

Functional Genomics Core Facility



During the last decade, molecular biology developed from a gene-by-gene analysis into a more comprehensive approach to study regulatory networks involving dozens to hundreds of interacting partners. For successful performance in this area, researchers require an increasing number of tools to either interrogate or alter genes on a genome-wide level. The Functional Genomics Core Facility provides state-of-the-art genomic tools for researchers at IRB Barcelona and other centres. These tools fall into two categories. The first is the genome-wide analysis of transcription, DNA polymorphisms, and chromatin immunoprecipitation (ChIP-chip). These analyses are performed using microarrays produced by Affymetrix and NimbleGen. For both analytical methods, the Facility provides a complete service, including initial consultation during the design of a project, quality control of starting material, sample and array processing, data analysis in collaboration with statisticians, and data interpretation and validation by real-time-PCR. The second category is the alteration of gene expression. For knock-down of gene expression, the Facility provides a genome-wide human and mouse shRNA library (Sigma), containing approximately 100,000 clones each, covering the majority of all known transcripts. For overexpression, we provide a human open-reading-frame library (Open Biosystems) containing 15,000 clones, covering three quarters of all human genes.

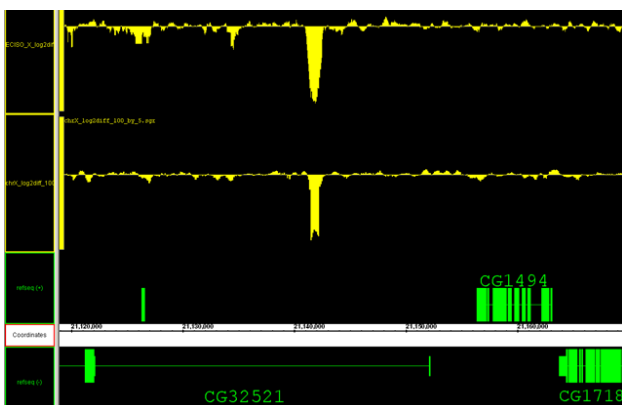


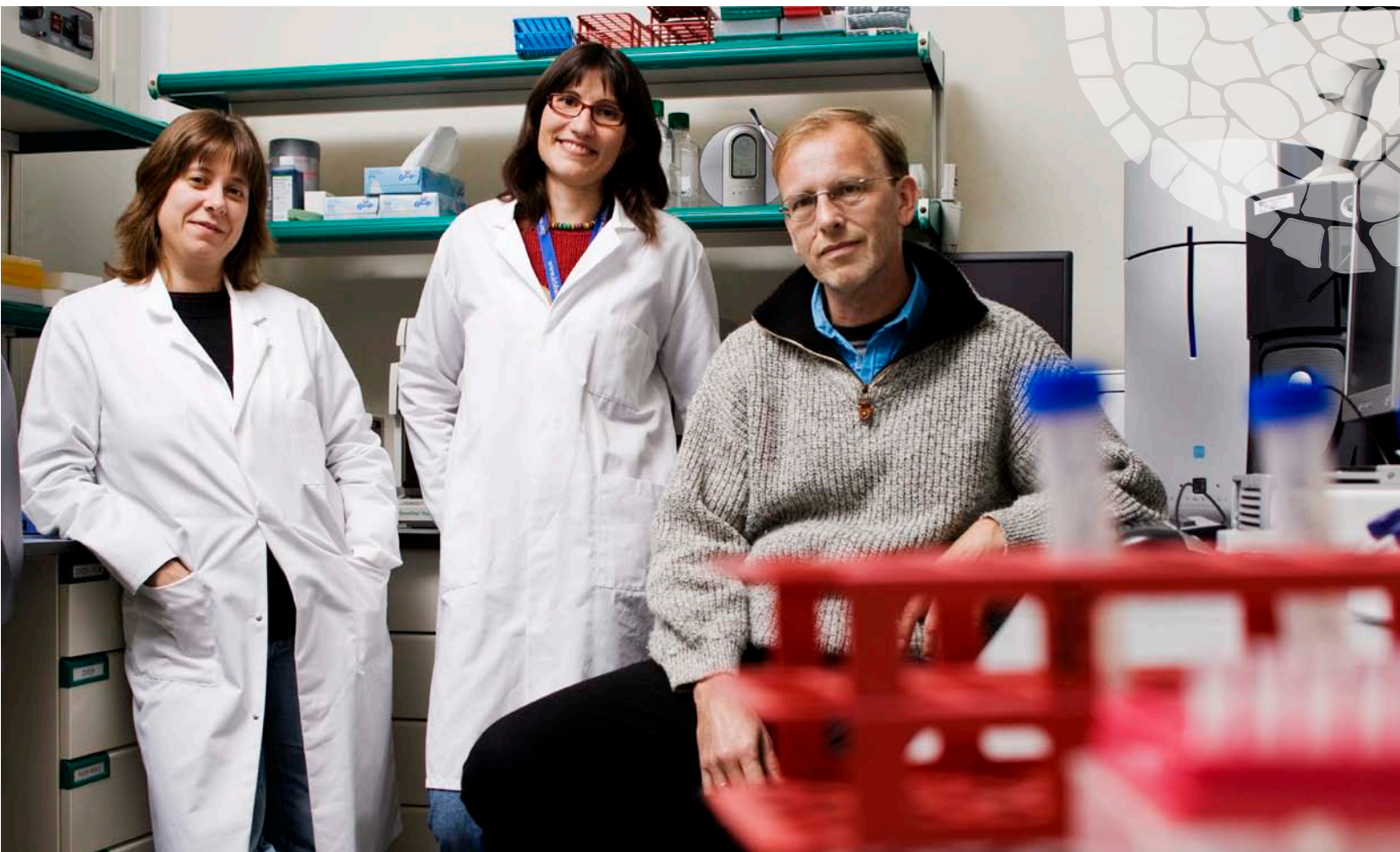
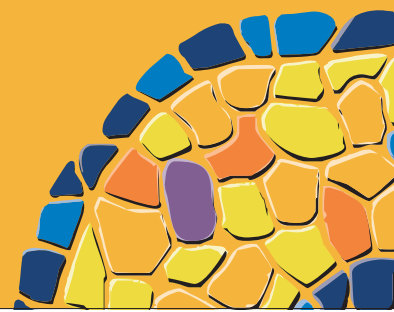
Figure 1. DNA copy number variation in Drosophila strains. The top line shows a homozygous deletion within the CG32521 gene in a Drosophila strain as measured by Next Generation Sequencing; the line below shows the result of the same chromosomal region measured by tiling arrays.

