Non-Invasive Liquid Biopsy

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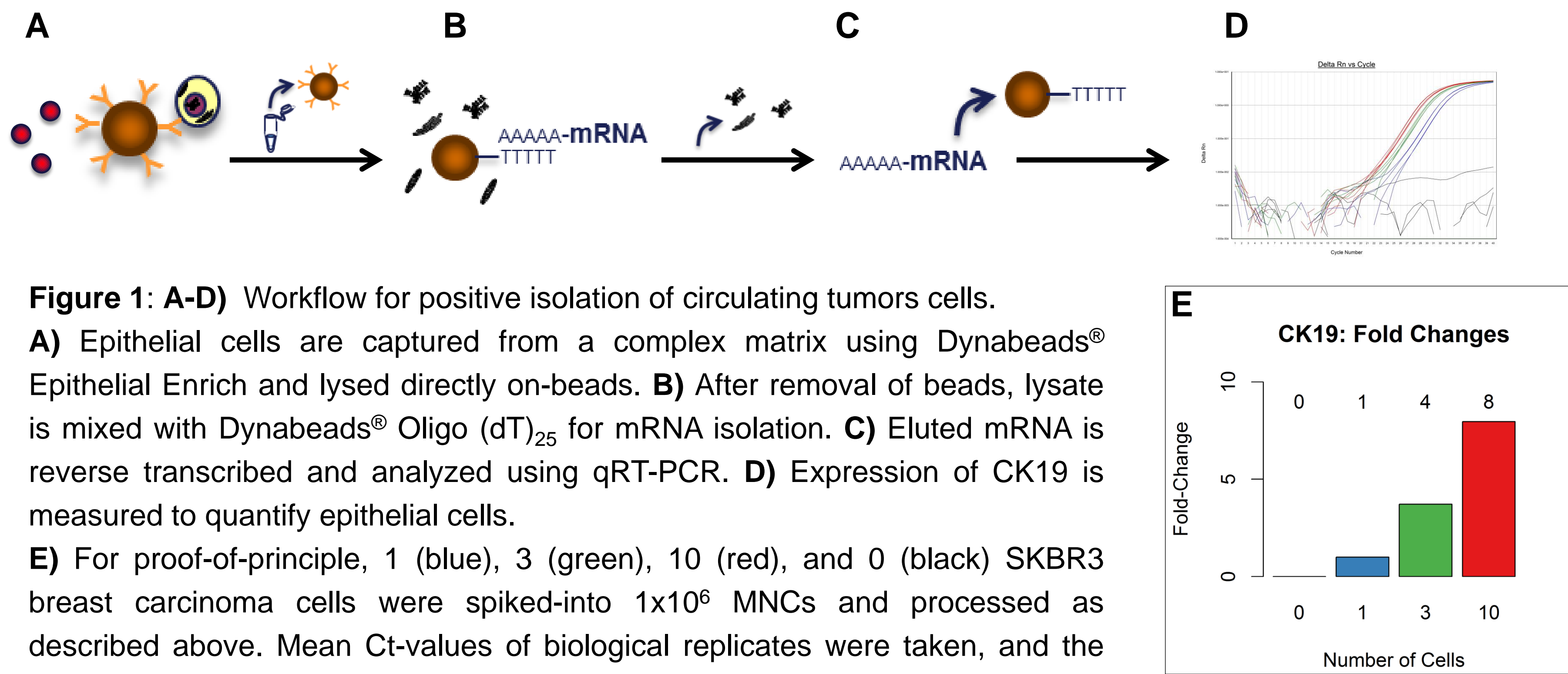[3] Centre for Cancer Biomedicine, University of Oslo, Oslo, Norway

Introduction

Invasive biopsies could often be replaced by simpler and safer non-invasive liquid biopsies using plasma or blood samples. A non-invasive liquid biopsy permits the analysis of multiple circulating biomarkers in one sample and facilitates the discovery of disease as well as treatment response monitoring in applications such as oncology. Here, we demonstrate that paramagnetic Dynabeads® provide a versatile and automation friendly tool for fast, sensitive, and high throughput isolation that can be tailored to isolate specific circulating biomarkers such as cells, exosomes, and cell-free nucleic acids.

