

Single CTC 3'RNA sequencing using VTX-1, cellenONE and cellenCHIP 384



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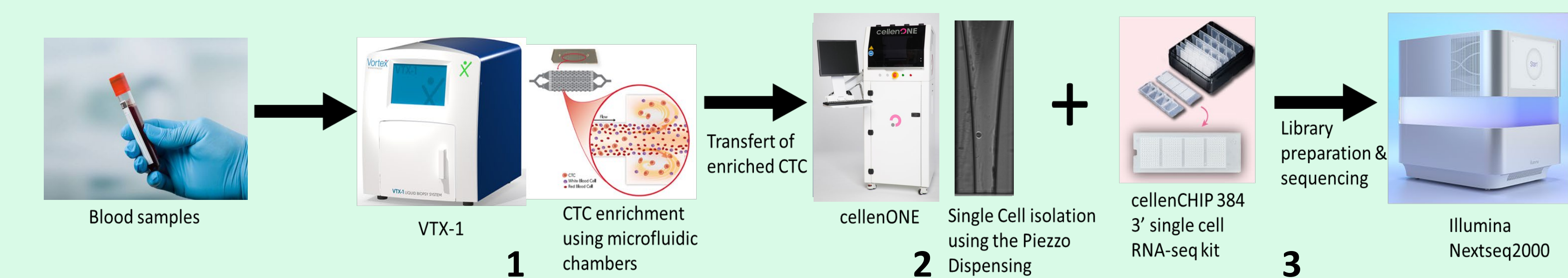


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Introduction

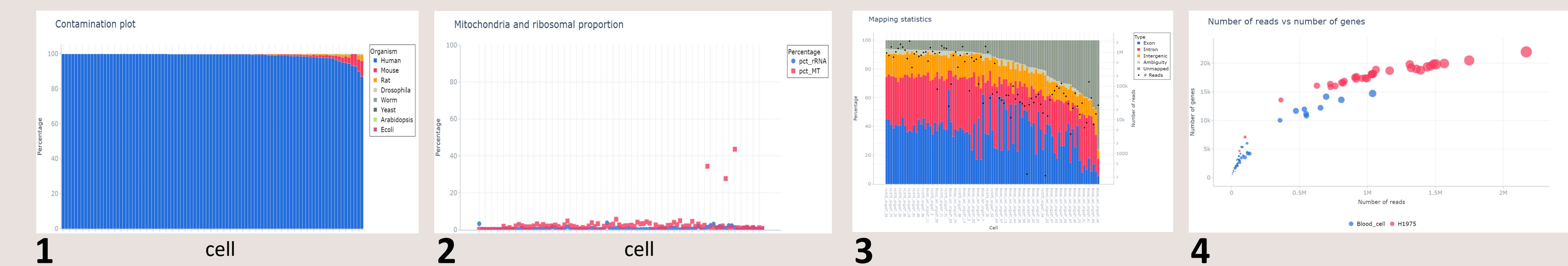
Isolation and genomic characterization of intact circulating tumor cells (CTCs) from cancer patient's bloodstream is thought to hold strong implications in cancer care management. However, accurate CTCs isolation remains difficult due to their low incidence in blood circulation and their extremely low number compared to white blood cells (WBC) (typically accounting for ~1 cell for every 10⁷ WBC). Here we present an integrated workflow for single CTC isolation and sequencing based on CTCs physical properties. CTCs are enriched from blood samples according to their size, shape, and deformability using the VTX-1 Liquid Biopsy System from Vortex Biosciences (1). Enriched CTCs are single isolated from remaining blood cells contaminants according to size and elongation criteria using the cellenONE instrument in enrichment mode (isolates even if there is more than one cell of interest) (2). Single CTC mRNA transcripts are then sequenced on Nextseq2000 following library preparation using the cellenCHIP 384-3'RNA-Seq Kit (3).



