A fluorescence microscopy image showing a dense population of cells. The cells are stained with three different dyes: green, red, and blue. The green staining highlights the cytoplasm and some organelles, the red staining highlights other organelles or structures, and the blue staining highlights the nuclei. The overall appearance is a complex, multi-colored cellular network.

Among the most important assets of the CNB are its core facilities. They provide access to leading-edge technology in the areas of structural and cell biology, genomics, proteomics and bioinformatics. The centre also stands out for its research installations, which include a specific pathogen-free animal facility, a greenhouse, and one of the few high-level biocontainment (BSL-3) laboratories currently operative in Spain. Specialised personnel offer technical support in many other facets of the centre's scientific activities.

Scientific Services

STRUCTURAL AND CELL BIOLOGY

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Sylvia Gutiérrez

Cryoelectron microscopy

Rocío Arranz

Electron microscopy

Cristina Patiño

Macromolecular X-ray crystallography

César Santiago

Flow cytometry

M^a Carmen Moreno-Ortiz

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Belén Pintado

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Bioinformatics for genomics and proteomics

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Radioactive facility and biosafety level 3 laboratory

Fernando Usera

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Cell culture, washing and sterilisation

Rosa María Bravo

Instrumentation

Ismael Gómez

Photography

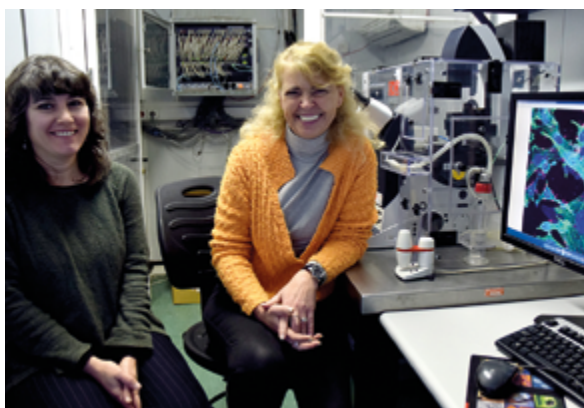
Inés Poveda

Radiation protection and biological safety

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Workshop

Daniel Pastora



94 SCIENTIFIC SERVICES

Advanced light microscopy

HEAD

Sylvia Gutiérrez Erlandsson

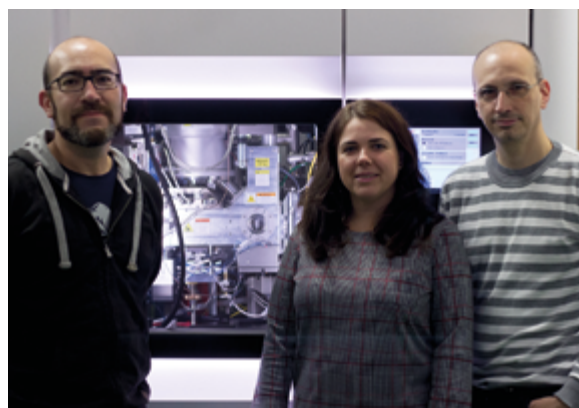
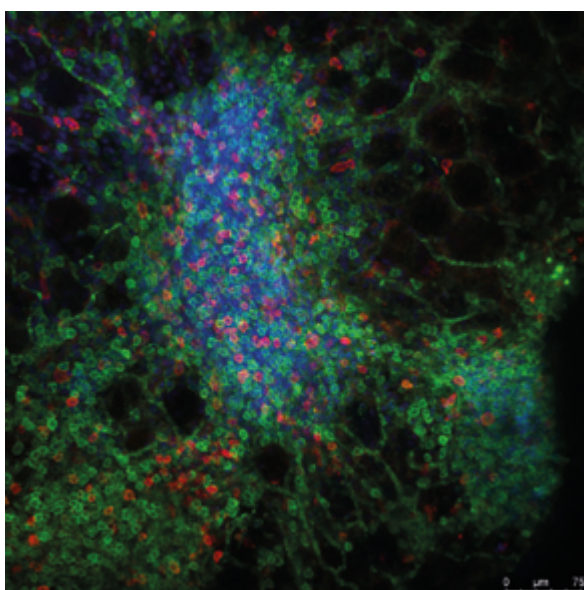
PERSONNEL

Ana Oña Blanco

The presence of fluorescent markers in the sample allows location of cell components in single sections and various experimental approaches, involving single or multiple fluorescent labelling in fixed cells and tissues.

The facility provides infrastructure for epifluorescence, confocal laser scanning microscopy, TIRF, STED, SMLM nanoscopy and image processing tools, covering most experimental light microscopy imaging approaches.

The equipment and services are available to all CNB personnel as well as to researchers from the public and private sectors. Technical staff offers assistance and training in the use of equipment, experimental methods and image processing and analysis procedures. The facility also provides cell culture support and aliquots of probes and secondary antibodies with broad use in fluorescence microscopy applications.



