8 / Scientific Services

One of the most important assets of the CNB is its platform of scientific-technical services. They provide leading-edge technology in the fields of structural biology and image processing, cell biology, genetically modified mouse and plant models, genomics and proteomics, as well as bioinformatics and computational biology. The centre also stands out for large research installations, such as its animal facility, greenhouse and one of the few high-level biocontainment (BSL-3) laboratories currently operative in Spain.

Electron microscopy

HEAD OF SERVICE:
Cristina Patiño Martín

PERSONNEL:
Rocío San Andrés Cervilla
Ana Beloso Quiñones
Javier Bueno Chamorro

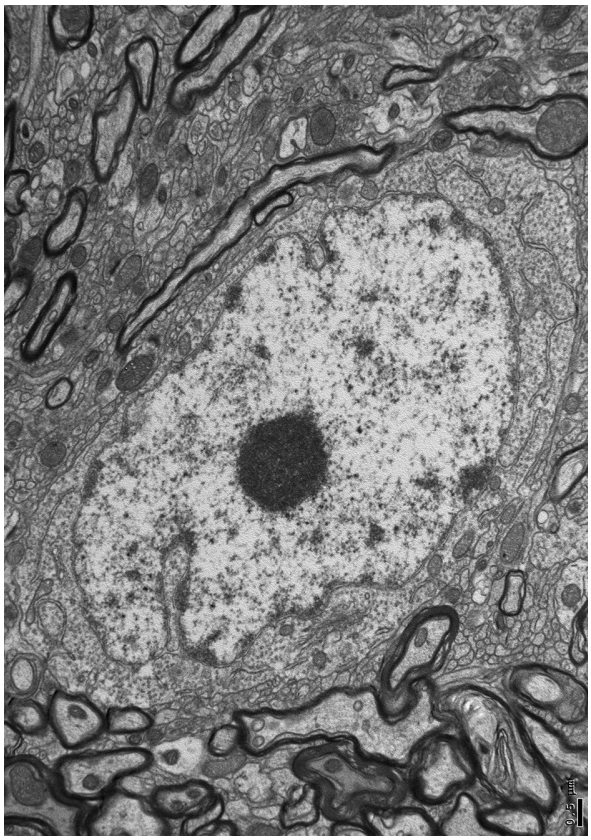
The electron microscopy service offers a variety of equipment and techniques for the preparation, processing and analysis of biological samples (cell and bacterial cultures, cell fractions, proteins, viruses, animal and plant tissues) by transmission electron microscopy.

The technical staff provides support to users in the correct use of equipment and methodologies. We offer regular training in the techniques and methods available. We also take care of the sample preparation, if required, and image acquisition and provide support for data interpretation.

Techniques offered include chemical fixation and inclusion in epoxy and acrylic resins, cryofixation (plunge freezing, high pressure freezing), freeze substitution and inclusion in low temperature resins, ultramicrotomy, negative staining, immunonegative staining, immunolabelling, *in situ* hybridisation, conventional transmission electron and low dose electron microscopy.

Specialised equipment

- JEOL JEM 1011 transmission electron microscope with Gatan ES1000W camera
- Leica Ultracut UC6 cryo-ultramicrotome
- Reichert Ultracut E ultramicrotome
- Automatic cryosubstitution system Leica EM AFS2
- High-pressure vitrification system Leica EM PACT2
- Sample trimmer Leica EM TRIM
- Knifemaker Reichert
- Carbon coating system Leica EM MED020

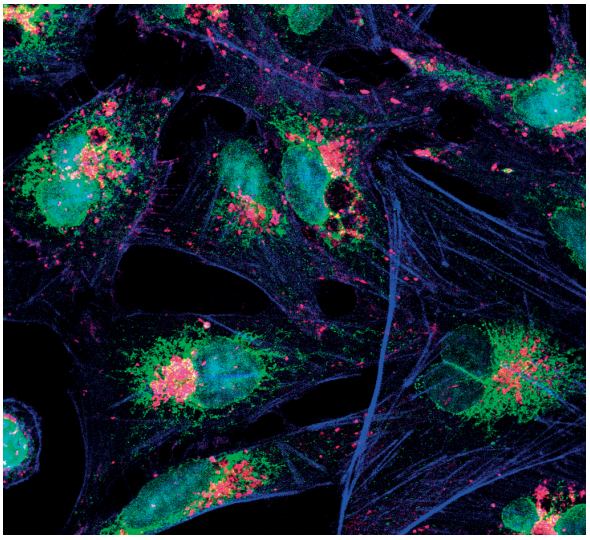


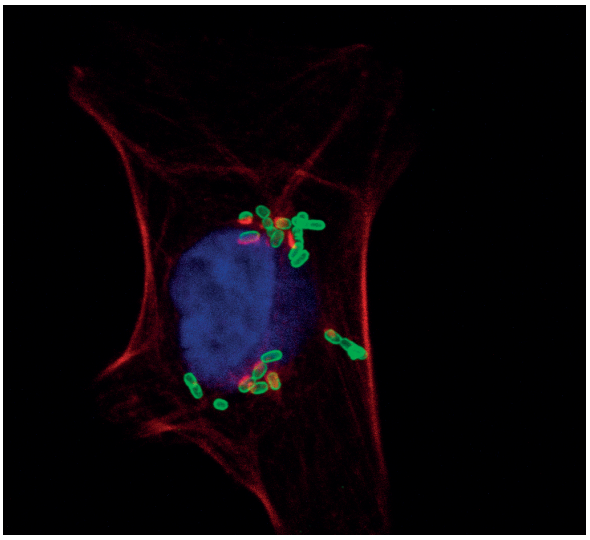
Confocal microscopy

LEAD SCIENTIST:
Sylvia Gutiérrez Erlandsson

PERSONNEL:
Susana Hernández García

Confocal microscopy imaging techniques use lasers and electronic systems of digital image capture to provide optical sections of the material. 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 service provides infrastructure for fluorescence, confocal laser scanning microscopy and image processing tools, covering most light microscopy applications, with technical assistance to all its users. The equipment and services are available to all CNB personnel as well as to researchers from the public and private sectors. The technical staff offers training and advice about equipment use, available methods, and for image processing, quantification and analysis if required. Aliquots of secondary antibodies and probes with broad use in fluorescence microscopy applications are also provided.