Circulating Tumor Cells (CTCs)
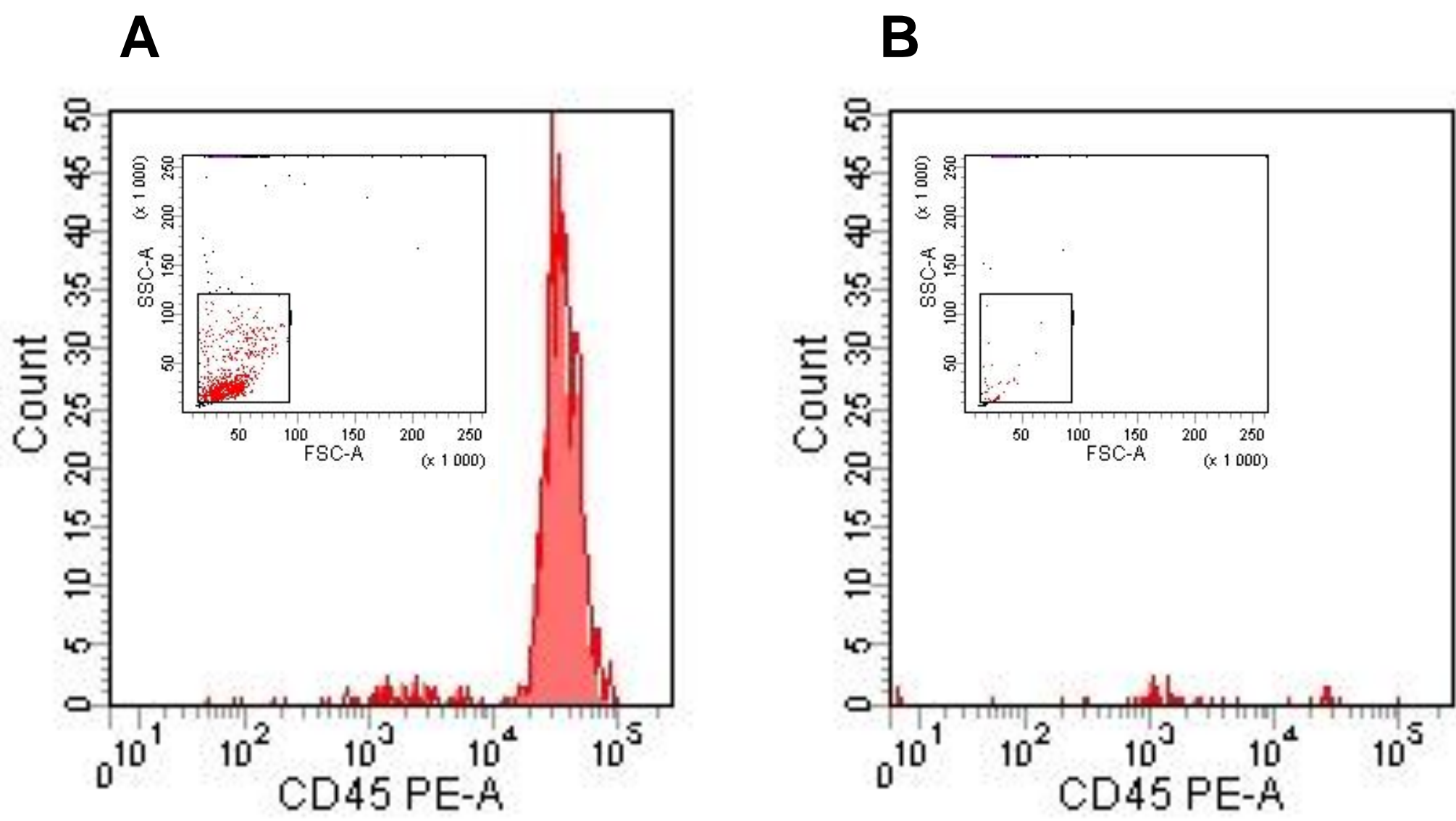
Positive Isolation
For the positive isolation, Dynabeads® coupled with a monoclonal antibody targeting EpCAM (epithelial cell adhesion molecule) are used to capture CTCs from whole blood samples. For downstream applications, mRNA can be isolated using Dynabeads® Oligo (dT)$_{25}$ and analyzed using gene expression analysis (Figure 1E) or sequencing.

