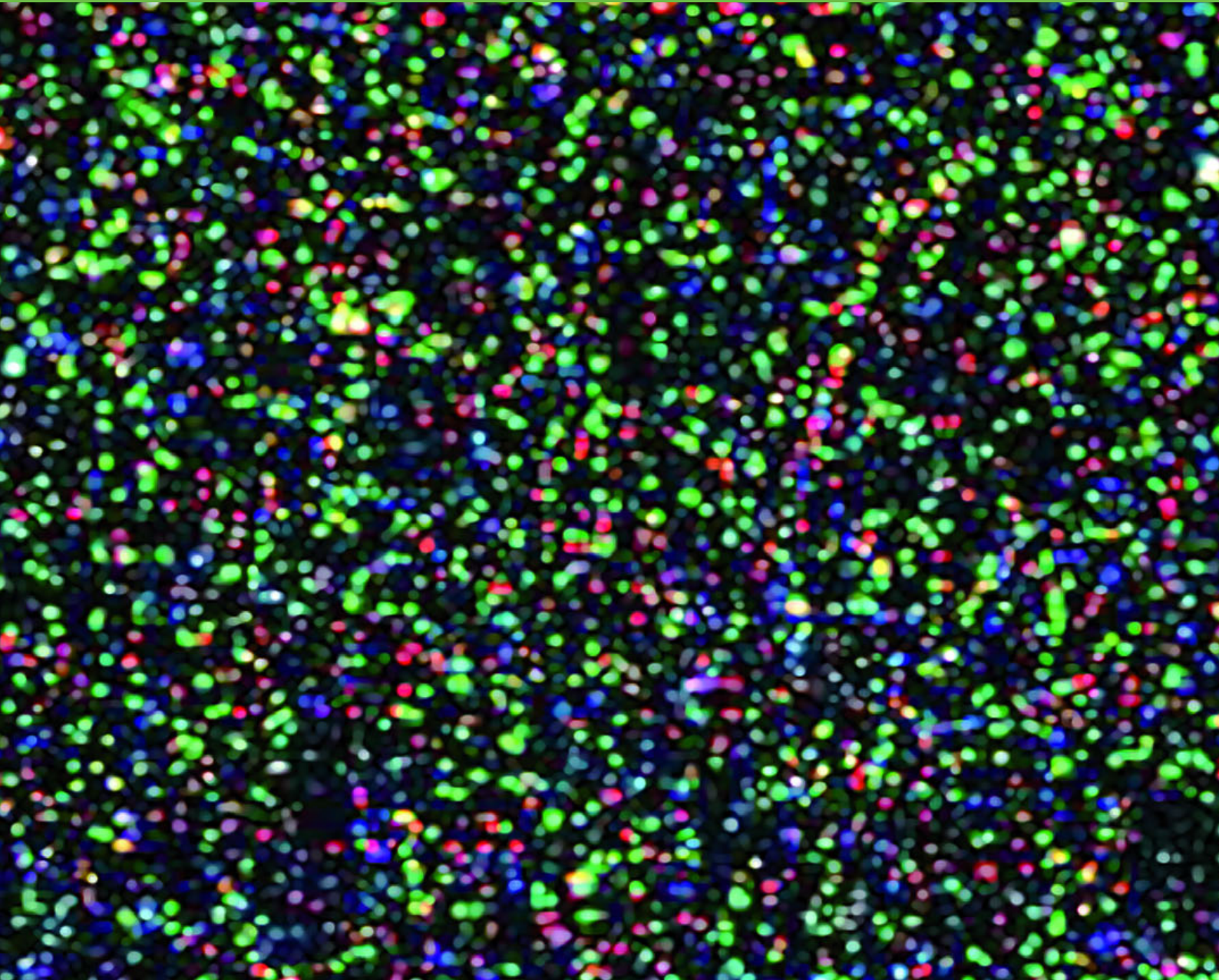


6 Technical Units



Microscopy and Dynamic Imaging


Head of Unit:

Valeria R. Caiolfa

Support Scientists:

Moreno Zamai
Christian Hellriegel
Antonio Manuel Santos Beneit
Elvira Arza

Postdoctoral Researchers:

Antonio Manuel Santos Beneit
Elvira Arza

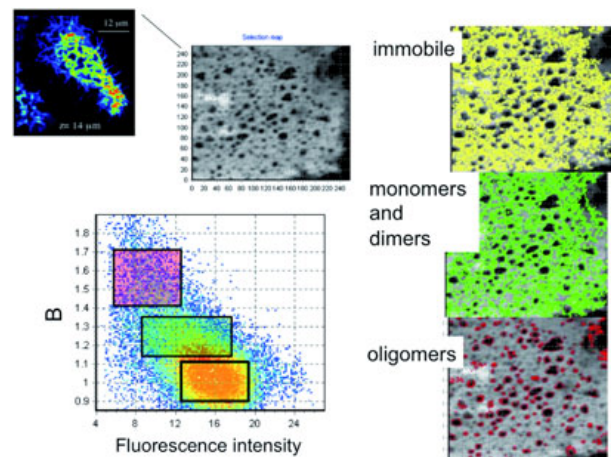


RESEARCH INTEREST

The Microscopy and Dynamic Imaging Unit, established in 2008, provides state-of-the-art expertise and training in optical microscopy to scientists at the CNIC and beyond. Resources are maintained for spectroscopy, microscopy, biochemistry, cell culture and data analysis. Areas of expertise include multi-dimensional (multi-D) imaging, immunolabeling, time-lapse, multi-color TIRFM, and 3D cross sectioning. We also provide capabilities in the tracking of single molecules, intracellular vesicles and cells, and in fluctuation analysis techniques such as FCS (fluorescence correlation spectroscopy), RICS (raster image correlation spectroscopy) and N&B (number and brightness analysis). These approaches are used to quantify diffusion of single proteins, monomer-dimer-oligomer equilibrium, stoichiometry of protein-ligand binding, etc.

In addition to providing customized training and data analysis, the Unit is also involved in several collaborative projects that require the development of new technologies and new analytical protocols. Two-photon FRET-FLIM is currently being applied in collaborative projects with the Vascular Biology and Inflammation Department and external groups. We have also started a new project for mapping stem cell differentiation *in vivo*, in collaboration with the Cardiovascular Developmental Biology Department.

More than 130 internal researchers routinely use the facility, and in 2010 services were opened to external users. During the year, the Unit organized four internal courses for beginners and one advanced international workshop with more than 40 participants from the CNIC and other institutes.



Example of N&B analysis by which we can determine the oligomerization of labeled proteins in living cells. The figure shows the basal membrane of HEK293 cells transfected with a GPI-anchored membrane receptor that was fused to mEGFP as fluorescent tag. N&B counts the fluorophore molecules that diffuse together in the cell by measuring their brightness (B). The higher is the B value, the higher is the oligomerization state of the protein. B values around 1 are indicative of immobile molecules. In the figure, the different forms of the receptor are localized in the image according to their B values (yellow = immobile; green = monomers and dimers; red = higher oligomers).



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Transgenesis



Head of Unit:

Luis-Miguel Criado Rguez.

Support Scientist:

José María Fernández Toro

Technician:

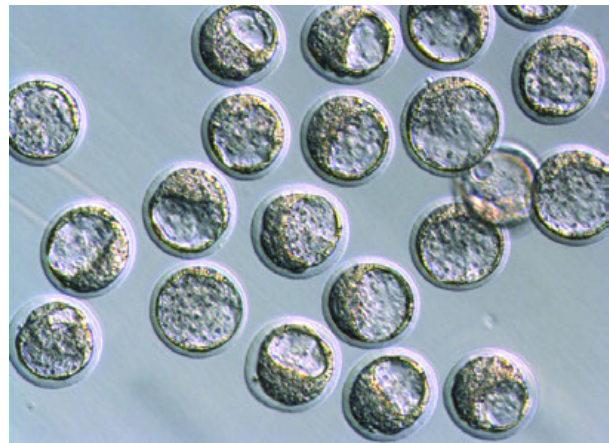
David Esteban Martínez



RESEARCH INTEREST

The Transgenesis Unit provides a range of services for the production of genetically-modified mice -so called transgenic mice- to serve the needs of the CNIC research groups. The interest is two fold: to understand how genomic activity translates into the complexity of a whole organism, and to generate mouse models of human cardiovascular disease.

Transgenic mice are produced in the Unit by the established methodologies of microinjection of DNA in solution into zygote pronuclei (pronuclear microinjection) or of recombinant lentiviruses beneath the zygote zona pellucida (subzonal or perivitelline microinjection). Chimeric mice for the generation of knockout and knockin mice are produced by a variety of techniques, but mainly by microinjection of genetically modified mouse embryonic stem cells into eight-cell embryos or blastocysts. Other key services and techniques include rederivation of mouse strains by embryo transfer, cryopreservation of mouse strains (frozen embryos or sperm), in vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI).

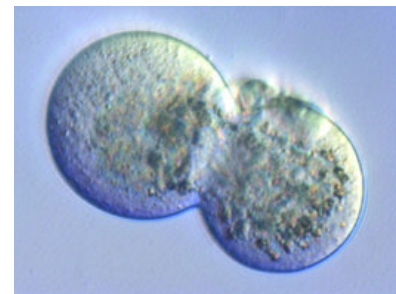


C57BL/6J01aHsd murine blastocysts used for microinjection with murine stem cells (mES-Cells) for the production of chimeric mice.



Mechanical enucleation of a mouse zygote. The two pronuclei can be seen inside the enucleation needle (right).

Production of heteroplasmic mouse embryo. Electrofusion of a NC (NZB mtDNA-C57BL/6J01aHsd gDNA) cytoplasm with a C57BL/6J01aHsd zygote. The zonae pellucidae have been removed.



