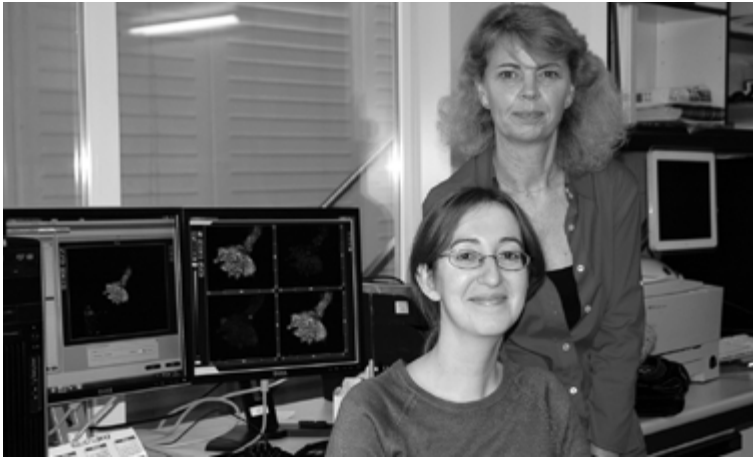


**08**

Scientific Services



## Confocal microscopy service

### LEAD SCIENTIST:

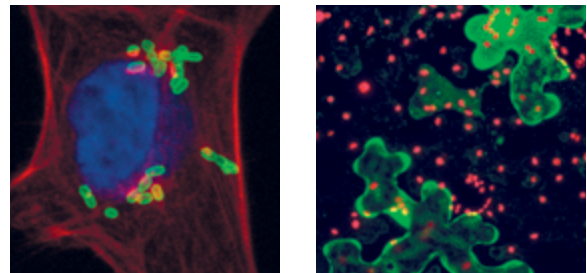
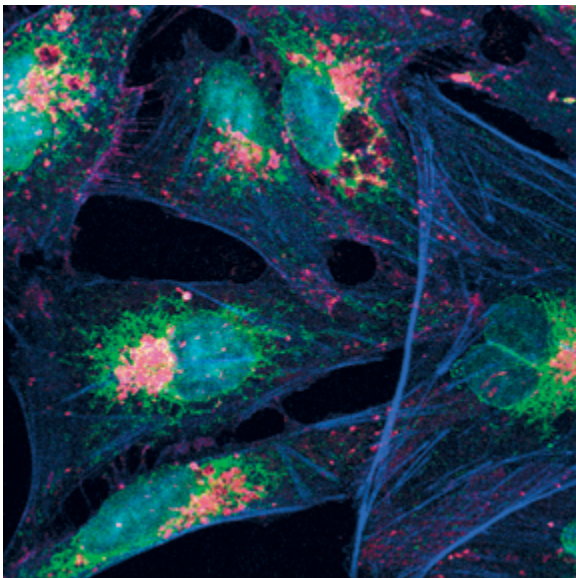
**Sylvia Gutiérrez Erlandsson**

### 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, Image J, Laser Pix, Huygens, Imaris)
- Auxiliary equipment: CO<sub>2</sub> 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



## Proteomics

**LEAD SCIENTIST:**

**Juan Pablo Albar**

**PERSONNEL:**

**Alberto Paradela**  
**Rosana Navajas**  
**Silvia Juarez**  
**Sergio Ciordia**  
**Marisol Fernández**

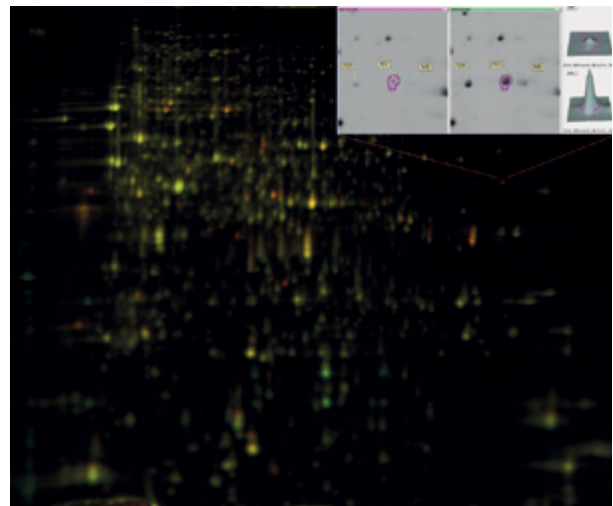
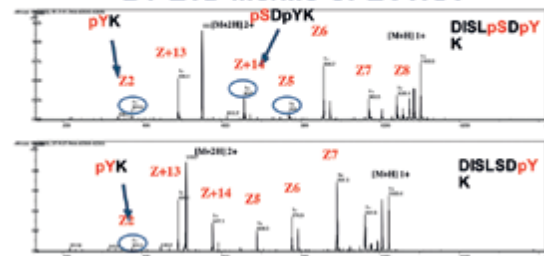
**Fernando Roncal**  
**Manuel Lombardía**  
**María del Carmen Mena**  
**Alberto Medina**

