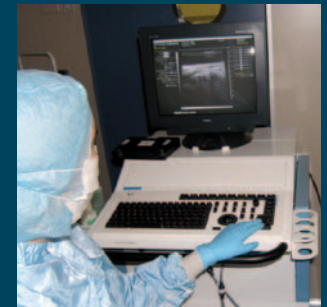


The CIC bioGUNE's Technology Platforms

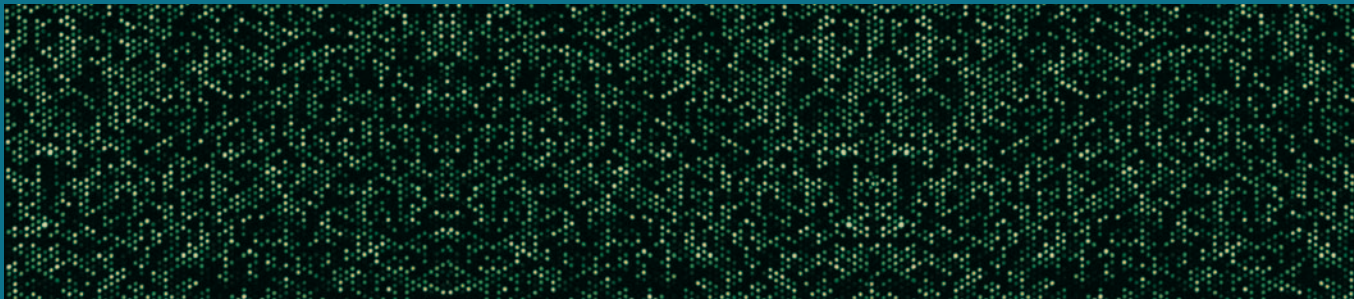
EXPERT PARTNERS FOR YOUR RESEARCH



INTRODUCTION

CIC bioGUNE (Center for Cooperative Research in Biosciences, www.cicbiogune.es), a non-profit biomedical research organization founded at the initiative of the Basque Government, has invested over 35 million in state-of-the-art research infrastructure, including genomics, gene silencing, proteomics, metabolomics, NMR, electron microscopy, X-ray diffraction, computer and animal facilities, to accelerate innovative research of the center as well as of other academic

institutions, technology centers and companies by providing highly specialized services, equipment, and expert consultation. To this end, CIC bioGUNE has created this catalog of core facilities where the main equipment and services are listed. Policies regarding services, access, fees and prioritization vary by facility. For more information please contact the core facility directly, by accessing our web site.



STRUCTURAL BIOLOGY UNIT



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MACROMOLECULAR CRYSTALLOGRAPHY PLATFORM

CIC bioGUNE enjoys state-of-the-art X-ray crystallography facilities for in-house users as well as external users: as a service or collaborative research.

CIC bioGUNE's Macromolecular Crystallography Platform offers competitive services in structure determination for in-house users as well as external users.

Today there are more than twenty structural genomic (SG) groups around the world working in the high-throughput (HT) determination of protein structures. Much effort has been invested in the development of protocols for automatization of all the steps during protein structure determination process (cloning, expression, crystallization, data collection, processing and phasing). CIC bioGUNE enjoys state-of-the-art X-ray crystallography facilities that today can support HT crystallization experiments and conventional in-house data collection.

Our aim is to promote the use of the crystallization facility as a HT service, offering most of the commercial crystallization methods and customized solutions for the specific user's requirements. The image analysis platform allows users to check their crystallization experiments on-line. This feature might be appealing for external users since they will be able to track their own experiments remotely.

There are many scientists that may be interested in incorporating crystallographic studies in their own projects but do not have the infrastructure or the expertise to perform the experiment; the Macromolecular Crystallography Platform at CIC bioGUNE can offer them the infrastructure as a service, including training in proper use of the equipment and advice on the preparation of high quality crystallization samples.

Within collaborative research we can also offer: data collection, data processing, structure determination, validation and coordinates deposition in the PDB data bank.