SELECTED PUBLICATIONS

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Genomics


Head of Unit:

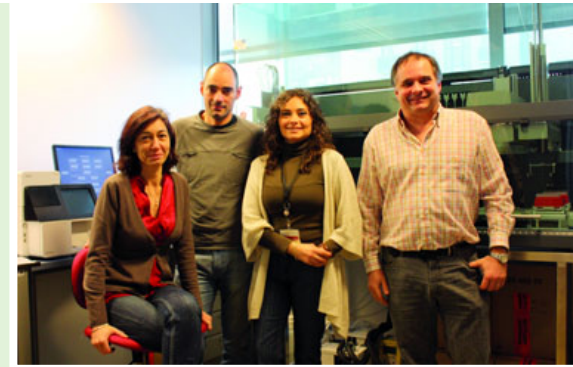
Ana Dopazo

Support Scientists:

Sergio Callejas
Alberto Benguría
Fátima Sánchez Cabo

Technician:

Rebeca Álvarez



RESEARCH INTEREST

The Genomics Unit is dedicated to providing high-quality genomic technology to the scientific community at the CNIC and beyond. The Unit is equipped with Agilent and Affymetrix microarray platforms, the world's leading DNA chip technologies. Microarray applications include whole-genome differential gene expression analysis (including at the exon level using Exon arrays), microRNA expression analysis and CGH arrays.

The Unit's capabilities expanded in 2010 to incorporate the new technology of next-generation DNA sequencing, with the acquisition of an Illumina Genome Analyzer platform. This enables CNIC scientists to undertake ultra-deep sequencing projects that, because of their size, would not otherwise be possible. The Unit has also begun to offer high-throughput microRNA sequencing services in addition to the already existing microRNA microarray profiling service, thus allowing researchers to obtain a complete picture of microRNA expression in biological samples.

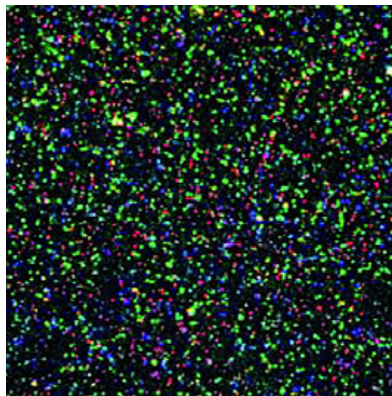
Other services include the maintenance and management of real-time PCR instruments (one AB 7000 and two ABI 7900HT machines) and a TaqMan array processing service. The Unit also provides user advice and training on topics related to its activity.

The Genomics Unit actively participates in the ongoing CNIC IM-JOVEN clinical study. IM-JOVEN is part of a large, multicenter case-controlled study aimed at identifying the clinical, genetic and demographic characteristics that determine the occurrence of myocardial infarction in young women.

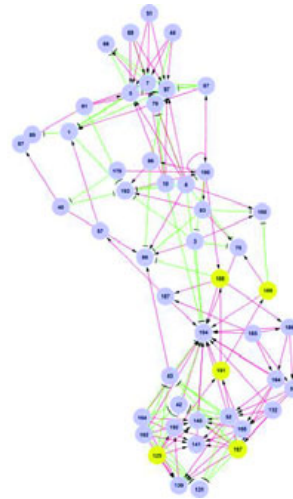


Hierarchical clustering of microarray data

6 Technical Units



DNA clusters for analysis by next-generation sequencing



IPA gene network

SELECTED PUBLICATIONS

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Lamas JR, Rodriguez-Rodriguez L, Vigo AG, Alvarez-Lafuente R, [Lopez-Romero P](#), Marco F, Camafeita E, [Dopazo A](#), [Callejas S](#), Villafuertes E, Hoyas JA, Tornero-Esteban MP, Urcelay E and Fernandez-Gutierrez B. **Large-scale gene expression in bone marrow mesenchymal stem cells: a putative role for COL10A1 in osteoarthritis.** *Ann Rheum Dis* (2010) 69: 1880-5

Lopez-Huertas MR, [Callejas S](#), Abia D, Mateos E, [Dopazo A](#), Alcami J and Coiras M. **Modifications in host cell cytoskeleton structure and function mediated by intracellular HIV-1 Tat protein are greatly dependent on the second coding exon.** *Nucleic Acids Res* (2010) 38: 3287-307

Luna-Zurita L, Prados B, Grego-Bessa J, Luxan G, Del Monte G, [Benguria A](#), Adams RH, Perez-Pomares JM and de la Pompa JL. **Integration of a Notch-dependent mesenchymal gene program and Bmp2-driven cell invasiveness regulates murine cardiac valve**

Pluripotent Cell Technology



Head of Technical Service:

Giovanna Giovinazzo

Support Scientist:

Francisco Gutierrez

Technician:

Mara Angeles Sanguino



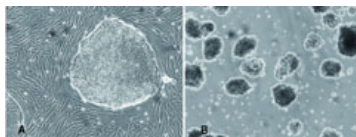
RESEARCH INTEREST

The Pluripotent Cell Technology (PCT) facility provides centralised support with the culture and manipulation of mouse and human pluripotent stem cells. The comprehensive range of support services offered also includes expert advice and training and the development and implementation of new technologies.

