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 collaborate with groups from other research institutions as well as with private companies.

This year, the Biotechnology Programme was able to keep up and running all the operations necessary to guarantee the preservation of the Centre’s activities. In fact, the overall activity of our Core Units during 2021 has already returned to the levels achieved in 2019, before the pandemic.

Regarding the projects led by the Core Units, the Mouse Genome Editing Core Unit has been working on the development of mouse models expressing humanized ACE2 to be used for preclinical studies. Having initially received funding through a call for projects focusing on SARS-CoV-2 and Covid-19 launched by the ISCiii in 2020, the project was expanded to incorporate a collaboration with the CNIO Telomeres and Telomerase Group and Dr Luis Enjuanes’ Group at the CNB-CSIC, and is now financed through a Synergy Project from the Comunidad Autónoma de Madrid. Coordinated by Sagrario Ortega, Head of the Unit, this research uses mouse models to study the effect of short telomeres on the severity of the disease.

On the other hand, our technological capabilities were upgraded during 2021. One example is the acquisition of a high-resolution ultrasound system (Vevo 3100) in the Molecular Imaging Unit, used for imaging studies in animal models, mainly for pancreas, prostate, heart, kidney, and other soft organs. Also, a flow cytometry spectral analyzer (Cytek Aurora) was purchased that will expand the technical capabilities of the Flow Cytometry Unit by making it possible to run cytometry protocols involving more than 40 markers.

This year Javier Muñoz, Head of the Proteomics Unit since 2012, left the CNIO to join the Instituto de Investigación Sanitaria BioCruces Bizkaia, as group leader. During his time at the CNIO, Javier did a fantastic job, bringing proteomics to the core of the projects undertaken by the CNIO Research Groups, as well as developing his own projects and collaborating with groups outside the CNIO. We thank him for his efforts and excellent work, and wish him the very best for his scientific career from now on.

As usual, the Core Units were active in attracting funding from external sources through innovation-related activities, including contracts and agreements with private companies and public institutions based on the technologies mastered by several of our Core Units. The royalties derived from the sales of the antibodies produced by the Monoclonal Antibodies Unit continue representing a significant funding source for the CNIO. In 2021, and for the first time in the history of the CNIO, the total income derived from this concept exceeded €1 million.

Last but not least, 2021 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 more than 30 publications co-authored by members of the Units, many of them published in top journals.
All tumours, even those of the same type and sharing a similar fate, are different and heterogeneous at the genetic level. By employing a distinct set of methodologies with the capacity to analyse a wide number of genetic loci, or even whole genomes in a single assay, genomics reveals basic molecular programmes and helps to dissect biological mechanisms. The Genomics Unit, with its array of molecular services, contributes to the dissection of these biologically complex mechanisms in research projects conducted by multiple CNIO research groups.

For genomic-wide level analysis, we use NGS-based technologies, performed mainly on the Illumina platform. NGS constitutes the final readout for a great variety of different explorations at both the structural and functional levels, including detailed genome or exome tumour characterisations, mutation repertoires, location of bound protein factors, variations in chromatin folding, or on/off functional states. Transcriptional profiles reflect functional choreographies at the genomic level and are useful to decipher tumour compositions, uncover therapeutic targets, or predict disease outcome and guide treatment decisions. Transcriptomes are characterised either from tissue — even from archived FFPE samples — or from cell culture extracts. Transcriptomes can also be obtained at single cell resolution, through prior separation of individual cells in microdroplet emulsions using the 10xGenomics Chromium platform. A recent implementation at single cell resolution is the multiomic profiling of gene expression and open chromatin regions, opening up new perspectives into the underlying gene regulatory mechanisms that drive cell differentiation and development.

At the single locus level, we provide other services. A traditional DNA capillary sequencing service, based on a 3730xl DNA Analyzer from Applied Biosystems, is being used to find and confirm mutations in candidate genes, or to verify cloned genes or inserts. A relatively simple cell authentication service provides confidence in the identity of the cell lines used for experimentation. The Unit overseas a transgenic mouse genotyping service as well. Its current catalogue includes over 150 genetic modifications, all assayed by custom allele-specific, real-time PCR for a quick and efficient turnaround time.

