Demonstration of RNA Sequencing of Circulating Trophoblast Cells from Maternal Blood

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Abstract # T-194

BACKGROUND

During pregnancy, rare circulating trophoblasts (CTBs) can be identified in maternal circulation. Isolated CTBs have potential to provide a "liquid biopsy" sample of placenta, and thus present a novel opportunity to investigate normal and abnormal placental function via transcriptomic analysis. Here, we present a cutting-edge platform to isolate, collect and sequence CTBs from maternal blood samples.

METHODS

After informed consent, we enrolled healthy participants at 24-42 weeks gestation with no complications at the time of peripheral blood draw (Swedish Medical Center, Seattle). For the current analysis, 40 mL peripheral blood samples were collected in EDTA blood collection tubes. Nucleated cells were isolated (AccuCyte® sample preparation system) and immunomagnetic enrichment of CTBs was performed using a cocktail of antibodies against trophoblast surface proteins. To identify CTBs, the enriched sample was spread on slides, fixed in methanol and stained using a targeted immunofluorescence detection panel. Slides were scanned with the CyteFinder® instrument. CTB identification was based on nuclear morphology, defined staining pattern for cytokeratin (CK) and absence of CD45 (marker of white blood cells, WBC). A cell was classified as "indeterminate" (IND) with minor deviation from the defined criteria or "hybrid" (HYB) with CD45 staining along with CK and other CTB characteristics. Cells were mechanically retrieved from slides using the integrated CytePicker® module, deposited individually into tubes and processed for sequencing following a previously published method (FRISCR). Paired end sequencing was performed on unique dual indexed libraries at an average depth of ~50 million reads, followed by alignment to the human genome/transcriptome and quantification using DRAGEN RNA and Salmon, respectively. Cells were verified as being CTBs or CTB- WBC Hybrids at a molecular level based on detection of a set of 36 literature derived trophoblast markers and the presence or absence of canonical WBC markers. Molecularly verified CTBs with at least 5e+5 total counts were then compared to WBCs using DESeq2 for differential expression analysis.

RESULTS

Pregnant participants (n=48) were enrolled at a median gestational age (GA) of 33 weeks (range 25-40). One participant developed preeclampsia subsequently (blood draw at 25 5/7 weeks GA, preeclampsia diagnosis and delivery at 36 0/7); noted with a * in Figure 1. We identified 48 CTBs that met our criteria for molecular certification as CTBs based on their paucity of WBC marker expression and presence of expression for *KRT8, KRT18, KRT19, EGFR, CGA, MSX2, XAGE2, PGF, DLX3,* and many other genes reported in the Human Protein Atlas[3] and the POPS Placental Transcriptome Databases[4] to have trophoblast specificity. 8 cells met the criteria for CTB-WBC hybrids. Unique genes detected per cell ranged from ~3000 to ~17000 with an average of ~6500 protein coding genes per cell. Differential expression analysis, comparing the top 29 CTBs based on QC metrics to live WBCs sequenced in parallel, revealed 109 genes at a p-adj value of <1E-03. PCA analysis clustered WBC separately from CTBs while live single cells of the JEG3 trophoblast cell line were more similar.