The facility's equipment includes:

• Confocal multispectral Leica TCS SP5 system. Laser lines: 405, 458, 476, 488, 514, 561, 594 and 633 nm. Incubation system for *in vivo* studies

• BioRad Radiance 2100 confocal system. Laser lines: 457, 476, 488, 514, 543 and 637 nm

• Fluorescence microscope Leica DMI6000B with incubation system for *in vivo* studies and OrcaR2 monochrome digital camera for image detection

• Two epifluorescence microscopes (Leica DMRXA and Zeiss Axiophot) with colour digital cameras and one Leica stereomicroscope

• The unit also provides offline computer workstations for fluorescence and confocal image processing and analysis (LAS AF, MetaMorph, ImageJ, Laser Pix, Huygens, Imaris)

• Auxiliary equipment: CO_2 incubator, centrifuge, laminar flow chamber, freezer

Laser scanning confocal microscopy applications:

• Multichannel confocal imaging + transmission imaging of living cells or fixed samples (2D, 3D, 4D imaging)

• High speed confocal microscopy

• Multidimensional *in vivo* time-lapse experiments

• FRET, FRAP, photoactivation, photoswitching, lambda scan, calcium imaging

• Subcellular colocalisation studies

Widefield applications:

• Multichannel fluorescence imaging + transmission imaging (BF, DIC, phase contrast)

• Multidimensional *in vivo* time lapse experiments (wound healing, infection, etc.)

• Tile scan imaging

Macromolecular X-ray crystallography

LEAD SCIENTIST:
César Santiago

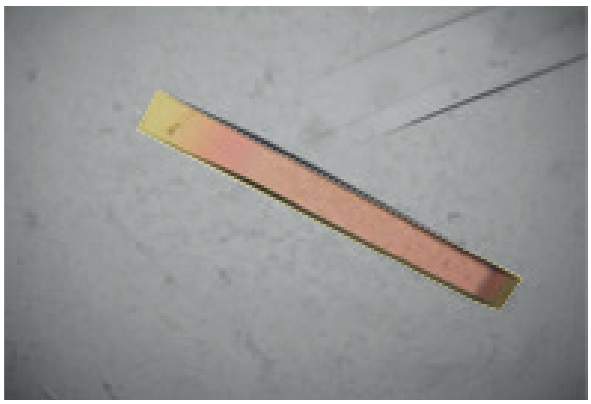
Protein X-ray crystallography is a high-resolution technique that allows us to study protein structure at atomic level. This method provides a detailed view of protein function, ligand and protein interactions, supramolecular organisation and mutants related to human diseases. Great improvements both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

The macromolecular X-ray crystallography facility provides the following techniques:

- Advice and supervision of protein production from cloning to expression in bacterial, yeast and eukaryotic systems
- Support and training in protein purification to obtain crystal-grade protein
- Automated macromolecular crystallisation
- Optimisation of crystallisation conditions applying standard and in-house techniques
- Crystal mounting, access to synchrotron beam time, X-ray diffraction data collection
- Data processing and structure resolution and analysis

Service equipment:

- Three temperature-controlled crystallisation rooms
- Genesis RSP 150 workstation (Tecan Trading AG nanodispenser robot
- Rigaku Desktop Minstrel system for automated visualisation of crystallisation plates
- CrystalTrak database suite for crystallisation screening and improvement of positive trials



Proteomics facility

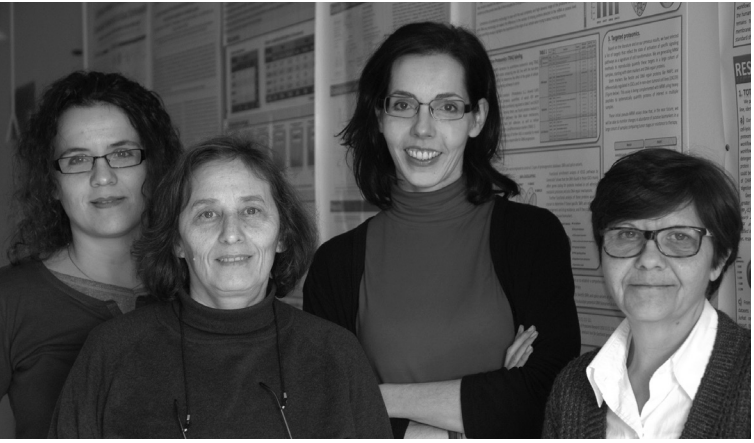
LEAD SCIENTISTS:
Juan Pablo Albar
Alberto Paradela

PERSONNEL:
Gema Bravo
Sergio Ciordia
Manuel Lombardía
Alberto Medina
Mari Carmen Mena
Rosana Navajas

Created in 1999, the CNB Proteomics Facility maintains a technological platform suitable for large-scale protein identification and characterisation, offering its services to the CNB scientific community as well as to external researchers. Massive protein identification and characterisation is performed by multidimensional nano-HPLC chromatography coupled to a nano-electrospray ion trap mass spectrometer (MS), to a TripleQ-TOF MS, or to a MALDI TOF/TOF MS (LC-MS/MS). For differential proteomics (quantitative proteomics), we use metabolic or chemical stable isotope labelling (ICPL, SILAC, iTRAQ,TMT) combined with LC-MS/MS analysis. We also offer targeted and, in combination with AQUA peptides, absolute quantitative protein analysis by selected/multiple reaction monitoring (S/MRM-MS). Phosphorylation analysis is performed through specific phosphopeptide enrichment procedures followed by LC-MS/MS analysis, using CID (collision-induced dissociation) or ETD (electron transfer dissociation) fragmentation methods. Prolamin detection and characterisation by ELISA and mass spectrometry are also offered in our analysis portfolio. For educational purposes, we organise practical courses on topics such as quantitative proteomics and bioinformatics. The CNB Proteomics Facility is a member of the Proteored-ISCIII Platform (Plataforma en Red de Proteómica-Carlos III).