Cryoelectron microscopy

HEAD

Rocío Arranz

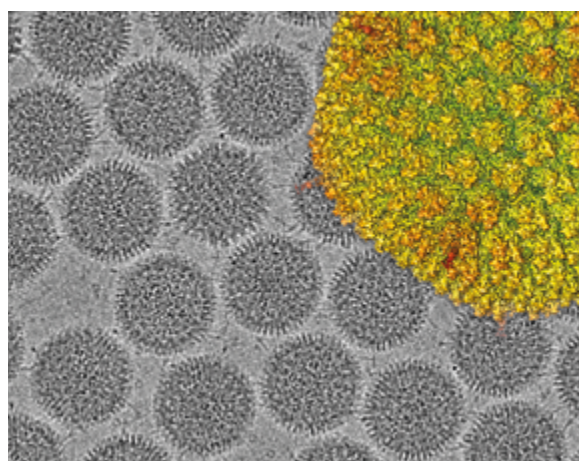
PERSONNEL

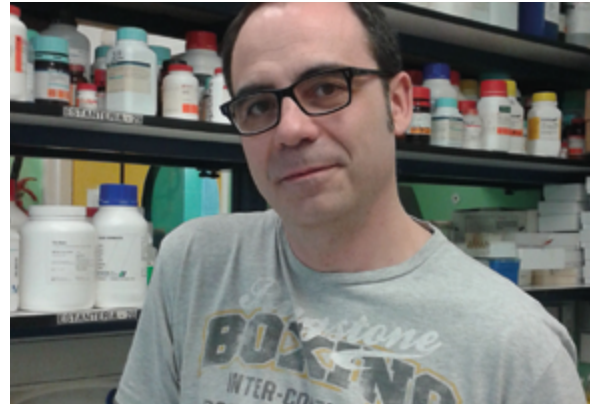
Francisco Javier Chichón
Rafael Núñez (CIB-CSIC)

The cryoelectron microscopy core facility is located at the CNB and jointly operated with the Centro de Investigaciones Biológicas (CIB-CSIC). The services offered by the facility include sample preparation and image collection for cryoelectron microscopy.

The facility provides access to two microscopes for cryoelectron microscopy of unstained biological material. One of them, a 200 kV FEI TALOS Arctica, equipped with an autoloader and with a Falcon III direct electron detector, is ideally suited for the collection of large amounts of high-resolution data. The other microscope, a 200 kV FEI Tecnai G2, equipped with a CCD camera, is available for general routine cryoEM.

The service also has two different apparatus for specimen vitrification, a FEI Vitrobot and a Leica EM CPC.





Electron microscopy

HEAD

Cristina Patiño Martín

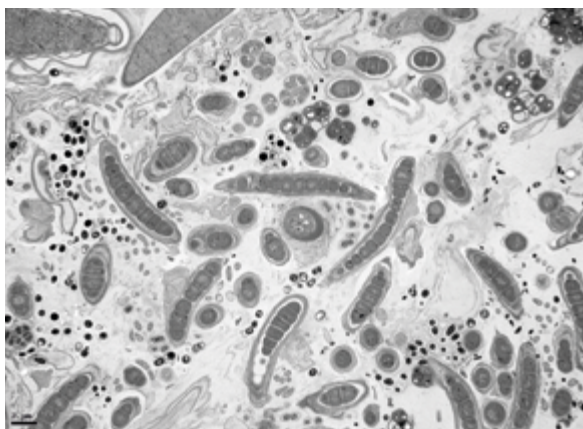
PERSONNEL

Beatriz Martín Jouve

The transmission electron microscope (TEM) operates on many of the same optical principles as the light microscope but uses electrons as the illumination source. Their much shorter wavelength makes it possible to obtain much higher resolution, which allows to study ultrastructure of organelles, viruses and macromolecules.

The aim of this facility is to deliver scientific and technical support to researchers at the CNB and to users from public or private institutions by providing access to the necessary equipment, advising on the most appropriate techniques, offering specialised sample preparation, performing microscopy, data collection and support for the interpretation of the obtained data.

The facility is equipped with a 100 kV transmission electron microscope with a digital camera. For sample processing, the facility disposes of a Reichert ultramicrotome, Leica UC6-FC6 cryo-ultramicrotome, Leica AFS2 automatic freeze substitution system, Leica AC 600 carbon coating equip and Leica EM PACT2 high-pressure vitrification unit.