Figure 1: A-D) Workflow for positive isolation of circulating tumors cells.
A) Epithelial cells are captured from a complex matrix using Dynabeads® Epithelial Enrich and lysed directly on-beads. **B)** After removal of beads, lysate is mixed with Dynabeads® Oligo (dT)$_{25}$ for mRNA isolation. **C)** Eluted mRNA is reverse transcribed and analyzed using qRT-PCR. **D)** Expression of CK19 is measured to quantify epithelial cells.
E) For proof-of-principle, 1 (blue), 3 (green), 10 (red), and 0 (black) SKBR3 breast carcinoma cells were spiked-into 1x10^6 MNCs and processed as described above. Mean Ct-values of biological replicates were taken, and the fold-changes of cDNA content were calculated in relation to 1 cell.
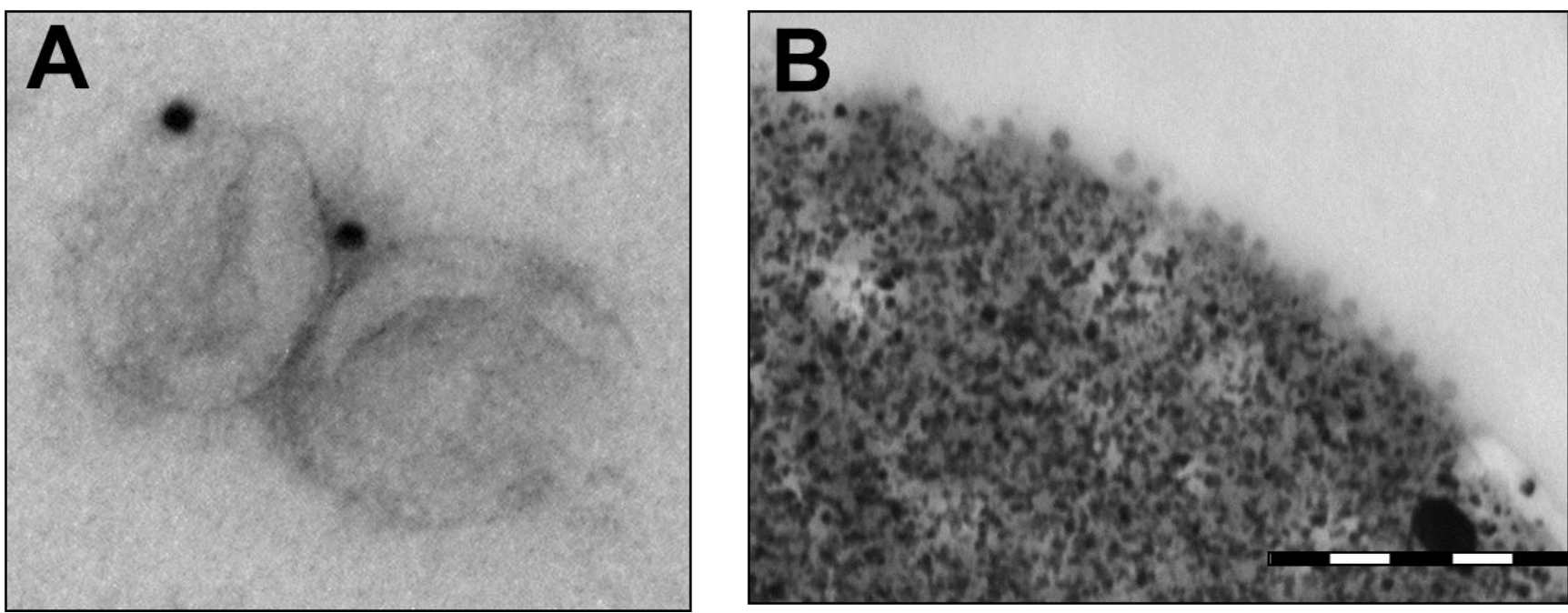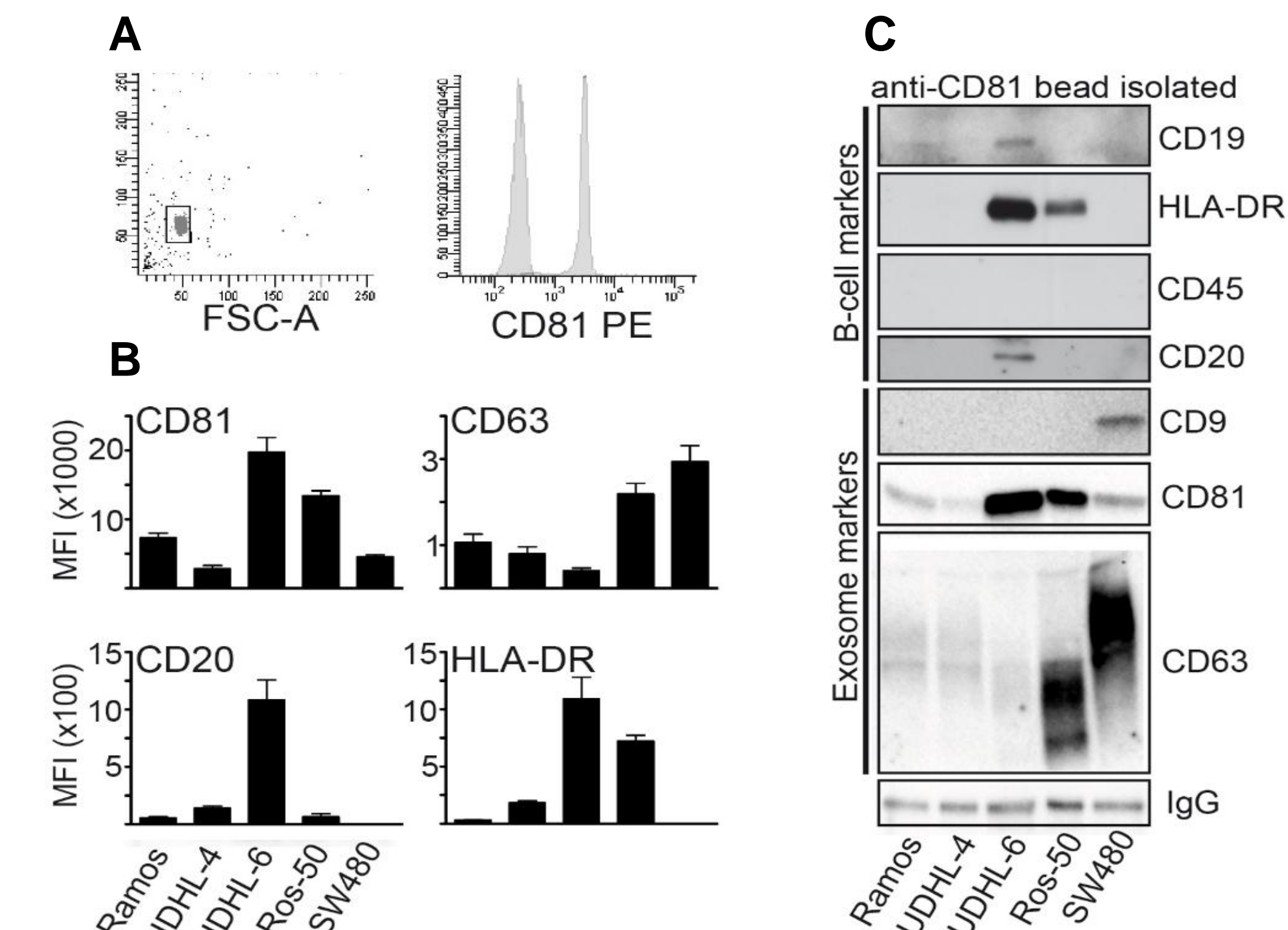
CK19: Fold Changes

