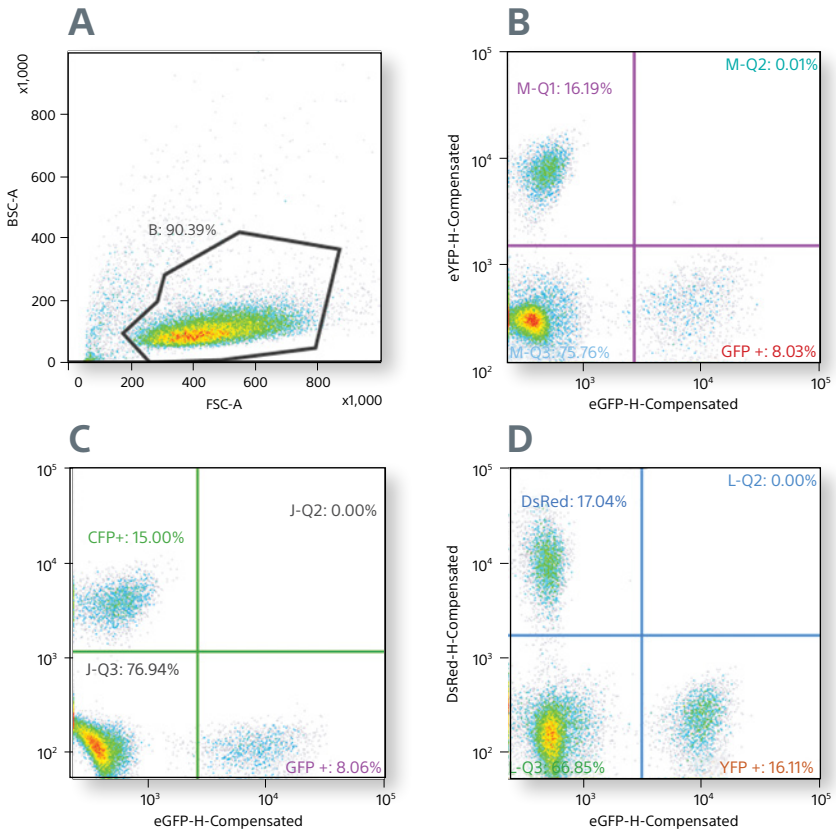
Sorting of Fluorescent Protein Expressing Cells using the SH800S Cell Sorter

Fluorescent proteins (FP) are used as reporter molecules in a variety of studies analyzing protein expression and gene editing properties of CRISPR and other nucleases. Since the characterization and sequencing of the green fluorescent protein (GFP), a wide pallet of spectrally distinct FPs have been discovered. This broad range of FPs allow researchers to generate cells expressing a unique fluorescent protein or combinations of them.

A fluorescent protein(s) expressing population can be further characterized by staining it for surface epitopes using fluorochrome conjugated antibodies. Viability dyes such as DAPI may be used to stain dead cells.

In the experiments below, Jurkat cells were co-transfected with Green fluorescent protein, Yellow fluorescent protein, dsRed and Cyan fluorescent protein. Using the SH800 cell sorter, cells expressing a single protein or combinations of the proteins were analyzed using specific optical filters and sorted. The optical filter sets used are shown.

