

## Isolation of Stably Transfected Fluorescent Cells with the S3™ Cell Sorter

Derrick Magdefrau and Kelly Kroeger  
Bio-Rad Laboratories, Inc., Hercules, CA 94547

### Introduction

Many fields of research, from immunology to neurobiology, require the isolation of cells based on extracellular or intracellular phenotypes. A way to accomplish this with exquisite accuracy is through fluorescence-activated cell sorting (Arun 2005, Maric and Barker 2004). This technology allows researchers to isolate live cells based on the expression profile of one or multiple proteins of interest. Cells can be identified by using antibodies conjugated to fluorophores or by expressing fluorescently tagged proteins.

Until recently, cell sorters were complicated, expensive, and typically required an experienced operator in a core lab setting. The S3 and S3e™ Cell Sorters, however, can be run by any researcher due to key automated features such as the AutoGimbal™ System and ProDrop™ Technology. Sorts can be carried out reliably and with high purity. Here we show that stably transfected GFP<sup>+</sup> HEK 293 and RFP<sup>+</sup> HEK 293 cells can be sorted to greater than 98% purity from a mixed population using the S3 Cell Sorter.

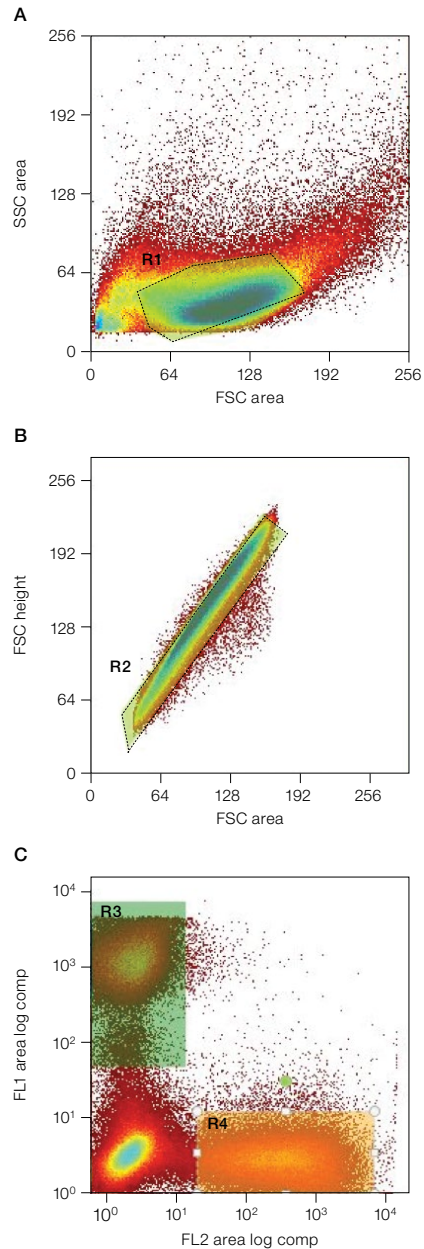
### Results

HEK 293 GFP<sup>+</sup> and HEK 293 RFP<sup>+</sup> cells were sorted to high purity on the S3 Cell Sorter. Figure 1 illustrates the experimental setup for this two-way sort. Cell debris was excluded from whole cells by creating region R1 (Figure 1A) for whole cells using forward and side scatter parameters, which serve as indicators of relative particle size and complexity. Examining the relative signal height vs. signal area of cells in this R1 gate, we were able to exclude doublets from the sort by selecting only cells that fell in region R2 (Figure 1B). Using only cells that fell into these two regions, R2 < R1, live single cells expressing GFP and RFP were gated for sorting by creating regions R3 and R4 (Figure 1C). HEK 293 GFP<sup>+</sup> and HEK 293 RFP<sup>+</sup> cells were sorted simultaneously using left and right sorting channels, in Purity mode.

A small amount of each sorted sample was re-analyzed on the S3 Cell Sorter in order to assess purity. Each sorted population (HEK 293 GFP<sup>+</sup> and HEK 293 RFP<sup>+</sup>) was greater than 98% pure (Figure 2A and B).

### Discussion

HEK 293 cells expressing GFP or RFP were sorted at high purity (>98%), illustrating the utility of the S3 Cell Sorter in performing high purity sorts of adherent cells that express intracellular fluorescent proteins. While the present study focused on sorting of mammalian cells based on GFP and



**Fig. 1** Gating strategy applied to selectively sort the HEK 293 GFP<sup>+</sup> and HEK 293 RFP<sup>+</sup> cells. Whole cells were identified in region 1 (R1) of the SSC area vs. FSC area plot (A). Single cells were then selected by excluding doublets in region 2 (R2) of the FSC height vs. FSC area plot (B). Using these gates, cells expressing GFP (R3) and RFP (R4) were selected for sorting (C).

