High throughput single cell gene expression analysis using SH800Z cell sorter

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Background

Conventionally, flow cytometry has involved analysis of cellular phenotype at the population level rather than at the single cell level. Therefore, genetic analysis of populations sorted by flow cytometry used mean values from a heterogeneous population. But understanding of heterogeneity of cells and searching of molecular makers are difficult by the traditional flow cytometry population level analysis. The recent emphasis on developing methods for studying cells at a single cell level underlines the importance of single cell analysis. And high throughput methods that offer the ability to mine data on a larger scale are especially important for single cell analysis.

There are several ways to handle single cells, manual picking, robotic picking, capturing in micro fluids, droplet sorting and all that. Sorting of flow cytometry is one of the best, because a large amount of single cells which are selected by using specific biological markers can be sorted in high speed sorting. Only the problem was that sorting of flow cytometry was not easy for the users, adjusting the optical condition, detecting proper sorting conditions and targeting well plate precisely. But SH800Z cell sorter resolved these difficulties and offers the ability to achieve high throughput well sorting function. In this study we demonstrated the utility of sorting and single cell gene expression analysis of single cells in a 384 well plate format using SH800Z cell sorter. In the single cell analysis method, to avoid loss during RNA purification sample volume should be reduced to the minimum. As a result accuracy of sorting position is important for achieving accurate single cell gene expression analysis downstream of cell sorting.

Material and Methods

SH800Z plate sorting technology:

Angled Sorting Plate
To maximize the possibility to sort the sample into the lysis buffer SH800Z PCR plate holder is angled at 4 degrees(Fig1-(B)). In case lysis buffer volume is 0.4μl the margin to get the sample into tube is 0.738[mm] without angled holder, the margin to get the sample into tube is 0.738[mm] with angled holder.

Discussion

In this study we showed combination of SH800Z 384 well plate sorting function and amplification-free single-cell qPCR method provided high throughput single cell gene expression analysis method. Even for the small volume lysis (0.4μl) SH800Z could sort samples precisely and amplification-free single-cell qPCR method measured the absolute copy number stably. Ease-of-use of SH800Z allows wide range of users to do high throughput single cell analysis researches. This will bring them opportunity to discover new. As the next step by using combination of SH800Z 384 well plate sorting function and single cell sequencing method will provide high throughput single cell sequencing results.

Reference


(A) Comparison between vertical standing PCR plate cone and angled PCR plate cone. The margin of sort stream to sort the lysis in the cone, in case of vertical standing margin is 0.054[mm] very close to the edge of cone, and in case of angled all stream can hit into the lysis with 0.738[mm] margin. (B) picture of angled holder for PCR plate.

High accuracy position adjustment function

SH800Z performs automatic setup function to adjust the plate position. But in case of very strict experiment setting, for example single cell analysis of 384 well plates with very small lysis volume, expected accuracy will be over the auto setup position accuracy. Then SH800 software provides adjustment function to user. User can adjust plate position with 0.1[mm] increments in between using 4 corner wells of the plate.

High precision and high speed movement

The time required to sort into 384 wells is not dictated by sorting speed but by speed of plate stage movement and communication with the analysis software. SH800Z realizes high speed plate sorting with intelligent control software and high speed stage.

Figure 1: angled holder benefit

Figure 2: SH800Z plate sorting technology:

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Cell staining and sorting strategy:

**Cell staining**

Mouse embryonic stem (ES) cells were suspended in PBS and these cells were stained with PI (1μg/ml) to differentiate between live and dead cells.

**Sorting strategy**

FSC was used as a trigger (5% threshold) and live cells (PI-A histogram), and singlet live cells were identified (FSC-H vs. FSC-W). The target population was identified by gate G as singlet gate. Single cells from target population were sorted into 0.4μl of RNA lysis buffer of 384 wells. To evaluate the sorting accuracy and in this study each single cell was sorted into the staggered wells (total 192wells).

The qPCR plate was incubated at 95 ℃ for 15 minutes. Subsequently, qPCR was performed for 45 cycles, which consisted of 95 ℃ for 15 seconds, and 60 ℃ for 1 minute. The data were collected at 60 ℃. The standard curve for absolute values was constructed using a 5-fold dilution series of mouse genomic DNA as standard (3125, 625, 125, 25, 5, 1 copies).

**Evaluation of 384 well single cell sorting performance:**

To evaluate the performance of single cell sorting into 384 well plate by using SH800Z, we performed amplification-free single-cell qPCR analysis detecting Gnb2|1 (Accession No. NM_008143), a housekeeping gene of mouse ES cells. To evaluate success rate of single cell analysis single cells were sorted into 192 wells. To evaluate contamination rate by sorting error empty sheath droplets was targeted in the empty wells interspersed between the sorted wells. The sheath drops were set by sort test function of SH800 software function. Furthermore to evaluate absolute quantitative analysis performance of amplification-free single-cell qPCR analysis 32 of non-sorted wells (only lysis buffer) were prepared for non template control (NTC) of RT-qPCR. Single copy control sample was sorted into 4 wells (B10, B12, D10, D12), 5 copies control sample were sorted into 2 wells (F10, F12), 25 copies control sample were sorted into 2 wells (H10, H12), 125 copies control sample were sorted into 2 wells (J10, J12), 625 copies control sample were sorted into wells(L10, L12) and 3,150 copies control sample were sorted into wells(N10, N12) into 384 well plate. The results of amplification-free single-cell qPCR analysis demonstrated. That success rate of sorted cell was 96.8% (186/192). Error wells were M2, M10, O12, B11, J17 and H11. The amplification curves are shown (fig4-(B)). Moreover contamination rate from sorting samples was 0.0%, no gene expression was detected in sheath and NTC wells (fig4-(C), fig4-(D)). Since the sheath wells and NTC wells were located between the single cells wells, any inaccuracy in sorting position would be detectable. Such cross contamination was not detected. Absolute quantitative analysis of single cell gene expression showed good results, $R^2=0.9785$ efficiency=1.831. Absolute quantification using the standard curve method indicated that the expression level of Gnb2|1 was about 3000 copies per cell (mean = 3051.35, S.D = 995.50, CV = 0.33)(fig4-(G)). These results showed amplification-free single-cell qPCR method achieved analysis of absolute quantitative Gnb2|1 gene expression clearly. Moreover both row-wise and column-wise analysis demonstrated that this analysis method is effective (fig4-(H) and fig4-(I)).