Macromolecular structures and X-ray crystallography

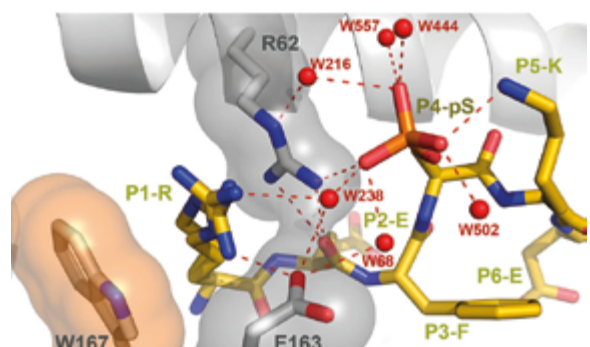
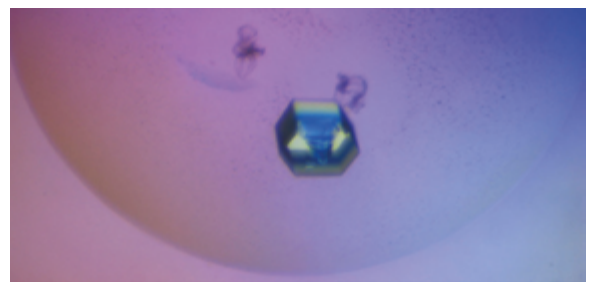
HEAD

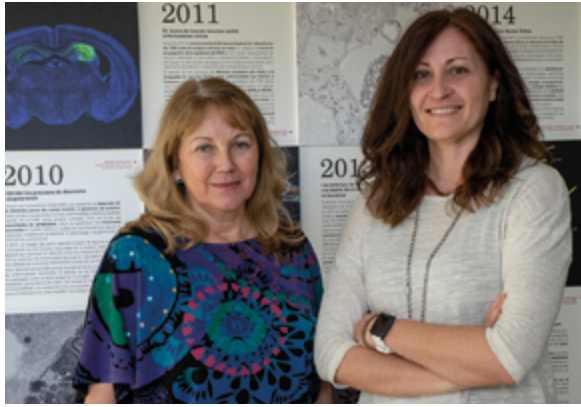
César Santiago

Protein X-ray crystallography is a high-resolution technique that allows to study protein structure at the atomic level. This method provides a detailed view of protein function, ligand and protein interactions, supramolecular organisation and mutants related to human diseases. Substantial improvements, both in crystallisation techniques and in software for structure resolution and refinement, have been achieved in the last decade, which increases the success of solving a macromolecule structure.

The facility provides both advice and supervision of protein production from cloning to expression in bacterial, yeast and eukaryotic systems, as well as support and training in protein purification to obtain crystal-grade protein for automated crystallisation using an automated nanodispensing robot.

The facility also offers crystal optimisation and freezing for diffraction data at the synchrotron. Structure resolution and interpretation are also provided for both data coming from X-ray crystallography or data produced with any other structural biology technique.





Flow cytometry

HEAD

M^a del Carmen Moreno-Ortiz Navarro

PERSONNEL

Sara Isabel Escudero García

The facility provides scientific and technological support to CNB groups and other researchers from the public and private sectors.

The facility offers training and advice on the principles and the applications of analytical flow cytometry, assistance to plan, design, and optimise flow cytometry experiments, including the implementation of new technologies and reagents as well as sample preparation, instrument operation and data analysis. The facility provides quantification of secreted cytokines by multiplexed assays, cellular isolation by cell sorting, antibodies and commonly used reagents. Analysis of results can be performed using specialised software packages.

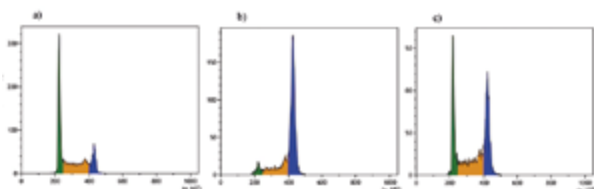
Equipment

1BD FACSCalibur: 4 colours. 2 Beckman Coulter CYTOMICS FC 500: 5 colors.1 BD LSRII: 8 colours. 1 Beckman Coulter GALLIOS: 10 colours. 1 Beckman Cytoflex: 13 colours. 1 Luminex 100 IS Multiparametric Analyzer. 1 Cell Sorter Beckman Coulter Moflow XDP: 10 colours.

Common applications

Cell viability, either fresh or fixed cells; apoptosis; cell cycle and ploidy levels in eukaryotic cells; mitotic population studies, proliferation assessment using BrdU, EdU, CFSE or CELLTRACE, gene expression of fluorescent proteins; immunophenotyping up to 13 colours; intracellular signalling; cell migration; calcium mobilisation; intracellular cytokines, quantitation of soluble molecules by multiplexed assays and cell sorting.

Fluorescence intensity (propidium iodide) to measure T Cell arrest at G2M phase after treatment with nocodazole: a) untreated cells, b) after nocodazole treatment, c) cell division recovery after nocodazole withdrawal.



