Transgenic Mice *Core Unit*

Sagrario Ortega

Unit Head

Sagrario Ortega was born in Toledo (Spain). She obtained her PhD in Biochemistry in 1987 at the *Universidad Complutense de Madrid* for her thesis work on the molecular mechanism of initiation of prokaryotic DNA replication at the *Centro de Investigaciones Biológicas* (CSIC), Madrid.

From 1987 to 1990 she was a Fulbright Scholar at Merck Sharp and Dohme Research Laboratories, Rahway (USA), during which time she studied fibroblast growth factors (FGFs), their function in the control of cell proliferation and their therapeutic applications.

In 1992 she joined the New York University (NYU) Medical Center (USA) as a Research Associate where she studied the *in vivo* function of members of the FGF family through the generation of knockout mice. In 1998 she co-directed the Gene Targeting and ES cell Culture Facility with A. Joyner at NYU Medical Center.

In September 1998 she joined the CNIO as a Staff Scientist in Mariano Barbacid´s lab to study cell cycle control in mammals generating and characterising gene targeted mice for the cyclin-dependent kinases (Cdks) and their regulators.

Since 2002 she has been Head of the Transgenic Mice Unit at the CNIO.

Summary

Our Unit offers state-of-the-art technology for the manipulation of the mouse genome. It is a combined facility for both gene targeting in embryonic stem (ES) cells and mouse transgenesis. Other available techniques in our laboratory include the establishment of ES cell lines from mouse embryos and *in vitro* differentiation of ES cells. We are also responsible for the cryopreservation of genetically modified mouse strains through both sperm and embryo freezing.

Our Unit is also in charge of the rederivation of mouse lines as well as management of tool strains (Cre and Flp transgenic lines and reporters among others) in collaboration with the CNIO Animal Facility. We also provide support to CNIO Groups working on projects involving mouse models, ES cells, induced pluripotent stem (iPS) cells and embryos.

Main Objectives

- Provide CNIO Groups with the most up-to-date transgenic technologies for the manipulation of the mouse genome
- Cryopreserve genetically modified mouse lines generated at the CNIO or imported from external sources
- Develop new genetic tools to study cancer using the mouse as a model system
- Support CNIO Research Groups in the handling and manipulation of ES cells and embryos

Graduate students: Rodrigo Diéguez and Inés Martínez (until September). **Technicians:** María del Carmen Gómez, Javier Martín, Jaime A. Muñoz, Patricia Prieto and Marta S. Riffo.

Highlights

In 2010 the Transgenic Core Unit generated 21 new gene targeted mouse lines including knockout, knockin and conditional models. In addition, 30 new mouse lines have been introduced into the CNIO Animal Facility by rederivation and 89 lines have been cryopreserved by sperm (71 lines) and/or embryo (18 lines) freezing.

The Unit is also creating a cryobank of all the genetically modified alleles generated at the CNIO by sperm or embryo freezing. This project involves freezing approximately 100 mouse lines per year.

Figure: Cre-reporter transgenic line expressing Katushka. Fluorescence over bright field live image showing two littermates born from a mating between a male carrying a germ-line Cre-recombined reporter transgene and a wild-type female. The pup with fluorescent signal has inherited the recombined reporter allele while the one without signal has not, as confirmed by PCR genotyping. Image acquired with an IVIS Spectrum (Xenogen Co.).

We have expanded the collection of parental mouse ES cell lines to be used for gene targeting by incorporating lines containing a C57BL/6N genetic background – as established by the International Consortium for Mouse Mutagenesis – including JM8.F6, JM8.N4 and JM8A3. The Unit is also collaborating with groups at the CNIO to establish and characterise new mouse ES cell lines and iPS (induced pluripotent stem cells) from genetically modified mice.

The Unit has developed a new Cre-reporter transgenic line that expresses the far-red fluorescent protein Katushka (Shcherbo D. et al., *Nat Methods* 2007) under the control of the strong ubiquitous pCAG promoter upon Cre-mediated excision of a lox-STOP-lox transcription termination cassette.

After germ line or tissue-specific Cre-driven reporter activation, Katushka expression is strong and ubiquitous (Figure) without toxic effects. The reporter allows for fluorescence detection in fresh and fixed samples derived from all tissues examined.

Moreover, fluorescence can be detected by *in vivo* non-invasive whole-body imaging when Katuhska is exclusively expressed in a specific cell population located deep within the animal such as pancreatic beta cells.

Thus, this reporter model enables early, widespread and sensitive *in vivo* detection of Cre activity and should provide a versatile tool for a wide spectrum of fluorescence and live imaging applications (Diéguez R. et al., *Genesis Online* 2010).