Negative Isolation
CTCs can also be enriched by applying negative isolation. The advantage is that it is independent of surface marker expression (e.g. EpCAM) on the CTCs. To deplete white blood cells, Dynabeads® CD45 are used. These beads deplete more than 95 % of the leukocytes from mononuclear cells (MNCs) (Figure 2), whole blood, and buffy coat. Addition of Dynabeads® CD15 could further increase cell depletion. Following negative isolation, downstream applications could include cell culturing and molecular analysis such as qPCR and sequencing.

Figure 2: Fluorescence histograms for CD45-stained MNCs with inlaid scatter plots, measured before **(A)** and after **(B)** depletion of CD45-positive cells using Dynabeads® CD45.

Exosomes

Figure 3:
Flow cytometric (FC) and Western immunoblot analysis of exosomes isolated with Dynabeads® coated with anti-human CD81 antibodies. Exosomes were immunoisolated from pre-enriched exosomes using magnetic beads coated with anti-human CD81. Exosomes were stained for B-cell surface antigens (CD20, HLA-DR) and exosomal antigens (CD63 CD81) followed by FC analysis and presented as median fluorescence intensity (MFI).
A) Scatterplot and fluorescence histogram of anti-CD81 magnetic beads in the absence or presence of exosomes labeled for CD81.
B) CD81+ exosomes were stained for CD20 and HLA-DR in addition to the exosomal antigens CD81 and CD63. One of 3 representative experiments is shown.
C) CD81+ exosomes were lysed and processed for Western immunoblotting analysis. Expression of the B-cell surface proteins (CD19, HLA-DR, CD45, and CD20) and exosome markers (CD9, CD81, and CD63) were analyzed. Ref. Oksvold et al., 2014, Clinical Therapeutics Vol. 36 (6).
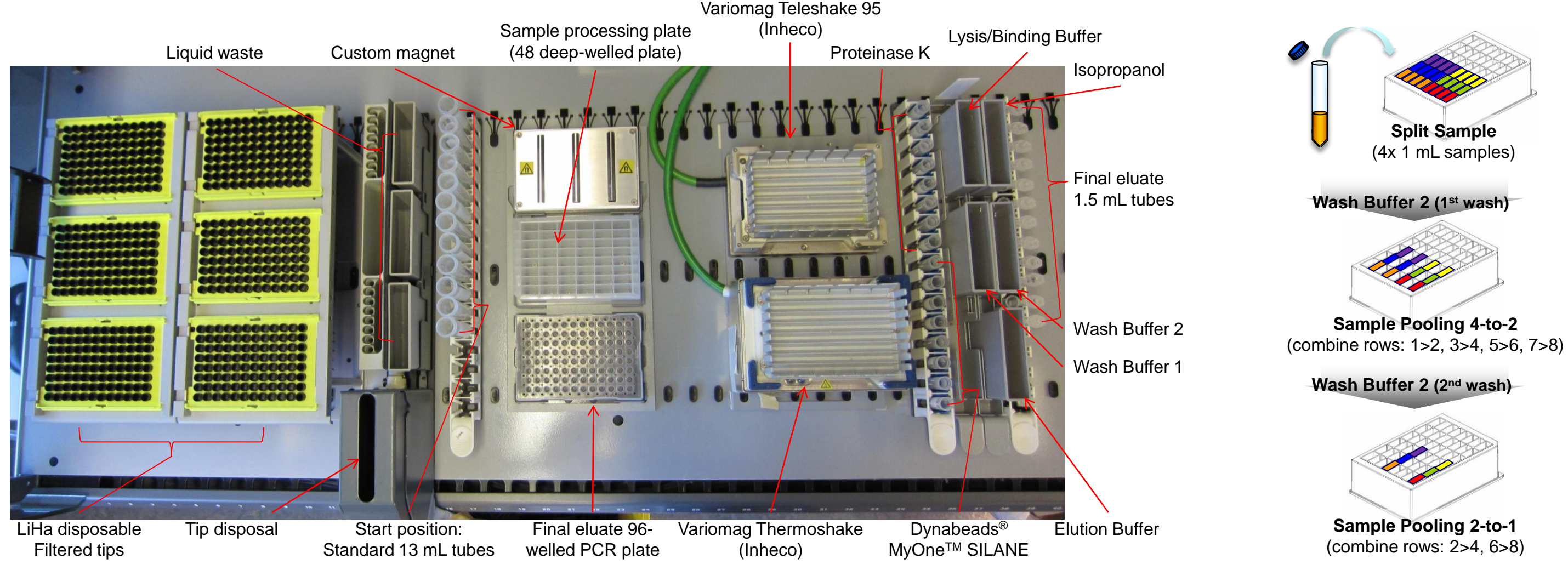