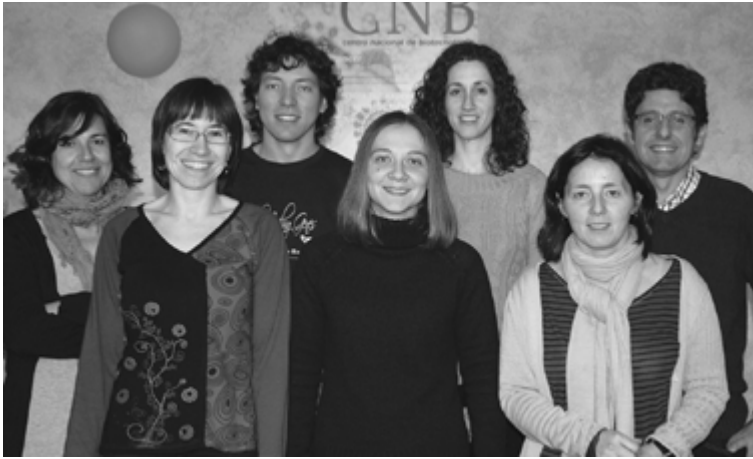
Created in 1999, the CNB Proteomics Facility maintains a technological platform suitable for large-scale protein identification and characterisation, offering its services to the CNB scientific community as well as to external researchers. Massive protein identification and characterisation is performed 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). Differential proteomics (quantitative proteomics) is done by analysis of fluorescent-labelled samples and differential 2D-electrophoresis (2D-DIGE), as well as by stable isotope labelling (ICPL, SILAC, iTRAQ) in combination with LC-MS/MS. SELDI-ToF MS (surface enhanced laser desorption-ionisation-time of flight mass spectrometry) is used to obtain protein expression profiles. We also offer targeted and quantitative protein analysis by selected/multiple reaction monitoring (S/MRM-MS). Prolamin detection and characterisation by ELISA, quantitative PCR 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 head of the CNB Proteomics Facility (Dr JP Albar) is also the General Coordinator of Proteored-ISCI III (Plataforma en Red de Proteómica-Carlos III).

**SERVICES:**

- Two-dimensional gel electrophoresis/differential proteomics (2D-DIGE)
- Protein identification and characterisation by MALDI-TOF/TOF, TripleQ-TOF, ProteinChip/SELDI-TOF and ESI MS/MS mass spectrometry
- Selected/multiple reaction monitoring (S/MRM-MS)
- Protein profiling, purification and biomarker determination by SELDI-TOF MS
- Identification and characterisation of post-translational modifications
- Peptide synthesis and membrane-bound peptide array design
- Gluten analysis by ELISA, PCR and mass spectrometry

### CHARACTERIZATION OF PHOSPHORYLATION SITES BY ETD MS/MS SPECTRA





## Genomics unit

### LEAD INVESTIGATOR:

**José Manuel Franco Zorrilla**

### PERSONNEL:

**Irene López-Vidriero**  
**Gloria García Casado**  
**Marta Godoy**  
**Juan Carlos Oliveros**

**Iria Calvete**  
**Luis Almonacid**  
**Eduardo Gil**  
**Beatriz Martín**

The Genomics Unit at the CNB focusses on gene expression analysis using microarrays (or DNA “chips”). This technology allows the study of gene expression from different biological samples, interrogating the activity of thousands of genes or complete genomes at once, which will help to elucidate the genetic basis of biological processes under study. We routinely hybridise and analyse one- and two-channel microarrays. The platforms we support now include Affymetrix, Agilent and custom microarrays.

We offer our services to CNB and external researchers; they include microarray printing, RNA integrity analysis and microarray hybridisations. We also provide statistical analysis and bioinformatic support. Raw data are statistically analysed using state-of-the-art algorithms and filtered results are supplied to customers in an easy-to-use, web-based tool developed in our unit. We offer advice and support in the use of several bioinformatic tools for functional analysis of genes and genomes, helping customers with the biological interpretation of the results.

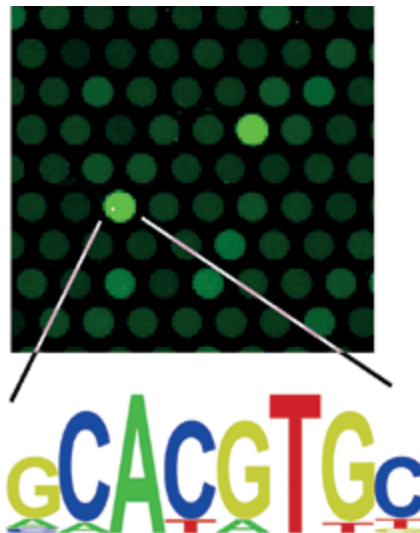
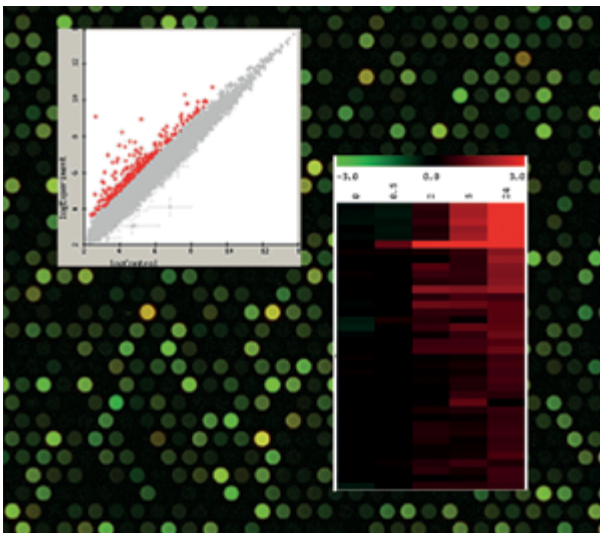
We also offer the possibility of validating gene expression data by real time qPCR analysis.

Through the Genomics Unit, the CNB participates in the CSIC-PCM Ultrasequencing Platform, physically located at the Parque Científico de Madrid installations. This platform can perform massive sequencing experiments using Genome Analyzer or Genome FLX systems, and allows the sequencing of complete genomes, transcriptomes, small RNAs or DNA/RNA-protein interactions.

Research projects are constantly being developed by our personnel to implement new services and technologies for customers. These include microarray-based technologies such as a new DNA chip for studying DNA-protein interactions, analysis of the translatoome, and new strategies for analysis of the miRNA-guided degradome.

