CRT Technology Core (CRT-TC)

Technology Core of the Institut Pasteur Center for Translational Science (*Centre de Recherche Translationnelle*) supports translational and fundamental research projects by providing access to 30 state-of-the-art technologies. Our technologies are selected to permit cell phenotyping and sorting, molecular profiling and experimentation at single cell level. The technologies are installed in six dedicated laboratories, enabling optimal experimental setup and sample analysis. A significant part of the equipment is situated within two fully equipped Bsl2+ cell culture laboratories allowing manipulation of human and infectious material. Different approaches in the fields of molecular medicine and biomarker discovery are supported. Since 2011 the Technology Core is ISO9001 certified providing quality assurance of our service. Our affiliation to the Center for Translational Science allows a close collaboration with the Clinical Coordination team and a support in regulatory aspects of clinical projects engaged at the CRT Technology Core.

Our support for technologies involves training for autonomous use of the equipment; help with data analysis and data management; advice; technical support; scientific collaborations and service (for a selection of the technologies). In order to facilitate the analysis of large-scale data generated by our technologies, the CRT-TC provides training and access to different data analysis software, as well as an adapted bioinformatics support.

The CRT-TC hosts projects and teams from all departments at the Institut Pasteur, International Network of the Institut Pasteur as well as collaborators from academia and industrial partners.

Below please find an extensive list and short description of our technologies and software.

Protein Identification & Quantification

Application	**Technology** (in blue if situated in Bsl2+ environment)	**Description**
Medium-throughput Protein quantification	Bioplex 200(BioRad)	Bead-based multiplex ELISA technology xMAP that utilizes cytometry technology for simultaneous quantification of up to 100 analytes in a single microplate well.
Ultrasensitive digital ELISA	Simoa HD-1 Analyzer (Quanterix)	Fully automated instrument for running up to 4-plex immunoassays. Digital readout of ELISA, trapping and sealing individual immunocomplexes on paramagnetic beads in thousands of femtoliter sized wells. Allows protein biomarkers detection as low as fM
Medium-throughput Protein quantification (sample preparation)	Curiox	DropArray plate (hydrophobic surface with 96 hydrophilic spots). Use in combination with the BioPlex200 system to perform immunoassays on drop. Less Reagents and less samples used.

Cell Phenotyping & Sorting (part I)

Application	Technology	Description
Flow cytometry (analyzers)	LSR Fortessa (BD)	Equipped with five laser lines (355nm 405nm, 488nm, 561nm and 640nm) and 18 fluorescent detectors
	LSR II (BD)	Equipped with five laser lines (355nm, 405nm, 488nm, 561nm and 640nm), 18 fluorescent detectors and an HTS unit for high-throughput screening in 96-well plates
	Gallios (Beckman Coulter)	Equipped with three laser lines (405nm, 488nm, 638nm)
	Cytoflex (Beckman Coulter)	Equipped with four laser lines (405nm, 488nm, 561nm, 638nm) and a plate reader
	Symphony A3 (BD)	A powerful tool for identification and analysis of distinctive phenotypes in heterogeneous populations based on a simultaneous measurement of up to 28 different parameters of a single cell. Equipped with five laser lines (355nm 405nm, 488nm, 561nm and 640nm) and 28 fluorescent detectors. The only cytometer able to detect up to 8 violet parameters and 6 UV parameters.
	SP6800 (Sony)	Emerging technology that revolutionizes the cytometry by identifying specific fluorochrome "signatures" and by eliminating need for compensation. Equipped by 3 lasers (488nm, 635nm and 405nm), SP6800 collects the entire fluorescence spectrum emitted from a sample and deconvolutes it to allow a detection of up to 25 parameters per cell. The sensitivity and specificity of measurement are increased by an efficient removal of cell auto-fluorescence;
Imaging cytometry	ImageStreamX , ISX (Amnis)	Integrates flow cytometry (3 lasers) and microscopy (60x magnification) to simultaneously produce 12 images of each cell directly in flow, at rates exceeding 15 000 cells/min. Enables quantification of cell shape changes, internalization, apoptosis, nuclear translocation, co-localization, cell-cell interactions, and much more.
Live cell imaging	Incucyte (Essen Bioscience)	Quantitative live cell imaging in a standard incubator using any traditional culture plate/dish. IncuCyte supports HD phase-contrast, green fluorescence and red fluorescence at 20x magnification. With its remote control and access to the real-time data it is a perfect tool for high content screening of effects of drugs, viruses, cytokines and other effector molecules.