Services

- 01 Advice on crystallization procedures and quality sample preparation.
- 02 High-throughput screening with more than 1000 commercial or customized crystallization solutions for specific user requirements.
- 03 Crystallization plates storage at 4°C, 10°C, 18°C, 27°C.
- 04 Crystallization plates storage and imaging at 21°C in a Bruker Crystal Farm. Images will be accessible via web-interface.
- 05 Personal training in data collection using the X-ray home system.
- 06 Support and training in quikcryosoaking 1 technique for in-house derivatization.
- 07 Individual and group training in all the steps of the procedure; from data collection to structure determination.
- 08 Within collaborative research we offer data collection, data processing, structure determination, validation and coordinates deposition.

Equipment

- 01 TECAN liquid handling.
- 02 MOSQUITO crystallization robot.
- 03 BRUKER Crystal Farm and image analysis platform.
- 04 Two crystallization rooms: at 4°C and 18°C.
- 05 Two temperature controlled incubators (Molecular Dimensions).
- 06 X8-PROTEUM system from BRUKER with two detectors, a CCD and Mar345 image plate.
- 07 Two Oxford Cryosystems 700.
- 08 Storage dewar for crystal samples.

Selected publication:

Nagem, RPA, Ambrosio ALB, Rojas, AL, et al. *Getting the most of X-ray home sources*. Acta Crystallographica D. D61, 1022-1030 (2005).



· MACROMOLECULAR CRYSTALLOGRAPHY PLATFORM
· ELECTRON MICROSCOPY PLATFORM
· NUCLEAR MAGNETIC RESONANCE (NMR) PLATFORM

STRUCTURAL BIOLOGY PLATFORMS

STRUCTURAL BIOLOGY UNIT



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ELECTRON MICROSCOPY PLATFORM

CIC bioGUNE enjoys state-of-the-art electron microscopy facilities for in-house and external users; as a service or a part of collaborative research.

The Electron Microscopy (EM) Platform at CIC bioGUNE offers high-tech instrumentation, competitive service, specialized training and support in projects requiring high-resolution transmission electron microscopy (TEM) to determine the structure of large macromolecular complexes.

To achieve these goals EM platform provides two transmission electron microscopes and the auxiliary equipment required for cryo-electron microscopy. Our most sophisticated microscope is the JEM-2200FS/CR, equipped with a 200kV field emission gun (FEG) and an OMEGA in-column energy filter. The energy filter included in the JEM-2200FS electron microscope allows the examination of thick samples, necessary for cryo-tomography experiments. The other microscope is the JEM-1230, equipped with a 120kV thermionic gun. This microscope is available for in-house and external users trained in TEM. This high-tech equipment allows us to make use of advanced methods of image processing and computation to investigate at different levels the biological complexity of large macromolecular structures, using single particle analysis or electron tomography techniques.

There are many scientists that may be interested in incorporating EM studies in their own projects; the EM

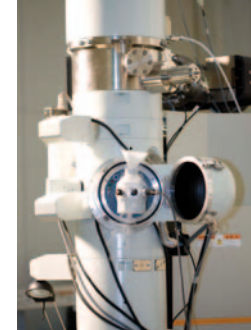
platform at CIC bioGUNE offers the infrastructure as a specialized service, including sample preparation, data collection and data processing for the specific user's requirements.

Services

- 01 Advice on quality sample preparation for specific user requirements.
- 02 Support and training in negative staining technique for electron microscopy.
- 03 Support and training in vitrification techniques for cryo-electron microscopy.
- 04 Individual and group training in the use of the JEM-1230 microscope and the ORIUS CCD digital camera.
- 05 EM data collection for qualitative and descriptive interpretation of images with the JEM-2200FS/CR microscope.
- 06 EM data collection for structure determination with the JEM-2200FS/CR microscope.
- 07 Within collaborative research we offer sample preparation, data collection, data processing and structure determination by single particle analysis or electron tomography techniques.

Equipment

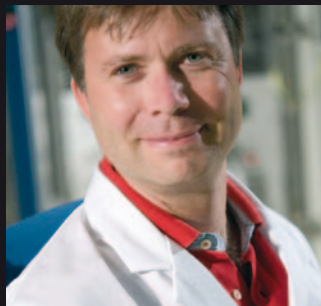
- 01 JEM-2200FS/CR transmission electron microscope (JEOL, Japan), equipped with an ULTRASCAN 4000 SP cooled slow-scan CCD camera (GATAN, UK).
- 02 JEM-1230 transmission electron microscope (JEOL, Japan), equipped with an ORIUS SC1000 cooled slow-scan CCD camera (GATAN, UK). This microscope can be booked by trained users.
- 03 VITROBOT: automated vitrification robot (FEI).
- 04 3 cryo-transfer holders used for cryo-electron microscopy.
- 05 1 ultra-high tilt specimen cryo-transfer holder used for cryo-electron tomography.
- 06 Darkroom and ZI PHOTOSCAN scanner (ZEIS).
- 07 High vacuum coating system (MED 020 BALTEC) for carbon evaporation and glow discharge.
- 08 HP Workstations (Linux) for data processing.



