TRANSGENIC MICE CORE UNIT

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OVERVIEW

Genetically engineered mice are an essential tool for analysing the molecular mechanisms underlying tumour development and cancer biology. Modelling cancer by modifying the germ line of the mouse has become a crucial component of drug discovery as well as for the assessment of experimental therapies at the preclinical stage. The Transgenic Mice Unit at the CNIO offers state-of-the-art technology for the manipulation of the mouse genome. Using classical transgenesis, homologous recombination in embryonic stem cells and genome editing by targeted nucleases, the Unit has generated more than 300 mutant alleles of cancer related genes in the mouse germ line. The Unit also provides support and collaborates with CNIO researchers in many aspects related to research with embryonic stem (ES) cells, induced pluripotent stem (iPS) cells, and embryo- and mouse model-based research. Finally, the Unit also leads its own research projects focused on the generation of mouse models to study tumour biology, as well as on the screening of cancer-related genes.

"In 2016, the Unit generated over a dozen GEM strains containing knockout and knockin mutations, using the CRISPR/Cas9 system of *S. Pyogenes*. The Unit contributed to 8 peer-reviewed articles, in collaboration with CNIO and external groups, including the description of a new mouse strain for conditional gene targeting of the lymphatic system."

RESEARCH HIGHLIGHTS

The CNIO Transgenic Mice Unit is dedicated to the generation, cryopreservation and derivation of genetically engineered mouse strains. We have created over 200 mutant strains, including knockout, knockin and conditional alleles, by gene targeting in embryonic stem (ES) cells, and over 100 mouse strains by conventional transgenesis. The Unit currently maintains a cryopreserved stock of over 1000 mouse strains, frozen at the Unit as sperm or embryos. This stock represents an invaluable resource of engineered strains for modelling and studying cancer in the mouse. Through our Unit, the CNIO shares part of this stock with EMMA (the European Mouse Mutant Archive) in order to make these models more accessible to the wider scientific community. We acknowledge the CNIO Animal Facility for their constant help and collaboration to make all these achievements possible.

The CRISPR/Cas9 system of *Streptococcus pyogenes* has expanded the currently available set of mammalian genome engineering tools, providing an easy, efficient, flexible and versatile method for creating targeted mutations in mammalian genes. We use the CRISPR/Cas9 system to generate knockout and knockin mice by introducing the components of the system, the guide CRISPR RNA and the Cas9 nuclease (either as messenger RNA or as protein) directly into mouse zygotes (FIGURE). In our experience, this system has proven to be extremely efficient for introducing new additional mutations in strains that are already carrying several engineered alleles, such as some mouse models of lung and pancreatic cancer that are used at the CNIO. We have also used the system to generate knockin alleles (point mutations) and tag insertions with efficiencies close to 20% directly in zygotes. The characteristics of the CRISPR system – efficient, fast and easy to implement – make it extremely useful for creating constitutive mutations in the mouse and to test certain biological questions before embarking on the creation of conditional or more sophisticated alleles. For these types of alleles, gene targeting in ES cells may still be the method of choice and we are currently optimising the use of CRISPR in ES cells to increase the efficiency of this technology. ■

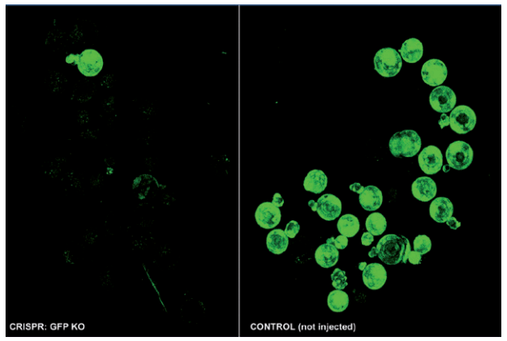

Figure Efficiency of GFP knockout via CRISPR in mouse embryos. Embryos are collected from B6.CBA females, crossed with 129Gt(ROSA)26Sortm(CAG-EGFP) Luo (KI/KI) males, at E0.5. Embryos are injected with gRNA_GFP97 (50ng/µl) and commercial Cas9 protein (100ng/µl) in the cytoplasm at the zygote stage and cultured *in vitro* for 3 days up to the blastocyst stage. Confocal images (maximal projection) of GFP fluorescence.

PUBLICATIONS

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