In Vivo Imaging Service for basic research and preclinical studies

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PLAISANT

Animal Facility Outsourcing & Experimental Research Support

The bio-imaging is a very powerful system to monitor molecules and drugs distribution and to observe kinetic of tumor growth. The same mouse can be observed many times, instead of sacrifice it and collect tumors at different kinetic times, this is according to the 3R's (reduction) rule. The IVIS (In Vivo Imaging System) gives a good comparison of manual measurement methods (calibration) and photon release. This can reduce distress in mice due to the restrain and give a major accuracy of data, minimising risk of errors.

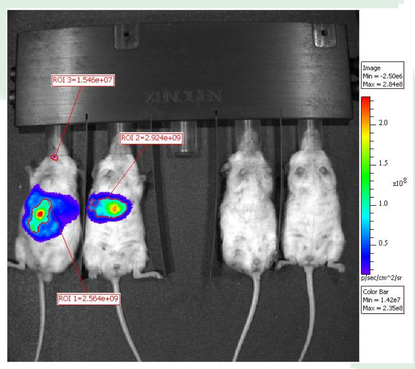
The mice are inoculated with solid tumor cells and leukaemia cells, genetically modified to express luciferase. 10 minutes before acquisition, the mice are inoculated with luciferase substrate that is metabolised giving luminescence to the cancer cells. Mice are anesthetised by gas anaesthesia (isoflurane) for a maximum of 3-5 minutes and observed. Isoflurane is quickly metabolized and doesn't give any suffering to the animals. Leukaemia cells and solid tumour cells administration is performed by intravenous or intraperitoneal injection and by subcutaneous or orthotropic (Colon, liver etc.) injection respectively.

Plaisant "Castel Romano", conducts In Vivo Imaging on different research projects and preclinical studies for different customers. Most of our IVIS experiments are conducted for OPBG (Ospedale Pediatrico Bambino Gesù).

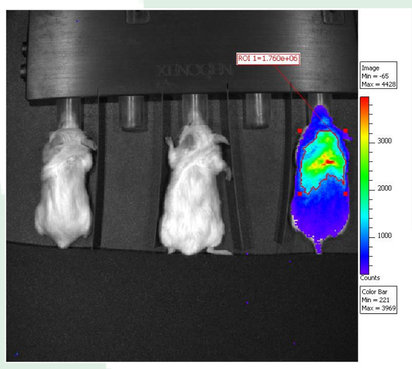
IVIS UNIT

Adoptive T-cells therapy for tumor disease (Leukemia and solid tumors)

Solid Injection



Intravenous Injection



Quinatarelli C., De Angelis B., Caruana I. (OPBG)

Solid Injection

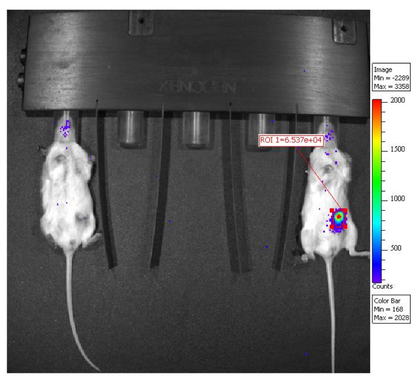
Image Number	ROI	Image Layer	Total Flux (p/s)	Avg Radiance (p/s/cm2/sr)	Stdev Radiance	Min Radiance	Max Radiance
LT20170126095716	ROI 1	Overlay	2,56E+09	1,12E+08	4,35E+07	6,88E+07	2,84E+08
LT20170126095716	ROI 2	Overlay	2,92E+09	1,02E+08	4,16E+07	5,37E+07	2,16E+08
LT20170126095716	ROI 3	Overlay	1,55E+07	9,62E+06	3,80E+06	5,22E+06	2,07E+07

Intravenous Injection

Image Number	ROI	Image Layer	Total Counts	Avg Counts	Stdev Counts	Min Counts	Max Counts
LT20170127162912	ROI 1	Overlay	1,76E+06	1,88E+03	6,29E+02	1,06E+03	4,43E+03