· MACROMOLECULAR CRYSTALLOGRAPHY PLATFORM
· ELECTRON MICROSCOPY PLATFORM
· NUCLEAR MAGNETIC RESONANCE (NMR) PLATFORM

STRUCTURAL BIOLOGY PLATFORMS

STRUCTURAL BIOLOGY PLATFORM



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NUCLEAR MAGNETIC RESONANCE (NMR) PLATFORM

CIC bioGUNE features a most modern and versatile high-field Nuclear Magnetic Resonance facility for the full range of advanced biomolecular NMR studies.

The CIC bioGUNE's NMR Platform is equipped to play a leading role among biomolecular NMR facilities in Europe, and aims to interact with a broad, inter-disciplinary community - both in academic organizations and in the industry.

NMR spectroscopy is the most versatile analytical technique to study molecules with atomic resolution. For structure elucidation, it complements X-ray crystallography ideally by probing the solution state, covering also highly flexible molecules. Yet, NMR can provide unique information far beyond this principal objective of structural biology. For example, it is the only technique to study molecular dynamics at atomic level, and to probe directly proton interactions and protonation states.

NMR spectroscopy also offers unique capabilities for functional proteomics, hit discovery, and rational drug development. NMR screening exploits its extreme sensitivity to register even the weakest binding between components added to a solution. NMR can thereby detect and distinguish binding sites, and discriminate between site-specific and unspecific binding. Then the interactions can be studied to guide the assembly of novel high-affinity ligands from weakly binding molecular fragments.

The CIC bioGUNE's NMR Platform has the most advanced hardware to carry out the full range of solution-state NMR studies. Our 800 MHz high-field spectrometer is optimal for biomolecular investigations with the highest sensitivity and resolution, while the 600 MHz spectrometer has ample accessories to reach far beyond standard applications. The platform is supported by a specialized laboratory to produce proteins with the required isotope labeling (2H, 13C, 15N).

The bioGUNE's scientists have long international experience in the various fields of biomolecular NMR and protein biosynthesis. Their research projects cover NMR methods development, analysis of protein structure and dynamics, and interaction studies. The bioGUNE's NMR Platform offers its expertise, assistance and resources for the full range of (biomolecular) solution-state NMR: from protein expression and isotope labeling, implementation of NMR experiments, spectra recording, spectral assignment, small-scale NMR screening, to analysis of molecular structures, dynamics and interactions. We also offer consultations, training and teaching, and set-up services for protein biosynthesis and NMR spectroscopy.

Services

- 01 **NMR consulting:** defining, developing and supervising NMR projects (also at customer facilities).
- 02 **NMR programming:** designing, programming and implementing customized NMR experiments (also at customer facility).
- 03 **NMR teaching and training** (also at customer facility).
- 04 **NMR experiment time** for independent use (experienced users only).
- 05 **NMR measurements** on request.
- 06 **NMR interaction studies:** hit validation, small-scale screening (particularly by ^{19}F -NMR), elucidation of binding sites and modes.
- 07 **NMR structural studies** of small molecules, amenable proteins, and amenable ligand-protein complexes (analysis of binding modes for rational drug design).
- 08 **NMR spectral assignment** of amenable target proteins (e.g., as precursor to NMR screening and hit validation using HSQC fingerprint spectra).
- 09 **Specialized protein biosynthesis** for NMR: expression of proteins with various stable isotope labels (^2H , ^{13}C , ^{15}N).

