Streamlined Workflow for Cell Sorting Using Microfluidics Chip Based Sorter

Emi Ikeda, Rie Serita, Yoshitsugu Sakai Medical Business Unit, Sony corporation, Tokyo, Japan

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Background

Keywords: Streamlined workflow for cell sorting using microfluidics chip based sorter

A growing range of applications need that cells be sorted in controlled conditions. In many cases, there is a need to minimize carryover from sample to sample. Here we demonstrate a streamlined approach to sorting of cells without cross-contaminating successive samples using an integrated approach that uses exchanging sorter components that come in direct contact with sample, sterile source of sheath fluid and a biosafety and aerosol management system to enclose the sorter.

Material and Methods

System: Exchangeable fluidics cell sorter FX500 cell sorter with 3 laser configuration 488nm, 561nm and 638nm and plate sorting option was used in this study. The sorter was setup in a Class II biosafety cabinet BCC300 from Baker Co. (Figure1)

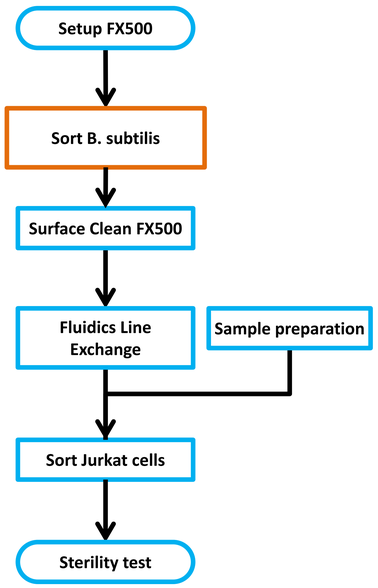

Figure1:FX500 setup inside biosafety cabinet BCC300 from Baker Co.

Sorting of bacteria: *Bacillus subtilis*, a spore forming microorganism was chosen for this study. This is a standard organism used routinely as a positive control in sterility testing procedures. Bacterial sample stained with SYBR Green™ and propidium iodide was sorted with 100um microfluidics sorting chip at 20psi. Chip alignment, droplet calibration, side stream adjustment and drop delay were setup automatically using Sony Corefinder™ technology. SYBR Green+/PI- population singlet cells of *B. subtilis* were gated and 6x10^6 cells were sorted using Normal purity mode in the software.

Cleaning: After sorting of *B. subtilis*, the sheath line and sample line were removed following steps outlined in a software wizard. The surface of FX500, chip loading area and interior of sorting chamber was decontaminated using 70% ethanol. The waste catcher and sample chamber were washed with water and set to dry. Deflection plates were wiped 70% ethanol.(Figure2)

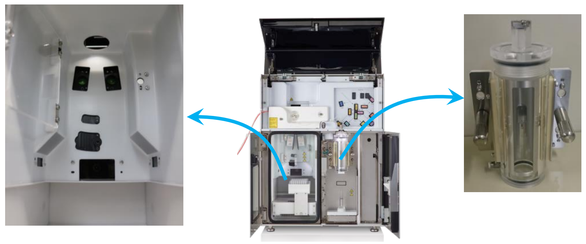

Figure 5: FX500 sort collection area and sample loading chamber

Fluidics Line Exchange: A new and unused gamma irradiated sheath fluidics line was connected to FX500 sorter following the steps outlined in the software wizard for fluidics line setup. (Figure3)All open connections of the sheath line to the sheath bags and chip were made inside the BSC.(Figure4,5)

Figure 3: FX500 exchangeable fluidics- Irradiated sheath line kit, sample line, sorting chip and Autosetup beads

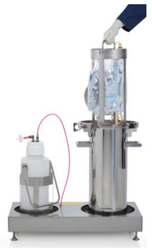

Figure 4: FX500 sheath tank with sheath rack and saline bags

Figure5: FX500 chip loading

Mammalian cell sorting: Jurkat cells were stained with CD45 FITC and 7AAD and singlet FITC+/7AAD – population was sorted. Six million sorted cells were incubated post sort in RPMI with 10% FCS at 37°C/ 5%CO_2.

Sterility test: Sorted cells of *B. subtilis* and Jurkat were tested in liquid culture using a test based on JP pharmacopeia16th edition. This test is harmonized with the European Pharmaopeia and the U.S. Pharmacopeia. Each sample was seeded into two types of medium - Soybean casein digest medium (SCD) and thioglycollate medium (TGC). *B. subtilis* was used as positive control and sterile sheath fluid was used as negative control. Samples seeded in SCD medium were cultured at 25°C and samples seeded in TGC medium cultured at 30°C.The growth of *B. subtilis* was monitored by measuring its density using OD600 (GeneQuant Pro; GE Healthcare) at 1, 7 and 14 days.