One of the unit's core tasks is to facilitate the generation of genetically modified mice through homologous recombination in mouse embryonic stem cells (mESCs). Our staff take charge of all the key steps of the gene targeting protocol: electroporation of the targeting vector, selection, karyotyping, culture, and the preparation of cells for blastocyst injection. If required, we also advise on

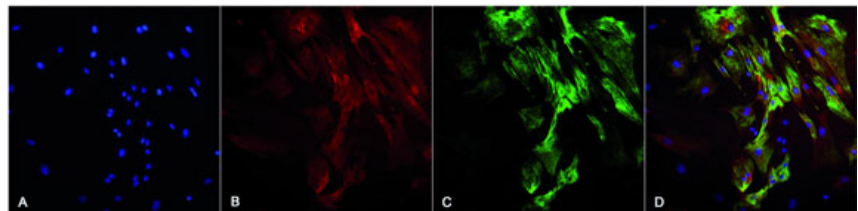
appropriate targeting and screening strategies. The technology developed in the unit has achieved efficient transmission of targeted mESCs to the germline, allowing us to generate numerous lines of genetically modified mice.

Recent collaborations with CNIC research groups have involved us in the derivation of mutant homozygotic mESC lines and the differentiation of mESCs to cardiomyocytes. Throughout the last year we also focused on the design and fine-tuning of protocols for the routine culture of human pluripotent stem cells. This pioneering technology will underpin the use and application of cutting-edge human pluripotent cell technologies by CNIC researchers.



A, Human embryonic stem cells at day 5 in culture. **B**, Human embryoid bodies at day 1 of differentiation.

Immunofluorescence staining of cardiac cells differentiated from human embryonic stem cells. **A**, DAPI. **B**, Troponin I. **C**, Actinin. **D**, Merge.



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Proteomics

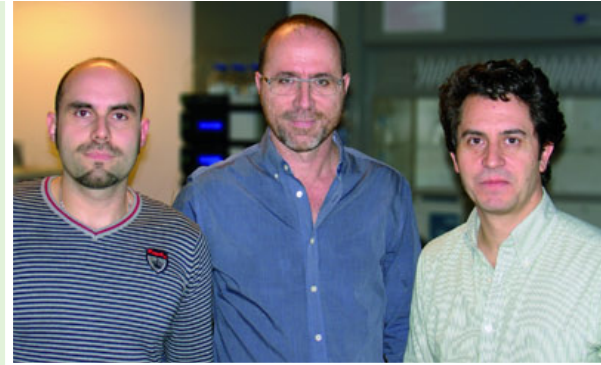


Head of Unit:

Juan Antonio López

Support Scientists:

*Enrique Calvo
Emilio Camafeita*



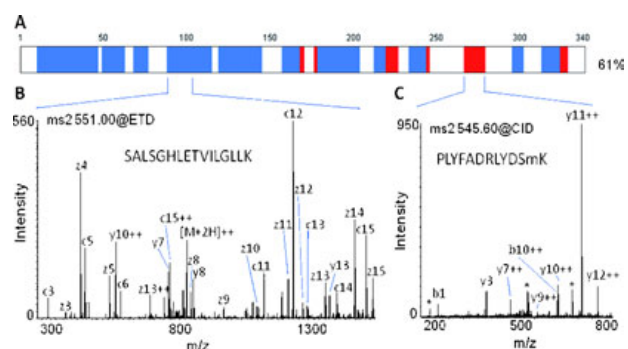
RESEARCH INTEREST

The Proteomics Unit has broad experience in proteomics approaches to the separation, quantification, identification and characterization of proteins in biological systems, and maintains a program of continuous technological development and improvement to meet the demanding requirements of the research community. Over the last year substantial progress was made in the initial steps of sample preparation, especially relating to the selection of specific subproteomes (for example based on protein activity) and fishing for proteins that interact with selected baits for interactome analysis.

We have made continuous improvements in the separation and quantitative analysis of differential protein expression by gel-based separation (2D-DIGE) and "gel-free" technologies based on nanoHPLC coupled to mass spectrometry. Proteins and peptides and their post-translational modifications are identified and characterized by MALDI-TOF/TOF and ESI mass spectrometers, the latter comprising a hybrid triple quadrupole (QqQ) and a linear ion trap coupled to an Orbitrap high resolution mass analyzer. Particular progress has been made in post-acquisition analysis and data visualization of the spectra through the use of combined validation technologies included in the Scaffold (Proteome Software) program.