During 2008 the Facility performed projects with over 20 research groups from four programmes at IRB Barcelona and from other institutions throughout Barcelona, Catalonia and Spain.

Using products provided by Affymetrix, the Facility performs genome wide expression analysis at the gene and exon levels as well as comparative genome hybridisation (CGH) analysis. These technologies are provided for over 20 organisms including all standard model organisms and humans. For CGH analysis, resolution is further increased by use of tiling arrays. These arrays provide probes tiled across the entire genome without prediction of genes; therefore, this type of array offers the most comprehensive picture of genomic alterations currently available in microarray technology.

Since summer 2008, the Facility also offers services based on NimbleGen microarray products. NimbleGen technology provides longer probes than Affymetrix and therefore higher specificity. In addition, NimbleGen microarray production is extremely flexible and consequently facilitates the design of customised microarrays, even for small projects. This technology is currently used for expression and CGH analysis.

Facility Manager Herbert Auer Senior Research Officers Eva González, Silvia Rodríguez



The tools for altering gene expression, namely the shRNA libraries and the open-reading-frame library, contain over 200,000 clones. These are centrally stored and a database has been developed for clone administration. It also provides information about knock-down efficiency and accuracy of clone annotation.

Services for IRB Barcelona researchers

DNA/RNA quantification and quality control

Various analyses are provided for the assessment of purity, integrity and concentration of nucleic acids. These analyses include specific quantification of DNA and RNA using nuclei acid-specific fluorometric assays, spectrophotometric assessment of contamination, quantitative measurements of fractions of RNAs, like small RNAs, mRNA and rRNAs, and the evaluation of RNA integrity.

Expression profiling

Genome-wide analysis of transcripts is provided at three levels of resolution:

- 3' biased arrays containing one probe set per gene; these arrays are available for more than one hundred organisms.
- Exon arrays containing one probe set per exon; these arrays are currently available for human, mouse and rat.
- Tiling arrays interrogating the entire genome at a 35-base-pair resolution; these arrays are currently available for human, mouse, *Drosophila*, *S. cerevisiae* and *S. pombe* from Affymetrix and can be customised via NimbleGen for every sequenced organism.

DNA polymorphism analysis

Genome-wide analysis of DNA polymorphisms comes in two forms:

- For over 20 organisms, DNA copy number variation (CNV) is measured at the resolution of individual genes. For organisms where exon or tiling arrays are available (see above) even higher resolution can be provided. For CNV analysis, arrays can be customised via NimbleGen for every sequenced organism.
- For human DNA, up to one million single nucleotide polymorphisms (SNPs) can be measured in parallel with the same number of CNVs across the genome.