Equipment

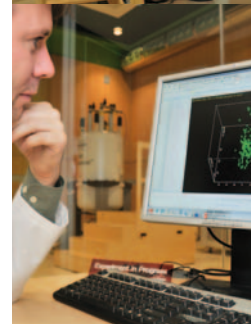
01 800 MHz BRUKER AVANCE III spectrometer:

- Equipped with a TCI cryo-probe (with z-gradients), where both ^1H and ^{13}C coils are cryo-cooled for direct detection with highest sensitivity.
- Back-up probe-head: QXI (^1H , ^2H , ^{13}C , ^{15}N , ^{31}P) with x,y,z triple-axes gradients.

02 600 MHz BRUKER AVANCE III spectrometer:

This machine is equipped with a BCU Xtreme cooling unit to run experiments at low temperatures ($< -50^\circ\text{C}$), and features a vast range of probe-heads for various requirements:

- QXI (^1H , ^2H , ^{13}C , ^{15}N , ^{31}P) with x,y,z triple-axes gradients.
- TXI (^1H , ^2H , ^{13}C , ^{15}N) with x,y,z triple-axes gradients.
- TXI (^1H , ^2H , ^{13}C , ^{15}N) with x,y,z triple-axes gradients, tuned for high salt concentrations ($> 1\text{ M}$).
- TBI (^1H , ^2H , ^{13}C , BB) with z-gradients and tunable BB coil (^{31}P - ^{109}Ag).
- SEF (^1H , ^2H , ^{19}F) with z-gradients.
- QXI (^1H , ^2H , ^{13}C , ^{15}N , ^{19}F) with z-gradients.
- HR-MAS TXI (^1H , ^2H , ^{13}C , ^{15}N).



· MACROMOLECULAR CRYSTALLOGRAPHY PLATFORM
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STRUCTURAL BIOLOGY PLATFORMS

GENOME ANALYSIS PLATFORM



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CIC bioGUNE employs the most recent technologies for whole genome analysis using microarrays and massive sequencing. Both external services and collaborations are on offer.

The main goal of the Genome Analysis Platform is the optimization of sample processing protocols for a set of sophisticated, state-of-the-art, high-throughput genome analysis technologies. The principal methodologies used deal with the characterization of variants at gDNA sequence level (mainly Single Nucleotide Polymorphisms –SNPs and Copy Number Variations – CNVs), changes at mRNA or smallRNA expression level and epigenetic modifications. Recently, the group has been also establishing appropriate work-flow procedures for the data analysis, as well as developing new bioinformatics tools.

Joint unit



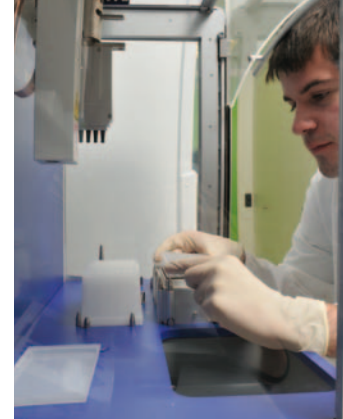
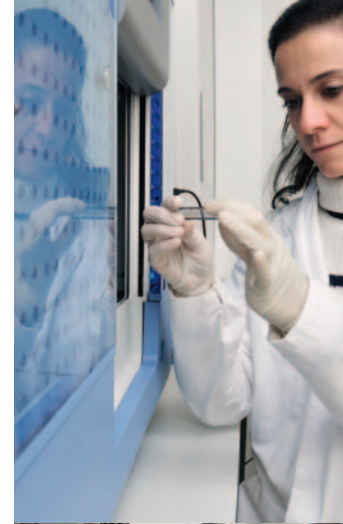
Services

- 01 High-throughput genotyping:
 - Standard panels from 10 thousand up to 1 million SNPs.
 - Customized Panels from 96 to 1536 SNPs.
- 02 High-throughput gene expression analysis:
 - Whole genome transcription analysis of human, mouse and rat tissues using standard chips.
 - Customized Panels for up to 700 genes.
- 03 Characterization of methylation induced epigenetic modifications:
 - 27 000 CpG islands (for human DNA).
 - Customized Panels for up to 1536 CpG islands.
- 04 Next-generation sequencing:
 - De novo sequencing and resequencing of genomic DNA.
 - BAC (bacterial artificial chromosome) end sequencing.
 - Genomic regions of interest.
 - Complete genes.
 - Complete chromosomes.
 - Complete genomes.
 - DNA methylation analysis at the complete genome level.
 - Expression analysis (complete transcriptome).
 - Identification and analysis of microRNA and smallRNA.
 - DNA-protein interactions (ChIP-Seq).
- 05 Assistance in designing of the customized SNP (for genotyping projects) or gene panels (for differential expression projects).