High-Throughput *In Vivo* Bioluminescence Imaging System

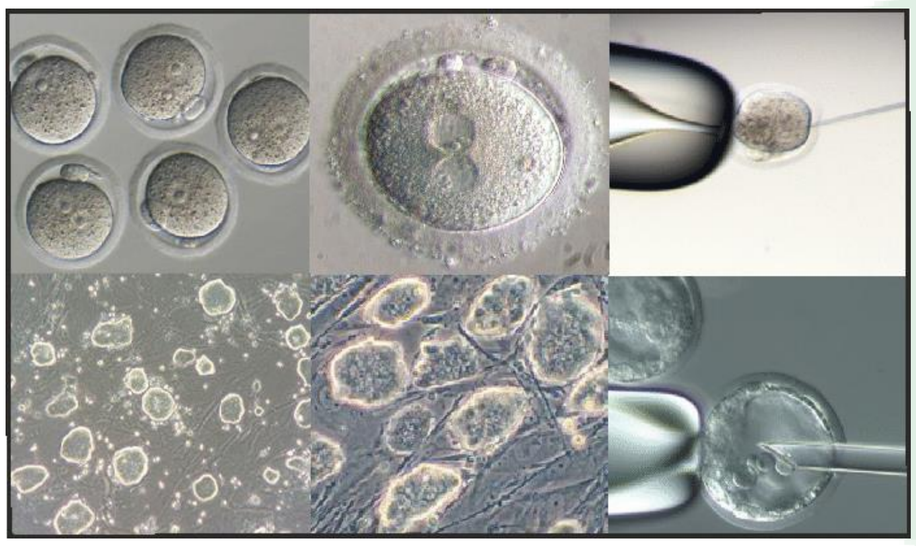
Ortotopic Injection of Colon Cancer cells



The IVIS® SpectrumBL is an advanced high-throughput 2D and 3D optical imaging system designed to improve quantitative outcomes of bioluminescence, chemiluminescence and Cerenkov *in vivo* imaging. The SpectrumBL supports 10 mice simultaneous imaging for true high-throughput imaging for longitudinal studies to support large cohorts of mice. It uses unique optical imaging technology to facilitate non-invasive longitudinal monitoring of disease progression, cell trafficking and gene expression patterns in living animals.

Image Number	ROI	Image Layer	Total Counts	Avg Counts	Stdev Counts	Min Counts	Max Counts
LT20160928103425	ROI 1	Overlay	6,54E+04	7,78E+02	6,61E+02	2,70E+02	3,36E+03

TRANSGENIC FACILITY



Pronuclear Injection

A plasmid DNA is injected into the pronuclei of fertilized mouse eggs to produce transgenic mice in which the transgene is randomly integrated into the mouse genome. The embryos are transferred into foster mothers and the resulting pups will be genotyped by PCR to identify founder mice for the presence of the transgene.

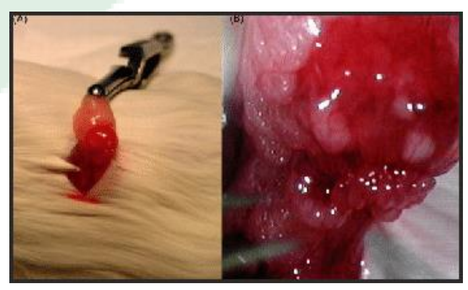
Gene targeting in ES-cells

Embryonic Stem (ES) cells are electroporated with a DNA targeting vector to introduce a specific mutation in the mouse genome by homologous recombination in the correct locus. Selected clones after antibiotic selection, are picked for screening and freezing. Correctly modified clones are expanded for blastocyst injections. The resulting mouse is a chimera of two different cell types: the host embryo and the injected ES cells. The chimeric mice are mated with wild-type mice to transmit the desired mutation to the offspring, introducing the desired mutation to the germ line.

Cryopreservation

Cryopreservation purpose is to protect against loss of valuable unique mouse lines and cost reduction of maintaining mouse lines that are not in use. Our facility offer sperm freezing as a default method for Cryopreservation for its rapidity and need of a small number of animals.

Re-derivation of mouse strains



The presence of any type of mouse pathogen (whether it causes clinical disease or not), changes the physiology of the mouse, which can lead to false experimental results. Mouse strains harboring pathogens can be 'rederived' to a pathogen-free status by transferring pre-implantation embryos from a contaminated mouse to a pathogen-free foster mother. The resulting pups will then also be pathogen-free.

We offer homozygous and heterozygous mouse re-derivation.