Technological validation of single cell expression analysis

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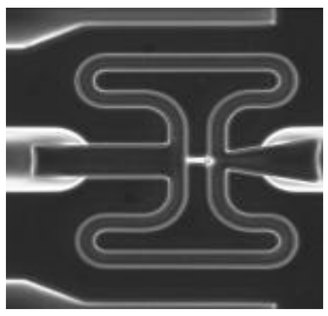
Toulouse « Genome and Transcriptome » core facility (France) acquired and validated the Fluidigm C1 Single-Cell auto prep system. This technology allows separating and preparing up to 96 individual cells for transcriptomic analysis. The workflow, shown here below, includes mRNA extraction from individual cells, reverse transcription and pre-amplification (Specific Target Amplification). The pre-amplified individual cDNAs are then harvested into collection wells and transferred to the BioMark System for quantitative PCR analysis.

TECHNOLOGICAL VALIDATION

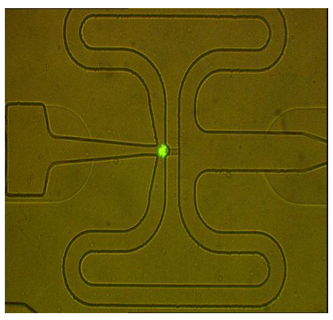
We validated the C1 single-cell auto prep system for:

(A) Detection of the viability of the injected cells.
(B) Cell-capture performance, reproducibility and absence of contamination.
(C) Correlation between single cell expression median and cellular pool expression (used as a positive control).