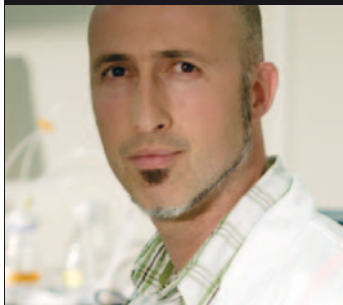
- 06 Results can be delivered as raw data or in a processed form: the basic statistical analysis of the data can be performed.

Equipment

- 01 BeadStation 500 GT/GX (Illumina, Inc.).
- 02 Genome Analyzer II (Illumina, Inc.).
- 03 All the equipment required to perform various molecular biology experiments, such as Experion (BioRad) and Bioanalyzer (Agilent Technologies) systems, EpiMotion (Eppendorf) liquid handling workstation, centrifuges, electrophoresis apparatus, gel imaging systems, etc.



PROTEOMICS AND METABOLOMICS PLATFORM



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The Proteomics and Metabolomics Platform is chiefly focused on the analysis of proteins and metabolites by mass spectrometry.

The CIC bioGUNE established a group of technical experts using the state-of-the-art technologies to support research in proteomics and metabolomics areas. The Proteomics and Metabolomics Platform provides services to the in-house groups and external users.

Proteomics

By analyzing the abundance of proteins under different physiological or experimental conditions, it is possible to identify groups of proteins that are important to those specific conditions. Differential in-gel electrophoresis (DIGE) is nowadays the most powerful technique for gel-based differential proteomic analysis.

During the last decade the genomes from many organisms, including human, have been sequenced. The availability of genomic sequences has boosted the proteomic research as the data obtained by analyzing proteins and peptides by mass spectrometry can be directly linked to the genomic information, using bioinformatics methods. Continuous optimization of sample preparation while employing the latest generation of tandem mass spectrometers, with their constantly improving high mass accuracy, high resolution and sensitivity, makes the protein identification process increasingly fast and accurate.

The Proteomics Platform provides services to in-house groups and external users and offers joining forces for collaborative research projects.

Member of



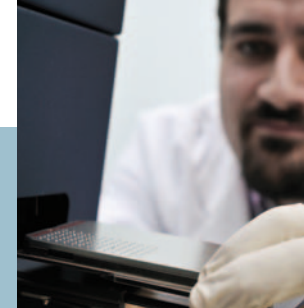
Metabolomics

Metabolomics employs the extraction and measurement of multiple small molecules from tissues or biofluids to produce metabolic profiles. The approach derives its success from the fact that disease, drugs or toxins cause perturbations of the concentrations and fluxes of endogenous metabolites involved in key biochemical pathways. Since mammalian metabolic control functions are dispersed across many cell types in topographically distinct locations, the metabolome has no compartmental constraints. The metabolomics techniques may therefore be regarded as a complete systems biology approach where the processes in separate organs and different cell types may be integrated into a holistic view of an organism.

CIC bioGUNE has established a mass spectrometry based metabolomics platform offering low concentration detection limits over an appropriate spectral range, in addition to comprehensive ion fragmentation tools for metabolite identification. The platform allows the study of the full range of body fluid types including serum, urine or bile. It is also possible to use cell culture extracts/ supernatants, tissue extracts and similar preparations. The CIC bioGUNE's Metabolomics Platform conducts

contract research for external organizations such as pharmaceutical and biotechnology companies and academic institutions.

Together, proteomics and metabolomics research efforts should help to get a more complete picture and supply the better understanding of the molecular events taking place in the cells.





Services

01 Molecular mass determination by MALDI TOF.

02 Protein identification by MALDI TOF/TOF.

The proteins previously purified or resolved on a gel can be identified by peptide mass fingerprinting (PMF). The process includes: protein digestion by trypsin, peptide extraction when required, sample preparation, analysis by MALDI TOF, data processing and database search by Mascot search engine. The sample, when possible, can also be analyzed by peptide fragment fingerprinting using MALDI TOF/TOF.