### EQUIPMENT

- Complete Affymetrix platform, including fluidics station, hybridisation oven and scanner (3000 7G)
- High-resolution scanner for 1- and 2-colour microarrays (Agilent Microarray Scanner)
- Hybridisation system for NimbleGen microrarrays
- Microarray spotter MicroGrid II (Genomic Solutions)
- Bioanalyser 2100 (Agilent) for analysis of RNA/DNA sample integrity
- Automated liquid-handling workstation (Biomek 2000, Beckman Coulter)
- Laser scanner for 2-colour microarrays (Axon 4000B)
- 7900HT Fast Real-Time PCR System (Applied Biosystems)







## TILLer service

LEAD INVESTIGATORS:

**Carlos Alonso-Blanco**  
**José M. Martínez-Zapater**

PERSONNEL:

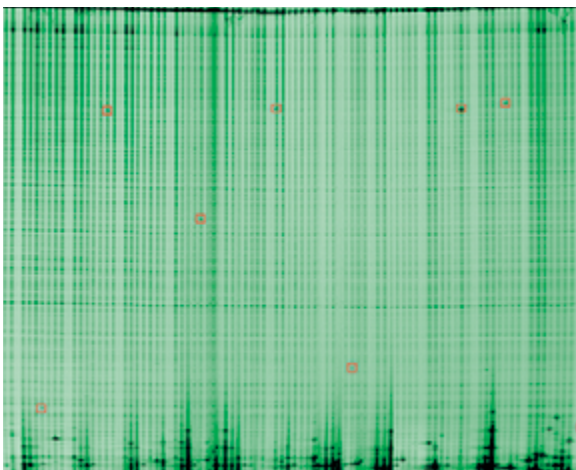
**Beatriz Martín Jouve**

TILLer Service is an international public service to search for EMS-induced mutants in the model plant *Arabidopsis thaliana*. TILLer is available through the web page (<http://www.cnb.csic.es/~tiller>) or through the international *Arabidopsis* web page (<http://www.Arabidopsis.org/>).

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 it has identified more than 500 mutants in those genes.

REFERENCE

Martin B, Ramiro M, Martínez-Zapater JM, Alonso-Blanco C (2009) A high-density collection of EMS-induced mutations for TILLING in *Landsberg erecta* genetic background of *Arabidopsis*. *BMC Plant Biology* 9:147



Detection of *Arabidopsis* TILLer mutant lines by LICOR 96-well denaturing gels (768 mutant lines) run after CEL1 digestion of heteroduplex DNA. Pools containing mutations are marked in red.

## Bioinformatics initiative

LEAD INVESTIGATOR:

**Alberto Pascual-Montano**

PERSONNEL:

**Juan Carlos Oliveros**      **José R. Valverde**  
**Alberto Medina**          **Carlos Óscar Sánchez Sorzano**  
**Mónica Chagoyen Quiles**

In an effort to improve the visibility and coordination of the existing bioinformatics services at the CNB, the centre has promoted the creation of the Bioinformatics Initiative, whose objective is to provide coordinated analysis services to all CNB research groups. The initiative also provides a series of monthly seminars, as well as training courses in various bioinformatics topics.

THE BIOINFORMATICS INITIATIVE IS COMPOSED OF SIX SERVICES:

- Computational Genomics: This service provides bioinformatics support for the analysis, visualisation and interpretation of genomics-related projects.
- Sequence Analysis and Structure Prediction Service: Provides bioinformatics support for sequence and structural prediction topics.
- Scientific Computing Service: Covers general scientific data analysis needs of the CNB and maintenance of the national EMBnet node.
- Computational Proteomics: This service is part of the Proteomics Facility; it provides interpretation, validation and reporting of data derived from proteomics experiments.
- Functional Analysis: This service is part of the Functional Bioinformatics group and provides functional analysis of high-throughput experiments such as microarrays and next-generation sequencing studies.
- Statistical Analysis: This service provides statistical support and consultancy, from experimental design to complex statistical data analysis.



## Computational genomics

LEAD SCIENTIST:

**Juan Carlos Oliveros Collazos**

*What would you do if you could sequence everything?*

Avak Kahvejian, John Quackenbush & John F Thompson, 2008

Current advances in genomics-related technologies such as DNA microarrays and, more recently, 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's 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

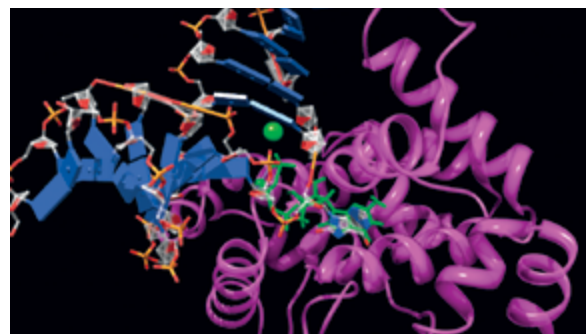
GROUP LEADER:

**José Ramón Valverde Carrillo**

The Scientific Computing Service provides support for the solution of scientific problems that require advanced computing solutions using clusters and supercomputers located at the CNB, CSIC and CESGA, as well as distributed grid infrastructures.

### CURRENTLY, OUR MAIN AREAS OF WORK ARE:

- NGS and metagenomics, with special emphasis on the analysis of biodiversity and *de novo* genome sequencing (with support from EU COST action SEQAHEAD, in which the service participates as national coordinator), pioneering the optimisation and development of analytic pipelines
- Nanotechnology and analysis of macromolecular interactions (protein-protein, protein-nucleic acids, biomolecule-inert substrates, drug-receptor) to the quantum chemical level (using techniques that include docking, molecular dynamics, quantum dynamics and quantum chemistry)
- Statistical analysis of scientific data using the programming language R
- Modelling of complex biological systems (bacterial communities, mutation dynamics, etc.) using advanced mathematical techniques
- Optimisation and reduction of research costs in the life and health sciences through the use of free software (coordinating CYTED Iberoamerican Network of Excellence 510RT0391 FreeBIT), through the organisation of meetings, courses, talks and presentations on free software, and promotion of scientific exchanges with Iberoamerica
- Delivery of web-based computing services and maintenance of various web sites ([www.free-bit.org](http://www.free-bit.org), [www.es.embnet.org](http://www.es.embnet.org), [sci.cnb.csic.es](http://sci.cnb.csic.es)) specialised in scientific computing and training
- The service is available to the wider international community





## Sequence analysis and structure prediction

LEAD INVESTIGATOR:

**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:

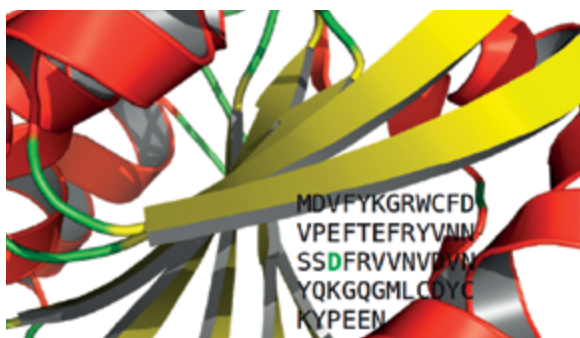
- Predict protein structure
- Search for homologous proteins
- Generate multiple sequence alignments
- Produce structural organisation drafts
- Study relevant residues for protein structure/function
- Extract sequence features from full proteomes

### 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.



## Macromolecular X-ray-crystallography

LEAD INVESTIGATOR:

**César Santiago**

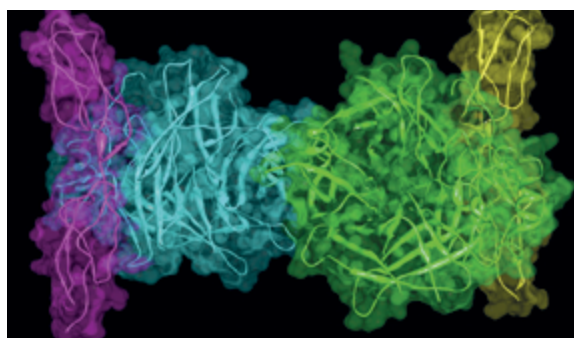
Protein X-ray crystallography is a high-resolution technique that allows us to study protein structure at the atomic level. This method provides a detailed view of protein function, ligand and protein interactions, supra molecular organisation and mutants related to human diseases. Great improvements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

### THE FACILITY PROVIDES THE FOLLOWING TECHNIQUES:

- Advice and supervision on protein production, from cloning to expression in bacterial, yeast and eukaryotic systems
- Support and training on protein purification to obtain crystal-grade protein for crystallisation
- Automated macromolecular crystallisation
- Crystallisation conditions optimisation, 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 crystallisation plate visualisation
- CrystalTrak database suite for crystallisation screening and improvement of positive trials







## CNB mouse embryo cryopreservation facility

### LEAD INVESTIGATOR:

**Lluís Montoliu**

### PERSONNEL:

**Julia Fernández Punzano**

**María Jesús del Hierro Sánchez**

**Óscar Javier Sánchez Sánchez**

**Marta Castrillo Labrador**

The CNB Mouse Embryo Cryopreservation Facility offers the possibility of cryopreserving transgenic and mutant mouse lines as frozen embryos and/or sperm. We also offer thawing of frozen mouse sperm and/or embryos and revitalisation of the cryopreserved mouse line. The cryopreservation of mouse lines is a highly recommended procedure to preserve animal models used in biology, biomedicine and biotechnology laboratories for long periods of time, safely and stably, without the need to maintain lines alive. This saves space and money and optimises the use of experimental animals, complying with current legislation on animal welfare.

We offer a variety of services and the latest methods in the field, including freezing 8-cell mouse embryos, freezing IVF-derived 2-cell mouse embryos, thawing mouse embryos and associated embryo transfer procedures to suitable pseudopregnant females for the revitalisation of mouse lines, freezing mouse sperm, thawing mouse sperm and *in vitro* fertilisation (IVF), storage of cryopreserved mouse embryos or sperm in liquid nitrogen. The CNB also hosts the Spanish node of the European EMMA project (European Mouse Mutant Archive, HYPERLINK <http://www.emmanet.org>), coordinated by Dr. Lluís Montoliu. The objective is the cryopreservation, organised archiving and coordinated distribution of mouse lines of interest to the biomedical research community. The current

EMMA project, EMMAservice (2009-2012), funded by the European Commission (7th Framework Programme), has been extended for four additional years under the new EU Project Infrafrontier-I3 (2013-2016), approved by the EU Commission in 2012.

In 2012, the CNB-CSIC and the CNIO joined forces and signed an agreement to archive and distribute mutant mice of interest in biomedical research, generated by CNIO investigators, through the EMMA project and its Spanish node at the CNB-CSIC.

Also in 2012, the CSIC and the University of Kumamoto signed a cooperation agreement to promote exchange of knowledge, personnel and information on mouse embryo and sperm cryopreservation and archiving activities undertaken by the Spanish EMMA node at the CNB-CSIC and the CARD archive, coordinated in Japan by Prof. Naomi Nakagata. The CNB Mouse Embryo Cryopreservation Facility is integrated within the INNOTEK Scientific-Technological Platform in support of research, part of the UAM+CSIC International Campus of Excellence.

### PUBLICATIONS

Kollmus H, Post R, Brielmeier M, Fernández J, Fuchs H, McKerlie C, Montoliu L, Otaegui PJ, Rebelo M, Riedesel H, Ruberte J, Sedlacek R, de Angelis MH, Schughart K. Structural and functional concepts in current mouse phenotyping and archiving facilities. *J Am Assoc Lab Anim Sci.* 2012 Jul;51(4):418-35

Montoliu L. Mendel: a simple excel workbook to compare the observed and expected distributions of genotypes/phenotypes in transgenic and knockout mouse crosses involving up to three unlinked loci by means of a  $\chi^2$  test. *Transgenic Res.* 2012 Jun;21(3):677-81

Montoliu L, Whitelaw CB. Using standard nomenclature to adequately name transgenes, knockout gene alleles and any mutation associated to a genetically modified mouse strain. *Transgenic Res.* 2011 Apr;20(2):435-40







