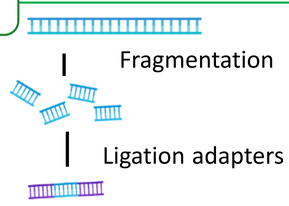
Next Generation Sequencing facility

Vanessa Rouglan, Frederic Martins, Alexandre Brochard, Alexandre Favereaux, Thierry Leste-Lasserre

Introduction

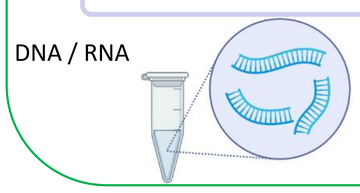
The platform was created in 2021 through a collaboration between IINS and the Neurocentre Magendie.
Its function is ensured by Thierry Leste-Lasserre (Platform Manager), Alexandre Brochard (Bioinformatician), Vanessa Rouglan and Frederic Martins (Technical Managers) and Alexandre Favereaux (Scientific Manager) and includes all steps from sample extraction to data analysis. The platform is at the forefront of technology and includes dedicated automated systems : one for library preparation and a second for quality check. We offer a turn-key service with a wide range of techniques from RNA analysis to DNA.

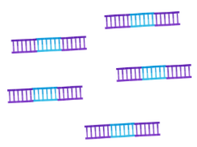
Overview

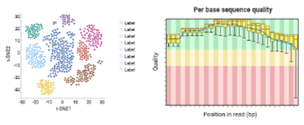


1.Project meeting → **3**.Libraries preparation → **5**.Sequencing

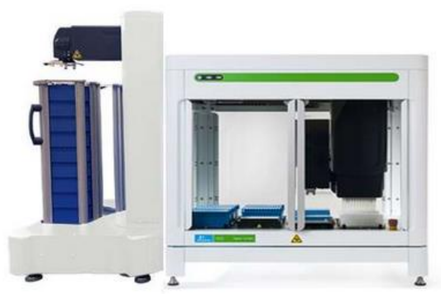
2.Extraction → **4**.PCR → **6**.Data analysis