03 Protein identification by liquid chromatography coupled on-line to tandem mass spectrometry.

04 Differential in-gel-electrophoresis (DIGE).

Due to the special nature of DIGE experiments, please contact the head of the unit.

05 The Metabolomics Platform strives to provide a top quality service for cutting-edge research, using strictly controlled analytical procedures and state-of-the-art facilities under expert supervision. On receipt of a request, advice will be provided to choose the right sample collection criteria, sample preparation procedures, analytical methodology and data processing methods for the project under consideration. All procedures are optimized to provide a high quality, fast turnaround, cost efficient final product.

Equipment (Proteomics Platform)

MALDI:

- 01 Proteiner DP robotic station (Bruker): allows automatic digestion of gel spots.
- 02 Autoflex III TOF/TOF (Bruker): this mass spectrometer can perform different types of analysis:
 - Intact protein molecular mass can be obtained for previously resolved and digested proteins.
 - Protein identification by peptide mass fingerprinting (MALDI-TOF) and/or peptide fragment fingerprinting MALDI TOF/TOF).

Liquid chromatography coupled to tandem mass spectrometry systems:

- 03 Nano Acquity UPLC chromatography system coupled on-line to Q-TOF Premier (Waters).
- 04 Agilent 1200 nano flow liquid chromatography system coupled on-line to LTQ-Orbitrap XL (Thermo-Fisher).

These two high sensitivity systems have the latest technical features for optimal identification of the proteins from complex protein mixtures.

Differential in-gel electrophoresis (DIGE):

- 05 Typhoon TRIO, spot picker and DeCyder.

DIGE is nowadays the most robust approach for gel-based differential proteomic analysis.

Equipment (Metabolomics Platform)

- 01 UPLC coupled to TOF (Waters).
- 02 UPLC coupled to Q-TOF (Waters).
- 03 UPLC coupled to Quad-IMS-TOF (Waters).

These systems offer selective, sensitive analysis with state-of-the-art ion fragmentation tools; ideal for novel metabolite identification experiments.

GENE SILENCING PLATFORM



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CIC bioGUNE offers the expertise of the Gene Silencing Platform to expand the potential of RNAi technology.

The Gene Silencing Platform (GSP) at CIC bioGUNE is a facility to develop and execute cell-based screening using RNAi. It has dedicated staff to manage platform resources and help in assay development and data analysis. The main goal of this platform is to make RNAi-mediated gene silencing as widely accessible as possible.

Within collaborative research, we can facilitate silencing of individual genes to elucidate their functions. Upon request, the GSP advises users on customer design of siRNAs to silence specific genes (mostly in cases of targets obtained from point mutations and/or chromosomal rearrangements).

An additional goal of the GSP is genome-wide or high-throughput, high-content screening to identify the genes implicated in particular biological processes. The GSP can also provide CIC bioGUNE investigators and their collaborators with sub-collections or sub-libraries targeting requested pathways or families of genes. We offer expertise in designing, developing, evaluating and implementing such screens. We can adapt our protocols (cellular models and biological assays) to meet user needs: human, mouse or *Drosophila* primary or tumor cells, for the analysis of stem cell differentiation, tumor invasion, cellular adhesion, angiogenesis, etc.

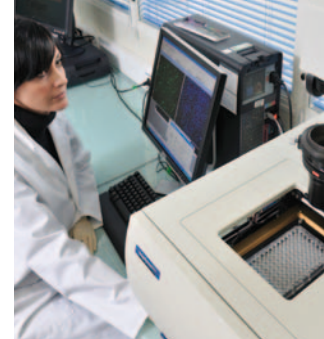
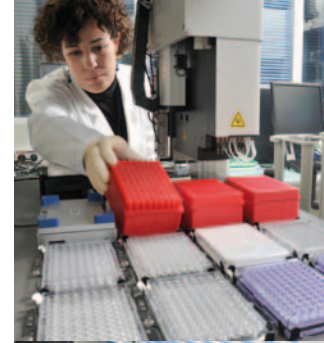
Because of the highly specific and efficient silencing of the target gene, RNAi emerges as a very promising technique for drug discovery, target validation in cell culture and also as a novel therapeutic strategy. The GSP at CIC bioGUNE can facilitate not only *in vitro* but also *in vivo* silencing and advise users on RNAi technology as a therapeutic approach.