HEAT MAP USING CURATED TROPHOBLAST / WBC GENE LIST BY MOLECULAR CLASSIFICATION

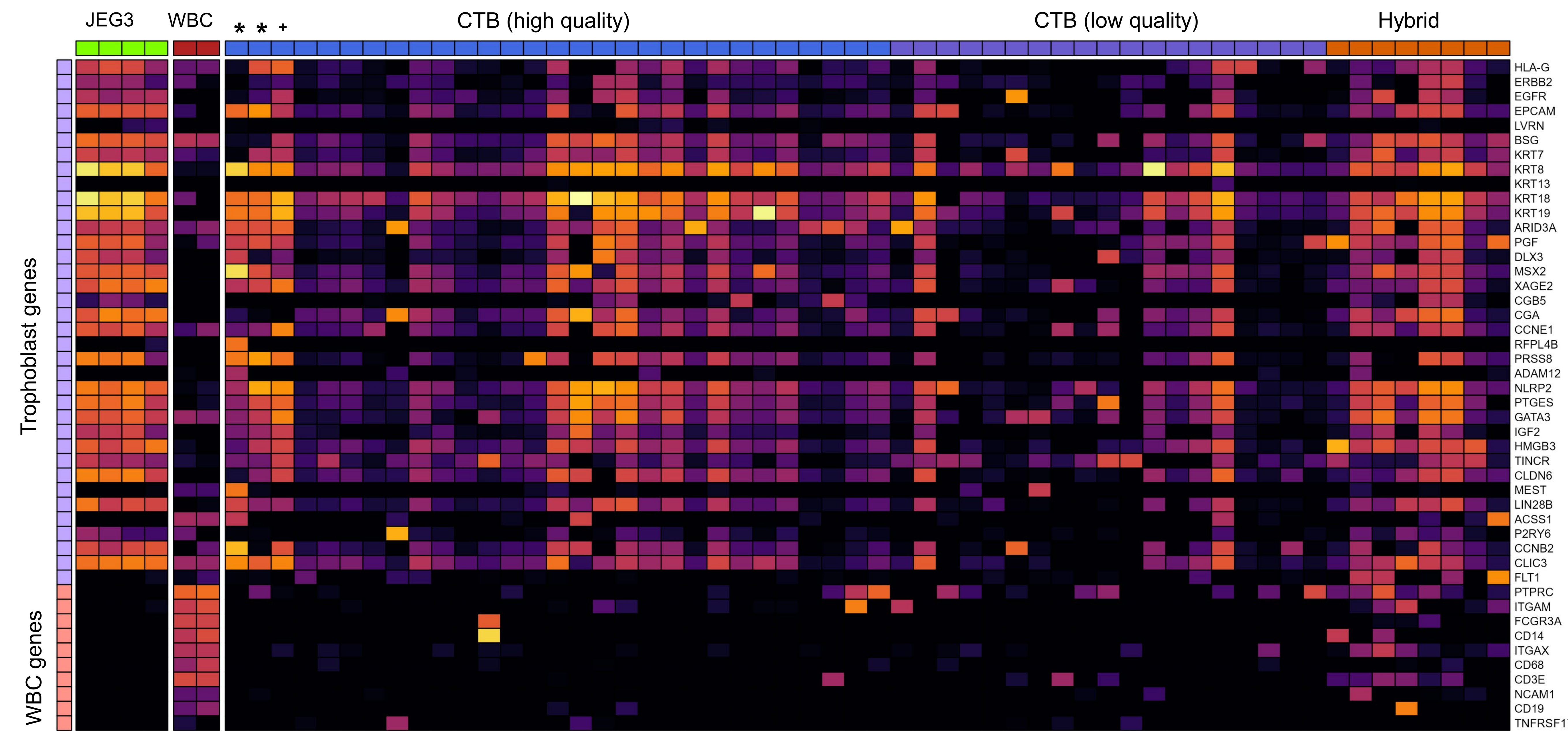


FIGURE1. Gene expression counts were normalized by CPM and log2(x+1) transformed for plotting. We generated a curated "Trophile" gene set made up of ~36 literature derived trophoblast enriched genes and 10 canonical white blood cell markers. Cells were classified as "molecular" CTB based on having >=50% of trophoblast and < 2 of 10 canonical WBC markers at 1 CPM or higher. Low quality CTB had less than 50% detection of trophoblast markers but equally low WBC content. Hybrid (HYB) cells showed expression of both trophoblast and WBC markers. We included both single live JEG3 (choriocarcinoma derived trophoblast cell line) and donor derived white blood cells as contrasting control groups for expression profiling and later DE analysis. +This cell was processed using a previous iteration of the current is
preeclampsia.

