Single cell sorting of CRISPR/Cas9-expressing cells using the Sony SH800S cell sorter

CRISPR (clustered regularly interspaced short palindromic repeats) is a popular tool used for editing genes. These edits are accomplished by introducing the Cas9 nuclease (in the form of DNA, RNA, or protein) and a guide RNA (gRNA) into the cell. Cas9, an RNA guided DNA endonuclease, is directed by gRNA to cleave DNA at a specific sequence. CRISPR/Cas9 tagged with GFP is transfected into cells. These cells are analyzed and sorted into 96-well plates using a cell sorter to isolate GFP+ cells. Positive clones are screened for CRISPR-edited sequences.

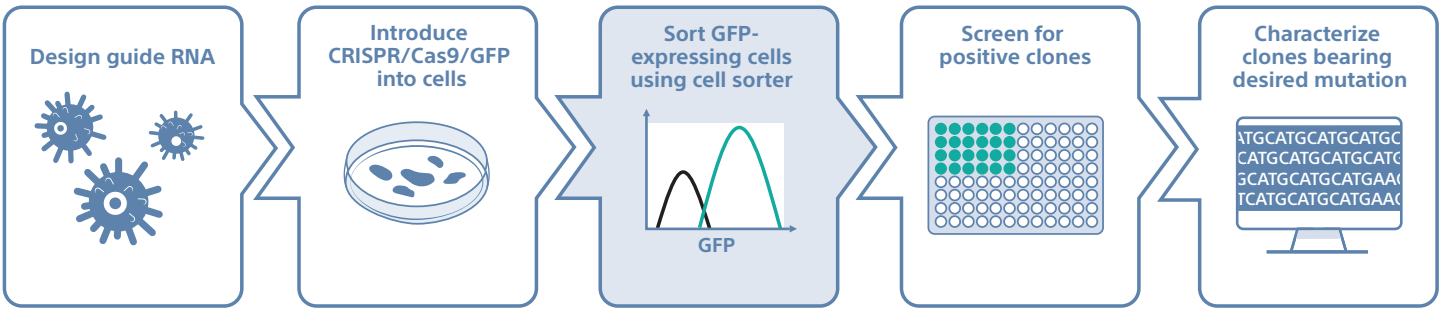

Figure 1. Screening CRISPR/Cas9-expressing cells

Sony SH800S cell sorter

The Sort Deposition System of the SH800S cell sorter facilitates high throughput single cell sorting and precise deposition of target cells into multiwell devices such as 96- and 384-well plates.

Features for single cell sorting

1. Automated and easy-to-use setup for single cell sorting
2. Custom angled plate holder for precise single cell deposition
3. Default onboard sample cooling
4. Index Analysis for recording the X and Y coordinate (well position) of each sorted event

Index sort analysis

This indexed information of sorted cells can be used for metadata analysis with downstream endpoint assays. It enables the user to correlate the phenotype of sorted cells such as GFP fluorescence and scatter and annotate it with clonality, gene expression profile, or functional results. Thus GFP fluorescence of the cell sorted into a given well may be compared to the metabolic, genomic, or growth properties of the clone obtained from that cell.

Sony SH800S cell sorter

Sorting workflow for CRISPR screening

HeLa cells were transfected with a vector containing a CRISPR/Cas9-EGFP construct. Post transfection, single cells were deposited into a 96-well plate using the the 100-micron sorting chip on an SH800 cell sorter. The index sorting data of the Cas9 EGFP expressing single cells was recorded.

Figure 2 shows a plate map with single cells deposited in a 96-well plate. When an array of wells is highlighted, the phenotype (e.g., GFP expression level) of single cells (blue dots) can be determined in the flow cytometry dot plot. Thus the flow cytometry data of each clone can be correlated with results from other endpoint assays.

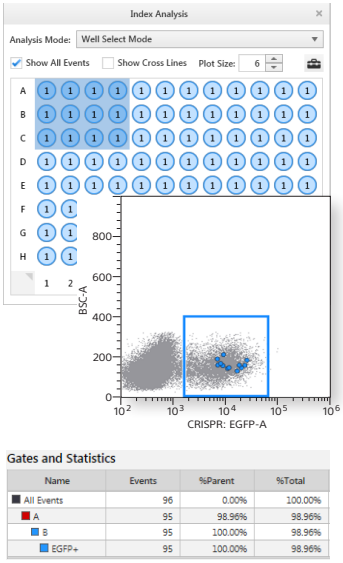

Figure 2: Single cell sorting and index data analysis of HeLa cells expressing CRISPR/Cas9 EGFP

Summary

With the ability to easily identify and isolate single cells from a heterogeneous population, the SH800S cell sorter is a useful tool for laboratories studying and applying the CRISPR technology as a tool for gene editing. The Sort Deposition System and Index Sorting software allow for precise cell deposition with high efficiency combined with multiparameter analysis of individual sorted cells. Analysis of index sort data is useful for a variety of applications in which single cell populations are desired, including gene expression, protein expression, and antibody production.

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