Label-free Enrichment and Integrated Full-length mRNA Transcriptome Analysis of Single Circulating Tumour Cells from Blood Sample

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Background

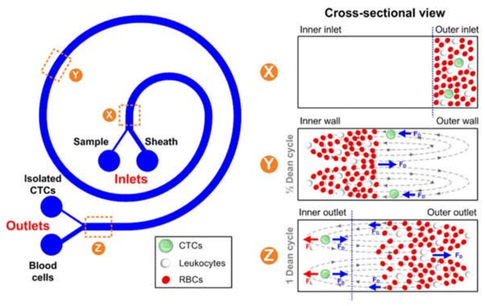
Understanding genetic and functional heterogeneity in tumour cells allows us to gain insight into the mechanisms underscoring drug resistance and tumour aggressiveness. In contrast to invasive primary tumour sampling, a liquid biopsy approach using circulating tumour cells (CTCs) provides accessible tumour material to assess the molecular and phenotypic changes of disseminated tumour cells. In this work, we developed a label-free workflow to isolate CTCs from a breast cancer patient for mRNA-seq analysis.

CTC Enrichment by ClearCell® FX System

ClearCell® FX

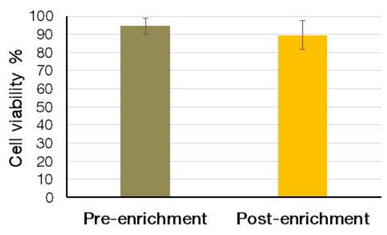
CTChip® FR1

- Label-free isolation method
- Enrichment of CTCs based on *size & inertia*
- Retrieval of wholly *intact & viable* cells
- High purity, *5log$_{10}$ depletion of WBCs*
- Process high blood volume of *7.5 mL*
- Fully automated CTC enrichment platform



Isolation by Size

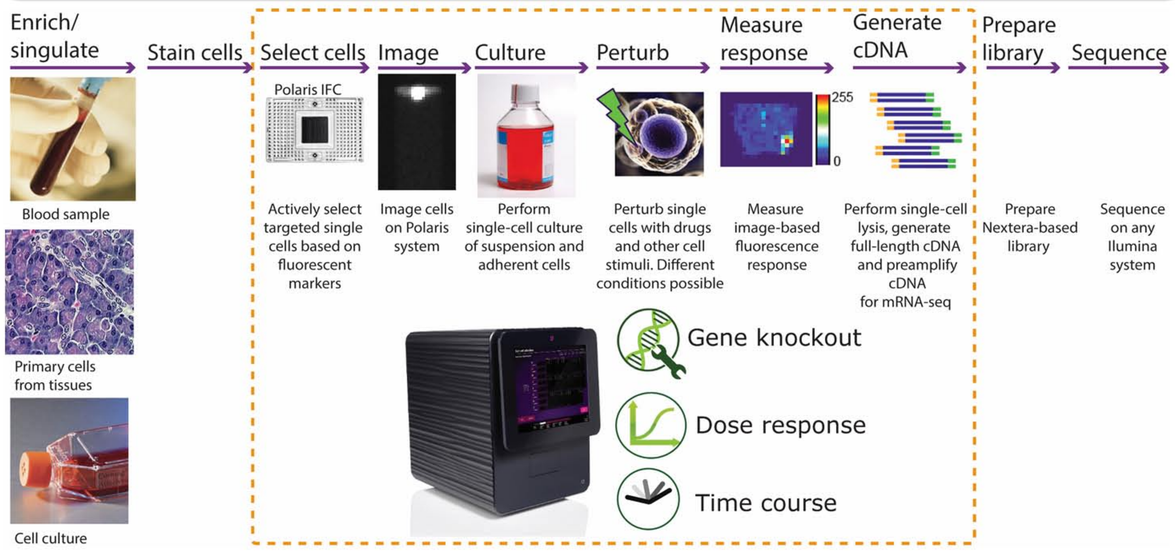
- Smaller blood cells [red blood cells (RBCs) ~8 µm; leukocytes ~8–15 µm] are affected by the Dean drag and migrate to outer wall.
- Larger CTCs (~15–20 µm) experience strong inertial lift forces as indicated by the red arrows and are focused along the microchannel inner wall.