**OVERVIEW**

The Genomics Unit provides core technological services in the fields of genomics and genetics. Seeking to uncover biological mechanisms, therapeutic targets or prognostic biomarkers, our services cover a broad range of applications. These technologies, with the capacity to interrogate whole genomes and their activities in a single assay, can reveal the entire package of structural features (mutation landscapes, chromosomal protein location, or chromatin structure) and molecular programmes (transcriptomic RNA profiles), even at the single-cell level. Next-generation sequencing (NGS) is a staple among them. More traditional methodologies like Sanger capillary DNA sequencing are also available. As a side activity, we oversee a genetically engineered mouse genotyping service.

“Our technology portfolio responds to the needs of CNIO’s scientists in the genetics and genomics fields and contributes to the understanding of disease and homeostasis at different levels of biological complexity.”

**FIGURE** Neural differentiation spotted at two time points for both wild type (wt) and Cdc14a/b-null (mut) embryonic stem cells. Simultaneous detection of chromatin accessibility patterns (ATAC) and mRNA transcriptomes (GEX) in the same individual cells, shown as dots clustered according to their similarities, illustrate the coordinated cellular fates of dynamic epigenomic and transcriptomic profiles uncovering gene regulatory programmes. Arrows indicate changes in cell subpopulations’ states between days 0 and 5. Data courtesy of Carolina Villarroya, Malumbres Lab, CNIO.
The term “cancer” encompasses a whole spectrum of extremely complex diseases. Genetic and epigenetic modifications in tumour cells lead to the acquisition of a “malignant” phenotype that enables them to escape normal physiological control. We can accurately reproduce many of these modifications in the mouse, creating animal models to study the disease. Tumour cells also interact, at different levels, with other cells in the body such as those of the tumour stroma, immune, cardiovascular or lymphatic systems, which, in turn, modulate tumour growth, invasion and expansion. The study of such complexity requires in vivo models that reproduce all the features of cancer in a “whole body” context, including the specific genetic alterations that lead to tumour development in each particular tumour. The precise, targeted and controlled modification of the mouse genome, using the most advanced genome editing tools, sustains the generation of genetic mouse models of cancer that are crucial for understanding the molecular basis of tumour development and the preclinical validation of new and efficient cancer therapies.

**PUBLICATIONS**


**RESEARCH HIGHLIGHTS**

**COVID-19 preclinical mouse models**

One limitation in COVID research is the lack of adequate models to study SARS-CoV-2 infection, especially animal models, where the complex interactions established between the virus and its host can be reproduced in a physiological context. During 2021, the Unit focused on generating and characterising mouse models for COVID disease.

The laboratory mouse is the most widely used animal model in biomedicine, but it is not a permissive species for SARS-CoV-2 infection. Structural differences between the human Angiotensin Converting Enzyme-2 (ACE2) protein, the cellular receptor for SARS-CoV-2, and its murine orthologue are the cause, at least in part, of the different sensitivity to viral infection in humans and mice. Supported by a dedicated grant from the Spanish Institute of Health Carlos III and a SANTHERA®-grant from the Madrid Local Government (CAM), the Unit has created “humanized” mouse models for the study of COVID19, in collaboration with the company Gen-H Genetic Engineering, Heidelberg (Germany).

Using the latest gene editing technologies, based on the CRISPR/Cas9 system, we created knockin mice in which the human ACE2 protein is expressed under the transcriptional control of the endogenous mouse Ace2 promoter, interrupting simultaneously the Ace2 coding sequence and resulting in the knockout of the mouse Ace2 gene. As an alternative, we used a RAG transgene approach to drive expression of human ACE2 under the control of the Ace2 promoter. In both cases, the expression of human ACE2 recapitulates the pattern and regulation of endogenous Ace2 expression.

During 2021, in collaboration with the Coronavirus Laboratory directed by Dr Luis Enjuanes at the Centro Nacional de Biotecnología (CNB/CSIC) in Madrid, we tested the susceptibility of our mouse models to SARS-CoV-2 infection. Intranasal inoculation of SARS-CoV-2 in the humanized knockin mice resulted in the accumulation of inflammatory infiltrate (CD45+ cells) surrounding lung alveoli and neighbouring blood vessels (CD31+). In the knockin mice expressing human ACE2 (brown, green arrowheads) in the lung of a knockin mouse, 3 days after intranasal virus inoculation, insert image (ACE2) expressing cells (black arrowheads in both images) lining the bronchioles.