## Transgenesis

### LEAD INVESTIGATOR:

**M<sup>o</sup> Belén Pintado Sanjuanbenito**

### PERSONNEL:

**Alfredo Serrano Montalbo**

**Marta García Flores**

**Verónica Domínguez Plaza**

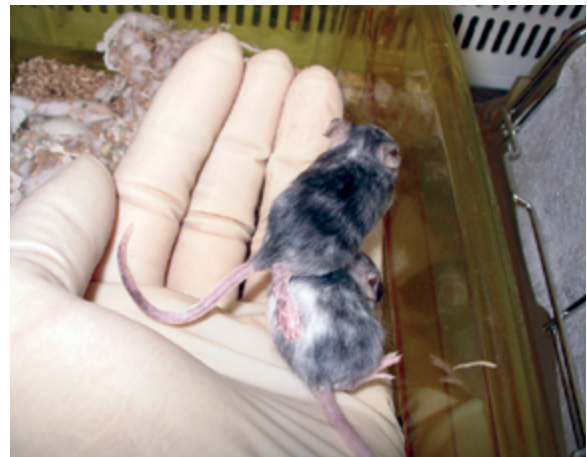
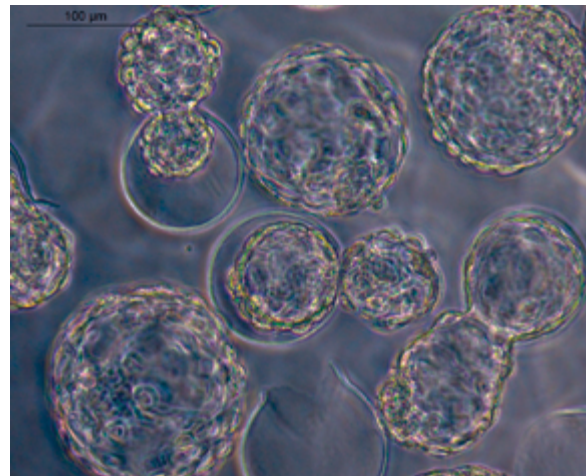
The CNB-CBMSO Transgenesis Unit provides support to researchers linked to the Platform CSIC-UAM in the creation, establishment and interchange of genetically modified mouse models. The unit offers technical and scientific advice on the best strategy to achieve the desired model, either by additive transgenesis 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.

### SERVICES:

- Advice in 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
- International consortia ES cell handling
- 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





## Histology

### LEAD SCIENTIST:

**Lluís Montoliu**

### PERSONNEL:

**Soledad Montalbán Iglesias**  
**Oscar Sánchez Sánchez**

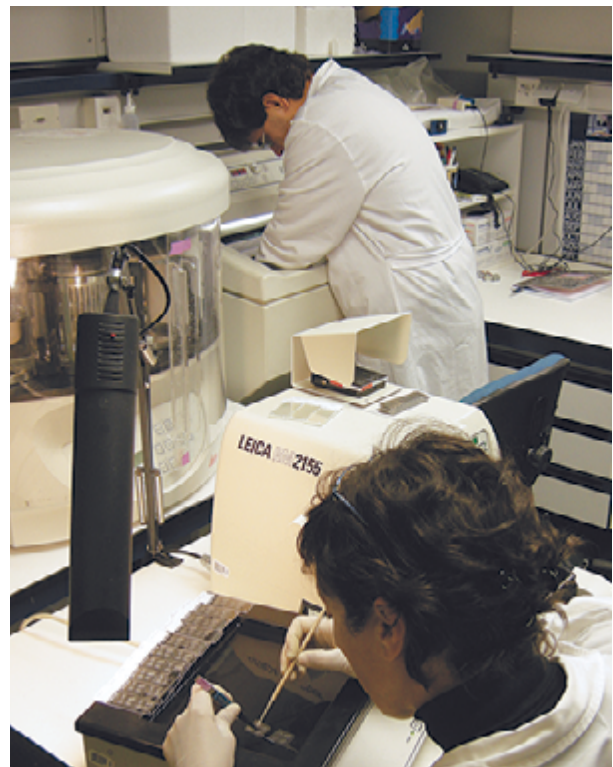
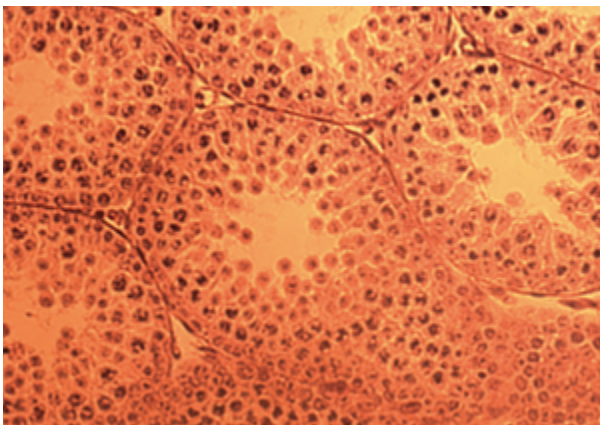
At the CNB Histology Facility, we offer methods for the histological analysis of animal and plant biological samples. Available methods include the preparation of paraffin/wax blocks and plastic (Histo-resin) for obtaining histological sections with the automated microtome, and the preparation of blocks for obtaining sections from frozen tissue with the cryostat. Sections can be counterstained or assigned for later analysis by immunohistochemistry.

The facility is equipped with a cryostat, two automated microtomes, a tissue processor carousel, a paraffin/wax embedding machine, two water baths, a stereoscope, an

oven, and additional small equipment to process all kinds of tissue samples. Our expertise is reflected by the large variety of tissue samples and species we have processed in the past, from animals and plants. An updated list of tissues processed by the CNB Histology Facility is available at [HYPERLINK http://www.cnb.csic.es/~histocnb/tabla.html](http://www.cnb.csic.es/~histocnb/tabla.html) <http://www.cnb.csic.es/~histocnb/tabla.html>

Since 2009, the CNB Histology Facility is associated with the IIB-UAM/CSIC Histology Facility ( [HYPERLINK http://www.iib.uam.es/servicios/patexperimen/intro.es.html](http://www.iib.uam.es/servicios/patexperimen/intro.es.html)). Both centres merged the operations of their facilities under the coordination of the CNB Histology Facility, offering CNB and IIB researchers increased processing capacity of histological samples.