Services:

- Two-dimensional gel electrophoresis
- Protein identification and characterisation by MALDI-TOF/TOF, ESI Q-TOF, ESI QQQ and ESI ion trap mass spectrometry
- Protein quantitation by 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 and mass spectrometry



Protein tools unit

LEAD SCIENTIST:
Leonor Kremer

SCIENTISTS:
María Teresa Martín (*Molecular Interactions*)
Mónica García-Gallo (*Immunobiology*)
Mercedes Llorente (*Immunochemistry*)

TECHNICIANS:
Tamara Rueda
Laura Martín
María Lozano

The Protein Tools Unit focusses on production and characterisation of custom monoclonal antibodies, immune response studies, design and development of immunoassays, protein labelling and molecular interaction analysis. The Unit is a founder member of the EuroMAbNet, the first European non-profit organisation of multidisciplinary academic laboratories specialised in mAb production, which provides researchers working in the field with a framework for exchange of knowledge, methods and materials.

Research tools and services are provided to scientists from the CNB, other CSIC institutes, universities, public research organisations and private companies. The laboratory offers expertise, technical assistance, advices with data analysis and interpretation, user training and introduction of new methods. The core facility also organises theoretical and practical courses and assists with preparation of manuscripts and oral presentations.

The facility is equipped with a surface plasmon resonance biosensor (Biacore 3000), which allows sensitive, reliable characterisation of biomolecular interactions and provides information such as kinetics and thermodynamic parameters of the binding events. The biosensor has been used for applications such as protein-protein interaction (a bacterial toxin-antitoxin system), antibody-antigen (monoclonal antibodies, nanobodies, trimerbodies), dsRNA-protein (RNA-VP3), lipid-protein (FAK-liposomes) and small molecule-protein (DREAM).

In this period, new monoclonal antibodies were raised and characterised against viral proteins (SARS-E, gp120), immunoglobulins (canine IgE), blood proteins (coagulation Factor V, CD5L), neurodegenerative disease-related proteins (DREAM, TAU, beta amyloid peptides), centrosomal proteins (CAP350), mitochondrial proteins (SCAM3), and chemokine receptors (CCR9).

Specialised equipment:

- ÄKTAprime plus chromatography system (GE Healthcare)
- SPR Biacore 3000 (GE Healthcare)
- EnVision 2104 Multilabel Reader (Perkin Elmer)
- Other equipment: biological safety cabinets, CO_2 incubators, centrifuges, microfuges, inverted fluorescence microscope (Zeiss Axiovert 40 CFL), thermal cycler, microplate reader, protein gel electrophoresis systems, Western blot systems and electrophoresis power supply units

Website: www.cnb.csic.es/index.php/ProteinTools

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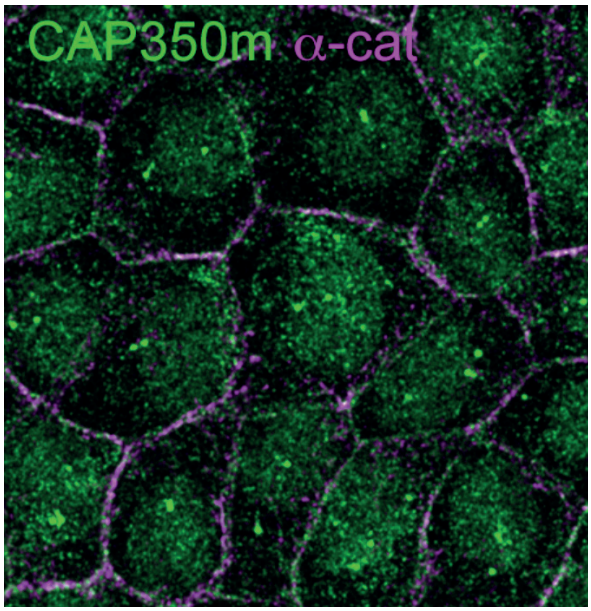

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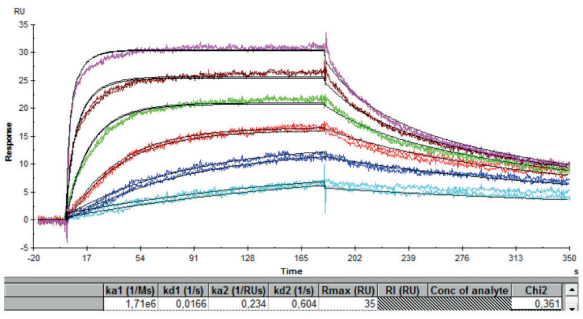


1 *Merged immunofluorescence image of MDCKII cells double-stained with α-catenin rabbit polyclonal and CAP350 mAb (figure kindly provided by María P. Gavilán and Rosa M. Rios, CSIC-CABIMER)*

2 *SPR analysis of an antibody-peptide interaction using a biosensor Biacore 3000. The antibody was used at concentrations from 0.41 nM to 100 nM, at 25ºC, with a flow rate of 30 µl/min. Data were collected for 180 s of the association phase and 180 s of the dissociation phase. Sensorgrams for different concentrations of analyte were overlaid, aligned and analysed with BIAevaluation Software 4.1*

Genomics unit

LEAD SCIENTIST:
José Manuel Franco Zorrilla

PERSONNEL:
Gloria García Casado
Irene López-Vidriero
Marta Godoy
Luis Almonacid
Beatriz Martín
Iria Calvete

The Genomics Unit at the CNB focusses on the analysis of gene expression from biological samples using microarrays, interrogating the activity of complete genomes in a single experiment, and helping to elucidate the genetic basis of biological processes. We routinely hybridise and analyse one- and two-channel microarrays, including Agilent, Affymetrix, and custom microarrays.

