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APPLICATION NOTE

NX One

Highly Efficient, Gentle, and Contamination-Free Single Cell Sorting

Introduction

Single-cell sorting is a critical technique in biological and medical research, enabling scientists to isolate and study specific cell populations precisely [1]. While traditional cell sorting methods have been effective, there is an increasing recognition of the importance of sorting one cell per well using a gentle sorting system when sorting single cells. The advantages of gentle, quick, and efficient sorting range from minimizing cell stress, enabling single-cell studies, evaluating gene editing efficiencies, and preserving rare cell populations to generating clonal cell lines for efficacy studies in the drug development pipeline [2].

Methods

Here, we demonstrate the sorting proficiency of the NX One system using Chinese Hamster Ovary (CHO) cells that endogenously express GFP. Other cell lines such as HEK293T, HeLa, COS7, and Jurkat stained with proliferation marker CytoTell UltraGreen demonstrate similar deposition accuracies, as reported in a Technical Note.

During multiple independent rounds of sorting performed outside a biosafety cabinet, only 0.37% of 2,772 wells showed signs of contamination after 7 days post-sorting when these cells grew in complete media supplemented with antibiotics.

Before initiating the sort, we gently triturated the cells to avoid clumping. The cells were loaded into the sample reservoir of a sterile cartridge at an approximate concentration of 100,000 cells per ml. As a control, we performed manual limiting dilutions to target a pipetting accuracy of 0.5 cells per well.

Results

The NX One system completed 7 independent 96-well plate single-cell sorting runs in 5:40 ± 0:2 minutes, with the cells subjected to 0.85 ± 0.05 psi sample pressure. After waiting for cells to settle into wells prefilled with 100µl media for 1 hr at 37°C, we imaged the plate to note the number of wells with either single cells dispensed, doublets (or multiplets), or empty wells (Fig. 1).

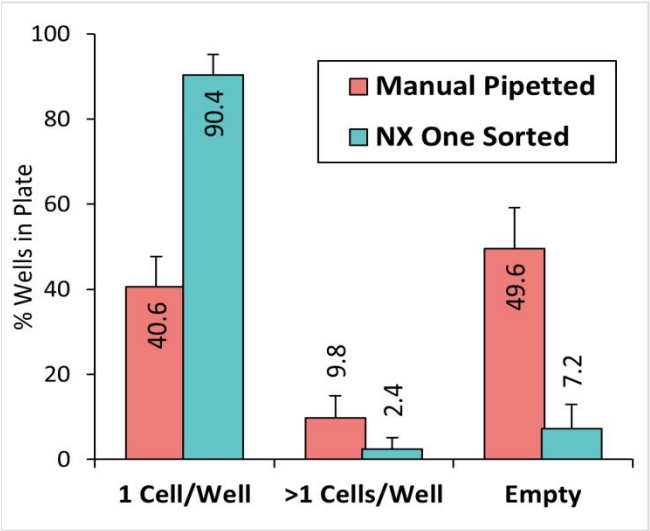


Figure 1. A higher percentage of wells contained a single cell sorted by the NX One system (90.4%) than the manual limiting dilution method (40.6%), and a lower number of empty wells (7.2%).

Results

We monitored and imaged the same plates on Day 7 to quantify the number of colonies grown in wells that contained a single cell on Day 0 (Fig. 2).

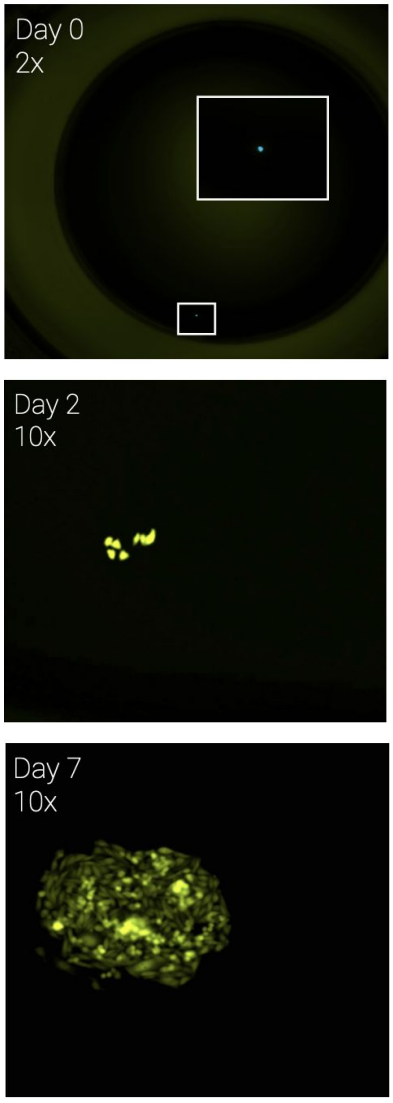
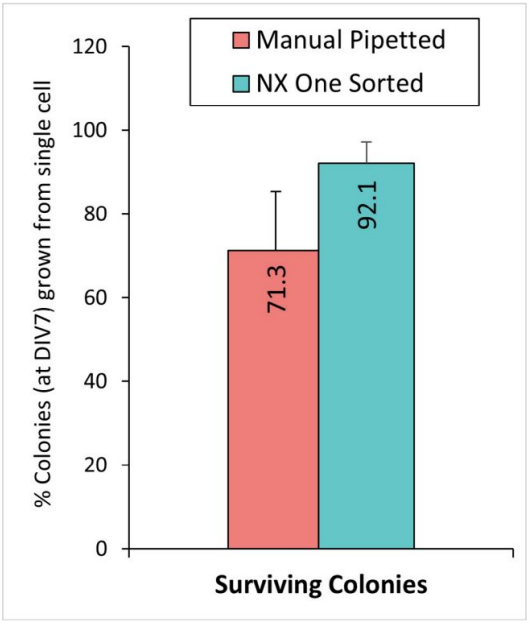


Figure 2. Images demonstrating colony formation from single cells sorted by the NX One system. Inset at Day 0 shows cell quantification mask in cyan. Colony formation from cells sorted by the NX One was higher than colony formation from the manual limiting dilution control.

Conclusion

The NX One system can operate both inside and outside of the biosafety cabinet; it facilitates accurate and quick single-cell sorting with minimal cellular stress (<0.90 psi sample pressure) and high reproducibility (4.76% SD), leading to a higher number of colony formation rate.



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References

1. Hu et al., Front Cell Dev Biol., 2016.
2. Gross et al., Int J Mol Sci., 2015.