The CNB Histology Facility is integrated within the INNOTEK Scientific-Technological Platform in support of research, part of the UAM+CSIC International Campus of Excellence. The CNB Histology Facility web site handles all submissions electronically.







## Electron microscopy

LEAD INVESTIGATOR:

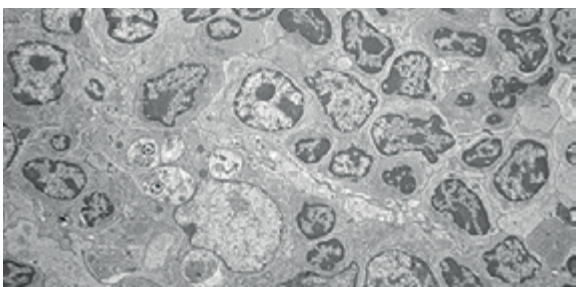
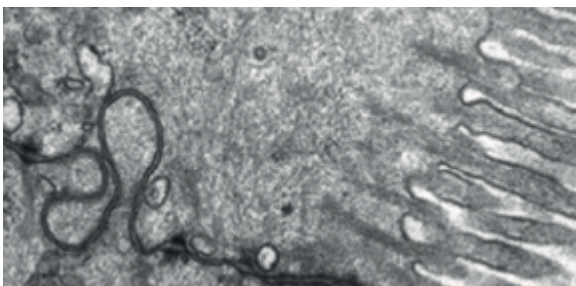
**Cristina Patiño Martín**

PERSONNEL:

**Javier Bueno Chamorro**

**Rocío San Andrés Cervilla**

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 carry out sample preparation, if required, as well as 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, immunolabeling, in situ hybridisation, conventional transmission electron and low-dose electron microscopy.



## In vitro plant culture

HEAD OF SERVICE:

**Raquel Piqueras Martín**

PERSONNEL:

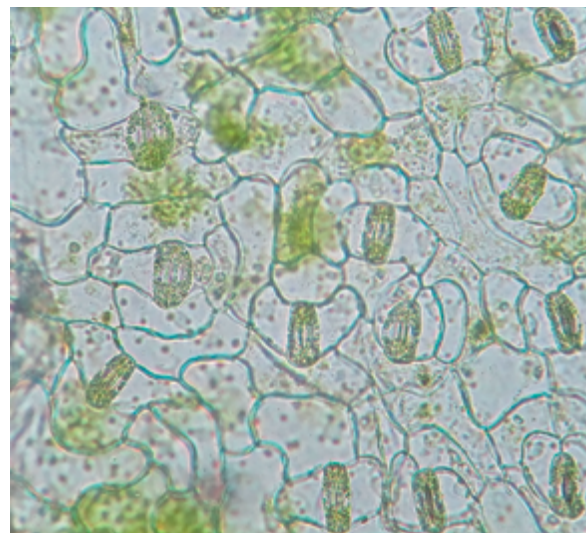
**María Luisa Peinado Vallejo**

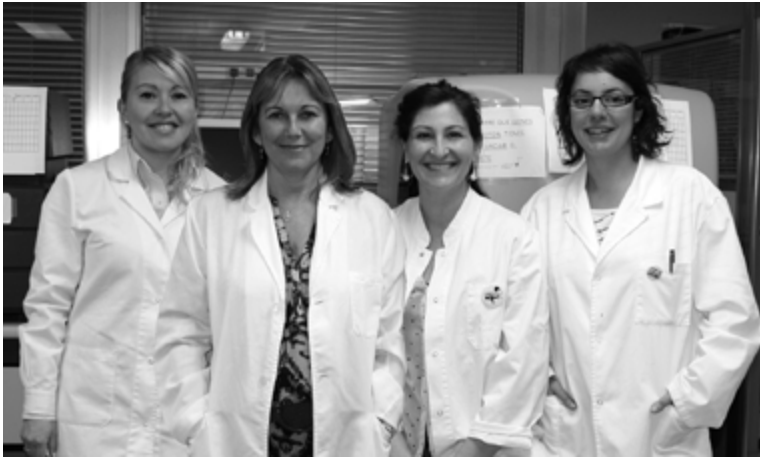
**Beatriz Casal López**

### TASKS PERFORMED BY THE SERVICE:

- Preparation of media
- Sterilisation of seeds and seed sowing in plates
- Maintenance of plants, plant cell cultures and plant callus
- Explant propagation
- Selection of *Arabidopsis* transformants
- Transformation of *Nicotiana* spp.
- Transformation of *Solanum* spp.
- Transformation of *Lycopersicum* spp.
- Transformation of *Oryza* spp.
- Induction of *Arabidopsis* callus, and induction of plant shoots and plant roots from callus
- Storage of wild seeds of the species most frequently used at the CNB
- Mesophyll protoplast preparation

These are the routine tasks carried out in the service, although we are available for many other types of laboratory work.





## Flow cytometry

### LEAD SCIENTIST:

**María del Carmen Moreno-Ortiz Navarro**

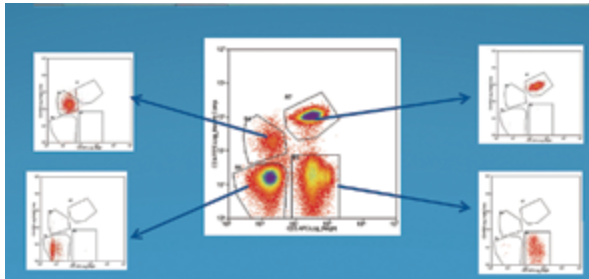
### PERSONNEL:

**Sara Escudero García**

**Sonia Rodríguez Murcia**

**Almudena González García**

The facility provides scientific and technological support to CNB research groups. We have the technical and human means to guarantee maximum quality and continuous improvement of our services.

