



Camille Fouassier
Manager of the platform



Michel Wassef
Scientific Director
Tel: +33 (0)1 56 24 65 57



Raphaël Margueron
Scientific Director
raphael.margueron@curie.fr
Tel: +33 1 56 24 65 51

The last few years have seen the emergence of new genome modification technologies. The most recent one, CRISPR/Cas9, allows, by its great flexibility, to introduce mutations on all known genes. Since September 2017, the CRISPRit platform takes advantage of the CRISPR technology to perform lentiviral-based genetic screens initially in cell lines. Current development aims at

applying this approach to organoids or patient-derived xenografts.

Genetic screens are powerful tools that enable the genome-wide interrogation of gene function, through CRISPR-based mutagenesis or transcriptional modulation (activation or repression).

The technology can be used to perform two types of screens:

- In positive-selection screens, the aim is to identify genes whose mutation, silencing or overexpression, gives a positive advantage over a given selective pressure (e.g., a drug). Positive screens are typically used to study the mechanisms of drug resistance in cancer cells. They can also serve in the study of various cellular processes as long as a positive pressure is robust (e.g., antibiotic selection, fluorescence-activated cell sorting – FACS).
- In contrast, negative-selection screens aim at identifying genes that are essential for survival or proliferation under defined conditions. Such screens are often used to identify context-dependent gene essentiality (e.g., synthetic lethal dependencies).

CRISPR libraries (genome-wide or custom) are delivered as a lentiviral pool onto cells and sgRNA representation is determined by high-throughput sequencing (HTS) before and after selection. Importantly, this pooled approach enables the use of both genome-wide and custom libraries (e.g., on a family of genes). A bioinformatic analysis then determines sgRNA enrichment (positive screens) or depletion (negative screens).

Missions

Because each project is unique, the CRISPRit platform advises scientists in their screening projects and oversees the whole screening procedure from library preparation to bioinformatic analysis. This entails several steps:

- 1- Library preparation (genome-wide or custom) and quality control
- 2- Virus production and titration
- 3- Infection of target cells and library preparation
- 4- HTS by the Next Generation Sequencing platform and quality control
- 5- Bioinformatic analysis

Collaborations

The CRISPR'it platform works in close collaboration with the NGS and Bioinformatics platforms of the Institut Curie. They are used to define candidates coming out of a screen.

The quality control and bioinformatics analysis are respectively carried out by Marc Deloger and Pierre Gestraud (see photo below).

