



Parsortix™

Cell Separation System

Small Volume Capabilities

Introduction

Mouse models are accepted and heavily utilized within the field of cancer research. The anatomy, relevantly short lifespan, and rapid development of mice make them desirable for a multitude of cancer associated models. This document describes a liquid biopsy method developed by ANGLE that enables the use of the Parsortix™ system to capture rare, cancer associated cells from small volumes (100 – 200 μ l) of murine blood. Such small volumes enable creative study designs involving multiple time-point blood draws on single mice. Once the sample is collected, the Parsortix™ system enables isolation of an enriched rare circulating cell population. Here we present data, using the Parsortix system with a small volume blood adaptor, which describes efficiency of the system's capture and harvest efficiency from such small volume murine blood samples.

Principle of Operation of the Parsortix™ System

The Parsortix™ cell separation system enables capture and harvest of rare circulating cells from whole blood samples. The Parsortix cell enrichment technology is epitope independent, and does not use any antibodies or magnets. The system uses a microfluidic cassette that captures cells on the basis of their size and deformability. Circulating tumor cells (CTCs) have a more rigid cytoskeletal structure and have a generally predictable size distribution. Taken together, the size and deformability of CTCs differ from white and red blood cells. The rare circulating cells are trapped on the cassette steps, at the cassette's critical gap, and can later be harvested from the system. The cassettes are available in several different sized critical gaps, depending upon the characteristics of cells targeted for capture. The Parsortix™ system was initially designed to separate larger blood samples, ranging between 1ml to 40ml. However, for small volume blood applications, ANGLE has developed a small volume blood adaptor that allows the system to process sample volumes as low as 100 μ l.

Methods

In total, fifty blood samples were processed over 7 days. Two Parsortix instruments identically configured were used for the study, and 25 samples were processed on each instrument. The blood samples were obtained from Healthy Normal Volunteer (HNV) human donors. Blood was drawn into standard 6ml EDTA vacutainer tubes and then 100 μ l of blood was dispensed into small cryovial tubes for the separations. The samples were spiked with a target number of 100 SK-BR-3 (ATCC) cells labeled with CFSE (CellTrace, Life Technologies, C34554).

To ensure that the numbers of cells being spiked were consistent between the samples processed throughout the 7 days, a control count was performed daily. The pre-labeled cell suspension was diluted to a concentration of roughly 100 cells per 10 μ l. Actual numbers of cells present in several 10 μ l aliquots were then counted, and the means of those counts was then taken to be the number of cells actually dispensed into the blood sample in a 10 μ l volume.

The samples were separated, using a semi-automated instrument procedure specific for low sample volumes, on the two Parsortix™ PR1 systems. The small volume blood adaptor (**Figure 1**) is mounted to the Parsortix™ instruments in a similar manner to larger sample tubes.



Figure 1: Prototype of Small Volume Blood Adaptor

Results

Figure 2 displays data related to the capture efficiency across fifty 100µl blood separations separated during a period of 7 days. The % capture is expressed as number of captured cells of the average control count (Figure 3). Data is presented for individual days, as well as with all separations together. Mean capture efficiency was ~84%.

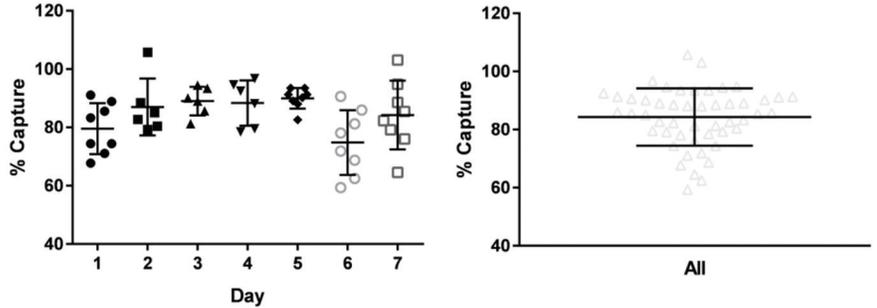


Figure 2: Capture Efficiency of SK-BR-3 Cells

Figure 3 shows the control count data pertaining to the cell spiking process on each day of the experiment. The aim was to spike approximately 100 cells into each sample. The mean values were used to determine % capture and % recovery for the experiments.

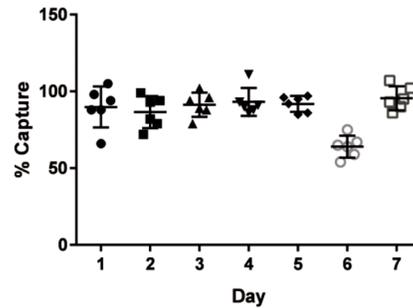


Figure 3: Control Counts for SK-BR-3 Cells

Figure 4 displays data related to the harvest efficiency across the fifty blood separations. The % harvest is expressed as number of harvested cells (i.e. captured cells successfully extracted from the cassette) divided by the mean spiked cell control count (figure 3). Data is shown for each day, as well as for all separations together. Overall, the mean harvest efficiency was ~58%.

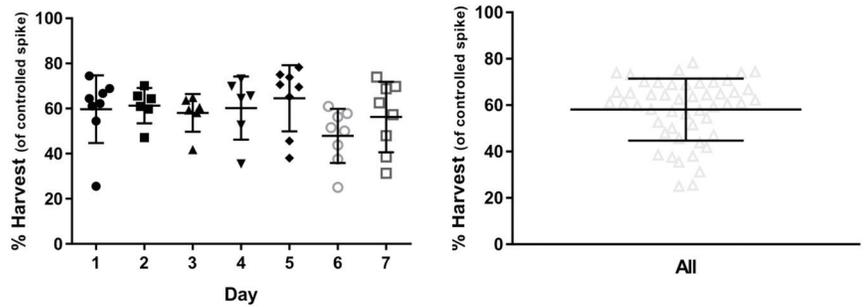


Figure 4: Harvest Efficiency for SK-BR-3 Cells

Figure 5 displays recovery efficiency data for fifty 100µl blood separations performed during a period of 7 days. Recovery is expressed as % of harvested cells divided by the numbers of captured cells, expressed as a percentage. Data is shown for each day, as well as for all separations together. Overall, the mean recovery efficiency was ~69%.

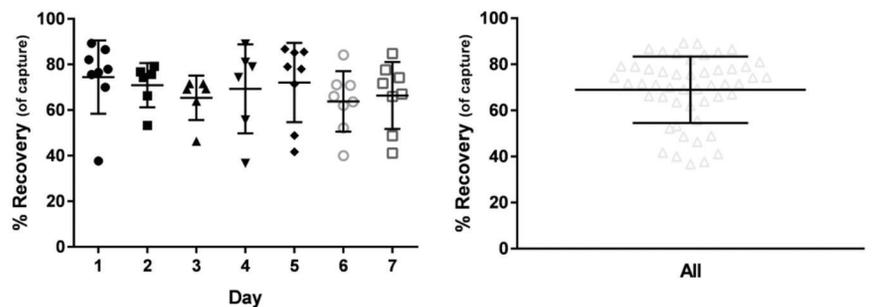


Figure 5: Recovery Efficiency for SK-BR-3 Cells

Conclusion

These results obtained using the small volume adapter on the Parsortix™ system open the door to new and exciting research studies. Use of smaller blood sample volumes will allow greater flexibility in study design, enabling researchers to extend their use of the Parsortix system. Specific to rodent models, small volumes enables multiple time-point blood draws on a single mouse, reducing the limits imposed by animal sacrifice. This promises improved research utility and cost savings.



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