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

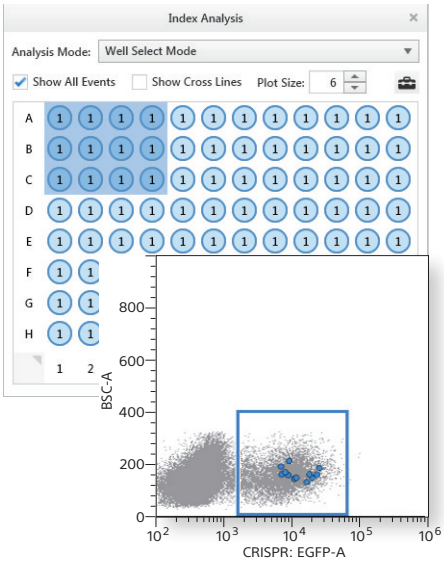
Sort Disposition Unit

96 well plate holder

Gates and Statistics

Name	Events	%Parent	%Total
All Events	96	0.00%	100.00%
A	95	98.96%	98.96%
B	95	100.00%	98.96%
EGFP+	95	100.00%	98.96%

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



CRISPR (Clustered regularly interspaced short palindromic repeats) is a popular tool for editing genomes. These edits are accomplished by introducing the Cas9 nuclease (in the form on 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.1 Cas9 and gRNA are often introduced into cells through transfection. Transfection efficiency varies widely across different kinds of cells and transfection methods. To identify the specific cells that have been successfully transfected with Cas9 and gRNA fluorescent proteins are frequently utilized.

The Sort Deposition System of Sh800S cell sorter facilitates high throughput single cell sorting and precise deposition of target cells into multi well devices such as 96 and 384 well plates. The index sorting feature of the software records the X and Y coordinate (well position) of each sorted event. This indexed information can be used for meta-data analysis to correlate the fluorescence and scatter phenotype of the sorted cells to any endpoint assay results. For example, the GPF 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.
In the experiment below HeLa cells were transfected with a vector containing CRISPR/Cas9-EGFP construct. Post transfection, single cells were deposited into a 96 well plate using the Sony SH800S cell sorter using the 100um microfluidics sorting chip. The index sorting data of the Cas9 EGFP expressing single cells was recorded. Figure A shows 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 when each of these cloned single cells develop into a colony, the the flow cytometry data can be correlated with results from other assays.

Conclusions

With the ability to easily identify and isolate single cells from a heterogeneous phenotypic population, the SH800 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 where single cell populations are desired including gene expression, protein expression, and antibody production.

References

[1] Doudna, Jennifer A., and Emmanuelle Charpentier. "The new frontier of genome engineering with CRISPR-Cas9." Science 346.6213 (2014): 1258096.

Publications using SH800 in CRISPR technology based studies are listed below.

1. Goto, Teppei, et al. "Hypomorphic phenotype of Foxn1 gene-modified rats by CRISPR/Cas9 system." Transgenic research (2016): 1-12.

2. Hayashi, Masayasu, et al. "Chd5 Regulates MuERV-L/MERVL Expression in Mouse Embryonic Stem Cells Via H3K27me3 Modification and Histone H3. 1/H3. 2." Journal of Cellular Biochemistry 117.3 (2016): 780-792.

3. Oakes, Benjamin L., et al. "Profiling of engineering hotspots identifies an allosteric CRISPR-Cas9 switch." Nature biotechnology 34.6 (2016): 646-651.

4. Tsunekawa, Yuji, et al. "Developing a de novo targeted knock-in method based on in utero electroporation into the mammalian brain." Development143.17 (2016): 3216-3222.

5. Morra, Rosa, et al. "Dual transcriptional-translational cascade permits cellular level tuneable expression control." Nucleic acids research (2015): gkv912.

6. Miyagawa, Shuji, et al. "Generation of α1, 3-galactosyltransferase and cytidine monophospho-N-acetylneuraminic acid hydroxylase gene double-knockout pigs." The Journal of reproduction and development 61.5 (2015): 449.

7. Nadler, Dana C., et al. "Rapid construction of metabolite biosensors using domain-insertion profiling." Nature Communications 7 (2016).

8. Schrage, Ramona, et al. "The experimental power of FR900359 to study Gq-regulated biological processes." Nature communications 6 (2015).

9. Sluch, Valentin M., et al. "Differentiation of human ESCs to retinal ganglion cells using a CRISPR engineered reporter cell line." Scientific reports 5 (2015).

10. Oakes, Benjamin L., Dana C. Nadler, and David F. Savage. "Protein engineering of Cas9 for enhanced function." Methods in enzymology 546 (2014): 491.

North America/International

1730 North First Street
San Jose, CA 95112 U.S.A.
Voice: +1 800-275-5963
FAX : +1 408-352-4130
sales@sonybiotechnology.com
http://www.sonybiotechnology.com

Japan

1-7-1, Konan, Minato-Ku,
Tokyo , 108-0075 Japan
Tel : +120-677-010
Fax :+120-388-060
sales_Japan@sonybiotechnology.com
http://www.sony.co.jp/LS

Europe

The Heights, Brooklands.
Weybridge, Surrey, KT13 0XW, UK
Tel: +44 (0) 1932 817448
sales_EU@sonybiotechnology.com