TRANSGENIC MICE CORE UNIT

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OVERVIEW

The laboratory mouse is the most widely used experimental model for genetic studies and preclinical drug development in cancer. The Transgenic Mice Unit is dedicated to the genetic edition of the mouse germ line and to the generation of genetically modified mouse strains. Hundreds of these strains have been created at our Unit. In many cases, they contain modifications that reproduce the genetic alterations found in human cancer and are introduced in the mouse to generate preclinical models of the disease, thereby contributing to the development of more efficient targeted therapies. Genetically modified mice are also created for testing *in vivo*, in a physiological context, hypothesis related to the molecular mechanisms that convert a normal cell to a malignant cell or that contribute to tumour expansion and invasion of distant organs, ultimately causing death. Cancer is an extremely complex disease that cannot be sufficiently well studied *in vitro* in a tissue culture plate. The generation of genetically modified mice is one of the basic pillars that sustain cancer research at the CNIO.

"CRISPR/Cas based genome editing technology has improved the generation of genetically edited mice, thereby promoting research progress and accelerating drug discovery in many fields including cancer."

Technicians
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**Titulado Superior* (Advanced Degree)

RESEARCH HIGHLIGHTS

CRISPR/Cas based tools have revolutionised the way we approach genetic studies. The Unit has incorporated CRISPR/Cas gene editing tools for mouse germ line precise modification, replacing, in many cases, gene targeting in embryonic stem cells (ES cells) for the generation of knockout and knockin mice with high efficiency. CRISPR reagents are introduced directly into mouse zygotes by pronuclear injection or electroporation, avoiding difficult and time-consuming ES cell culture and manipulation. Knockout allele generation by CRISPR is often around 80% to 90% efficient and bi-allelic knockout animals are frequently obtained. Point mutations or small tag insertions are also easily created by CRISPR-induced homologous recombination directly in zygotes, using single stranded oligodeoxynucleotides as donor DNA for repair. We have also developed strategies to increase the efficiency of CRISPR-mediated large (more than 2 Kb) knockin integrations using, in this case, circular plasmids as donor DNA. A high proportion of the pups born after zygote CRISPR microinjection carry targeted knockin inserts. Zygote electroporation is a good alternative to microinjection for gene knockout generation. Moreover, zygotes obtained by *in vitro* fertilisation can be edited the same day in a fast and efficient way through CRISPR electroporation.

The CRISPR gene editing system may also be used in forward genetics for genome wide screenings of new genes relevant in different aspects of cancer. In the Unit, we have established haploid ES cell lines from genetically modified mice generated at the CNIO, by induction of parthenogenetic division of unfertilised mouse eggs. Haploid ES cells are especially useful for the identification of recessive mutations in genome-wide screenings. We have set up several screenings based on genetic rescue of lethal mutations using lentiviral libraries of gRNAs in combination with the Cas9 endonuclease. We are also using activating CRISPR libraries based on the expression of the dead Cas9-VP64 fusion and modified gRNAs that bind transcription activators, targeted to the promoter regions of coding genes and microRNAs. This approach is being used in a genome-wide search for genes and pathways that activate expression of fluorescent reporters knocked-in in cancer related genes (FIGURE). ■

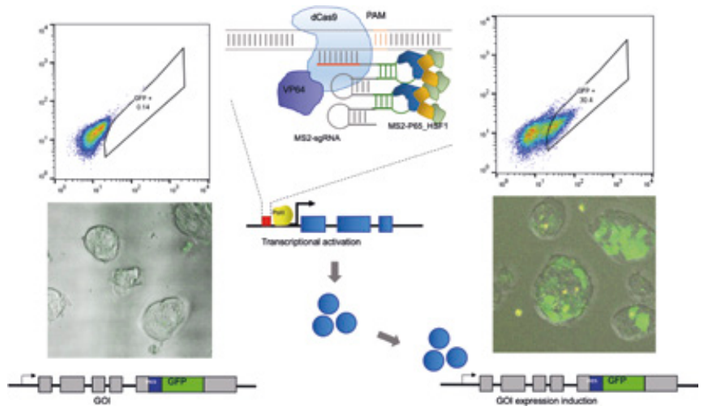

Figure Example of a genome-wide screening for genes and pathways that induce expression of a gene of interest (GOI) using transcriptional activating CRISPR/Cas gRNA libraries and embryonic stem cells containing a knockin GFP reporter in the GOI. Flow cytometry analysis and confocal images confirm increased expression of GFP upon infection with the CRISPR library.

PUBLICATIONS

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