Fluorochrome Protein	Filter
GFP	525/50
YFP	525/50
dsRed	600/60
CFP	450/50



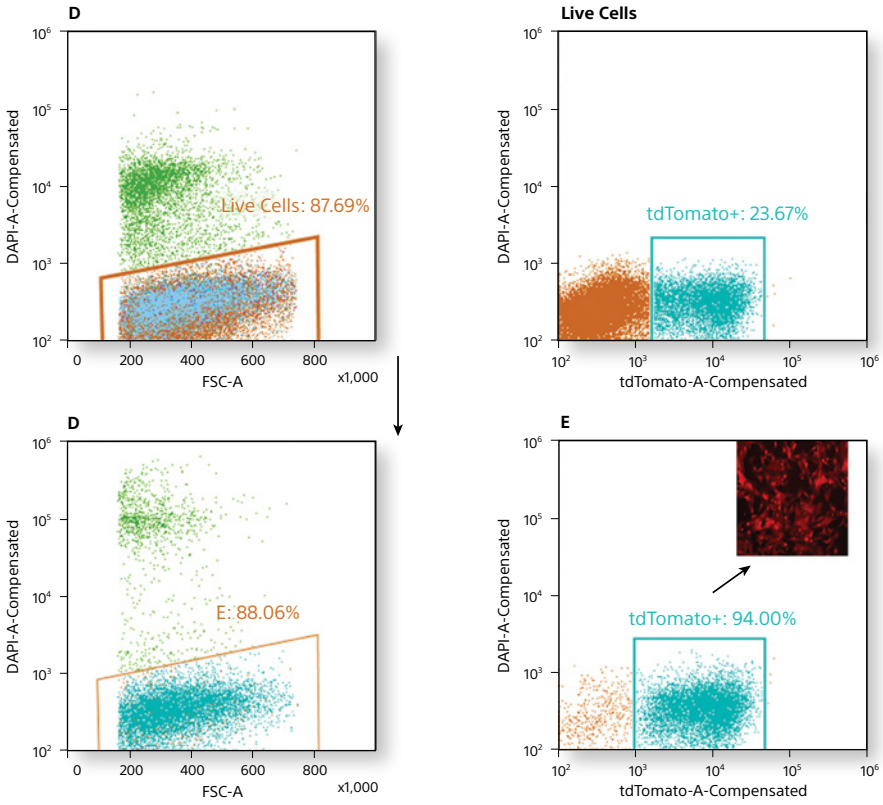
*(**A**) Cells were identified based on scatter. Upon spectral compensation, distinct populations of FP expressing cells could be identified from non-expressing cells. Approximately 16% of cells expressed YFP (**B**), 8% GFP (**B**), 15% CFP (**C**), and 17% dsRed (**D**) positive cells were identified.*

In the experiment below, mouse embryonic stem cells were transfected with Isl1Cre-tdTomato and stained with DAPI to determine cells viability. Isl1 is a transcription factor and potential cardiac progenitor marker.[1,2] Fluorescence of tdTomato is used to measure activation of Isl1.

To isolate viable cells expressing Isl1Cre -tdTomato, cells were sorted on a Sony SH800S cell sorter equipped with filter set 2. DAPI was detected with a 450/50 filter in the violet channel. tdTomato was detected off the yellow-green laser with a 600/60 filter. The purity of the sorted cells was analyzed post sort. The viability of the cells was determined 24h post sort by microscopy. The high recovery and viability of the transfected cells post sorting demonstrates that the sorting conditions used were suitable for this application.

Conclusions

The Sony SH800S is a useful tool for the analysis and sorting of fluorescent proteins. Populations of fluorescent cells are clearly distinguished. In reporter experiments, even with challenging cells, the SH800S delivers cells with high purity and viability.



*Mouse embryonic stem cells expressing Isl1Cre-rdTomato were sorted with the 100um chip for rdTomato positive cells. (**A**) Gate was set on DAPI negative (live cells) population. (**B**) tdTomato positive cells were sorted (88% live cells) (**C**) Post sort cells maintained a high viability of 88% (**C**) and a high purity of 94% (**D**).*

This data was kindly provided by Dr P. Andersen, Kwang Lab, Heart and Vascular Institute, John Hopkins University.

References

1 . Bu, Lei, et al. "Human ISL1 heart progenitors generate diverse multipotent cardiovascular cell lineages." Nature 460.7251 (2009): 113-117.

2. Lei, Ieng Lam, Lei Bu, and Zhong Wang. "Derivation of cardiac progenitor cells from embryonic stem cells." Journal of visualized experiments: JoVE95 (2015).