RFP expression, this technique is compatible with a wide range of fluorophores, dyes, and cell types. Using the S3 Cell Sorter, cells as diverse as yeast, bacteria, and plant cells can be sorted from complex mixed populations using up to four different fluorescent markers (Carter et al. 2013, Wernérus et al. 2002). This flexibility has led to the fast adoption of fluorescence-activated cell sorting across disciplines and is resulting in an ever increasing number of new applications for this technique (Gilsbach et al. 2014).

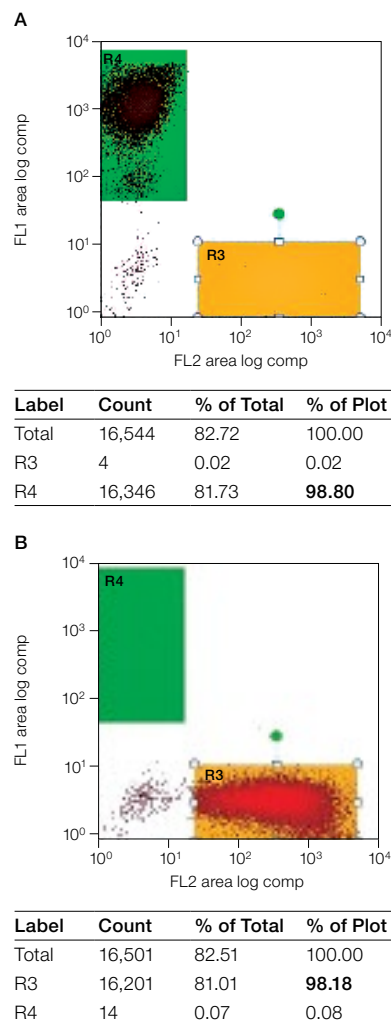
### Materials and Methods

HEK 293 GFP<sup>+</sup> (Cell Biolabs, Inc.) and HEK 293 RFP<sup>+</sup> (GenTarget, Inc.) cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% nonessential amino acids (NEAA), and 10 µg/ml blasticidin to positively select for stably transfected cells carrying the GFP or RFP expression plasmid. Negative control HEK 293 cells (ATCC) were cultured in DMEM supplemented with 10% FBS and 1% NEAA.

Four samples were prepared to run on the S3 Cell Sorter: HEK 293 (negative control), HEK 293 GFP<sup>+</sup>, HEK 293 RFP<sup>+</sup>, and a mixed population of HEK 293 GFP<sup>+</sup> and HEK 293 RFP<sup>+</sup> cells. To prepare each sample for sorting, cells were trypsinized, transferred to a conical tube, and centrifuged at 400 x g for 5 minutes. The cells were then washed once using phosphate buffered saline (PBS) supplemented with 3% FBS (sort buffer), resuspended in sort buffer, and filtered through a 70 µm filter to eliminate cell clumps and large debris. The cell concentration for the GFP<sup>+</sup> and RFP<sup>+</sup> samples was determined using Bio-Rad's TC20™ Automated Cell Counter. An equal number of cells were then combined to prepare the mixture of HEK 293 GFP<sup>+</sup> and HEK 293 RFP<sup>+</sup>. All cells were refrigerated until sorting.

Prior to running samples, QC was performed on the S3 Cell Sorter using ProLine™ Universal Calibration Beads. ProSort™ Software was used to generate five density plots: FSC (area) vs. SSC (area), FSC (height) vs. FSC (area), SSC (area) vs. FL1 (area), SSC (area) vs. FL2 (area), and FL1 (area) vs. FL2 (area). FSC and SSC parameters were collected using a linear scale while fluorescence parameters were collected in log scale. The HEK 293 negative control cells were then analyzed in cycle mode and the voltages for FSC, SSC, FL1, and FL2 were adjusted to ensure all events were collected on scale. Each sample was acquired at 2,000 events per second.

To compensate data prior to sorting, 20,000 events were collected from each sample and each FCS file was saved. In ProSort Software, the Autocompensation Wizard was run using a universal negative source (HEK 293 negative control cells), and two single fluorescent controls: a FL1 source (HEK 293 GFP<sup>+</sup>), and a FL2 source (HEK 293 RFP<sup>+</sup>). The compensation matrix was then applied to all relevant plots. Rectangular sort regions were set around the HEK 293 GFP<sup>+</sup> and HEK 293 RFP<sup>+</sup> populations within the FL1 (area) vs. FL2



**Fig. 2. Sorted cells at >98% purity. A**, analysis of the sorted HEK 293 GFP<sup>+</sup> population. The sort purity (percent of events in the R4 gate) was 98.80%; **B**, analysis of the sorted HEK 293 RFP<sup>+</sup> population. Sort purity (percent of events in the R3 gate) was 98.18%.

(area) plot. The sort logic was set to collect 50,000 events in Purity mode for both the HEK 293 GFP<sup>+</sup> and HEK 293 RFP<sup>+</sup> cell populations. To determine sort purity, 20,000 events of each sorted population were reanalyzed on the S3 Cell Sorter.

### References

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