**FIGURE 1** Lung inflammation in human ACE2 (hACE2) knockin mice, inoculated with SARS-CoV-2. CD45+ inflammatory cells (magenta, red arrowheads) in CD31+ endothelial cells (brown, green arrowheads) in the lung of a knockin mouse, 3 days after intranasal virus inoculation. Insert image: ACE2 expressing cells (black arrowheads in both images) lining the bronchioles.
Since the discovery of hybridoma technology by Caesar Milstein and Georges Köhler in 1975, monoclonal antibodies (mAbs) have become one of the most relevant methodological advances in biomedicine. mAbs have provided researchers with the ability to study biological processes reliably and with unprecedented accuracy, improvising our knowledge about the processes involved in tumour generation and development. Beyond their applications in improving our knowledge about the processes involved in biological processes reliably and with unprecedented accuracy, mAbs have provided researchers with the ability to study biomedicine. mAbs have become one of the most relevant methodological advances and have been hampered by the lack of specific mAbs to work across animal species.

For this reason, in collaboration with the Madrid Zoo, with several departments of veterinary sciences, and with the CNIO Histopathology Unit, we tested more than 100 mAbs in several domestic and wild animal species, generating an extended panel of mAbs able to detect and discriminate different lymphoid subpopulations by IHC. Our study will serve to facilitate further research needed to define the role played by lymphocyte subpopulations in immunological diseases and cancer in animal species.

Research activities

CD229 (Ly9). In collaboration with Professor Pablo Engel, from Barcelona University, we produced and characterised a new mAb against the cytoplasmic region of CD229 (Ly9) protein. CD229 is a homophilic receptor that belongs to the SLAM family of cell-surface molecules and acts as a signalling molecule, regulating lymphocyte homoeostasis and activation. In our study we investigated the expression of CD229 in normal tissues and B cell malignancies using tissue microarrays. We found CD229 to be restricted to haematopoietic cells, and it is strongly expressed in all cases of myeloma and splenic marginal zone lymphomas. CD229 represents a new biomarker of B cell malignancies, especially in myeloma.

Optimised panel of mAbs for the detection of lymphocyte subpopulations in animal species. Immunohistochemistry (IHC) has proved to be one of the most important ancillary techniques in the characterisation of neoplastic diseases in humans and, because oncologists demand such diagnostic specificity, it has become equally important in veterinary medicine. The number of immunohistochemical tests offered by veterinary diagnostic laboratories has increased exponentially over the last decade, but the use of this technique has been hampered by the lack of specific mAbs able to work across animal species.

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MOLECULAR IMAGING CORE UNIT

Molecular Imaging enables the visualisation of cellular function and the follow-up of the molecular processes in living organisms without perturbing them. Molecular imaging offers significant advantages to the scientist over traditional research paradigms. While traditional studies of tumour response to a therapeutic agent involve a large cohort of animals analysed at multiple time points, molecular imaging allows characterisation of tumour development and response to a therapy within the same small set of animals imaged longitudinally at multiple time points. This reduces the number of mice used and increases the statistical power of the study because each animal serves as its own control. Other advantages include the ability increasing the sensitivity and signal-to-noise ratio of the images. We are also helping to detect pregnancy at early stages, at only 6 weeks, to extract marine embryonic fibroblasts (MEFs) with better accuracy than only with abdominal palpation (FIGURE 1).

In 2021, we installed a new ultrasound imaging system, VEVO 3100, to replace one of the old ones, to perform diagnosis and follow-up of tumours, as well as to phenotype different models and organs. The system improves throughput diagnosis by increasing the sensitivity and signal-to-noise ratio of the images. We are also helping to detect pregnancy at early stages, at only 6 weeks, to extract marine embryonic fibroblasts (MEFs) with better accuracy than only with abdominal palpation (FIGURE 1).

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 to 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.

In 2021, the BBVA Foundation grant allowed us to work on theranostic applications of radiolabeled antibodies, looking for the best-matched isotope pairs for imaging and therapy and employing the pretargeting approach. We also continued with the rest of our ongoing grant projects. One of our projects, conducted in collaboration with CIEMAT, focuses on developing and labelling nanobodies produced by camelids following the ImmunoPET strategy, where we couple the high specificity and selectivity of the antibodies with the high sensitivity and quantitative capabilities of PET. Another grant project, the Spanish Network for Nanoparticles in Molecular Imaging for developing iron and silver-based nanoparticles for imaging, focuses mostly on optical imaging and multimodality (optical-MRI or PET-MRI) for the detection of primary tumours and distant metastasis.

The results of these research projects, in which the Molecular Imaging Unit is actively involved, will directly benefit CNIO scientists who will be able to use and test these new imaging tools in their own research. One example is a new NIR (near infrared) laser device bought with the RENIM budget.