Our services include microarray printing, analysis of RNA integrity and microarray hybridisations. Raw data are analysed statistically using state-of-the-art algorithms, and filtered results are supplied to scientists in an easy-to-use, web-based tool developed by the Unit. We offer advice and support in the use of several bioinformatic tools for functional analysis of genes and genomes, helping researchers with the biological interpretation of the results. Finally, we also offer the possibility of validating gene expression data by real-time qPCR analysis. Through the Genomics Unit, the CNB participates in the Parque Científico de Madrid (CSIC-PCM) Ultrasequencing Platform, located physically at the CSIC-PCM installations. This platform has the capacity for massive sequencing experiments of complete genomes, transcriptomes, small RNAs or DNA/RNA-proteins interactions.

Research projects are constantly being developed by our personnel, who implement new services and technologies for our users. These include new microarray-based technologies such as a new DNA chip for studying DNA-protein interactions.

Tiller service:

• Leaded by Dr Carlos Alonso Blanco, the TILLer Service is an international public service to search for EMS-induced mutants in the model plant *Arabidopsis thaliana.*

• In recent years, the TILLer Service has searched for chemically induced mutants by applying the TILLING (Targeting Induced Local Lesions in Genomes) technique in an EMS collection of 3712 mutants developed by the service for this purpose (Martin *et al.*, 2009). To date, the service has sought mutants in more than 25 genes derived from applications from several countries, and has identified more than 500 mutants in those genes.

Computational genomics

LEAD SCIENTIST:
Juan Carlos Oliveros Collazos

Current advances in genomics-related technologies such as DNA microarrays and more recent ultrasequencing methods allow life science researchers to gather huge amounts of genome-wide data in little time and at a relatively low cost. Transforming these (raw) data into results, and these results into relevant biological conclusions, requires integrating specific biology and informatics skills, and the use of special software and hardware.

The CNB Computational Genomics service provides researchers with global bioinformatics support for the analysis, visualisation, and interpretation of data obtained in their genomics-related projects.

Among other services we offer:

• Assistance in experimental design for ultrasequencing and DNA microarray projects

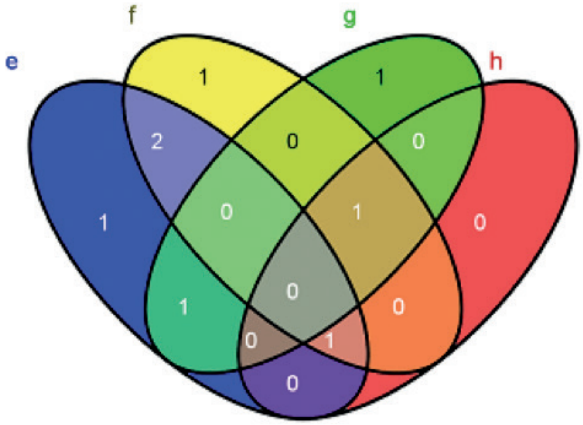
• Biostatistical support for the correct interpretation of genomics-related results

• Genomic data viewer development and maintenance

• Development of final user interfaces for third-party bioinformatics tools

• Organisation of periodic courses and tutorials on bioinformatics and genomics

In short, in the Computational Genomics Service we try to fill the gap between the complex outcome of the many powerful biostatistical methods available and the final user's needs that require placing these heterogeneous results in the context of their research projects.



Scientific computing

LEAD SCIENTIST:
José Ramón Valverde Carrillo

The Scientific Computing Service provides advanced support for scientific data analysis in bioinformatics and biocomputing through close collaboration and organisation of international courses (in-house and abroad).

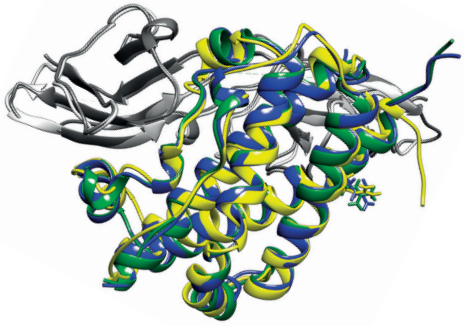
Our main areas of work include modern bioinformatics next-generation sequencing (NGS) data analyses, metagenomics and *de novo* genome sequencing for topics such as the effect of pesticides on the rhizosphere, sequencing novel *Escherichia coli* mutant strains, as well as offering courses on phylogeny analysis and metagenomics.

Computational biology includes binding pocket identification, docking, drug screening, *in silico* mutagenesis, molecular and quantum dynamics, QM and QM/MM (quantum mechanics/molecular mechanics) models and reaction modelling, which we use to study human growth hormone mutants, select natural compounds against cancer stem cells, resolve the reactions in the bacterial ε-ζ complex; we also offer courses on biocomputing.

Advanced biostatistical analyses include treatment of complex experimental setups related to new therapies, proteomics, non-linear longitudinal analyses, and an international course on biostatistics.

We provide support for many computer languages to produce dedicated software, and organise an international course on the Python programming language.