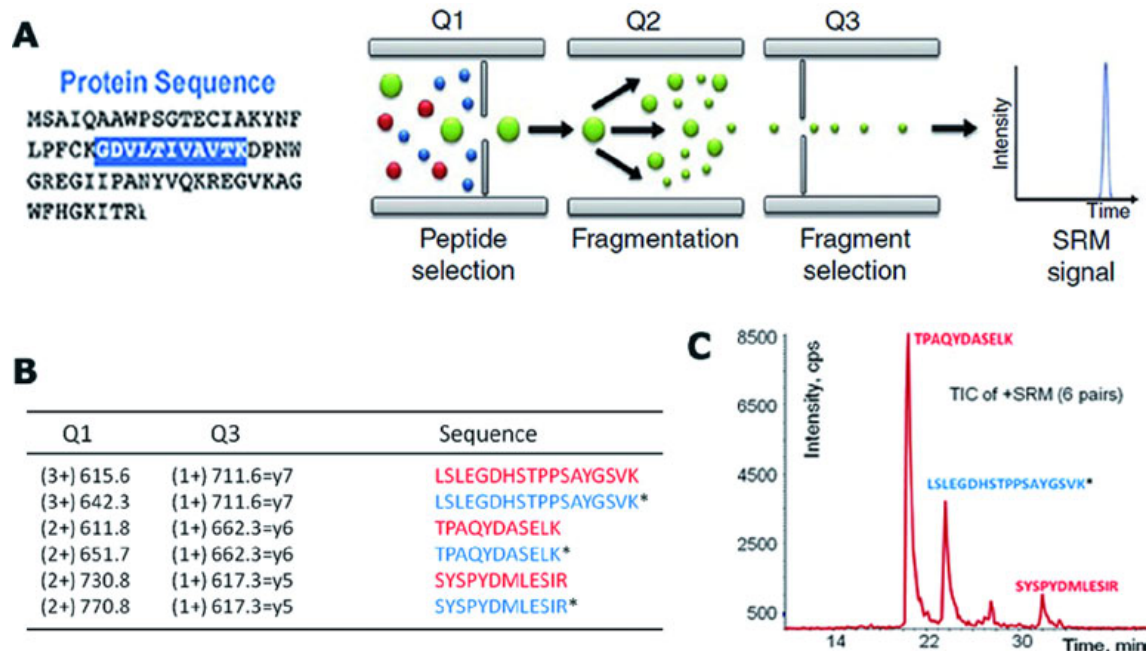
These approaches make use of shotgun and targeted proteomic analyses. While current approaches to global proteome profiling use high-throughput tandem mass spectrometry methods, these have limited sensitivity and are often unable to reliably detect and quantify low-abundance proteins in complex biological specimens such as a biopsy or cell extract. As an alternative we use directed approaches in which specific precursor/product ion transitions are selectively monitored (selected reaction monitoring; SRM) to improve overall detection sensitivity, reliability, and quantification.

This robust analytical platform, together with our recognized experience in the field, enables us to take on large and technically demanding research projects that require both qualitative and quantitative proteomic approaches for the detection of differential protein expression, chemical and posttranslational modifications, and protein-protein interactions in diverse biological systems.



Annexin A2 sequence analysis by combined digestion and activation on the LTQ Orbitrap ETD spectrometer. **A**, Annexin A2 sequence coverage using trypsin (blue boxes) and Lys-C (red) enzymes followed by LC-MS/MS analysis. **B**, Fragmentation spectra from an annexin-A2-derived tryptic peptide fragmented by electron transfer dissociation (ETD). **C**, Fragmentation spectra from an annexin peptide derived from Lys-C digestion fragmented by collision induced dissociation (CID). (From Fernandez-Garcia et al., 2010.)

6 Technical Units



Selected reaction monitoring (SRM). **A**, Setup of a triple quadrupole instrument such as the Applied 4000 QTrap. In the SRM mode, samples separated by nanoHPLC are injected into the mass spectrometer, but only selected precursor ions from the protein(s) of interest are allowed to enter q2 and undergo fragmentation. Q3 is then tuned to detect only selected fragment (product) ions. **B**, Selected masses from annexin A2 set at Q1 and Q3 for the analysis of phosphorylation at Thr19, Thr105 and Ser236. Masses corresponding to both the nonphosphorylated peptides (red) and phosphorylated peptides (blue) were monitored along the chromatogram in Q1, and a characteristic fragment ion was set in Q3 for each peptide. **C**, Total ion chromatogram (TIC) of the six precursor/product pairs selected. The sequences found are indicated next to their corresponding retention time. (Modified from Fernandez-Garcia et al., 2010.)

