



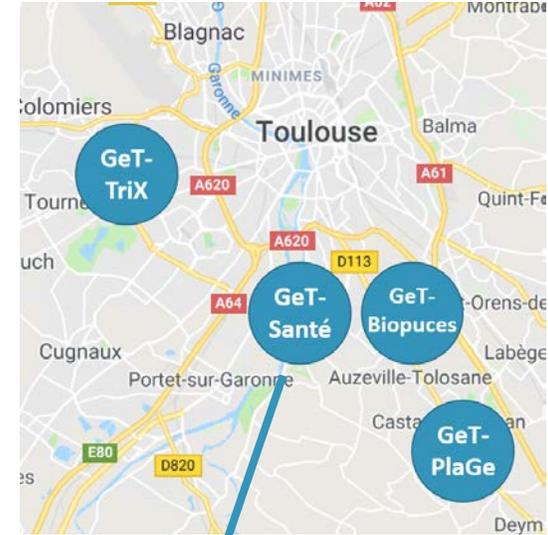
# Journée Single Cell

## 10/03/2020



⑤ Plateforme Génome et Transcriptome (GeT) de Genotoul, localisée sur 4 sites :

- GeT-Santé: *I2MC-CHU Rangueil*
- GeT-PlaGe: *INRA Auzeville*
- GeT-Biopuces: *INSA-TBI*
- GeT-TRIX: *ToxAlim- INRA- Saint Martin du Touch*



⑤ Une équipe de plus de 40 personnes

⑤ QC

⑤ QPCR, microarrays, ddPCR

⑤ NGS courts et longs fragments,

⑤ Single Cell...



GeT-Santé





GeT-Santé is one of the 4 sites of the Genome & Transcriptome core facility. Mainly focused on the health research community, this facility provides :

- Technological advising and realization of **Single-Cell omic analysis** and **Next Generation Sequencing projects**
- **Stand-alone use** after training to tools for the transcripts quantification and sample quality control

### Single cell omics

Technological advice, guidance, and realization of projects in Single-Cell analysis.

Technologies available :

C1 (Fluidigm) or Chromium (10x Genomics)

Applications : gene expression profiling, DNA-seq, mRNA-seq (3' or full length)

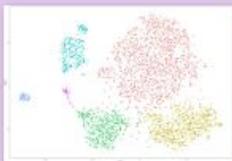
Possible interactions with others Genotoul facilities



#### 3'-scRNA-seq projects

15 projects already achieved

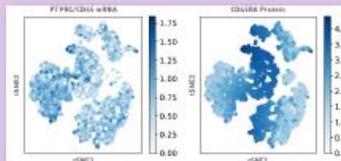
- on different cell types : monocytes, lymphocytes, blasts, stromal cells, stem cells, neurons...
- from variate organisms : human, mouse, pig, drosophila, zebrafish...



#### CITE-seq projects

Gene expression profiling and cell surface protein quantification

2 projects in progress on mice thymocytes and B lymphocytes



#### Upcoming developments

ATAC-seq, cell fixation improvement, spatial transcriptomics, immune profiling, full length RNA sequencing with Oxford Nanopore technology

Publications : Veerman K & al., Cell Reports, 2019; Pizzolato G & al., PNAS USA, 2019

### Next-Generation Sequencing

Custom project management and personalized support for unusual applications, using the wide range of NGS instruments available on GeT.



#### Genomic sequencing

Targeted sequencing with custom panels (AmpliSeq)

#### RNA sequencing

- meet the needs for RNA sequencing of total RNA with ribodepletion
- gene expression profiling dealing with low input RNA

#### ChIPseq projects

- support teams for pilot steps and libraries production
- creation of a working group on ChIPseq application and its variants