Histology

HEAD

Lluís Montoliu

TECHNICAL MANAGER

Soledad Montalbán

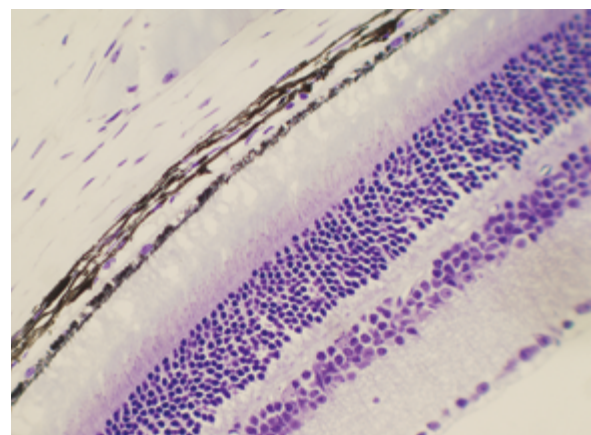
PERSONNEL

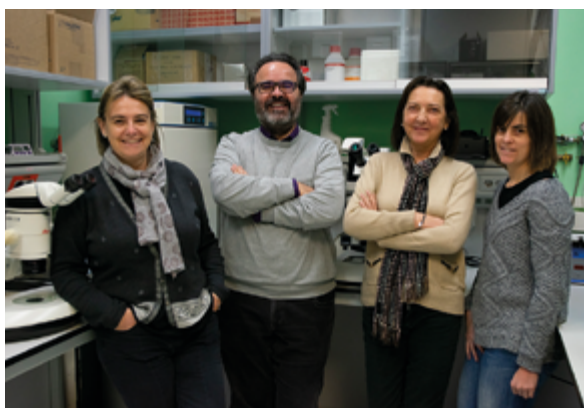
Óscar Sánchez

The CNB histology facility offers the preparation of animal and plant biological samples for their histological analysis. All requests are received and processed electronically, through the facility's web site, available in Spanish and in English.

Offered methods and procedures include the preparation of wax (paraffin) and plastic (resin) blocks with biological specimens embedded, and the corresponding generation of histological sections with one of the two available automated microtomes. The facility also offers the preparation and sectioning of frozen blocks with the cryostat. The orientation, width and arrangement of the sections can be specified by the user. All sections can be counterstained with any of the available staining procedures (haematoxylin/eosin, cresyl violet, PAS, Mason's trichrome, elastin fibers/Van Gieson/Sirius Red, etc.) or can be processed subsequently for immunohistochemistry. The facility implements new staining procedures or histological methods upon request. The CNB histology facility has an ample experience in processing a large variety of animal and plant tissues and organs.

The CNB histology facility coordinates a joint platform with the IIB-UAM/CSIC histology facility, offering to CNB and IIB researchers a larger processing capacity for histological samples.





Mouse embryo cryopreservation

HEAD

Lluís Montoliu

TECHNICAL MANAGER

Julia Fernández Punzano

PERSONNEL

María Jesús del Hierro Sánchez
Marta Castrillo Labrado,
Isabel Martín-Dorado Caballero

The CNB mouse embryo cryopreservation facility offers to researchers the possibility to freeze, maintain and rescue transgenic and knockout mouse lines in the form of embryos and/or sperm, hence contributing to current animal welfare recommendations and complying with the associated legislation on animal experimentation. Current methods available include freezing sperm, oocytes and/or embryos, the thawing of sperm, oocytes and/or embryos previously frozen and the subsequent revitalisation of the cryopreserved mouse lines through *in vitro* fertilisation, assessment and/or logistical support for importing/exporting frozen or refrigerated embryos or sperm, from and to the CNB, and quality controls and genotyping procedures.

The CNB hosts the Spanish node of the European scientific-technological infrastructure INFRAFRONTIER-EMMA, whose objective is the cryopreservation, organised archiving and coordinated distribution of mouse lines of interest for the scientific community in biomedicine.

The CNB mouse embryo cryopreservation facility has scientific cooperation agreements with the Spanish National Cancer Centre (CNIO) and the University of Kumamoto for the archiving and distribution of mutant mouse lines of interest in biomedical research.

Lluís Montoliu and Julia Fernández participate regularly as instructors in mouse cryopreservation workshops and courses, organized in collaboration with CARD-University of Kumamoto, INFRAFRONTIER-EMMA, ISTT and CIEMAT-SECAL, among other institutions.



Protein tools

HEAD

Leonor Kremer

PERSONNEL

María Teresa Martín
Mónica García-Gallo
Ana María García
Mercedes Llorente
María Lozano

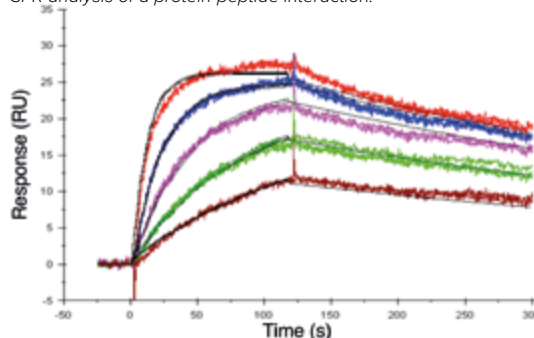
The CNB protein tools unit offers scientific services related to the design, generation and characterisation of custom monoclonal antibodies (mAb); immune response studies; customised immunoassays; antibody purification and labelling, and analysis of biomolecular interactions.