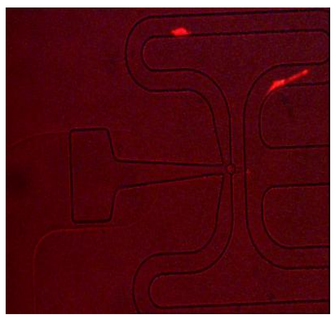
i. Capture site with cell



ii. Live cell properly loaded

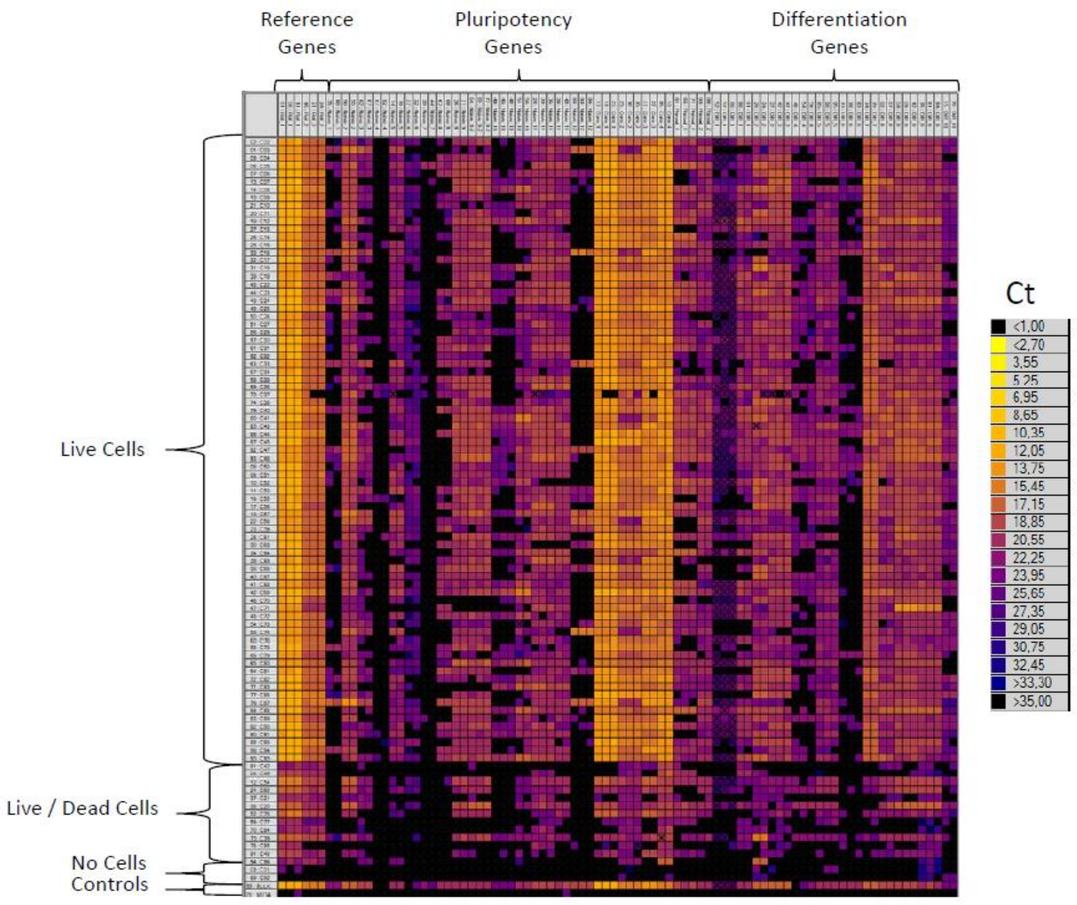


iii. Dead cells or remains



A . Detection of the viability of the injected cells.

Cells loaded in capture sites (i) are stained using LIVE/DEAD Viability Cytotoxicity Kit (LifeTechnologies). Green dye (ii) stains living cells whereas the red dye (iii) only stains cells with disrupted plasmic membrane



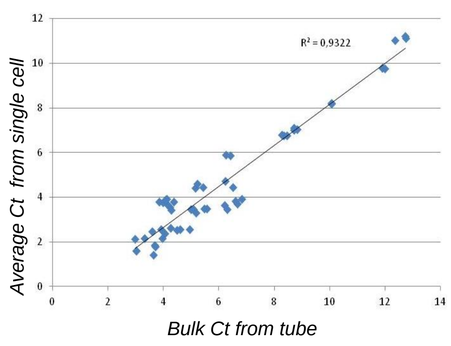
B. Cell-capture performance, absence of contamination and reproducibility

Heatmap represents individual cells and genes on rows and columns respectively. Heatmap colours summarize Ct-values (see legend).

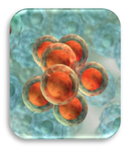
- Cell capture performance can be determined by the number of empty capture sites (« No Cells ») and the number of cells in which the transcription of the reference gene is incoherent (« Live/Dead Cells »). Seventy nine exploitable « Live Cells » remain, more than 80% of the chip.
- Absence of contamination is confirmed by the fact that chambers that do not contain captured cells have low or non-detectable gene expression (« No Cells » above).
- Reproducibility inter C1-array has been assessed by performing four runs on the C1-system with similar rabbit stem line cell. Occupancy rate is over 80% in all four runs.

C. Correlation between single-cell expression median and cellular pool expression.

Single-cell median expression correlates well with the bulk RNA tube control sample (R^2=0.93)