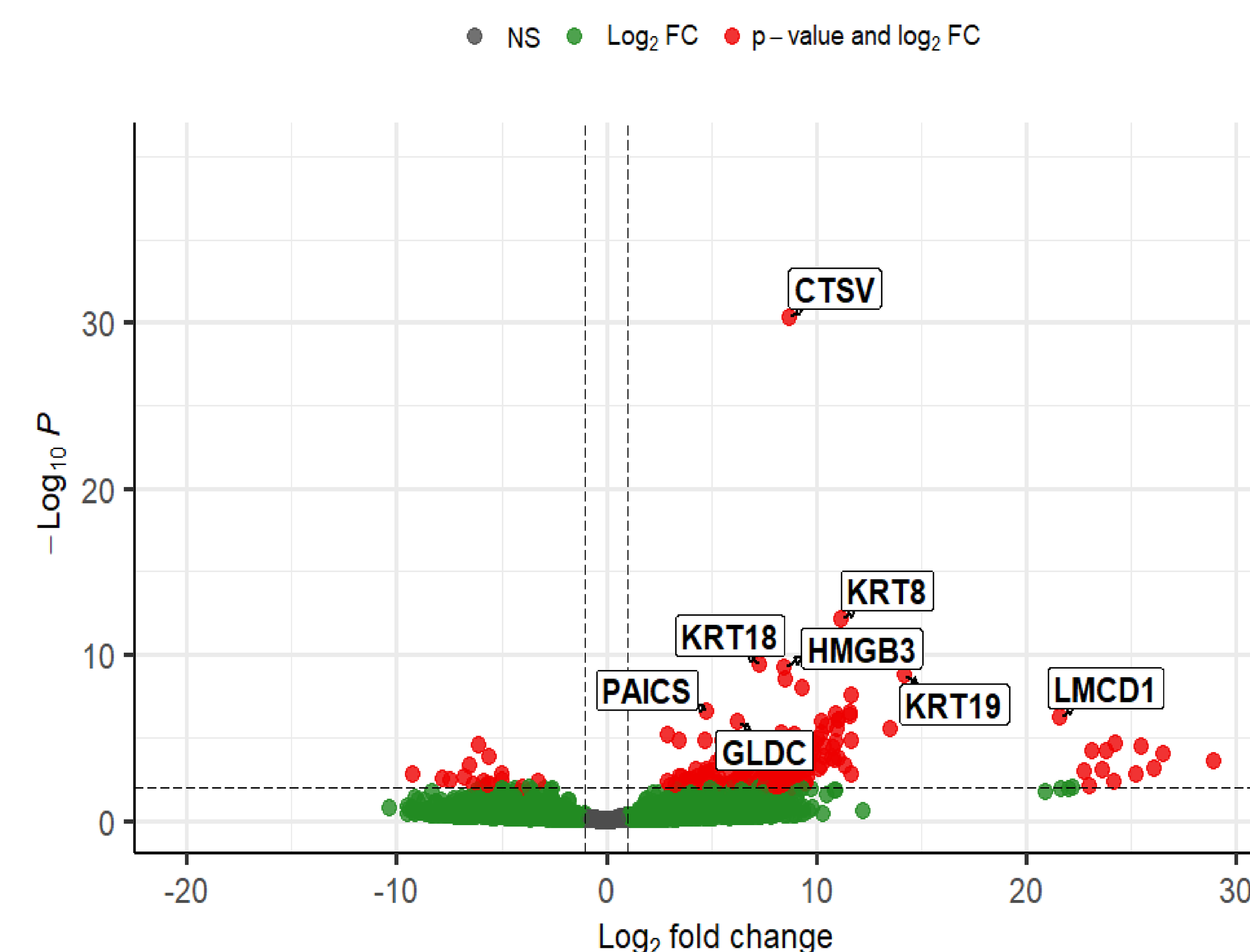


FIGURE 2. Volcano plot showing differentially expressed genes at a fold change of >2 and padj value of <1E-03, comparing the top 29 CTBs to 2 live WBCs.