Cell Phenotyping & Sorting (part II)

Application	Technology	Description
Cell metabolism	XFp Analyzer (Seahorse)	Simultaneous analysis of mitochondrial respiration and glycolysis in 96-well plates. With four programmable ports for the addition of liquid media during active assay measurements, it is ideal for the study of chemical-, adjuvant- or pathogen-induced stress.
Cell sorting	3 FACSAria III (BD) 1 FACSAria II (BD)	Equipped with 5 lasers (355nm, 405nm, 488nm, 561nm and 640nm), they allow bulk- or single-cell sorting into 96-well and 384 well plates with "index sorting" for downstream single cell applications.
	ViroFACS (BD)	FACSAria III sorter with a special configuration, designed for sorting of micro-particles and viruses. Equipped with four lasers: 633nm, 561nm, 405nm and a high power 488nm laser (400mW) that enables detection and sorting of micro-particles. A Special DFS (Digital Focusing System) module ensures optimized focalization for detection of elements with < 300nm of size.
	2 Astrios (Beckman Coulter) (under biosafety cabinet)	Equipped with 5 and 7 lasers / 7 pinholes configurable system; 355nm, 405nm, 488nm, 532nm, 561nm, 592nm, 642nm, it allows high speed, six-way sorting of animal, human samples and/or infectious material.
Magnetic cell sorting	1 AutoMACS (Miltenyi) 1 AutoMACS (Miltenyi)	rapid magnetic enrichment or depletion of cell populations

Molecular Phenotyping (part I)

Application	Technology (in blue if situated in Bsl2+ environment)	Description
DNA/RNA quantification	Qubit ® 2.0 Fluorometer (Life Technologies)	Sensitive quantification of DNA, RNA & proteins using fluorescent dyes. Qubit allows distinguishing between DNA, RNA or free nucleotides and the measurement is not affected by the presence of co-purified contaminants.
RNA and DNA quality/quantity	2100 Bioanalyzer (Agilent)	Chip-based capillary electrophoresis machine for quantification and quality control of DNA, RNA, & proteins. It allows RNA quality control measurements before downstream experiments.
Medium-throughput gene expression profiling	BioMark (Fluidigm)	Microfluidic-based technology for gene expression analysis (96 genes*96 samples), SNP genotyping, digital PCR, mutant detection and real-time PCR to qualify and quantitate samples prior to next-generation sequencing.
Single-cell cDNA preparation	C1 (Fluidigm)	Microfluidic-based technology that enables single cell preparation, RNA extraction, reverse transcription and PCR amplification using just one technology and thus reducing the variability caused by multi-platform technical errors. It supports multiple single-cell applications, allowing global transcriptome analysis, targeted gene expression, miRNA analysis and whole genome amplification for DNA sequencing.
Single cell functional assays	Polaris (Fluidigm)	Innovative technology for single cell functional genomic studies: it integrates cell biology and molecular analysis, by allowing single-cell selection, dose response, time course studies and quantitative gene expression preparation in a single chip. Correlation of single-cell gene expression with phenotypic information and exploration of molecular pathways that regulate cell function is supported. A selection of up to 48 single cells from a heterogeneous suspension is followed by culturing of cells (up to 24h) in an environmentally controlled chamber. The cells can be challenged with 6 various conditions (chemicals, microbes…). After the required incubation the single cell mRNA is isolated and cDNA synthetized for downstream single-cell gene expression profiling.
High-throughput gene expression and genotyping	iSCAN (Illumina)	A high-precision scanner for SNP genotyping, CNV analysis, DNA methylation, and gene expression profiling.

Molecular phenotyping (part II)

Application	Technology (in blue if situated in Bsl2+ environment)	Description
Gene expression profiling	nCounter Dx Analysis System (NanoString Technologies)	A digital color-coded barcode technology for direct multiplexed measurement of gene expression. It allows up to 800 mRNA, miRNA or DNA targets to be simultaneously profiled based on molecular "barcodes" and single molecule imaging, with high level of precision and sensitivity (<1 copy per cell), without a need for reverse transcription or pre-amplification.
Next generation sequencing	Ion Proton & Personal Genome Machine (Life technologies)	Ion semiconductor sequencing for exome, genome & transcriptome high throughput sequencing at reduced cost and complexity. The technologies are completed with the Ion Chef System to enable automated, high-throughput template preparation and reproducible chip loading. The sequencing run times of <4h and 200bp reads of up to 10Gb (Ion Proton) and <7h and 400 bp reads of up to 2Gb (PGM) are provided.
Medium-throughput transfection	Amaxa (Lonza)	Nucleofection in 96 well shuttle

Software available at the CRT-TC

Commercial software:

- Qlucore Omics Explorer (Qlucore) – a statistical software package optimized for gene expression studies and principal component analysis
- IDEAS (Amnis) – software developed for the analysis of ImageStreamX data; it evaluates over 35 features for each image and over 200 features per cell and integrates population statistics and imagery – every dot on a scatter plot links directly to a cell's images
- FlowJo (Tree Star) – the tool of choice for the manual analysis of flow cytometry data
- Kaluza (BCI) – flow cytometry analysis software solution designed for analysis of high content data (Radar plot/ Tree plot)
- Diva (BD) - for flow cytometer and application setup, data acquisition, and data analysis that help streamline flow cytometry workflows
- SP6800 analysis software (Sony) – the tool of choice for the manual analysis of spectral cytometry data

Open source software and data analysis pipelines:

- R/RStudio and specialized pipelines for (1) Microarray data analysis, (2) Unsupervised and Supervised Cytometry data analysis and (3) Single cell RNAseq data analysis.