Cell Viability

- High cell viability (89.7%) after sample enrichment of MCF-7 and H1975 cells by ClearCell® FX, as shown by Trypan blue assay (*n* =5). (Figure on left)

Fluidigm® Polaris™ System for Single-cell Functional Studies



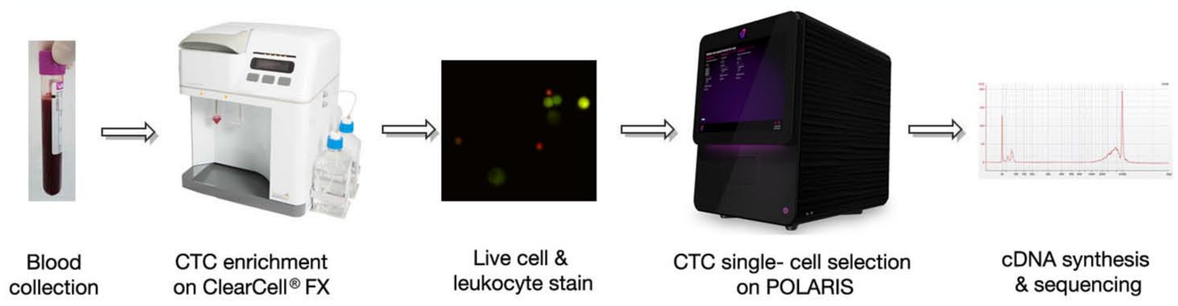
Cell Selection

- Cells can be selected based on universal tracker, viability stain, labeled antibody to cell surface receptor or fluorescent reporter gene.
- Select 48 target single cells (3% to 100% of target : non-target)
- Accepts input of 300–8000 cells per inlet (5 inlets/25 µL per inlet)

Single-cell Transcriptomics

- mRNA-seq analysis: Preamplified full-length mRNA transcriptome/cDNA

Integrated ClearCell® FX and Polaris Workflow



The CTCs were enriched from 7.5 mL of peripheral blood sample using ClearCell® FX, a label-free spiral microfluidics-based system. The enriched cells were stained with Calcein AM (live-cell marker), CellTracker™ Orange (universal marker), and Alexa Fluor® 647 conjugated antibodies for CD45 and CD31. Further selection of CTCs by negative depletion of leukocytes and endothelial cells was accomplished using the Fluidigm Polaris system. Following selection of CTC, the Polaris system generates full-length cDNA for mRNA-seq analysis. Sequencing libraries were constructed using a Nextera® kit and sequenced with Illumina® MiSeq™.

Results

Good-Quality cDNA and Sequencing Library Generated from Spiked SKBR3 Cells

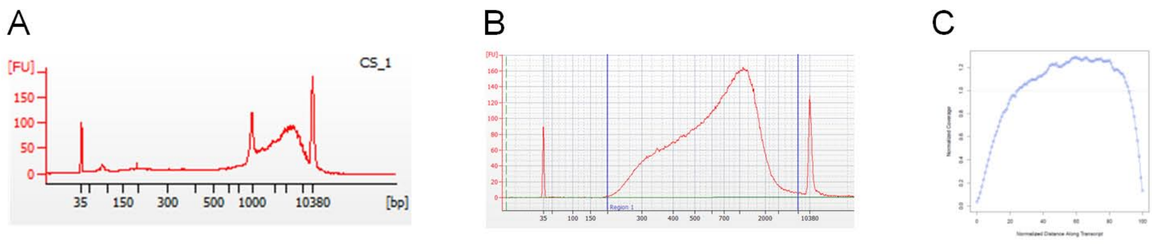


Figure 1. Quality metrics of single SKBR3 cells isolated from workflow and sequenced. (A) Bioanalyzer® (BioA) profile of cDNA generated from single SK-BR-3 cells shows high yield and large fragment size. (B) BioA profile of sequencing library generated from the cDNA shows high yield and expected size. (C) Sequencing coverage at a transcript site shows even coverage. (D) The number of genes mapped per cell was between 5000–9000.

