units from different Research Programmes.

Our Animal Facility has the capacity to house 19,000 type II cages. Our mouse lines are maintained and bred in the Facility’s barrier area, which assures Specific Pathogen Free (SPF) health status through a comprehensive health surveillance programme. Microbiological and environmental parameters in the animal areas are constantly monitored. All mouse strains housed in the barrier are either generated within the barrier or introduced by rederivation. We also have an additional area with a capacity for 1,800 type II cages dedicated for the use of non-replicative strains of adenovirus, lentivirus and retrovirus, as well as for xenograft models. In this area, mice are housed in ventilated racks with integration of Individually Ventilated Caging (IVC) units in the building ventilation systems. Mice are always manipulated in Type II biosafety cabins.

Daily operations and husbandry procedures are highly automated in order to safe-guard our personnel from any associated risks; robotic devices perform the potentially hazardous tasks such as the processing of dirty bedding, the washing and filling of cages and bottles, etc. These automated systems maximise the productivity and ensure the quality standards in our washing and sterilising areas. All records concerning breeding protocols and animal inventory are computerised and stored in a web-based application accessible via the CNIO intranet.

The Animal Facility currently harbours more than 40,000 mice representing more than 3,000 genetically modified mouse lines, either as live animals or as cryopreserved embryos or sperm, carrying close to 400 gene targeted alleles and more than 200 transgenic integrations. The Facility also provides access to more than 50 tool strains, including constitutive and inducible Cre strains, Flp strains, reporter strains, Tet transactivator strains and others.

The Animal Facility offers the possibility of running a broad number of experimental procedures in the premises, including the use of gamma irradiation, UV light and volatile carcinogenic agents, as well as surgical procedures, some behavioural studies, a non-invasive blood pressure system, and a lab animal monitoring system (Oxytel) that enables measuring a number of physiological parameters for metabolic profiling and phenotyping of mouse models.

Additionally, the monitoring of the mouse models through non-invasive imaging technologies is provided by the Molecular Imaging Unit, which has integrated all its image acquisition instruments within the Animal Facility. Likewise, the work of the Mouse Genome Editing Unit is performed in a laboratory inside the SPF barrier. Finally, the necropsy laboratory is equipped with instruments for the haematological and biochemical analysis of blood and urine, which complement the pathology and clinical diagnostics.

In addition to mice, the Animal Facility hosts a colony of rats for the generation of monoclonal antibodies directed against mouse antigens, as well as for a project of the Experimental Therapeutics Programme that aims to test the safety of some specific anti-tumour compounds.

All the work carried out by the Animal Facility complies with both national and EU legislation – Spanish Royal Decree RD53/2013 and EU Directive 2010/63/UE – for the protection of animals used for research experimentation and other scientific purposes. Experimental procedures and projects are reviewed and evaluated by the Research Ethics and Animal Welfare Committee of the Instituto de Salud Carlos III, as well as by the Institutional Animal Care and Use Committee (IACUC). The Orden EEC/566/2015 stipulates that all animal procedures are to be carried out by qualified people with the corresponding accreditation issued by the competent authority. The Animal Facility offers CNIO’s new staff a short course, focused on the work with laboratory animals, complementary to the online courses that are a requisite to gain access to the facility.

In accordance with our commitment to maintain the highest possible standards in relation to animal research issues, the CNIO has joined the Agreement on Openness on Animal Research, promoted by the Federation of Scientific Societies in Spain (COSCE) in collaboration with the European Animal Research Association (EARA), launched in September 2016. An institutional statement on the use of research animals can be consulted on the CNIO website.

In 2019 the Animal Facility’s Head was elected as President of the Spanish Society for Laboratory Animals (SECAL), for a 2-year period. SECAL is the most prominent scientific society in the field of laboratory animals in Spain, devoted to advancing the scientific understanding of the use, care and welfare of laboratory animals, as well as to promoting refinement, reduction and replacement strategies in research involving animal models.