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FLOW CYTOMETRY CORE UNIT

FLOW CYTOMETRY

Core Unit Head

Lola Martínez

Technicians

Julia García-Lantín (TS), Sara García García, Marite González Martínez (until February) (PEJ)

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

INNOVATION

BIOTECHNOLOGY PROGRAMME | FLOW CYTOMETRY CORE UNIT

OVERVIEW

Flow Cytometry is a fast and multiparametric technology, and a very valuable tool in the oncology field. It is an important workhorse for the identification, quantification and isolation of defined subpopulations of cells, based on the expression levels of fluorescent markers and their relationship to each other at the single cell level.

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 of flow cytometry data.

We currently have 5 analysers and 3 high-speed cell sorters with different optical configurations to cater to our users’ needs. We also have an automated magnetic bead separation system (AutoMACS), 2 automated cell counters (Countess) and a tissue homogenizer (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 and index sorting. We can accept human samples to sort under BSL2 regulations.

“...we incorporated a full spectrum cytometer to expand the number of parameters we could study per sample, increasing our knowledge on the role of different immune subsets in cancer progression, and elucidating new biomarkers with potential therapeutic value.”

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 relevant to their research projects. Some applications developed and validated at our Unit include:

- 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, Ca2+ flux, intracellular pH, etc.).
- Cytometric bead arrays to measure several cytokines from cell extracts and plasma.
- Platelet studies.
- Extracellular vesicles detection (microvesicles and exosomes).
- CTC detection and isolation.
- 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 using index sorting into 96 or 384 PCR plates to perform single OMICs techniques is now part of our routine portfolio. We also improved the performance characteristics of our instrumentation by creating volution templates in all our instruments to assess optimal voltage for each detector and expand our training capacities with many more workshops and small practical analysis sessions. This provides our users with more tools to successfully perform their flow cytometry experiments.

FIGURE

Immunophenotyping panel of mouse spleen combining antibodies coupled to AF647 and APC, a combination not possible with conventional cytometry. Full spectral analysis opens the door to increase panel complexity by allowing the use of fluorochrome combinations that were not possible before.

PUBLICATIONS

The Confocal Microscopy Unit continues to dedicate significant effort towards developing and implementing High Content Screening (HCS) technology at the CNIO. In 2021, the Unit renewed its equipment in this field, thanks to the funding obtained with a grant awarded through an infrastructures call of the Ministry of Science and Innovation. The Unit already had one Opera (Perkin Elmer) HCS system, which enables experiments to be run on fixed and live cells in multiwell plates, and the monitoring of cell dynamics (translocation, cell division, etc.) through the use of fluorescence. The acquisition of a new Opera Phoenix HCS microscope with a robotic plate handler will boost screening capacity, making 24/7 operation time possible. This new system will significantly reduce acquisition time and improve sensitivity and excitation flexibility. The platform is equipped with the latest analysis software, getting better results from 3D organoid campaigns and live-cell imaging assays.

In addition to this new system, the Unit is equipped with: 1 super resolution confocal microscope (sp8 STED super-resolution microscope with a white light laser and 3 depletion laser lines); 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 THUNDER system with computational clearing algorithms and 8-channel led excitation, and a Leica DMR6000 system, equipped with microscopy and microfluidics control). All the microscopes are automated and equipped with incubators for live cell imaging.

The Unit implemented the use of high-throughput technologies applied to confocal microscopy using not only the Opera system, but also through a sample navigation application integrated into the SP8 and SPS confocal systems. This enables high-throughput feeding of the instrument, both in multiwell plates and in tissue sections. These advances allow us to increase the level of information obtained from a sample, as well as carry out the automated screening of cell behaviour under different treatments.

The Unit is involved in promoting and helping its users with novel protocol development for sample preparation, bringing knowledge in tissue culture as well as in expansion microscopy. Moreover, microfluidics, used for live cell assays in perfusion chambers, has also experienced a great increase in performance and demand. Experiments of intra-vital microscopy are available, and we are now running several projects for studies of metastasis, skin alterations and immune system response.

#### FIGURE

Confocal image of cells in culture labelled for neurons (green) and astrocytes (red).

### OVERVIEW

Optical microscopy has traditionally been an indispensable tool in cell biology studies. In fact, one of the main challenges in oncology research is the study of specific markers, expression patterns or individual cells in the tumour environment.