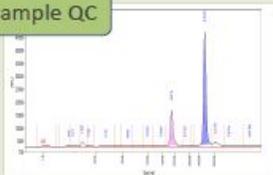
#### Upcoming developments

Handling very low input samples or degraded samples in NGS

Publication : Pichery M & al., Human Molecular Genetics, 2017

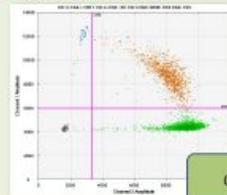
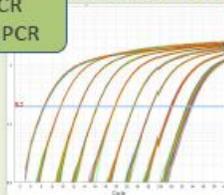
### Stand-alone use & training

Sample QC



Publication : Barquissau V & al., Cell Reports, 2018

qPCR  
HD qPCR



ddPCR

Training and events organization





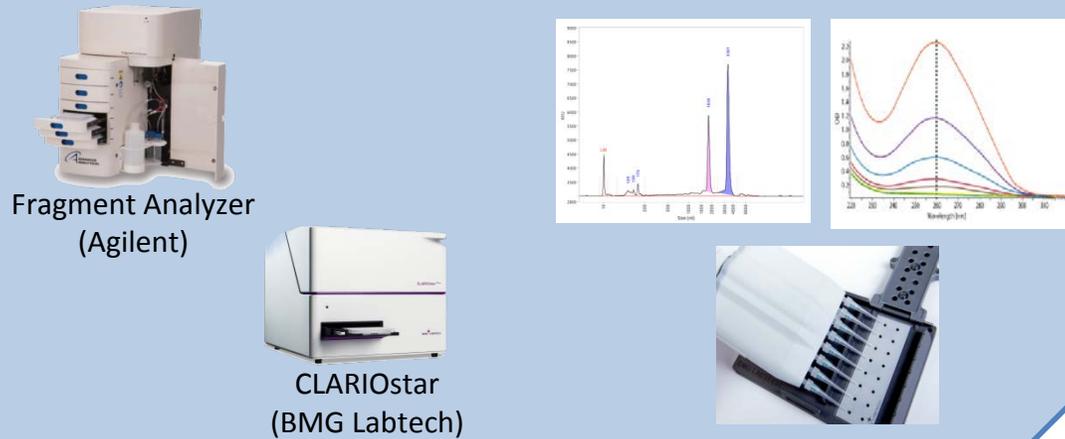
Pôle *tech*  
TECHNOLOGIQUE  
CRCT

**Pôle Technologique du CRCT**  
**9 plateaux**  
**15 personnes**

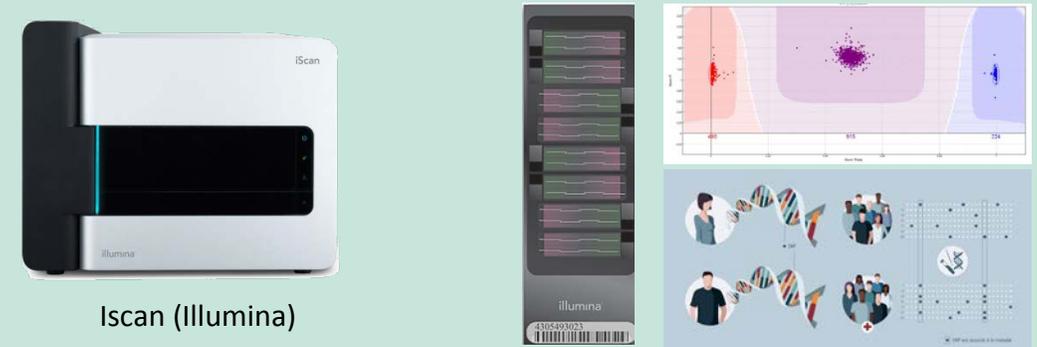
Génomique et Transcriptomique  
Bio Informatique  
Imagerie Cellulaire  
Cytométrie et Tri Cellulaire  
Protéomique  
Vectorologie  
Irradiateur  
Production d'Anticorps Monoclonaux  
Centre de Ressources Biologiques



✓ **Contrôle qualité des acides nucléiques (ADN, ARN)**



✓ **Etude du méthylome et du polymorphisme nucléotidique SNP, sur puces Illumina**