Enriched CTC are sorted in an automated manner with the CellenONE according to physical properties without the need of cell labelling

Single cell 3'RNA sequencing of mCTCs spiked in blood

A total of 100 fluorescently labelled non small cell lung cancer tumor cells (H1975) were spiked as mCTCs in 4 ml whole blood and processed using the single cell isolation workflow. Using fluorescence as a control, we determined that a total of 37 mCTC cells and 59 contaminant blood cells (including WBC, erythrocytes and platelets) were spotted in one array (96 wells) of a cellenCHIP 384 before subsequent library preparation and sequencing. A total of 42.5 million high quality reads were obtained following demultiplexing and analyzed using the cellenPIPE proprietary web application and an annotated sample sheet specifying "Blood cells" and "H1975" (<https://cellenpipe.cellenion.com/>).



The contamination plot indicated that for all cells most of the sequences mapped to human genome (1) and a low contamination rate of mitochondria and ribosomal-derived sequences was observed (2). Mapping statistics showed a low rate of unmapped reads for H1975 cells (3). A mean of 17653 and 2520 genes were detected from H1975 cells and contaminant blood cells, respectively, with a mean of 991661 reads for H1975 cells and 44631 reads for contaminant blood cells (4).

High quality sequencing data are produced on mCTCs following the full workflow VTX-1 + cellenONE + cellenCHIP 384 3'RNASeq kit allowing in depth analysis of transcriptomic profile of mCTCs.

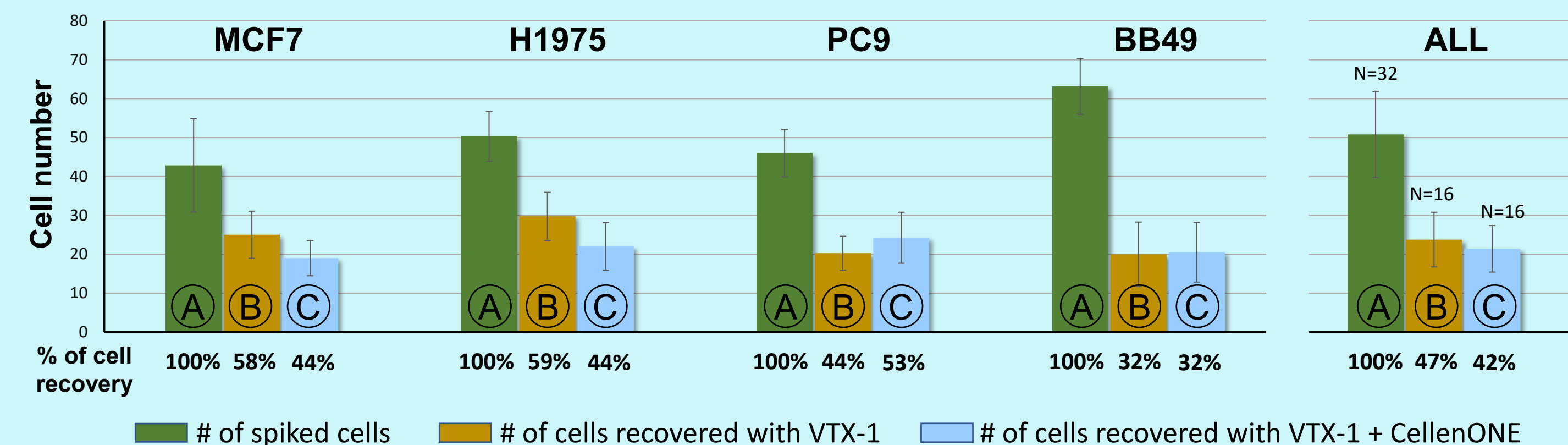
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Evaluation of the recovery of mCTCs