We coordinated the Iberoamerican Network on FLOSS for Biomedicine (CYTED 510RT0391), in collaboration with pioneering NGS groups, through COST action BM1006 (SEQAHEAD). We participate in EU CBRN CoE P35 and in EMBnet; with numerous external institutions including the University of Alcalá de Henares, we coordinate the Master in Bioinformatics of the University of Colombo (Sri Lanka), the Advanced Course in Genomics organised by ILRI in Nairobi, (Kenya), and many other courses in Europe, Latin America, Asia and Africa.



Sequence analysis and structure prediction

LEAD SCIENTIST:
Mónica Chagoyen Quiles

PERSONNEL:
Juan Carlos Sánchez Ferrero

Sequence analysis and protein structure prediction methods can explain, simplify and further guide experimental work.

We specialise in *ad-hoc* analysis of protein sequences to solve specific problems or questions.

In our analysis we commonly:

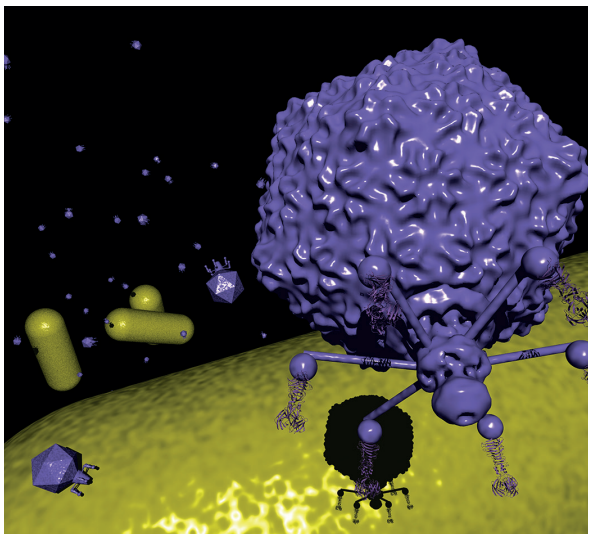
- Predict protein structure
- Search for homologous proteins
- Generate multiple sequence alignments
- Produce structural organisation drafts
- Study relevant residues for protein structure/function
- Extract sequence features from full proteomes

Additional services include:

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

In collaboration with other CNB services, we also organise periodic courses on bioinformatic approaches for sequence analysis and protein structure prediction.

The service is offered to the CNB-CSIC as well as to other academic institutions and private organisations.



Flow cytometry

LEAD SCIENTIST:
Mª del Carmen Moreno-Ortiz Navarro

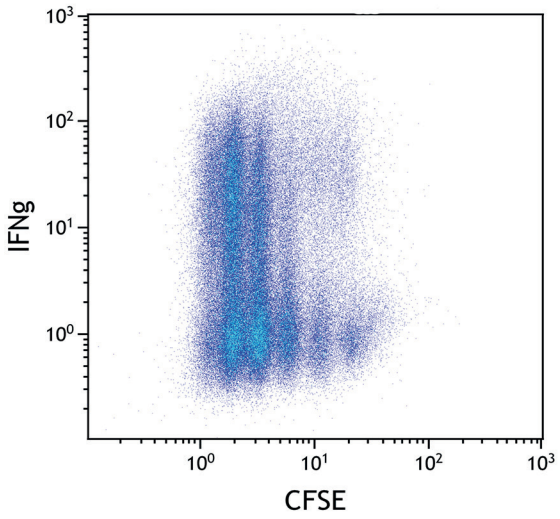
PERSONNEL:
Sara Escudero García

Flow cytometry is a technology of multiparametric cell analysis for detection and identification of molecules and cell structures using fluorescent markers and conjugated antibodies. Flow sorting is an extension of this technology, by which any cell or object measured can be separated selectively from the suspension based on properties measured in the flow stream. Flow cytometry techniques have undergone significant development in recent years in their ability to analyse thousands of cells per second and provide statistical information instantly.

The Flow Cytometry Unit provides scientific and technological support to CNB research groups, as well as to researchers from the public and private sectors.

The unit offers:

- Training and advice on flow cytometry principles and applications
- Development of new applications and experimental design, incorporating new technologies and reagents
- Quantification of secreted cytokines by multiplex assays
- Professional analysis with specific software
- Cellular isolation by cell sorting
- Maintenance of stock of antibodies and commonly used reagents



Equipment:

- BD FACSCalibur Analyzer: 4 colours, 2 laser (488 nm and 633 nm)
- Beckman Coulter EPICS XL-MCL Analyzer: 4 colours, 1 laser (488 nm)
- Beckman Coulter CYTOMICS FC 500 Analyzer: 5 colours, 2 laser (488 nm and 633 nm)
- Beckman Coulter CYTOMICS FC 500 Analyzer: 5 colours, 1 laser (488 nm)
- BD LSRII Analyzer: 8 colors, 3 laser (488 nm, 633 nm and 405 nm)
- Beckman Coulter GALLIOS Analyzer: 10 colours, 3 laser (488 nm, 633 nm and 405 nm)
- Luminex 100 IS Multiparametric Analyzer: A system that can be used to quantify multiple cytokines (up to 100) or any other soluble molecule from a single sample
- Cell Sorter Beckman Coulter Moflow XDP: 10 colours, 3 laser (488 nm, 633 nm and 405 nm)

The unit also provides 3 PC platforms for analysis with specific software: WindMDI, CXP, MultiTime, MultiCycle, DIVA, FlowJo, Summit, and Kaluza

Common applications performed routinely:

- Cell viability (IP, 7AAD, DAPI)
- Viability fixed cells; cell cycle and ploidy (IP, DAPI)
- Studies of mitotic populations (G2/M) with phosphohistone 3
- Ploidy levels in plants (IP)
- Proliferation assessment with BrdU or EdU
- Cell proliferation (CFSE, CellTrace)
- Apoptosis (SubG0/G1, annexin V, TUNEL)
- Gene expression using fluorescent proteins
- Intracellular signalling (phosphoproteins)
- Cell migration studies
- Intracellular Ca^{2+} mobilisation
- Immunophenophenotyping using multiparametric analysis up to 10 colours
- Study of T regulatory cells; intracellular cytokines
- Quantitation of soluble molecules, cytokines using multiplex assays
- Cell sorting

website: www.cnb.csic.es/~citometria

Greenhouse

HEAD OF SERVICE:
Tomás Heras Gamo

PERSONNEL:
Alejandro Barrasa Fustes
Esperanza Parrilla Carrillo

The Greenhouse Service takes care of the following facilities:

• A standard greenhouse with 8 cabinets (total growth surface: 180 m^2)

• A P2 safety level greenhouse with 4 cabinets (total growth surface: 83 m^2)

• 16 climate chambers

The Greenhouse Service 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

Animal facility

HEAD OF SERVICE:
Angel Naranjo

RESEARCH TECHNICIAN:
Javier Martín Torre

SHIPMENT COORDINATOR AND ADMINISTRATION:
Alberto García

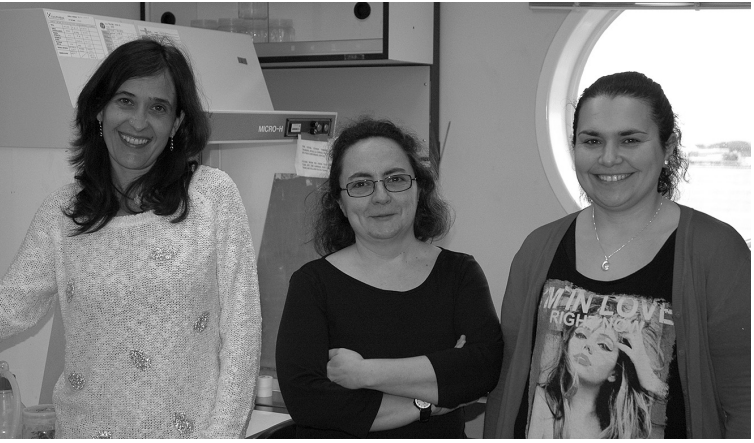
AREA AND COLONY MANAGERS:
Antonio Morales
Raquel Gutiérrez
Eladio Martínez

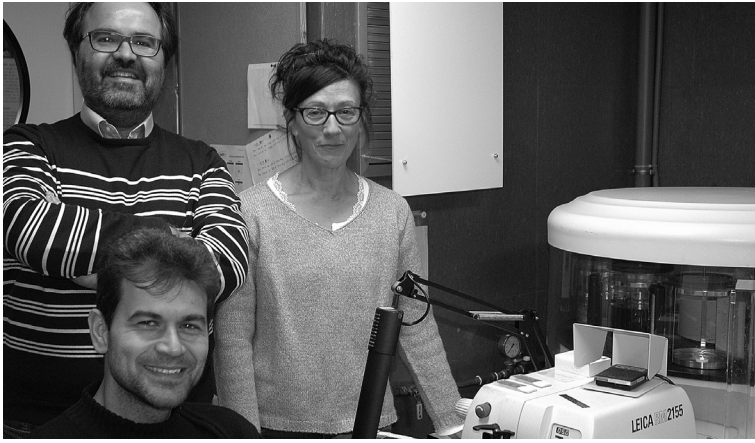
ANIMAL TECHNICIANS:
Angel Moreno
Israel López
Rebeca Acuña
Sergio Magallón
Alicia González
Rubí Jaramillo
Lola García
Susana Marcos
Patricia Sanz
Ivan Jareño
Raquel Castañera

The 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 and zebra fish. Our animal facilities and equipment are specially designed for these models. 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. We provide 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 gives service to laboratories for obtaining commercial lines and strains of animals, shipping animals for collaboration with other institutes, and 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. Veterinary staff gives research assistance in surgical techniques, selection of animal models, animal health surveillance, laboratory animal care, and animal well-being. We also organise courses to obtain accreditation for working with animals and manage colonies of genetically modified animals.

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





In vitro plant culture facility

HEAD OF SERVICE:
Raquel Piqueras Martín

PERSONNEL:
María Luisa Peinado Vallejo
Beatriz Casal López

The CNB *In vitro* Plant Culture Facility offers technical expertise and maintains a variety of equipment necessary for:

• sowing and growth of cells, tissues and seedlings of many plant species, including Arabidopsis, *Nicotiana sp,* tomato, potato and *Brassica sp*

• their maintenance and propagation

• generation of plant protoplast and cell cultures

We also assist in obtaining genetically modified plants by

• stable transformation of plants: using *Agrobacterium tumefaciens* as a transgene carrier

• transient transformation of plants using the particle delivery system

The *In vitro* Plant Culture Facility provides service to any researcher at the CNB and works very closely with members of the Plant Molecular Genetics Department. With appropriate authorisation, our services are also available to researchers from other institutions and companies.