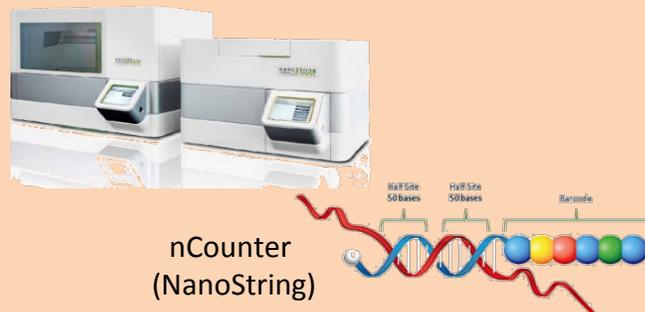
✓ **Mesure de l'expression des gènes (ARN)**

**Faible débit**  
 Appareils qPCR 96 puits  
 Quelques cibles



StepOne Plus (ThermoFisher)

**Moyen débit**  
 Quantification directe  
 Jusqu'à 800 cibles  
 Panels prédéfinis ou à façon



nCounter (NanoString)

✓ **Mesure de l'expression des gènes (ARN)**  
**Haut débit : Des milliers de cibles**

Puces Expression Transcriptome



GCS3000 (Affymetrix)

Production de bibliothèques RNAseq bulk et single cell RNAseq (CITE-seq, Dextramer...)



Chromium Single Cell (10X Genomics)

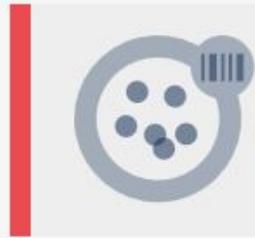
Séquençage NGS



Séquenceur NextSeq550 (Illumina)

# Applications Chromium 10x Genomics

## SINGLE CELL TRANSCRIPTOMICS



### Single Cell Gene Expression

3' gene expression profiling at scale with single cell resolution.

Now with Feature Barcoding technology!



### Single Cell Immune Profiling

V(D)J repertoires of T and B cells integrated with 5' Gene Expression.

Now with Feature Barcoding technology!

## SINGLE CELL EPIGENOMICS



### Single Cell ATAC

Chromatin accessibility and transcriptional regulation at the single-cell level.

## SINGLE CELL GENOMICS



### Single Cell CNV

Copy number variation and genomic heterogeneity at single cell resolution.

## SPATIAL TRANSCRIPTOMICS



### Spatial Gene Expression

Simultaneous analysis of molecular and imaging data from tissue sections.

1<sup>er</sup> tests : 23 Mars 2020

# Les types cellulaires

- **Types cellulaires humains :**
  - Myéloblastes, plasmocytes, lymphocytes T, cellules souches mésenchymateuses MO, lymphocytes B de rate, PBMC...
- **Types cellulaires murins :**
  - Lymphocytes T, macrophages résidents du foie, cellules stromales, macrophages TA et MO, neurones entériques, cellules dendritiques de ganglions, monocytes...
- **Cellules progénitrices de neurones de zebrafish**
- **Embryons de truies**
- **Bientôt du végétal ? Cellules souches d'Eucalyptus...**

# Chromium et Séquençage

## Chromium de la plateforme GeT (INRA Castanet)

- Responsable SC : Frédéric Martins
  - NovaSeq6000 de GeT

## Chromium du Pôle Technologique du CRCT (Oncopôle)

- Responsable : Carine Valle
  - NextSeq550 du CRCT

- 1 run 400M reads PE
- 16k cellules à 25k reads/cellule
- données brutes ou FASTQ

# Analyses Bioinformatiques

Si les équipes n'ont pas de forces en bioinformatique :

GeT > Delphine Labourdette, [delphine.labourdette@insa-toulouse.fr](mailto:delphine.labourdette@insa-toulouse.fr) (GeT-BioPuces)

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CPTP > Manuel LEBEURRIER, [manuel.lebeurrier@inserm.fr](mailto:manuel.lebeurrier@inserm.fr)

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