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Garrido-Gomez T, Dominguez F, Lopez JA, Camafeita E, Quiñonero A, Martinez-Conejero JA, Pellicer A, Conesa A, Simón C. **Modeling Human Endometrial Decidualization from the Interaction between Proteome and Secretome.** *J Clin Endocrinol Metab* (accepted)

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Lamas JR, Rodríguez-Rodríguez L, Vigo AG, Alvarez-Lafuente R, López-Romero P, Marco F, Camafeita E, Dopazo A, Callejas S, Villafuertes E, Hoyas JA, Tornero-Esteban MP, Urcelay E, Fernández-Gutiérrez B. **Large-scale gene expression in bone marrow mesenchymal stem cells: a putative role for COL10A1 in osteoarthritis.** *Ann Rheum Dis* (2010) 69: 1880-85

Cellomics



Head of Unit:	<i>María Montoya</i>
Support Scientists:	<i>José Manuel Ligos Hind Azegrouz</i>
Predoctoral Researchers:	<i>Begoña Díez Carmen Muñoz</i>
Technicians:	<i>Raquel Nieto Mariano Vitón M^a Montserrat Arroyo</i>



RESEARCH INTEREST

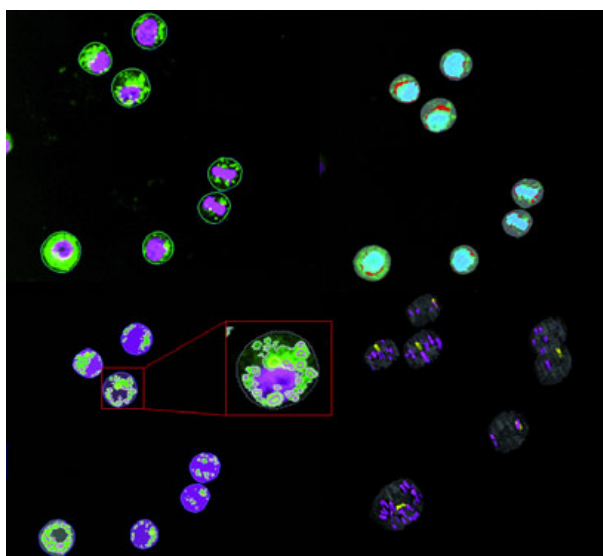
The fundamental unit of biological processes is the cell, and modern research is ever more dependent on the capacity to conduct sophisticated cell-based characterizations. The Cellomics Unit is dedicated to the two principal cell analytical techniques: flow cytometry and high content screening (HCS).

The Unit houses state-of-the-art flow cytometry and HCS equipment, and provides the necessary technical expertise in the use of this equipment to support the CNIC's research objectives. The Unit's staff assist researchers in experimental design and data interpretation for flow cytometry experiments, and design and perform high content screens, including miniaturization, automation, analysis and result validation. The Unit also provides training to CNIC research staff in flow cytometry and HCS technologies.

The Unit is equipped with

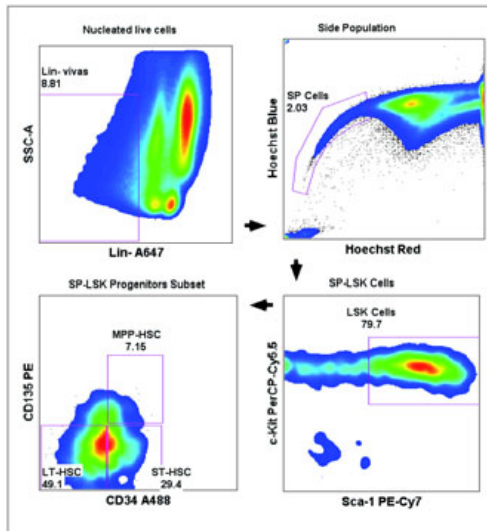
- Three latest generation digital analytical flow cytometers: two Becton Dickinson FACSCanto II machines and one Cyan (Beckman Coulter).
- Two high speed flow sorters: A MoFlo (Beckman Coulter) and a custom made FACS Aria II (Becton Dickinson).
- A liquid handling workstation connected to a cell culture incubator with 110 plate throughput (Freedom EVO, Tecan).
- An automated confocal microscope for microplate reading (Opera, Perkin Elmer).
- A full range of dedicated cytometry and image analysis software packages (Modfit, FlowJo, Acapella, Definiens, MatLab).

The Unit is involved in research into the regulation of membrane trafficking during cell migration. We are interested in the role of Rab8, a GTPase that regulates intracellular membrane trafficking to the plasma membrane, in cell migration and cytoskeletal rearrangements. A high content screen has been developed to identify novel molecules involved in the regulation of Rab8 activity.

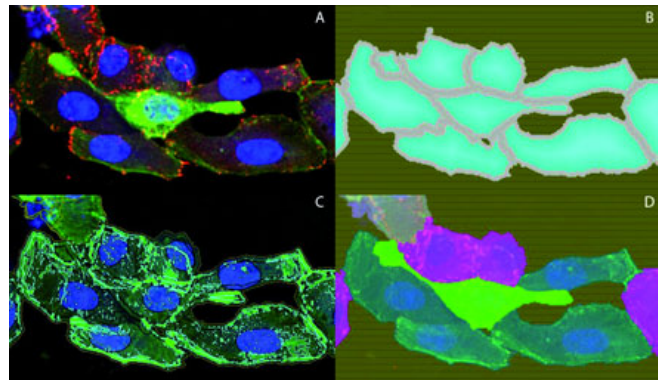


High content analysis of Cav internalization. HeLa cells expressing Cav1-GFP (green) are stained with Hoechst (pink). Confocal microscopy images were acquired using an automated microplate reader (Opera QEHS, PE) and then analyzed using a custom-made tool implemented in the Definiens Developer XD environment. Images show segmentation of nuclei (pale blue) and perinuclear space (red), and vesicles classified (green/pink/yellow) according to various parameters, both in 2D and 3D.