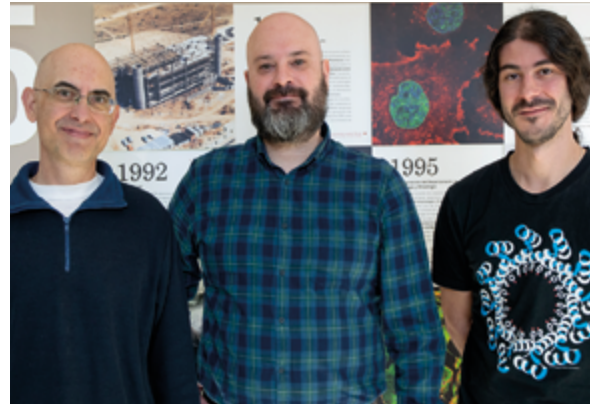
The facility has expertise in immunobiology and immunochemistry, and provides technical assistance, data analysis, training in specific techniques, introduction of new methodologies and technical advice. It also organises theoretical and practical courses and assists with the preparation of manuscripts and oral presentations.

The facility also has a surface plasmon resonance (SPR) based biosensor, Biacore 3000, that allows the characterisation of biomolecular interactions in real time and the determination of kinetic and affinity constants. SPR analysis can be applied to a wide range of molecules or particles such as proteins, nucleic acids, carbohydrates, lipids, low molecular weight compounds, liposomes and viruses.

Protein tools unit is a founder member of the EuroMAbNet, the first European non-profit organisation of multidisciplinary academic laboratories specialised in mAb production, which offers to researchers working in the field a framework for exchange of knowledge, methods and materials, recommendations and training in antibody validation. The facility provides research tools and services to scientists from the CNB, other CSIC institutes, universities, public research organisations and private companies.

SPR analysis of a protein-peptide interaction.





Transgenesis

HEAD

M^a Belén Pintado Sanjuanbenito

PERSONNEL

Verónica Domínguez Plaza (CBMSO)
Marta García Flores
Alicia Llorente

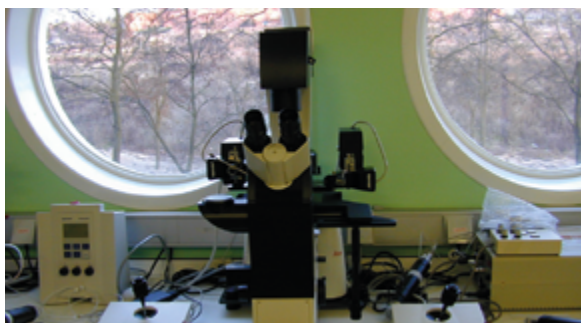
The CNB-CBMSO transgenesis unit is a joint scientific service shared between CNB and CBMSO. The facility is integrated in the scientific-technological platform INNOTEK (UAM+CSIC) and member of the Merck CRISPR Core Partnership Program to test new CRISPR/Cas9-related products.

It supports research groups in all the required steps to obtain the desired mouse model: from the creation of a genetically modified model to the establishment and management of lines in order to achieve the desired genotype. The facility provides the animal resources and technology to produce models based on additive transgenesis, targeted mutagenesis (KO, KI) or genome edition based on CRISPR/cas9 technology. The facility designs and tests procedures to produce KI and conditional mouse models in a single step, supporting and complementing the expertise of its customers.

The facility has two microinjection settings, dissecting microscopes, a standard molecular biology laboratory and a fully equipped laboratory for ES cells handling, as well as full access to the animal facilities of CBMSO and CNB.

Services

- Pronuclear microinjection of plasmidic, BAC and YAC DNA
- Genome editing based on CRISPR/cas9 technology, either by microinjection or embryo electroporation, including guide design and in vitro validation
- Injection of ES cell lines generated indoors or from international consortia
- Embryo rederivation through IVF or embryo transfer from external animal facilities



Bioinformatics for genomics and proteomics (BioinfoGP)

HEAD

Juan Carlos Oliveros

PERSONNEL

Rafael Torres-Pérez
Juan Antonio García-Martín

The BioinfoGP facility provides CNB's research groups with bioinformatics support for the analysis, visualisation and interpretation of both genomics and proteomics-related projects, including:

- Assistance in the experimental design of deep sequencing and DNA microarray experiments
- Biostatistical support for extracting quantitative results from genomics or proteomics projects
- Functional annotation of relevant lists of genes or proteins.
- Periodic courses and tutorials on bioinformatics

In short, the BioinfoGP facility aims to fill the gap between the complex outcome of the many powerful biostatistical methods available and the researchers' needs.





Genomics

HEAD

José Manuel Franco Zorrilla

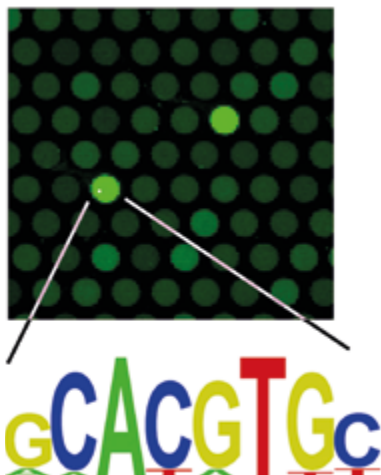
PERSONNEL

Irene López-Vidriero
 Marta Godoy
 Luis Almonacid
 Gloria García Casado
 Beatriz Martín

The genomics facility is focused on the analysis of gene expression from biological samples using microarrays, interrogating the activity of complete genomes in a single experiment, and contributing to the elucidation of the genetic basis of the biological processes. The facility routinely hybridises and analyses one- and two-channels microarrays, including Agilent, Affymetrix, and custom microarrays.