Histology facility

LEAD SCIENTIST:
Lluís Montoliu

PERSONNEL:
Soledad Montalbán
Óscar Sánchez

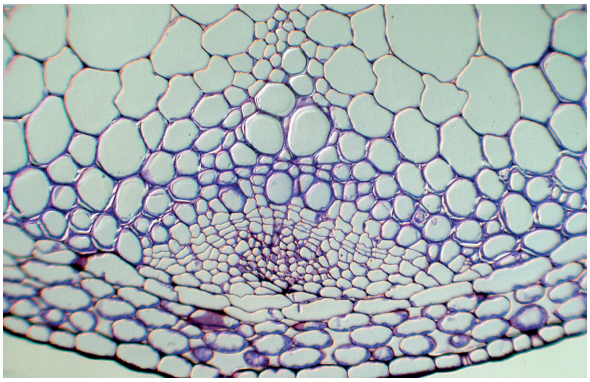
The CNB Histology Facility offers the preparation of animal and plant biological samples for histological analysis. All requests are processed electronically, through the facility's website, in Spanish and in English. Available methods include the preparation of wax and plastic (resin) blocks and the preparation of histological sections with an automated microtome. We also offer preparation and cryostat sectioning of frozen blocks. All sections can be counterstained with any of the various staining procedures available (including haematoxylin/eosin, crystal violet, PAS, Mason's trichrome, elastin fibres/Van Gieson) or can be used for immunohistochemistry. The Facility can implement new staining procedures or additional histological methods, according to the demand and user/researcher needs. The CNB Histology Facility has ample experience in processing a large variety of animal and plant tissues and organs.

The CNB Histology Facility is integrated within the INNOTEK technological platform of the Campus of Excellence UAM+CSIC, and is associated with the IIBm-UAM/CSIC Histology Facility, coordinated by the CNB Histology Facility, to offer CNB and IIBm researchers a larger processing capacity for histological samples. The CNB Histology Facility has joined the SEFALER technological platform of the CIBERER/ISCIII.

Publication

Chevalier F, Montalbán-Iglesias S, Sánchez OJ, Montoliu L, Cubas P. Plastic Embedding of Arabidopsis Stem Sections. Bio-protocol 2014; 4:e1261

Website: www.cnb.csic.es/~histocnb/



Mouse embryo cryopreservation facility

LEAD SCIENTIST:
Lluís Montoliu

PERSONNEL:
Julia Fernández Punzano
María Jesús del Hierro Sánchez
Marta Castrillo Labrado
Isabel Martín-Dorado Caballero

The CNB Mouse Embryo Cryopreservation Facility offers researchers the possibility of freezing and maintaining transgenic and knockout mouse lines in the form of embryos and/or sperm. Additional services available include the thawing of sperm and/or embryos and revitalisation of cryopreserved mouse lines, *in vitro* fertilisation, assessment and/or logistical support for importing/exporting frozen or refrigerated embryos or sperm to and from the CNB, quality controls and genotyping procedures. Cryopreservation of mouse lines is a highly recommended process for archiving animal models used in biology, biomedicine and biotechnology for long periods of time, The conditions are stable and safe, and dispense with the need to maintain mouse lines alive and breeding, therefore saving space, funds and reducing the number of animals in experiments, according to current animal welfare regulations. The National Centre for Biotechnology (CNB-CSIC) hosts the Spanish node of the European Project 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-CSIC and CNIO signed a scientific cooperation agreement to allow archiving and distribution of mutant mouse lines of interest in biomedical research, generated by CNIO researchers, through the INFRAFRONTIER-EMMA project. The CNB Mouse Embryo Cryopreservation Facility is integrated in the scientific-technological platforms INNOTEK, from the Campus of Excellence UAM+CSIC, and SEFALER of the CIBERER/ISCIII. The CSIC and the IRDA-University of Kumamoto signed an academic cooperation agreement to promote the exchange of knowledge, personnel and information on the mouse embryos and sperm archiving and cryopreservation activities undertaken at the Spanish node of INFRAFRONTIER-EMMA at the CNB-CSIC and at the CARD archive, coordinated by Dr Lluís Montoliu and Prof Naomi Nakagata, respectively. The first initiative derived from this cooperation took place in October 2013 with the International CARD-CNB Mouse Embryo and Sperm Cryopreservation Course, organised at the CNB by Naomi Nakagata and Lluís Montoliu.

Publication:

INFRAFRONTIER-providing mutant mouse resources as research tools for the international scientific community. INFRAFRONTIER Consortium. Nucleic Acids Res 2015; 43(Database issue):D1171-5

Website: www.cnb.csic.es/~criocnb/

Transgenesis

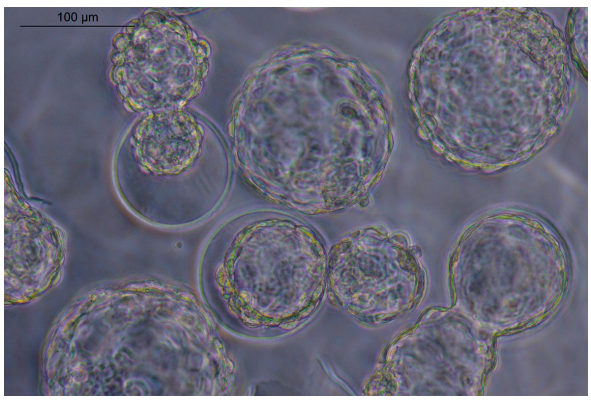
LEAD INVESTIGATOR:
Mª Belén Pintado Sanjuanbenito

PERSONNEL:
Alfredo Serrano Montalbo
Marta García Flores
Verónica Domínguez Plaza

The CNB-CBMSO Transgenesis Unit provides support to researchers linked to the CSIC-UAM Platform in the generation, establishment and interchange of genetically modified mouse models. The unit offers technical and scientific advice on the best strategy to achieve the desired model by additive transgenesis or targeted mutagenesis (KO and KI). We also facilitate the incorporation of those models already available from international consortia or as a result of scientific interchange when the health status of the original colony does not meet the requirements of our centres. In addition, support is provided for breeding schemes to ensure the most suitable genetic background.