6 Technical Units



FACS-based multiparametric detection of a hematopoietic stem cell subset. Long Term (LT-HSC: SP+, Lin-, Sca1+, c-Kit+, CD34-, CD135-), Short Term (ST-HSC: SP+, Lin-, Sca1+, c-Kit+, CD34+, CD135-) and MultiPotential (MPP: SP+, Lin-, Sca1+, c-Kit+, CD34+, CD135+). Hematopoietic progenitors were detected in mouse bone marrow efflux through a combination of Hoechst 33342 staining (side population) and five-fluorescence immunostaining.



High content screen development for genes that regulate Rab8 GTPase-induced cytoskeletal rearrangements. HeLa cells expressing Rab8Q67L-GFP were fluorescently stained for nuclei and cytoplasm with Hoechst and CellMask (blue), polymerized actin with phalloidin (green) and focal adhesions with anti-vinculin (red). Confocal microscopy images were acquired using an automated microplate reader (Opera QEHS, PE); an overlay of fluorescence images is shown in **A**. Images were analyzed using a custom made tool implemented in the Definiens Developer XD environment. **B** shows segmentation of cell membrane (white) and interior areas (blue). **C** shows segmentation of polymerized actin structures (green), and **D** represents classification categories (green/blue/pink).



MAJOR GRANTS

- Ministerio de Ciencia e Innovación. FIS (PS09/01028)



SELECTED PUBLICATIONS

Escobar B, de Cárcer G, Fernández-Miranda G, Cascón A, Bravo-Cordero JJ, [Montoya MC](#), Robledo M, Cañamero M, Malumbres M. **Brick1 is an essential regulator of actin cytoskeleton required for embryonic development and cell transformation.** *Cancer Res* (2010) 70: 9349-59

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Daudén E, Pedraz J, Pérez-Gala S, Muñoz C, [Vitón M](#), Onate MJ, García-Díez A. **Effect of mycophenolate mofetil therapy on the phenotypic profile of peripheral blood leukocyte populations in psoriatic patients.** *Eur J Dermatol* (2010) 20: 233-4

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Viral Vectors



Head of Technical Service:

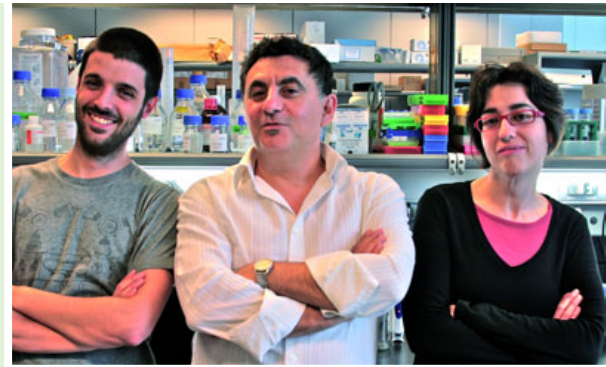
Juan Carlos Ramírez

Support Scientist:

Raúl Torres

Technician:

Aída García

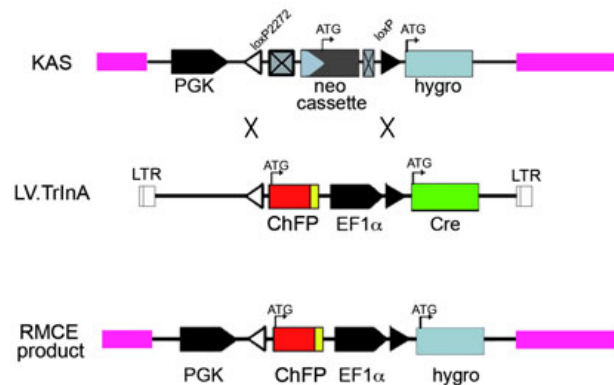


RESEARCH INTEREST

The Viral Vectors Unit provides CNIC researchers with replication deficient non-integrative adenovirus, HIV-derived lentivirus, and retroviral vectors. Virus stocks are produced, titrated and quality controlled by the rescue of replication-competent viruses and qPCR-quantification of particle/transduction units.