The services offered by the facility include microarray printing and design, analysis of RNA integrity and microarray hybridisations. Raw data are statistically analysed using “state-of-the-art” algorithms, and filtered results are supplied to customers in a web-based easy-to-use tool developed by the facility. The facility offers support in the use of several bioinformatics tools for functional analysis, helping customers in the biological interpretation of their results. The facility also offers the possibility of validating gene expression data by real time qPCR.

Research projects are being constantly developed by the facility with the goal to implement new services and technologies for customers, including novel high-throughput technologies for studying DNA-protein interactions to decipher the molecular basis of transcriptional regulation.



Proteomics

HEAD

Fernando Corrales

PERSONNEL

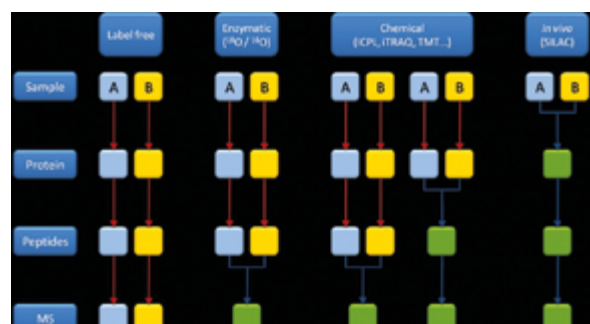
Adan Alpizar
 Gema Bravo
 Lorena Carmona
 Sergio Ciordia
 Manuel Lombardía
 Miguel Marcilla
 Rosana Navajas
 Alberto Paradelo
 Antonio Ramos

Created in 1999, the CNB Proteomics Facility maintains a technological platform suitable for large-scale protein identification and characterisation, with more than 3,000 proteomic analyses performed in 2017. Massive protein identification, characterisation (including posttranslational modifications) and quantification are performed by multidimensional nano-HPLC chromatography coupled to a nano-electrospray mass spectrometers (MS). Finally, prolamin detection and characterisation by ELISA, quantitative PCR and mass spectrometry are also included in our analysis portfolio.

For educational purposes, we organise practical courses on quantitative proteomics and bioinformatics. The head of the CNB Proteomics Facility (Dr. Fernando Corrales) also coordinates Proteored-ISCIII (Plataforma en Red de Proteómica-Carlos III).

Services

- Protein identification and characterisation
- Protein quantitation by label-free, metabolic and chemical stable isotopic labelling (SILAC, ICPL, iTRAQ, TMT)
- Selected/multiple reaction monitoring (S/MRM-MS)
- Identification and characterisation of post-translational modifications
- Peptide synthesis and membrane-bound peptide array design
- Gluten analysis by ELISA, PCR and mass spectrometry





Sequence analysis and structure prediction

HEAD

Mónica Chagoyen

Sequence analysis and protein structure prediction methods can explain, simplify and further guide experimental work. The facility specialises in *ad hoc* analysis of protein sequences to solve specific problems or questions.

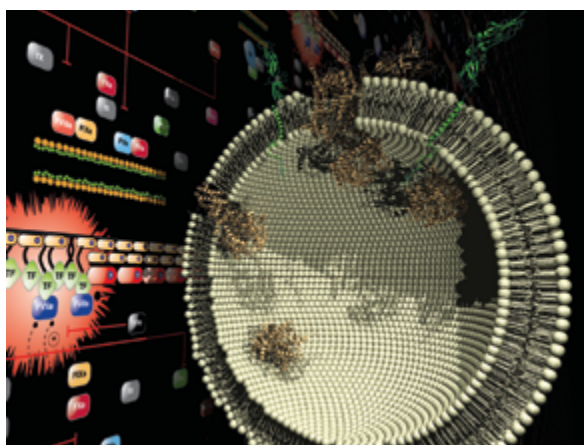
This analysis commonly aims to:

- Predict protein structure
- Search for homologous proteins
- Generate multiple sequence alignments
- Produce structural organisation drafts
- Study relevant residues for protein structure/function
- Extract sequence features from full proteomes

Additional services include:

- DNA/RNA motif discovery
- Consultancy on the use of sequence-based methods
- Generation of high-quality protein sequence/structure images for publication

These services are available to CNB researchers as well as to users from other academic institutions and private organisations.