Figure 4:
A) Transmission Electron Microscopy (TEM) of pre-enriched Sudhl4 exosomes labeled for CD63 [2]. CD63 was detected using anti-CD63 antibodies followed by protein A gold (10 nm).
B) TEM of exosomes isolated with Dynabeads® Human CD63 Isolation/Detection (human cell culture media)[1]. Bar 500 nm.

Circulating Nucleic Acids

In order to isolate sufficient amounts of cfDNA for downstream analysis, typically 4 to 10 mL plasma or serum is necessary to be processed into volumes below 100 µL. During processing, the work volumes can increase over 3 fold to that of the input volume. Processing is therefore mostly done manually and is highly work and skill intensive, while automation presents a serious challenge. Here we provide a solution using magnetic beads, Dynabeads® MyOne™ SILANE, in a highly sensitive, high-throughput automatable large volume sample processing.

Figure 5: Dynabeads® MyOne™ SILANE Automation on Tecan® Freedom Evo® 150

Figure 7: Four mL plasma samples from 10 individuals (A-J) and pooled plasma derived from Cell-Free DNA BCT tubes (St1 and 12) processed manually and on an automated setting (Figure 5). The resulting eluates were then quantified by qPCR with fragment specific probes.
A/B) Recovery rates of samples laced with 10^6 copies of 120 bp DNA **(A)** and 220 bp DNA **(B)** (~100%).
C) Recovery rates of endogenous cfDNA of manual and automated sample processing in comparison to that of a non-automatable Competitor. Dynabeads® MyOne™ SILANE yielded equally efficient recovery manually and automated, albeit yielding slightly lower recovery rates than that of Competitor (87% and 83% manually and on Tecan® instrument, respectively, to Competitor).
D) As a guideline for sample eluate purity, the total protein contents of Dynabeads® MyOne™ SILANE manual and automated extractions and Competitor were compared. The lowest mean total protein content was achieved with Dynabeads® MyOne™ SILANE manual processing. Competitor gave medium protein content but showed high sample to sample variation.

Figure 6: SILANE cfDNA Isolation from 4 mL Plasma or Serum