The Confocal Microscopy Unit provides the CNIO Research Groups with all the standard methodologies as well as the latest advances in microscopy, offering access to state-of-the-art equipment and software packages related to confocal microscopy, including technical and scientific advice and support to the CNIO scientists. The Unit is also actively involved in developing, testing, and implementing new microscopy technologies, tools and imaging applications that could be of interest to the Research Groups at the CNIO. Training activities are also an essential component of our mission.

**“The Confocal Microscopy Unit is fully committed to disseminating advanced microscopy methodologies that are useful for cancer research and society at large; we organised courses, talks and visits, always with the aim of increasing our understanding of cell biology and the disorders of cells that lead to cancer.”**

### PUBLICATIONS

Recent developments in “omics” technologies have revolutionized how biomedical research is conducted. These approaches enable unbiased analyses of biological samples and can be used to generate novel hypotheses. Proteins are the molecular effectors of cells, and mRNA assessment merely represents a proxy to estimate the final levels of the protein product. Moreover, genomics does not provide information about the post-translational modifications of proteins or their interactions. Thus, direct analysis of proteins is paramount to our understanding how biomedical research is conducted. These approaches enable systematic mapping of substrates of phosphoproteins, and metabolomics to dissect the series of molecular events that regulate the establishment of naïve pluripotency in embryonic stem cells. These data demonstrated the presence of post-transcriptional regulation, which fine-tune the levels of mitochondrial proteins and enhance their oxphos capacity. Finally, the Unit implemented novel methods aiming to reveal the true identity of proteins present in small extracellular vesicles (sEVs). This is based on high resolution density gradients in conjunction with proteome correlation profiling to deconvolute the origin of proteins (FIGURE 1).

Our data revealed that popular markers used to assess the purity of sEVs originate in non-vesicular fractions. This approach could have important applications for identifying potential biomarkers in liquid biopsies.
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 through a full range of services covering paraffin embedding and tissue sections to histochemical stains; research and diagnostic immunohistochemistry (IHC) testing; and antibody validation.

In 2021, the Unit was able to return to the standard levels of workload and services recorded before the pandemic and, in some specific areas, such as immunohistochemistry and image digitalisation and analysis, even exceeded expectations. Thus, more than 26,000 paraffin blocks of tissue samples were generated, and ca. 21,000 techniques were performed, including histological and IHC techniques (with dual and triple staining being increasingly in demand), in-situ chromogenic hybridization, tissue microarrays, slide scanning, etc.

During 2021, we made significant progress in digitalising our material, with approximately 35% of all the slides generated converted to digital files. In addition, 10% of these were subjected to image analysis and quantification.

We also consolidated the in situ hybridization technology for mRNA detection (RNAScope), with 160 cases analysed, some of them with double staining, using the Ventana-Roche automatic platform for IHC stains. This new technique enables 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 manifold, e.g., as an alternative to IHC whenever it is difficult to find specific antibodies working well on FFPE tissues, or to validate results from other technologies, among others.

The high quality of the techniques run by the Unit continues being endorsed by External Quality Assessment Schemes. In this respect, our histochemical techniques were evaluated by UK NEQAS. Similarly, NordiQC and SEAP have evaluated a subset of our IHC techniques under different modules, including general markers, breast cancer markers and PD-L1; these all obtained good scores.

Training and outreach activities are also a critical component of the Unit’s activities. Although some of the usual activities in this area were compromised due to the pandemic, the Unit was still able to participate in a Master’s course on oncology research, in online format, and we hosted a pre-doctoral student for a short training stay on immunohistochemistry techniques during the last quarter of the year.

In 2021, despite the Covid-19 pandemic, the Unit was able to return to its usual levels of workload and services, even exceeding expectations in some specific areas.

RESEARCH HIGHLIGHTS

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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 regulations regarding the use and care of animals in research is of paramount importance to the CNIO.

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 28 Research Groups, Sections and Units from different Research Programmes.

Our Animal Facility has the capacity to house 19,000 type IIL 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.

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/EU – 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 Animal Facility offers CNIO’s new staff a short course focused on work with laboratory animals, complementary to the online courses that are a requisite to gain access to the facility.

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 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. Recently, the Animal Facility’s Head was appointed as an ad hoc consultant for the site reviews performed by AAALAC.

In accordance with our commitment to maintain the highest possible standards in relation to animal research issues, the CNIO joined the Agreement on Openness on Animal Research, promoted by the Federation of Scientific Societies in Spain (COSEC) 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.

Until November 2021, the Animal Facility’s Head had served as President of the Spanish Society for Laboratory Animal Sciences (SECAL). 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.