Scientific computing service

HEAD

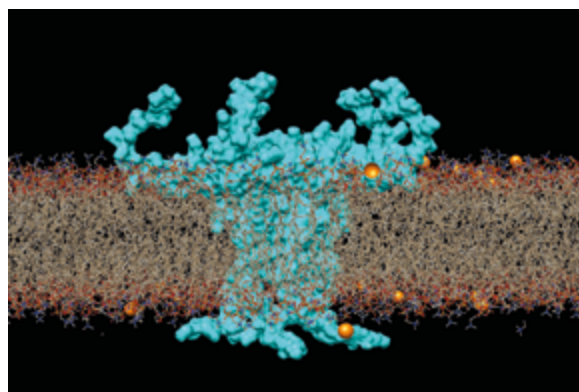
José R. Valverde

The Scientific Computing Service provides advanced support for scientific data analysis in Bioinformatics and Biocomputing through delivery of international courses, close collaboration with research groups and international institutions.

Our areas of expertise span Bioinformatics, Genomics, Metagenomics, Metabolomics, Biostatistics, Artificial Intelligence/Machine Learning, and Computational Biology, including molecular docking, drug screening, *in silico* mutagenesis, Molecular Dynamics, QM and QM/MM models, Quantum Dynamics and reaction modelling, as well as computer programming in numerous languages.

Sample applications of these techniques involve cancer diagnosis, the effect of pesticides on soil, identifying ecological indicators, sequencing new bacterial strains, dynamic metabolic networks for improving biotechnological processes in *S. lividans*, activity of the ϵ - ζ toxin-antitoxin system, analysis of viral structures and virus-host interactions in Phage T7, human SARS-CoV, and HIV for the development of new antiviral therapies.

The service has participated in several Networks of Excellence in Bioinformatics, Grid computing, Genomics and CBRN, and advised students' theses at Graduate, Master and PhD levels from U. Autónoma of Madrid, U. of Alcalá de Henares, U. Politécnica of Madrid, U. of Colombo in Sri Lanka. Additionally, the service collaborates in academic activities organised by other institutions in numerous countries in Europe, Latin America, Asia and Africa.





Animal facility

HEAD

Ángel Naranjo

RESEARCH TECHNICIAN

Javier Martín

SHIPMENT COORDINATOR AND ADMINISTRATION

Alberto García

AREA AND COLONY MANAGERS

Antonio Morales
Raquel Gutiérrez
Eladio Martínez

ANIMAL TECHNICIANS

Sergio Magallón
Ruví Jaramillo
Lola García
Ivan Jareño
Raquel Castañera

The CNB laboratory animal facility is an area dedicated to the production and maintenance of experimental animals, aiding in research, essential techniques, and legal support for this duty. Most of the experimentation is carried out with genetically modified mice. The laboratory animal service provides a controlled environment for the animals, with periodic control of diet, water, temperature, air, housing, and husbandry conditions. The unit is separated into several areas: quarantine, conventional, and specific pathogen-free (SPF), depending on the microbiological status of the animals. The facility provides special housing conditions for conventional, genetically modified, and immunodeficient animals, depending on the experimental objectives. At the same time, a totally isolated biosafety area is dedicated to *in vivo* experiments using biological agents.

The animal facility staff delivers services to laboratories for obtaining commercial lines and strains of animals, shipping animals for collaboration with other institutes, as well as maintenance, breeding, and generation of transgenic, knock-out and knock-in animals. These services allow control of the microbiological and genetic quality of the animals used in experimentation. The animal facility staff provides services for various techniques used in mouse research models, research assistance in surgical techniques, selection of animal models, animal health surveillance, laboratory animal care, and animal well-being. The facility also organises courses for continued education and to obtain accreditation for working with animals and manage colonies of genetically modified animals.

The facility's goal is to achieve research excellence following the 3R principles: reduction, refinement, and replacement of animal experiments.

Greenhouse

HEAD

Tomás Heras Gamo

PERSONNEL

Alejandro Barrasa Fustes
Joaquín Rivera Cuesta

The CNB greenhouse takes care of the following facilities specific for plant cultivation:

- A standard greenhouse with 8 cabinets (total growth surface: 180 m²)
- A P2 safety level greenhouse with 4 cabinets (total growth surface: 83 m²)
- 16 climate chambers

The facility carries out the following tasks:

- Growth and propagation of plants under controlled environmental conditions
- Growth and propagation of mutant and transgenic lines under controlled environmental conditions
- Identification, selection and phenotypic analysis of mutant and transgenic plants





Radioactive facility and biosafety level 3 laboratory

HEAD

Fernando Usera Mena

PERSONNEL

María Teresa Bartolomé Jiménez
 Aranzazu de la Encina Valencia
 Jessica Gaspar Navarro

This unit directly operates the centre's gamma irradiator, radioactive facility and biosafety level 3 laboratory.

Radioactive facility

The CNB radioactive facility is a category 2, non-encapsulated sources type installation, equipped with all the required means of shielding, containment and detection of ionising radiation.

The following equipment is available for users:

- Two cabinets for radioisotopes beta and gamma
- Biosafety class IIA cabinet
- CO2 incubator
- Ultracentrifuge
- Centrifuges and microfuges
- Vacuum concentrator (SpeedVac)
- Hybridisation oven

Biosafety level 3 laboratory

The laboratory has three sub laboratories with all the necessary equipment for safely handling biological agents included in risk group 3.