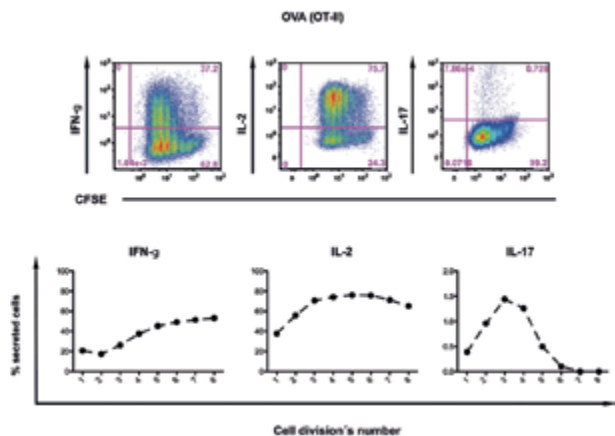


### THE FACILITY OFFERS:

- Training in and advice on the principles and applications of analytical flow cytometry, to obtain maximum advantage
- Development and optimisation of applications that incorporate new technologies and reagents. In the past two years, the unit has optimised CFSE incorporation in cytokine expression assays
- Quantification of secreted cytokines by multiplexed assays
- Results analysis using specialised software
- Cell isolation by cell sorting (sorting of cell populations including cell suspensions derived from any animal organ and from cell lines)

### THE FACILITY'S EQUIPMENT INCLUDES:

- BD FACSCalibur Analyser: 4 colours, 2 laser excitation (488 nm and 633 nm)
- Beckman Coulter EPICS XL-MCL Analyser: 4 colours, 1 laser excitation (488 nm)
- Beckman Coulter CYTOMICS FC 500 Analyser: 5 colours, 2 laser excitation (488 nm and 633 nm)
- Beckman Coulter CYTOMICS FC 500 Analyser: 5 colours, 1 laser excitation (488 nm)
- BD LSRII Analyser: 8 colours, 3 laser excitation (488 nm, 633 nm and 405 nm)
- Beckman Coulter GALLIOS Analyser: 10 colours, 3 laser excitation (488 nm, 633 nm and 405 nm)
- Luminex 100 IS Multiparametric Analyser: A system that can be used to quantify multiple cytokines (up to 100) or any other soluble molecule from a single sample
- Recently, the unit acquired a Cell Sorter Beckman Coulter Moflow XDP: 10 colours, 3 laser excitation (488 nm, 633 nm and 405 nm)
- The facility also provides the computer science system to analyse the results obtained: 2 PC platforms running specialised software packages (WindMDI, CXP, MultiTime, MultiCycle, DIVA, Flowjo, Summit, Kaluza)







## Protein tools unit

LEAD INVESTIGATOR:

**Leonor Kremer**

PERSONNEL:

**María Teresa Martín** (Molecular Interactions)    **Tamara Rueda** (Technician)  
**Mónica García-Gallo** (Immunobiology)        **Laura Martín** (Technician)  
**Mercedes Llorente** (Immunochemistry)        **Lucio Gómez** (Animal Facility)

The Protein Tools Unit (PTU) is focussed on the design, production and characterisation of custom monoclonal antibodies (mAb), immune response studies, development of specific immunoassays, protein labelling and biomolecular interactions analysis.

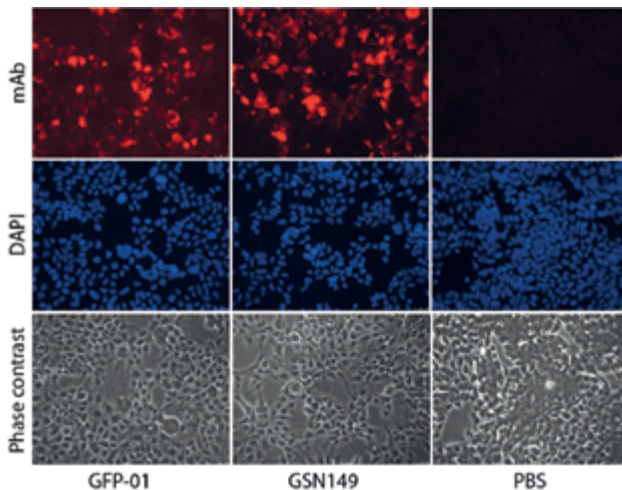
A wide panel of mAb against different types of antigens has been developed, including fluorescent proteins (GFP), blood proteins (coagulation Factor V), neurodegenerative disease-related proteins (TAU, beta amyloid peptides),

membrane raft proteins (MYADM), chemokine receptors (CCR9), FERM-containing proteins (Protein 4.1R) and nuclear proteins (Dido).

The facility has a surface plasmon resonance biosensor for the characterisation of biomolecular interactions in real time and determination of kinetic and affinity constants. This technique is applied to a wide range of samples such as proteins, antibodies, nucleic acids, carbohydrates, lipids, low molecular weight compounds, liposomes and viruses.

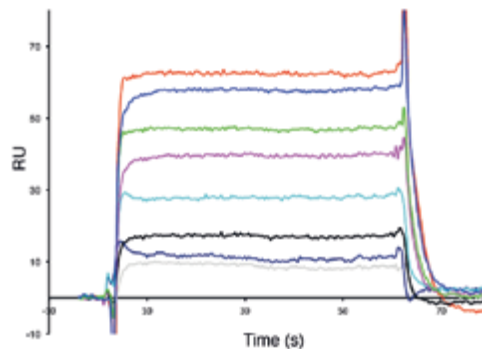
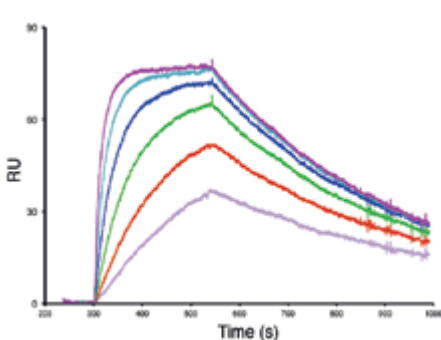
Research tools and services are provided to scientists from the CNB, other CSIC institutes, universities, public research organisations and private companies.