WORKFLOW

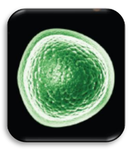


Isolate And Enrich

Pipette into C_1 Array

Load and Stain

Capture Single Cells



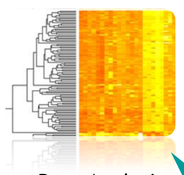
Image

Lyse, Preamplify, and Harvest

Pipette into Dynamic Array

Load IFC

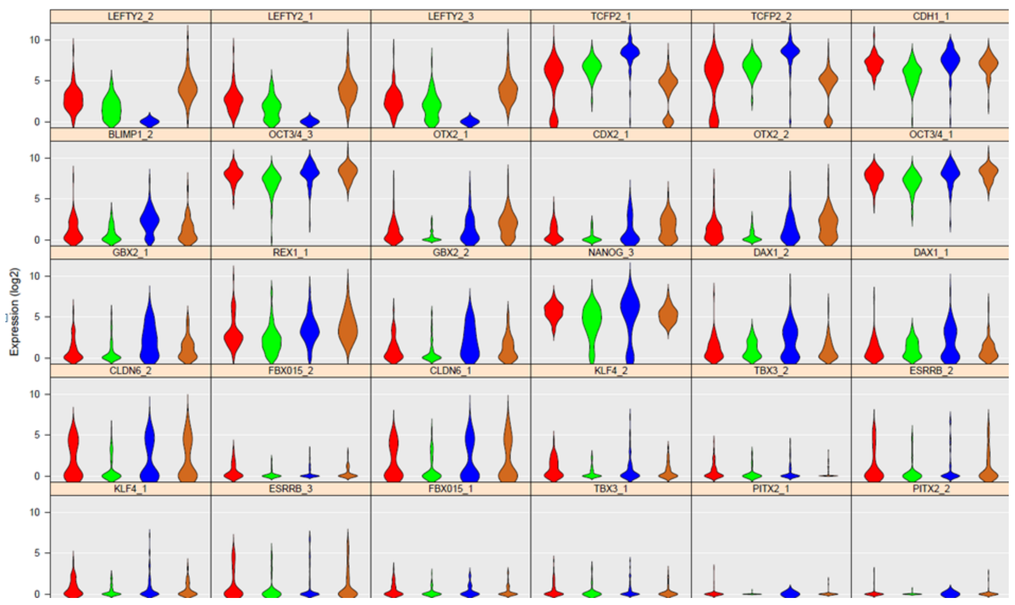
Run on Biomark



Data Analysis

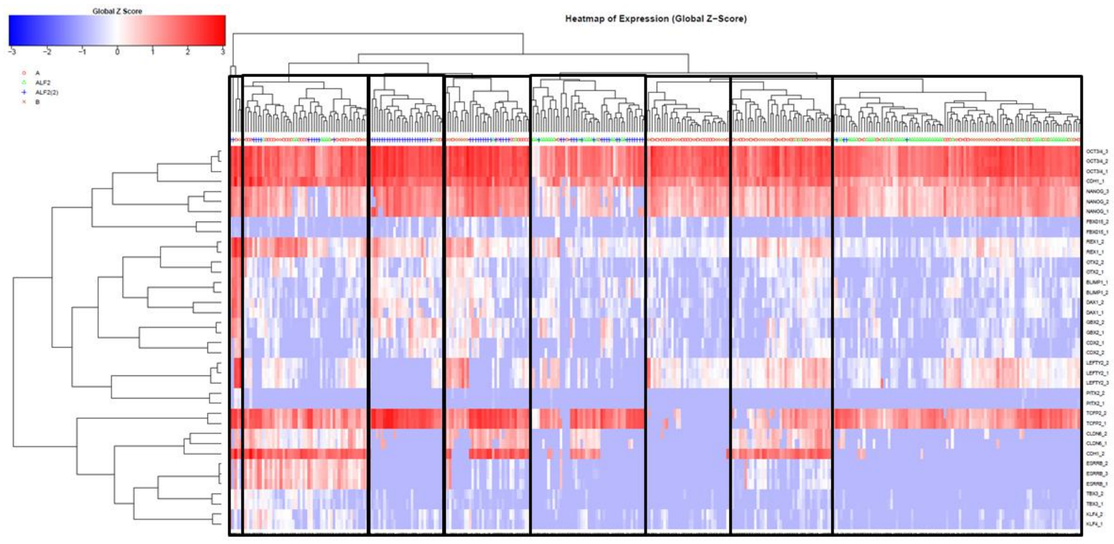
PROJECT

Four rabbit embryonic stem cells lines have been studied. These cells display all the pluripotent stem cells characteristics but also heterogeneity in surface markers and functional characteristics. The goal of this project is to study the heterogeneity of these stem cells lines at the transcriptional level, especially the pluripotency and differentiation genes, using a single cell - qPCR approach.



Data visualisation with violin plots

Violin plots depict the distribution of individual gene expression. Each color corresponds to a cell line. Bimodal distributions indicate that some pluripotency and/or differentiation genes are differentially expressed in at least two subpopulations.



Hierarchical cluster analysis of single cell gene expression

More than 300 cells have been captured on the C1-system and analyzed together. Hierarchical cluster highlights 8 gene expression patterns. The two first on the left bring together cells in which most of the pluripotency genes are expressed : this pattern represents 18% of total cells. Rabbit embryonic stem cells constitute a mosaic population.

CONCLUSION

Results obtained during this validation step are promising.

- Data produced by this system are quantitative, reproducible and consistent with the cellular pool expression.
- The analyzed data reveal a transcriptional heterogeneity within nominally homogeneous cell populations.

PERSPECTIVES

- Achieve single cell approach on a variety of cell types including plant cells or small cells as sperms
- Explore embryonic stem cells and development as well as heterogeneity in tumor progression

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