Transcriptome Profiling of CTCs from Breast Cancer Patient Sample

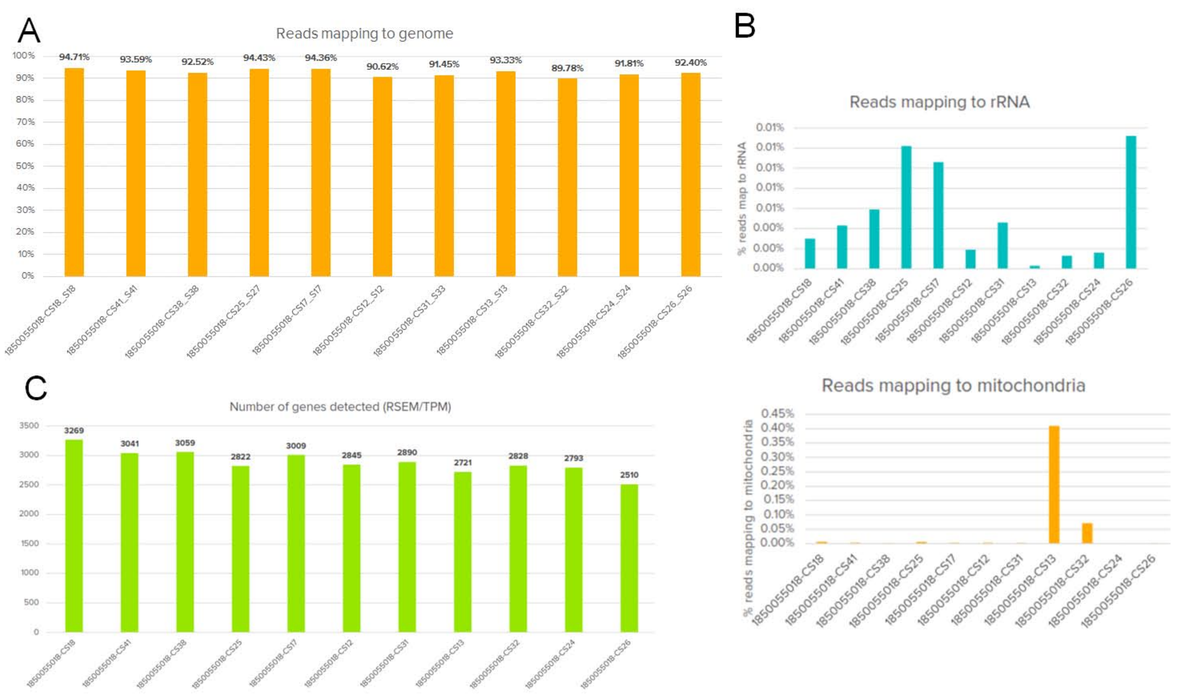
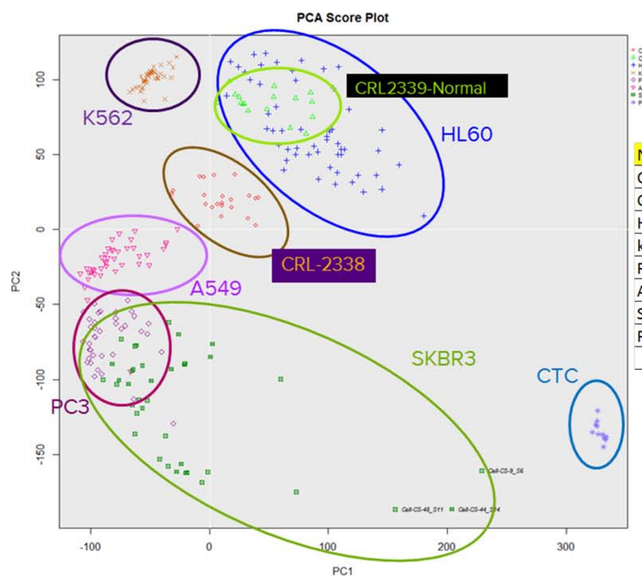


Figure 2. RNA sequencing of single CTCs from breast cancer patient sample. Eleven CTCs from a breast cancer patient blood were singly isolated and sequenced using the ClearCell® FX-Polaris workflow. (A) Percentage of total reads mapping to genome. (B) Low percentage of reads were mapped to ribosomal RNA and mitochondria RNA (contaminants). (C) Number of genes detected per cell.



Name	Cancer type	# single cells
CRL-2338_breast carcinoma	Breast cancer	22
CRL-2339_Paired_Normal	Paired normal for above	17
HL60	Promyelocytic leukemia	54
k562	Myelogenous leukemia	87
PC3	Prostate cancer	38
A549	Lung cancer	41
SKBR3	Breast Cancer	39
P1_CTC	CTC from TNBC patient	11
		309

Figure 3. Principal component analysis (PCA). Cluster plot of CTCs with sequencing results from other cancer cell-lines. Breast cancer cell line, SKBR3, has the closest gene expression profile to the CTCs compared to the other cell types.

Conclusion

We have demonstrated a workflow to isolate CTCs and perform high-quality single-cell mRNA sequencing. The workflow captures viable CTCs individually and could potentially allow CTC culture and functional perturbation studies to be performed at the single-cell level.