Validation of microarray results by real-time PCR

For real-time PCR validation of microarray data, assays are designed and performed and data are analysed for differential expression.

Alteration of gene expression

Bacterial clones are provided for the knock-down of almost all well characterised human and mouse transcripts. Multiple clones targeting the same transcript are available to assess off-target effects. For overexpression, one open-reading-frame clone is available per human gene. Clones are centrally managed at the Facility and are provided as bacterial stocks. The clone database is available online and clones can be requested electronically.

SCIENTIFIC OUTPUT

Publications

Cuscó I, del Campo M, Vilardell M, González E, Gener B, Galán E, Toledo L and Pérez-Jurado LA. Array-CGH in patients with Kabuki-like phenotype: identification of two patients with complex rearrangements including 2q37 deletions and no other recurrent aberration. *BMC Med Genet*, 11, 27 (2008)

Font-Burgada J, Rossell D, Auer H and Azorin F. *Drosophila* HP1c isoform interacts with the zinc-finger proteins WOC and Relative-of-WOC to regulate gene expression. *Genes Dev*, 22(21), 3007-23 (2008)

Rodríguez-Mulero S and Montanya E. Islet graft response to transplantation injury includes up-regulation of protective as well as apoptotic genes. *Cell Transplant*, 17(9), 1025-34 (2008)

Salgado R, Toll A, Espinet B, González-Roca E, Barranco CL, Serrano S, Solé F and Pujol RM. Analysis of cytogenetic abnormalities in squamous cell carcinoma by array comparative genomic hybridization. *Actas Dermosifiliogr*, 99, 199-06 (2008)

Singh S, Robinson M, Ismail I, Saha M, Auer H, Kornacker K, Robinson ML, Bates CM and McHugh KM. Transcriptional profiling of the megabladder mouse: a unique model of bladder dysmorphogenesis. *Dev Dyn*, 237(1), 170-86 (2008)

Collaborations

Allele specific gene expression
Jorge Ferrer, IDIBAPS (Barcelona, Spain)

Alterations of glycogen metabolism in pathological conditions
Joan Guinovart, IRB Barcelona (Barcelona, Spain)

Characterisation of genes implicated in mitochondrial dynamics
Antonio Zorzano, IRB Barcelona (Barcelona, Spain)

*Characterisation of nucleosome positioning in *S cerevisiae**
Modesto Orozco, IRB Barcelona (Barcelona, Spain)

Characterisation of yeast mutants used in wineries
Ricardo R Cordero Otero, Rovira i Virgili University (Tarragona, Spain)

Compartment boundary formation: identification of new genes and properties
Marco Milán, IRB Barcelona (Barcelona, Spain)

Copy number variation in metabolic diseases
Luis Castaño, Hospital de Cruces (Basque Country, Spain)

Development and evaluation of next generation sequencing algorithms
Karl Kornacker, Nationwide Children's Hospital, Columbus (Ohio, USA)

Evaluation of miRNA analysis platforms
ABRF Microarray Research Group

*Gene expression in *S pneumoniae**
Adela González de la Campa, Caubet-Cimera Foundation, Joan March Hospital (Bunyola, Spain)

*Genomic and transcriptomic characterisation of cancer in *Drosophila**
Cayetano González, IRB Barcelona (Barcelona, Spain)

Intestinal stem cells and colorectal cancer stem cells
Eduard Batlle, IRB Barcelona (Barcelona, Spain)

Molecular characterisation of mutants relevant to the cerebral cortex
Eduardo Soriano, IRB Barcelona (Barcelona, Spain)

Molecular characterisation of neural development in chicken
Marian Martínez Balbas, IBMB, CSIC (Barcelona, Spain)

Molecular mechanisms causing metastasis
Roger Gomis, IRB Barcelona (Barcelona, Spain)

Roles of the nuclear receptor LXR in macrophage biology
Antonio Celada, IRB Barcelona (Barcelona, Spain)

TGF beta signalling in colorectal cancer
Elena Sancho, IRB Barcelona (Barcelona, Spain)

The cross-talk between GR, PPAR- γ and the JNK/AP-1 pathway
Carme Caelles, IRB Barcelona (Barcelona, Spain)

The role of DNA binding proteins in chromatin structure
Ferran Azorín, IRB Barcelona (Barcelona, Spain)

The role of the GAGA factor in the regulation of chromatin structure and function
Jordi Bernués, IRB Barcelona (Barcelona, Spain)