

“Transgenesis, recent technical developments and applications” Nantes, 8th June 2009

On June 8 2009 was held in Nantes, France, the international meeting “Transgenesis ; recent technical developments and applications” ([see web site for more information](http://www.ifr26.nantes.inserm.fr/ITERT/TRM/index.php?num=0) <http://www.ifr26.nantes.inserm.fr/ITERT/TRM/index.php?num=0>). This meeting is the second one of its kind to be organized by the Transgenic Rats Nantes facility (<http://www.ifr26.nantes.inserm.fr/ITERT/transgenese-rat/>) from the INSERM UMR 643 and Biogenouest. The meeting was supported by several academic institutions, as well as private companies. The meeting received support and co-sponsorship from the International Society for Transgenic Technology (ISTT). Around 100 participants, from France but also in significant proportion from different European countries and Canada, attended the meeting and registration prices were kept as low as possible to facilitate the participation of students. The meeting aimed to provide an update on recent technical developments in the generation of transgenic animals and in some of their applications. It was intended for Master, PhD and medical students with a background in molecular biology and genetics as an introduction to future work in these rapidly developing areas of research. It was also intended for post-docs and scientists already working in certain of these fields and who are interested in expanding their knowledge on the potential applications of these new techniques to their models.

Attendees were welcomed by **Ignacio Anegon**, director of the INSERM UMR 643 and transgenic rat core facility.

Louis-Marie Houdebine (INRA, Jouy-en-Josas, France) who described with in depth pros and cons of most of the currently used techniques to generate genetically modified animals. He paid particular attention to techniques applicable in mammals other than mice and on the very useful models of genetically modified rabbits to analyse dislipidemias and genetic diseases.

Michel Cohen-Tannoudji (Pasteur Institute, Paris, France) reviewed technical aspects and applications of knock-out and knock-in strategies using ES cells to generate mutant mice. He covered with the knowledge given by personal experience several of the molecular systems currently used with mouse ES cells such as the Cre-lox system and oestrogen receptor and tetracycline-inducible promoter systems. He also described the methods recently used to generate ES cells in rats (although no KO have been yet described) that could also be applied to other species in which ES cells are lacking.

Kader Thiam (GenOway, Lyon, France) discussed the use of RNAi-mediated gene knockdown for that he presented the use a knockin strategy with the HPRT or ROSA 26 loci in ES cells and the advantages/disadvantages in relation to knock-out strategies. This technology has been extended to the Cre/lox system to produce inducible gene knockdowns.

Toni Cathomen (Hannover Medical School, Hannover, Germany) covered the generation of zinc finger nucleases as tools with great potential for mutating genes. He has made important contributions to the optimization of this hybrid molecules composed of a zinc finger domain recognizing targeted DNA sequences fused to the FokI nuclease. He also described the application of zinc finger nucleases delivered using viral gene vectors coupled to the delivery of DNA donor sequences to correct genetic defects.

Roland Buelow (Open Monoclonal Technology, Palo Alto, California, USA) showed the application of designed zinc finger nucleases to generate knock-out rats via embryo pronuclear microinjection. This is the first application of zinc finger nucleases to generate mutant mammals and the first generation of targeted knock-out rats. The results showed that this method was rapid, efficient and cost-effective. The gene targeted was the IgM heavy chain locus and the aim is to create a platform for the generation of humanized monoclonal antibodies.

Daniel Boujard (CNRS/University Rennes 1, Rennes, France) detailed the techniques use to generate transgenesis in species models fish and amphibian, in particularly *Xenopus laevis*. Among these species, the rapid determination of germ cells does not authorize stem cell approach and zinc finger nucleases have great potential to generate

Edwin Cuppen (Hubrecht Institute, Utrecht, The Netherlands) explained the technique of gene inactivation by mutagenesis using N-ethyl-N-nitrosourea by which he has generated a large panel of mutated rats. This technique has been optimized to be applicable in a large scale and coupled to massive sequencing has the potential of being of great help in the generation of loss of gene knock-out but also of subtle changes in aminoacid sequence very useful to decipher gene function.

Cesare Galli (Istituto Sperimentale Italiano L. Spallanzani, Cremona, Italy) covered the cloning by nuclear transfer in farm animals. Somatic cell nuclear transfer is still subject of investigation because it offers a model of genome reprogramming to restore totipotency and produces many abnormal phenotypes. The success of the procedure can only measured by the birth of live animals and it is the result of a technically simple but a biologically complex procedure. His experience in cloning pigs gave to his talk great interest and a flavour of the difficulties in the generation of this important model for biomedical research in general and xenotransplantation in particular.

Luis Montoliu (National Center of Biotechnology, Madrid, Spain) described the important advantages of using yeast and bacterial artificial chromosome-type transgenes as compared to short sequences as well as the techniques that are needed for efficient and safe handling of these large sequences. The use of these large genomic-type transgenes is therefore the method of choice in order to overcome chromosomal position effects, commonly associated with standard-type transgenes (i.e. plasmid based), with a much smaller size.

Marie Malissen (Centre d'Immunology de Marseille Luminy, Marseille, France) presented data on the application of immunomonitoring techniques to analyse mutant mice. Her research not only on knock-out genes but also of subtle gene mutations using ES cells and conditional knock-outs very nicely exemplified the potential of these models for unravelling immune mechanisms. She presented results of LAT (Linker for activation of T cells) “knock-in” mutant mice harboring single or combined point mutations in the last four tyrosine residues of

the LAT cytoplasmic tail which were found to develop lymphoproliferative disorders involving polyclonal T-cells that produce high amount of Th2 cytokines.

Ignacio Anegon (INSERM UMR 643, Nantes, France) covered lentiviral transgenesis applied to different species and in particular showing own data on the generation of transgenic rats. Lentiviral transgenesis has become an established, useful and alternative technology to DNA microinjection in particular in species or strains of mice or rats with low transgenic efficiencies.

Laurent Lescaudron (INSERM UMR 643, Nantes, France) overviewed rodent models of neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD). He then focused on 2 models used in his work, the transgenic HD rat which carries a truncated huntingtin cDNA fragment with 51 CAG repeats under control of the native rat huntingtin promoter and the new transgenic PD rat, generated in "Transgenic Rats Nantes" Facility, bearing the human A30P and A53T mutations of the α -synuclein under control of the tyrosine hydroxylase promoter.

Jean-Louis Guénet (Pasteur Institut, Paris, France) delivered in first an overview of the knowledge geneticists have of the mouse genome sequence. Second, he provided some examples where the availability of the sequence has been beneficial to the design of experiments (positional cloning, quantitative genetics, gene copy number variations, etc.). Finally, he makes some predictions about a likely future for mouse genomics and proteomics based on the very rapid development of the sequencing techniques.

Time between presentations and during coffee breaks and lunch gave opportunity for discussions and exchanges. The overall very positive balance of this meeting both in the quality of scientific presentations and the number of participants are excellent reasons to renew the experience in two years.

The local organizing committee hopes to welcome many participants in 2011 to the 3rd transgenesis meeting organized by the Transgenic Rats Nantes facility.

Ignacio Anegon & Séverine Menoret