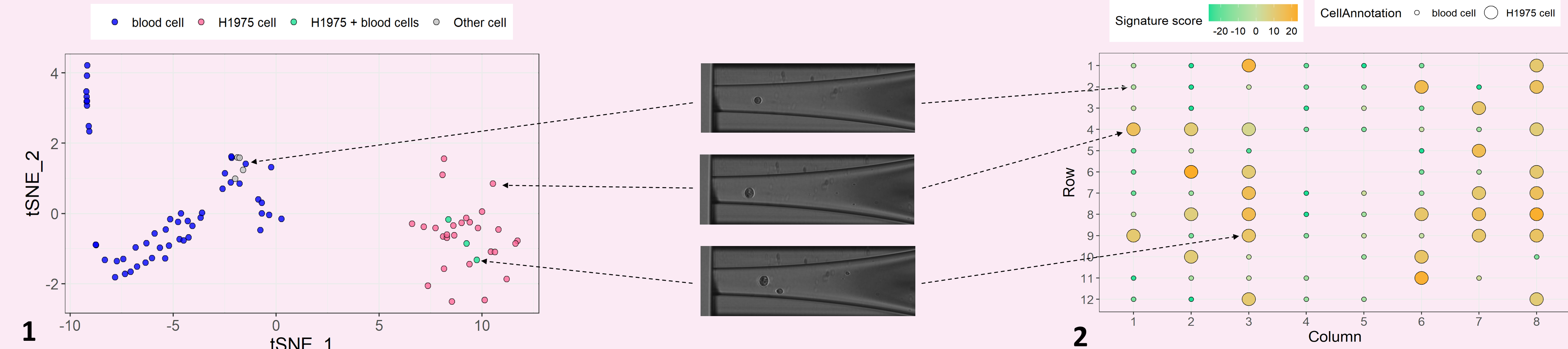
The CTC isolation workflow was validated with cell lines of cancers of various origin (MCF7, H1975, PC9 and BB49) referred as mimicking CTC (mCTC) and spiked in healthy donor blood. For each cell line, 8 replicates containing an average of 50 fluorescently labeled cells spiked in 4mL of blood were processed using the VTX-1 liquid biopsy system (A). Four enrichment replicates were used for direct fluorescent mCTCs enumeration (white arrows) (B), and four enrichment replicates were recovered in 20 µL of PBS+BSA, loaded in the cellenONE for single cell isolation and direct enumeration using the fluorescence signal acquired by the cellenONE in the PDC (C).



- The average recovery of the VTX-1 was 47%
- The average recovery of the cellenONE was 85%
- The average recovery of the full workflow VTX-1 + cellenONE was 42%
- A total of 108.8 (CV=41.4%) contaminating blood cells were also isolated by the cellenONE for each replicate

Identification of mCTCs in spiked blood by sequencing

The transcriptomic profile analysis differentiate 3 groups of cells each consisting mainly of spiked H1975 cells or WBC. Three transcriptomic profiles positive for both H1975 and WBC signatures were visually identified as wells spotted with both a H1975 cell and a WBC (1). A specific gene expression signature score has been used to efficiently differentiate spiked H1975 from white blood cells. Concomitant localization of the 37 fluorescently labeled H1975 in the cellenCHIP was achieved by direct visualization of spotted cells and following bioinformatic analysis based on the specific signature score (2).



Contaminant blood cells were accurately discriminated from mCTCs following tSNE analysis and the cell signature obtained by sequencing was correlated to the visual identification of cells spotted in cellenCHIP enabled by the cellenONE.

Conclusion

The workflow presented here combines the VTX-1 Liquid Biopsy System and the cellenONE for CTC enrichment, automated isolation and single cell 3'RNA sequencing. This workflow is based on physical parameters of the cell and does not require specific labeling of CTC prior to isolation which renders it suitable for exploratory studies. The percentage of recovery of the workflow was in average 42% of mCTCs' spiked-in blood samples. A total of 37 mCTCs were recovered out of 100 from spiked-in blood samples and were subsequently analyzed at the transcriptomic level using cellenCHIP 384 3'RNAseq. Data produced were of very good quality, allowing easy clustering of mCTC vs. other cells. Granting access to this quality of data to scientist is opening an avenue for in depth analysis and characterization of CTCs from any cancer type.