The installation is equipped with:

- Three biosafety class IIA cabinets
- Three cell culture incubators
- Microbiological culture incubator
- Refrigerated ultracentrifuge
- Three bench-top refrigerated centrifuges
- Three refrigerated microfuges
- Three inverted optical microscopes
- Fluorescence microscope
- Liquid nitrogen tank with capacity for 6,000 samples
- Double door steam steriliser
- Pass through box for biological inactivation of small items
- Air lock for biological inactivation of large items
- Five ultra-freezers (-80°C)
- Data transmission network (computers and telephone)
- Several alarm systems to alert of incidents, accidents or malfunctions

Cell culture, washing and sterilisation

HEAD

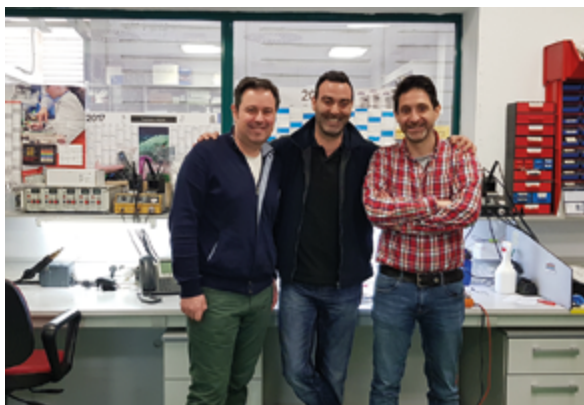
Rosa M^a Bravo Igual

PERSONNEL

Carmen Berdeal
 Esther Dorado
 Carlos Enríquez Casas
 Margarita Felipe Hombrados
 Isabel Martín-Dorado
 Ana Montero
 Ana Isabel Nieto Jiménez
 Josefa Pérez Alfaro
 Rosa Ramos Hernández
 Aránzazu Rodríguez Martínez
 Sonia Rodríguez Murcia
 Anunciación Romero
 M^a Angeles Sánchez
 Ángel Valera

Services

- Preparation of cell culture media
- Routine cell culture procedures
- Washing, sterilisation and replacement of laboratory material



Instrumentation

HEAD

Ismael Gómez López

PERSONNEL

Juan Ignacio Golpe de la Fuente
Carlos González Redondo
Rodrigo López Manzano

Services

- Calibration and validation of scientific instrumentation
- Maintenance and repair of scientific instrumentation
- Technical advice during the acquisition of scientific-technical equipment
- Supervision of the installation of scientific-technical equipment
- User training for scientific-technical equipment



Photography

HEAD

Inés Poveda

The CNB photography service supports scientists with the photographic material necessary for their research and the dissemination of their results.

Photos are taken on a reprographic table with continuous lighting or with studio flashes against an adjustable background, and illumination with white or ultraviolet light, as needed.

The photography service also manages image processing and, when required, photo retouching; digital images are made accessible to clients on dedicated servers.

The service offers digital color printing of large format posters and, on request, also provides advice for graphic and image design.



Radiation protection and biological safety

HEAD

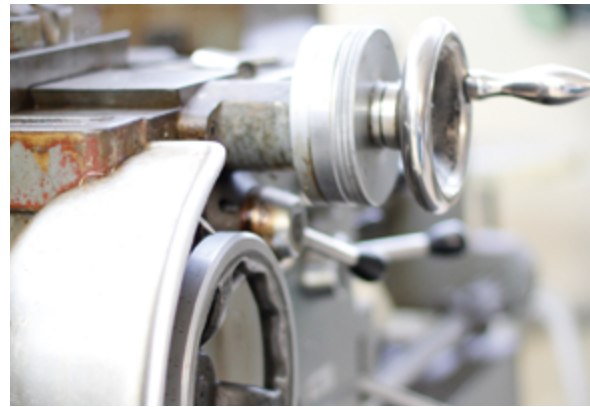
Fernando Usera Mena

PERSONNEL

María Teresa Bartolomé Jiménez
 Aranzazu de la Encina Valencia
 Jessica Gaspar Navarro

Services

- Evaluation of biological, chemical and radiological risks
- Management of official authorisations for research facilities
- Management of the acquisition of radioisotopes and equipment for protection
- Design of laboratories and other facilities potentially exposed to risks
- Issuing of the CNB Basic Guide, the CNB Safety & Health Manual and other safety guidelines and standard operating procedures
- Training of personnel related to specific risks in research laboratories
- Control of the accomplishment of safety and health rules related with research activities
- Management of medical and dosimetry of the exposed personnel in the laboratories
- Management of accidents and emergency situations following established procedures
- Managing of biologic, toxic and radioactive waste



Workshop

HEAD

Daniel Pastora

Services

- Machining metal and plastic parts
- Custom manufacture of metal structures
- Welding and repair of steel carts

Equipment

- Parallel lathe
- Milling machine
- Power welding set
- Spot welding equipment
- Mitre saw
- Reciprocating saw
- Automatic slitter
- Bending machine
- Grinding machine
- Column drilling machine