The unit provides the following services:

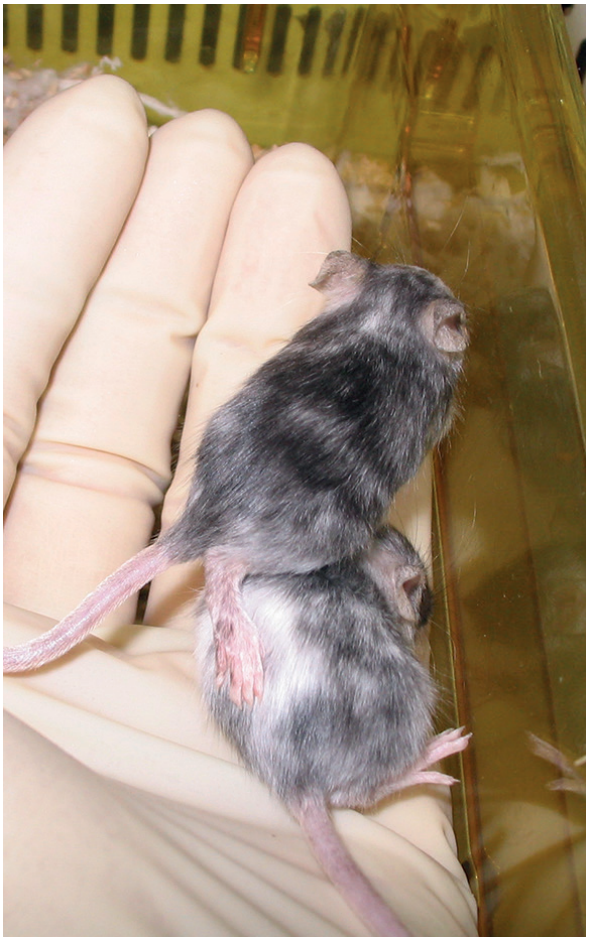
- Advice on the design of target vectors or constructs for microinjection
- Pronuclear microinjection of plasmid, BAC or YAC DNA
- Vector electroporation in R1 or G4 ES cell lines
- Zinc finger nuclease injection
- Handling of ES cells from international consortia
- ES cell injection or aggregation to generate chimaeras
- Embryo rederivation through IVF or embryo transfer
- DNA purification and founder identification by PCR on request
- Reproductive biotechnology to solve breeding problems of genetically modified mice
- Support in the generation, establishment and management of genetically altered mouse lines



These activities are combined with training and education on demand, and applied research to develop and refine reproductive technologies to enhance transgenic production efficiency or colony management.

Specialised equipment:

- Two microinjection systems with hydraulic micromanipulation system and Eppendorf femtojet injector
- One electric microinjection system with piezo drill
- Dissecting microscopes
- Microforge and pipette puller
- Thermocycler and electrophoresis equipment
- Fully equipped laboratory for ES cell handling



Radiation protection & biological safety

HEAD OF SERVICE:
Fernando Usera Mena

SUPERVISOR:
Sonia Calvo Ladrero

PERSONNEL:
Jessica Gaspar Navarro
Aránzazu de la Encina Valencia

Tasks and services:

- Preliminary evaluation and periodic control of biological, chemical and radiological risk
- Management of radioisotope acquisition and the means, equipment and instruments for prevention and protection
- Design of biosafe labs and other facilities
- Issuing the CNB Basic Guide, the Safety & Health CNB Manual, and specific associated procedures
- Handling and administration of reports and documents related to start-up procedures or legally required operating conditions
- Safety and health seminars for personnel exposed to potential risk agents
- Classification and signalling of risk areas and laboratories, and control of compliance with safety and health rules
- Cooperation in the management of medical and dosimetric surveillance of exposed personnel. Maintenance of medical and dosimetric records of exposed personnel
- Action in incidents, accidents and emergency situations following previously established procedures
- Managing the production and conditioning of biosanitary, toxic and radioactive waste at the sources, internal handling and storage until transfer to an authorised waste management facility

Facilities

The service surveys the operational risks in all CNB labs and facilities, and directly operates the gamma irradiator, the radioactive facility central premises, and the biosafety level 3 laboratory.

Radioactive facility

The CNB radioactive facility is a Category 2, non-encapsulated source type and is equipped with all required means of shielding, containment and detection of ionising radiation. It also has the following equipment:

Two cabins for radioisotopes

- One biosafety class II cabin
- One CO_2 incubator
- Ultracentrifuge, centrifuges and microfuges
- One speed vac
- One hybridisation oven

Biosafety level 3 laboratory

The lab has three *in vitro* cell culture sublaboratories with all necessary equipment for safe handling of biological agents included in Risk Group 3 and to perform activities with genetically modified organisms that require such a degree of confinement. It is equipped with:

Three biosafety class II cabins

- Two cell culture incubators
- One microbiological culture incubator
- One double door steam steriliser
- One SAS for biological inactivation of small items
- One SAS for biological inactivation of large items
- One refrigerated ultracentrifuge
- Three portable refrigerated centrifuges
- Three refrigerated microcentrifuges
- Three inverted optics microscopes
- One liquid nitrogen tank
- Three ultrafreezers (-80ºC)
- Data transmission network (computers and telephone)

Several alarm systems to alert of incidents/accidents or malfunctions

Website: www.cnb.csic.es/index.php/biosafety

Tissue culture, washing and sterilisation

HEAD OF SERVICE:
Rosa María Bravo

MEDIA PREPARATION:
Ana Montero
Concepción Cobeña Chivato

CELL CULTURES:
Sonia Rodríguez Murcia

WASHING AND STERILISATION:
Enrique Méndez
Anunciación Romero
Margarita Felipe Hombrados
Josefa Pérez Alfaro
Carlos Enríquez Casas
Arancha Rodríguez Martínez
Carmen Berdeal Mera
Ángeles Sánchez Pérez
Ana Isabel Nieto Jiménez
Rosa Ramos Hernández
Ángel Valera López