Possibilities of collaborative researches

- 01 *In vitro* and *in vivo* silencing of individual genes.
- 02 Customer design of siRNAs.
- 03 Adapted cell-based screening using sub-libraries targeting requested pathways or families of genes.
- 04 Genome-wide screening using the *Drosophila* library.

Equipments and Facilities

- 01 The Human retroviral shRNA^{mir} library developed by Greg Hannon (CSHL), Stephen Elledge (Harvard) and colleagues. The library includes about 62.500 microRNA30-based hairpin constructs targeting the whole human genome.
- 02 Silencer[®]*Drosophila* RNAi library distributed by Ambion/Applied Biosystem. This library is a collection of 13.071 dsRNAs targeting the best-annotated *Drosophila* genes.
- 03 Sciclone ALH 3000 (liquid handling system) + Twister II.
- 04 Cytomat 2C (CO₂ incubator with plate shuttling).
- 05 Detection systems:
 - Microplate reader (Synergy HT).
 - Automated wide-field microscope (ImageXpress) including MetaXpress Analysis Application Modules for cell scoring, translocation, cell cycle, etc.



ANIMAL FACILITIES UNIT



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The Animal Unit is equipped with state-of-the-art facilities and a team in continuous training to develop projects in accordance with the most stringent ethical and legal standards in the use of animals in research.

The CIC bioGUNE's Animal Unit (AU) is an AAALAC accredited facility. It includes a Specific Pathogen Free (SPF) area to house rodents from commercial sources and to produce and keep some strains of genetically engineered mice (GEM). The AU works on continually improving their services and develops new services through the collaboration with researchers from different areas of interest. Our main objective for the near future is focusing on the use of ultrasounds (echography) to detect liver lesions in murine models of steatosis and hepatic tumors. We are also developing preliminary echocardiography assays in order to assess the heart function in new strains of GEM.

The main functions of the AU are:

- Covering the CIC bioGUNE users' needs by providing them with the assessment and equipment necessary to carry out their research on laboratory animals.
- Providing the care and looking after the welfare of laboratory animals and carrying out periodic health monitoring.
- Ensuring observance of all legal and ethical standards concerning the use of animals in research and for other scientific purposes.

Joint unit

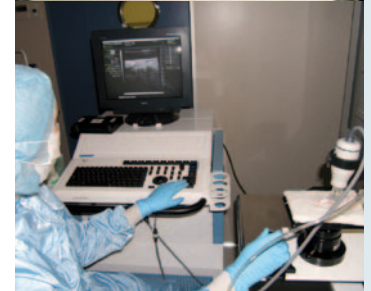
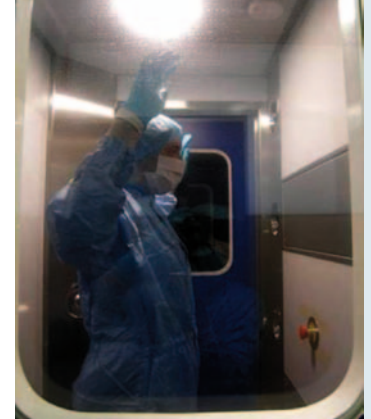


Services

- 01 Ordering of animals (from vendor to the cage).
- 02 Animal export/importation to/from overseas institutions for research use (paperwork, shipment).
- 03 Consultations and technical support (experiment design, techniques, equipment).
- 04 Animal maintenance.
- 05 Breeding of genetically engineered mice.
- 06 Health monitoring.
- 07 Equipment provision.
- 08 Administration of substances.
- 09 Sample collection (blood, urine, faeces).
- 10 Autopsy procedures.
- 11 Tissues/organs extraction.
- 12 Biochemical analysis.
- 13 Hematological analysis.
- 14 Hepatic echography.

Equipment

- 01 7 racks for individual ventilated cages (IVC).
- 02 15 racks for automatic watering cages.
- 03 Portable machine for inhalatory anesthesia.
- 04 SPINLAB 100 for biochemical analysis.
- 05 ABACUS VET for hematological analysis.
- 06 Vevo-770 for small rodents' echography.
- 07 KODAK FX PRO for *in vivo* X-ray and luminescent imaging.





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bioGUNE
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