The core facility offers expertise in immunobiology and immunochemistry, technical assistance, data analysis, training in specific techniques, introduction of new methodologies and advice. In addition, the core facility organises theoretical and practical training courses.



### EQUIPMENT:

- Biological safety cabinets (Nuair 437-400E)
- Centrifuges and microfuges (Hettich)
- Inverted fluorescence microscope (Zeiss Axiovert 40 CFL).
- CO2 incubators (Thermo Steri-Cult)
- ÄKTAprime plus chromatography system (GE Healthcare)
- SPR Biacore 3000 (GE Healthcare)
- EnVision 2104 Multilabel Reader (Perkin Elmer)
- Thermal cycler (Eppendorf AG)
- Microplate reader (Bio-Rad 680)
- Protein gel electrophoresis and Western blotting systems (Mini-PROTEAN 3 and Mini Trans-blot cells)
- Electrophoresis power supply units (Bio-Rad PowerPac Basic and Universal).





## Radiation protection & biological safety

### HEAD OF SERVICE:

**Fernando Usera Mena**

### SUPERVISOR:

**Sonia Calvo Ladrero**

### TECHNICIANS:

**Jessica Gaspar Navarro**  
**Aránzazu de la Encina Valencia**

### FEATURES AND SERVICES:

- Risk assessment
- Acquisition of security materials
- Design of laboratories and facilities
- Acquisition and management of radioisotopes
- Editing health and safety manuals
- Processing of legal documentation for undertaking activities and operating conditions
- Training and information in chemical, biological and radiation for staff
- Classification and signposting in laboratories
- Control of compliance with health and safety norms, and of operation and use of facilities
- Control and management of staff medical and dosimetry surveillance; records maintenance
- Intervention in accidents and emergencies
- Control of production and processing of hazardous waste
- Internal transport and storage of waste for transfer to authorised or controlled disposal

### FACILITIES:

The Service supervises hazardous operations in CNB laboratories and directly manages the gamma irradiator and the central radioisotopes laboratory, which has:

- 2 safety cabinets for radioisotopes
- CO<sub>2</sub> incubator
- Biosafety cabinet
- Ultracentrifuge, centrifuges and microcentrifuge
- Speed vac
- Hybridisation oven

The Service manages the level 3 biological containment laboratory, consisting of three sublaboratories for in vitro culture with all necessary equipment for safe handling of Risk Group 3 biological agents and contained use of genetically modified organisms.



### THE LABORATORY HAS THE FOLLOWING EQUIPMENT:

- Three biosafety cabinets
- Two incubators for animal cell culture
- A tissue culture incubator
- A double-door autoclave
- A SAS for biological inactivation of small materials
- A SAS for biological inactivation of large materials
- A refrigerated ultracentrifuge
- Three refrigerated benchtop centrifuges
- Three refrigerated microcentrifuges
- Three inverted optical microscopes
- A liquid nitrogen tank
- Three ultra-freezers (-80°C)
- Communications systems: computer network and telephony
- Various alarm systems in case of malfunction, incident or accident



## Animal facility

### LEADING SCIENTIST:

**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**

**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, supporting 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.

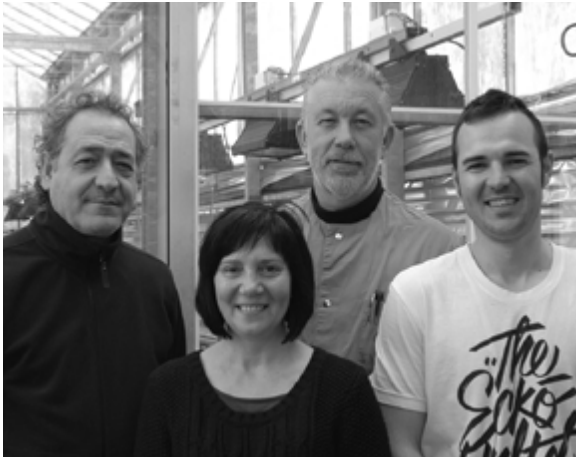
The animal facility staff gives service to laboratories for obtaining commercial lines and strains of animals, shipping animals for collaboration with other institutes, and maintenance, breeding, and generation of transgenic, *knock-out* and *knock-in* animals. These services allow control of the microbiological and genetic quality of the animals used in experimentation.

The animal facility staff provide services for various techniques used in mouse research models. Veterinary staff give research assistance in surgical techniques, selection of animal models, animal health surveillance, laboratory animal care, and animal well-being.

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







## Greenhouse

HEAD OF SERVICE:

**Tomás Heras Gamo**

PERSONNEL:

**Alejandro Barrasa Fustes  
Esperanza Parrilla Carrillo  
Raúl Pedraza León**

SERVICE:

- A standard greenhouse with 8 cabinets (total growth surface: 180 m<sup>2</sup>)
- P2 safety level greenhouse with 4 cabinets (total growth surface: 83 m<sup>2</sup>)
- 18 climate chambers

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



## D10 tissue culture

HEAD OF SERVICE:

**Rosa María Bravo**

MEDIA PREPARATION:  
**Ana Montero**

CELL CULTURES:  
**Anunciación Romero**

WASH AND STERILISATION:  
**Enrique Méndez**



## Washing & sterilisation

HEAD OF SERVICE:

**Rosa María Bravo**

PERSONNEL:

**Arancha Rodríguez Martínez  
Carmen Berdeal Mera  
Angeles Sanchez Pérez  
Rosita Ramos Hernández  
Ana Isabel Nieto Jimenez  
Josefa Pérez Alfaro  
Carlos Enrique Casas**

**Margarita Felipe Hombrados  
Concepción Cobeña Chivato  
Josefa Pérez Alfaro  
Laura Muñoz Calvo  
Margarita Felipe Hombrados  
María Concepción Cobeña Chivato  
María Trinidad Mba Ondo**