Equipment

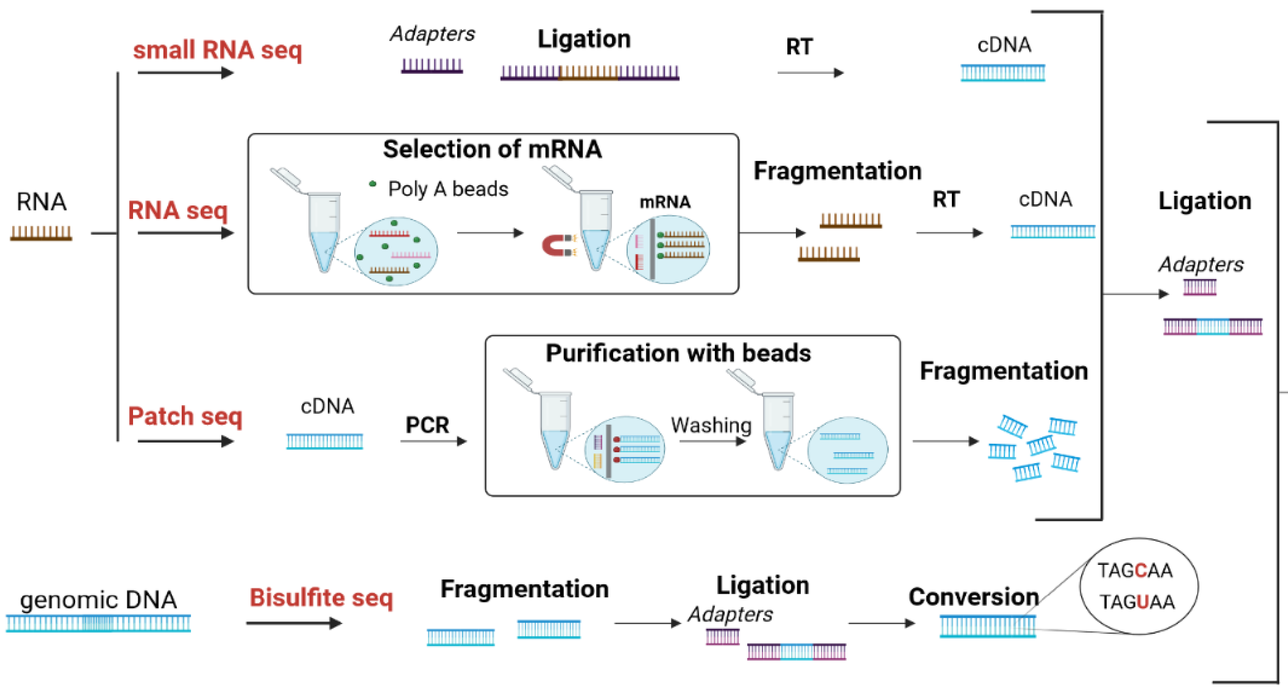


Zephyr NGS IQ – PerkinElmer

Labchip GX-touch - PerkinElmer

*The **Zephyr** is a robot used for library preparation, extraction and PCR*
*The **Labchip** is a robot used to check the quality of cDNA, RNA and libraries*

Sample Preparation



Types of analysis:

- **Analysis of micro-RNAs**: **small RNA-seq**
- **mRNA analysis**: **RNA-seq**
- **Single neuron analysis**: **Patch-seq**

Association of Patch-clamp and RNA-seq techniques at the single cell scale. Patch-clamp is a technique that uses a glass pipette adhering to the surface of the cell and allows the content of the cell to be aspirated

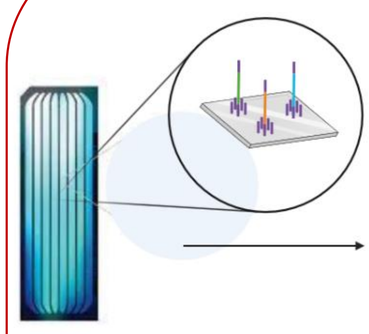
- **DNA methylation analysis**: **Bisulfite-seq**

This technique consists of treating the gDNA with sodium bisulfite, which converts Cytosine into Uracil and then into Thymine after amplification allowing the detection of methylation in the genome

Types of samples:

- **Fresh tissue**
- **Frozen tissue**
- **Bulk tissue**
- **Brain slices**

Sequencing



Flow Cell

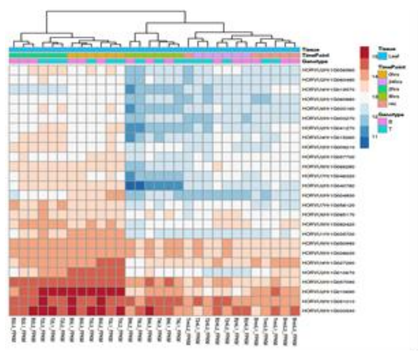
NextSeq 2000

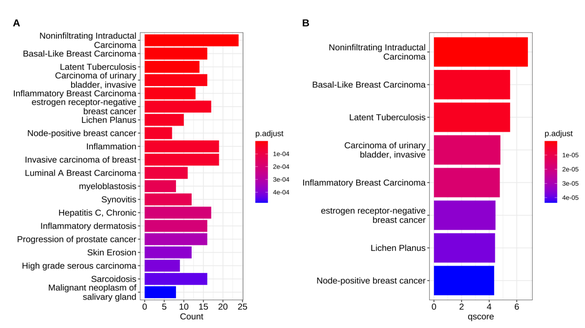
- Sequencing consists of the identification of each nucleotide, within a sample
- Each fragment has a unique index which allows us to combine different samples, this process is called multiplexing
- The next generation sequencing (NGS) allows various samples to be sequenced faster and at the same time
- The platform works in collaboration with the Platform Genome Transcriptome of Bordeaux (PGTB) to sequence the libraries. It has been equipped since November 2021 with a NextSeq 2000 sequencer

PGTB SEQUENCAGE | GENOTYPAGE

Data analysis

The first step of sequence alignment and read counting requires the use of a computing cluster. We use the Curta cluster provided by the Mesocentre de Calcul Intensif Aquitain (MCIA), with automated scripts allowing faster results.
The second analysis is based on the comparison of groups of samples to a reference group, and use the module DESeq2 to detect differentially expressed genes and provide fold changes and p-values to determine the most significant differences. From this analysis, the results can be represented with several types of graphs to help with quality control (PCA, Heatmap...) or the search for biological information (Gene Ontology, Pathway…).





Data analysis is performed by Brochard A., Martins F. and Favereaux A.

They trust us

Contacts

ngs.u1215@inserm.fr
Frédéric Martins
Tél: +33 (0)5 57 57 36 79

thierry.leste-lasserre@inserm.fr
Tél: +33 (0)5 57 57 36 67
Neurocentre Magendie - INSERM U 1215
146, rue Léo Saignat - 33077 Bordeaux cedex