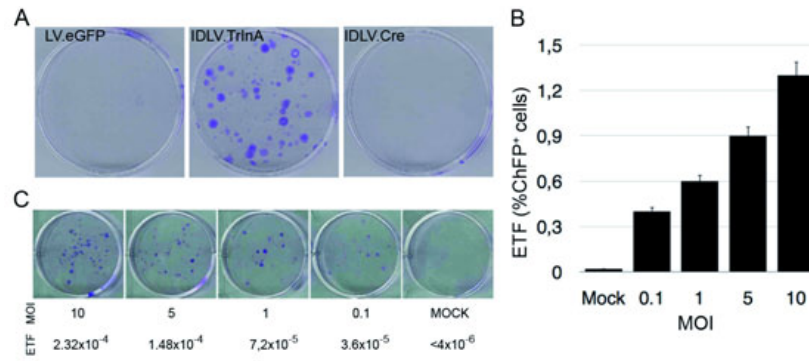
Over the last year our vector library grew to include close to a hundred viral backbones, matching the diverse expression requirements at the Center. Promoters driving constitutive (EF1alpha, CMV, PGK, Ubq, CAG), heart-specific (MHC) or tetracycline-inducible expression are assembled together with Picornaviridae IRES or 2A peptides for polycistronic mRNA synthesis of genes of interest in combination with selectable markers (PuroR, hygRO, neoR) or fluorescent reporter genes (green, cerulean, tomato, cherry, orange, td-tomato).

The Unit's research and development program has recently focused on two main areas. Our recent work on the role of the chemokine SDF-1gamma demonstrated that this cardiac-specific isoform targets the nucleolus; we are now collaborating with the Pluripotent Cell Technology facility to generate isoform-specific knockout mice. Secondly, we are working on a novel strategy to promote homologous recombination in genome-targeted cells by combining recombinant-mediated cassette exchange (RMCE) and integration-defective lentivirus (IDLV) technologies (*Kas-Trina* system). Cells targeted by a zinc-finger nuclease in the AAVS1 site with the *Kas* cassette are transduced with IDLV (*Trina*), and the self-limiting expression of the *cre* recombinase from the virus promotes RMCE at high frequency (up to 10%) and in a MOI-dependent manner. RMCE is revealed by promoter trapping of selectable markers and fluorescent proteins. We are currently testing the feasibility, reliability and simplicity of this system in human primary cells and established cell lines.



Cre recombinase-mediated insertion and cassette exchange strategy. Structure of the genetic landing pad (*KAS*) and the incoming construct (*LV.TrInA*), showing the most important elements: promoters *PGK*, *EF-1 α* and *SV40* in the *neo* cassette (black arrows), reporter genes (coloured boxes), recombinase *cre* (green box), translation start site (cornered arrows), and polyA signals (crossed boxes). Triangles represent sites for *loxP* (black) and *lox2272* (white). Homology arms are pink and lentiviral LTRs are open boxes flanking the *KAS* and *TrInA* cassette. The expected RMCE product is depicted below.

6 Technical Units



Randomly integrated chromosomal copies of the genetic landing pad KAS are targeted by IDLV expressing the TrlnA cassette. HEK293A cells resistant to G418 (293AKAS) were pooled and transduced with a low MOI (2 transduction units/cell) of IDLV-TrlnA. **A**, Transduced cells were selected with hygromycin and the colonies counted. The figure shows stained plates after selection of cells that were MOCK-transduced (LV.eGFP, left) or transduced with IDLV-TrlnA (center) or IDLV-Cre (right). **B**, Quantification of RMCE frequency in 293AKAS cells by FACS analysis of ChFP⁺ cells upon transduction with IDLV-TrlnA at different MOIs. **C**, Quantification of RMCE frequency in 293AKAS cells by culturing under selection conditions with hygromycin after transduction at different MOIs with IDLV-TrlnA as in B. ETF = Effective Targeting Frequency, expressed as the ratio between ChFP⁺ cells (B) or hygro^r cells (C) and the total number of integrants (G418^r).



MAJOR GRANTS

- Ministerio de Ciencia e Innovación (IPT-010000-2010-040)

Comparative Medicine

The Comparative Medicine Unit supports *in vivo* work at the CNIC, and is organized into five core work areas:

- **Animal Husbandry.** This area is staffed by dedicated animal technicians, managers and veterinarians who take charge of the daily husbandry and welfare of animals. Housing and husbandry conditions conform to European and national regulations for the use of animals for experimental and other scientific purposes, including the provision of mandatory training to researchers involved in animal experiments.
- The **Pathology Core (PC)**, run by an on-site laboratory animal pathologist. The PC has established collaborations with the Comparative Pathology Laboratory of the Weill Cornell Medical College and the Memorial Sloan-Kettering Center in New York, and with the Phenotyping Core at the Department of Molecular and Comparative Pathobiology, Johns Hopkins Hospital in Baltimore.
- The **Phenotyping Core (PhC)**, which provides a comprehensive cardiovascular phenotype evaluation service.
- The **Veterinary Medicine and Experimental Surgery Core (ESC)** provides highly specialized expertise in animal medical problems, disease follow-up, surgical procedures, minimally invasive intervention, and life support.
- The **Quality Control Core (QCC)** is run by a senior microbiologist and monitors the health and the genetic status of the animals on site.

The PC and PhC services combine *in vivo* evaluation, imaging strategies, and clinical and anatomic pathology to characterize complex phenotypes—including multisystemic phenotypes or syndromes—for the development and validation of genetically engineered mouse models.



Nano PET/CT apparatus for imaging studies in rodents

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