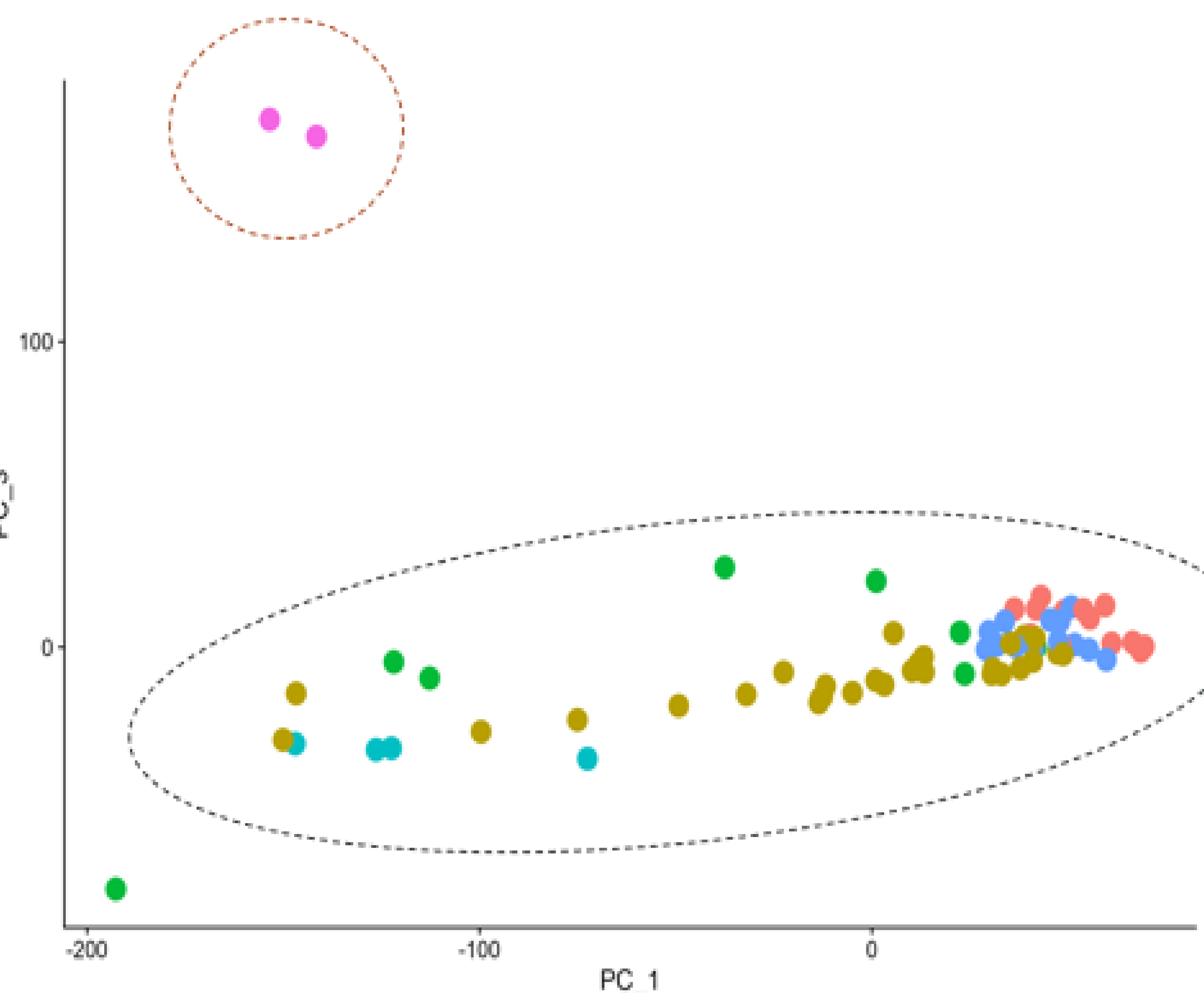
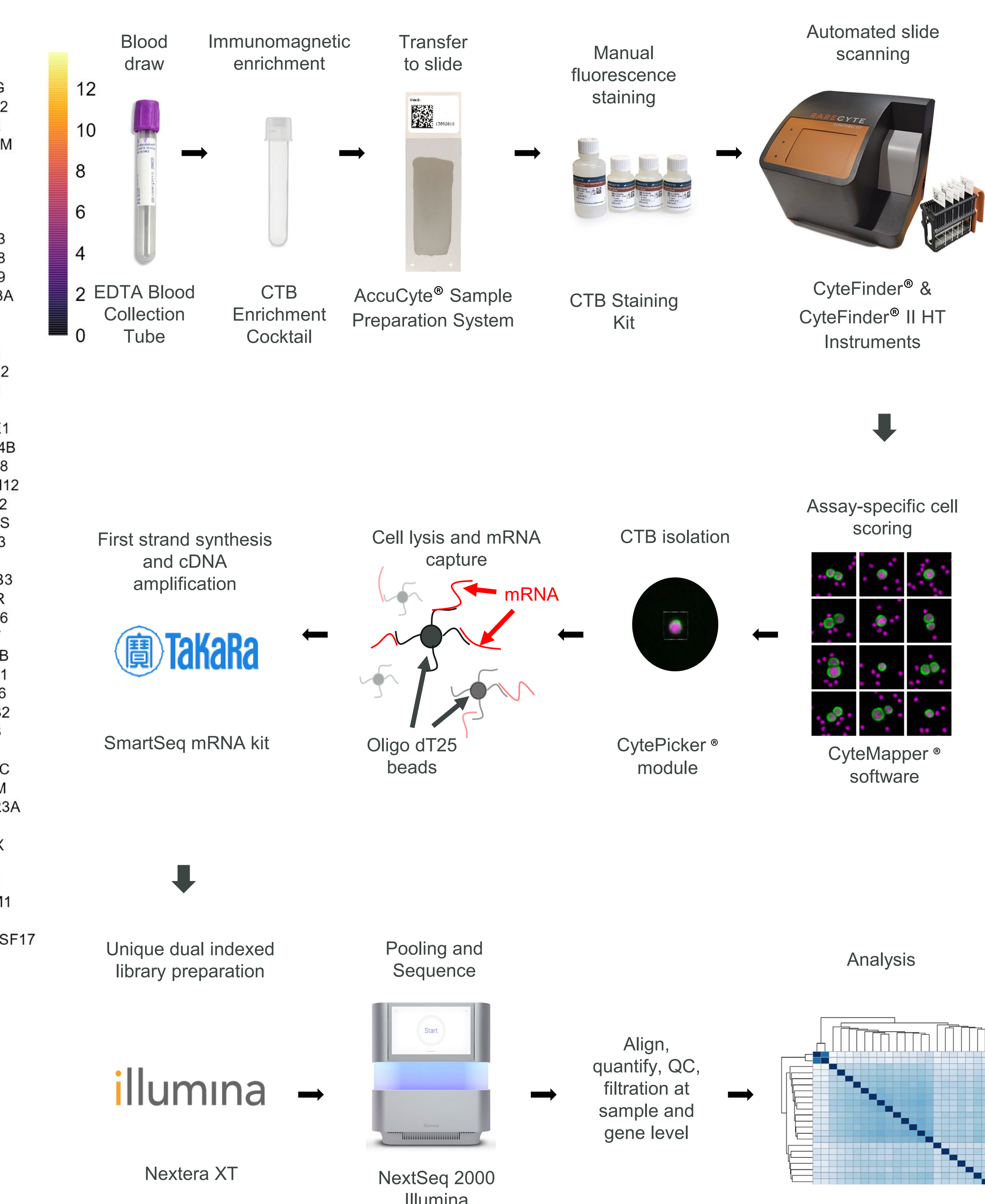


FIGURE 3. PCA plot of 2 WBC, 4 JEG3, and 29 certified CTB cells. Gene set filtered for low expressing, mitochondrial, ribosomal, and long intergenic non-coding RNAs.

SAMPLE ANALYSIS WORKFLOW



CONCLUSIONS

We report here the first successful identification, retrieval and single-cell transcriptional profiling of fixed circulating trophoblasts from third-trimester maternal blood samples. This demonstrates proof-of-concept that CTBs can provide real-time data reflecting placental function and may be used to investigate placental physiology in normal and abnormal gestation.

ACKNOWLEDGMENT

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