Endotoxin test: Sorted Jurkat cells were tested endotoxin level by Japan Electron Beam Irradiation Service Co.,Ltd., using the gel clot method

USP class test: Biological Reactivity of FX500 Exchangeable Fluidics Sheath line were tested based on USP 39"<88> Biological Reactivity Tests, In Vivo" by Japan Food Research Laboratories.

Results

We performed sorting using above method in triplicate and did not detect any contamination of *B. subtilis* in sorted Jurkat samples and negative control. In contrast, *B subtilis* sorted sample and positive control showed growth of bacterial cells. Our results demonstrated that exchange of fluidic lines of the cell sorter is effective in preventing carryover and cross contamination between samples.

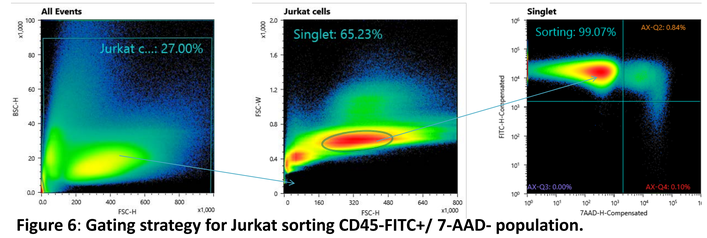

Figure 6: **Gating strategy for Jurkat sorting CD45-FITC+/ 7-AAD- population.**

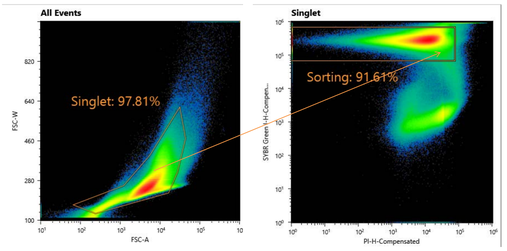

Figure 7: Gating strategy for *B.subtilis* sorting SYBR Green+/ PI- population.

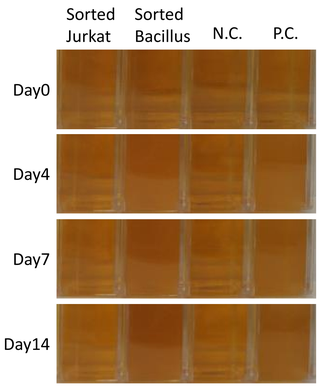

Figure 8A: Photo of each sample in SCD Medium

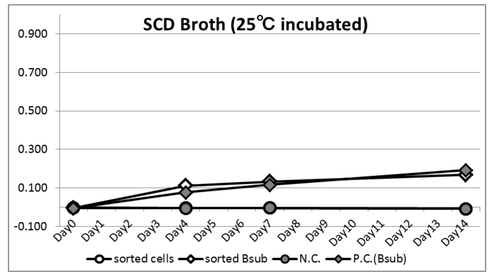

Figure 8B: Change in OD of SCD Broth containing Jurkat, *B.subtilis* and sheath fluid measured over 14 day period

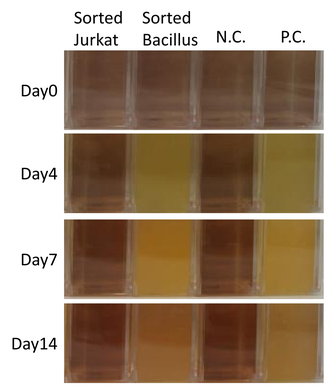

Figure 8C: Photo of each sample in TGC medium

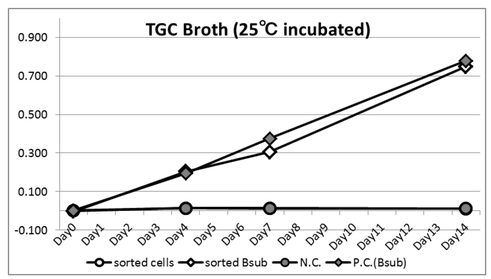

Figure 8D: Change in OD of TGC Broth containing Jurkat, *B.subtilis* and sheath fluid measured over 14 day period

Figure 8: Sorted Jurkat samples and sterile sheath fluid (negative control) did not show any turbidity till day 14 of incubation in microbiological growth medium. Sorted sample of *B. subtilis* and spiked culture of *B. subtilis* (positive control) showed growth at day 4 in bacterial growth medium. This growth increased till day 14 as measured by the absorbance of the growth medium. In contrast, absorbance of the growth medium containing sorted Jurkat samples and negative controls did not change till day 14.