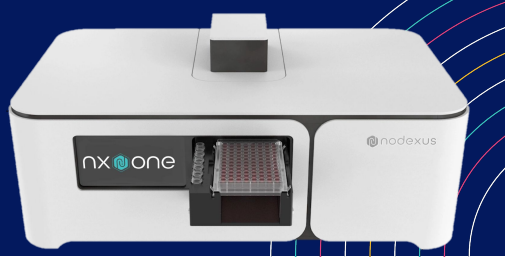
nodexus

APPLICATION NOTE

Neuronal Patterning of NX One Sorted Pluripotent Monoclonal iPSCs

nx one



Introduction

Human Induced Pluripotent Stem Cells (hiPSCs) are immensely valuable in research and drug discovery. These cells offer a unique platform to study disease mechanisms, model patient-specific conditions, screen potential drug candidates, and test the efficiency of gene editing strategies like CRISPR. Obtaining monoclonal iPSC lines thus becomes crucial in achieving consistency and reliability in research and therapeutic applications. Monoclonal lines derived from a single iPS cell exhibit uniform genetic and phenotypic characteristics, thereby minimizing the variability between experiments and enhancing the reproducibility of results, which is extremely important when studying disease mechanisms or testing the efficacy of drug candidates. Equally important to clonogenicity, an iPS cell must maintain its pluripotency, i.e. the ability of these cells to differentiate into other cell types. Monoclonal iPSC lines with robust pluripotency exhibit consistent differentiation potential, allowing researchers to generate a wide range of cell types.

This application note demonstrates that single iPS cells sorted by the Nodexus NX One benchtop single cell sorting and dispensing platform carry the clonogenic ability to grow into a pluripotent colony. Using two previously published patterning protocols (**Figure 1**), these sorted iPSC colonies also showed similar differentiation potential as the parent iPS line in generating cortical neurons.

Methods

A footprint-free human iPSC line (Alstem Bio #IPS11) was cultured as described previously. After sorting, the single iPS cell was incubated for 14 days before transferring the colony to one well of 12-well plate.

Two colonies were selected for the pluripotency assay and neuronal patterning. Pluripotency was determined using the live-cell TRA-1-60 Alexa Fluor™ 488 conjugate staining (Thermo Fisher Scientific #A25618) and following the manufacturer's instructions. For neuronal patterning, we followed two patterning protocols using the small molecules as described in the publications shown (**Figure 1**).

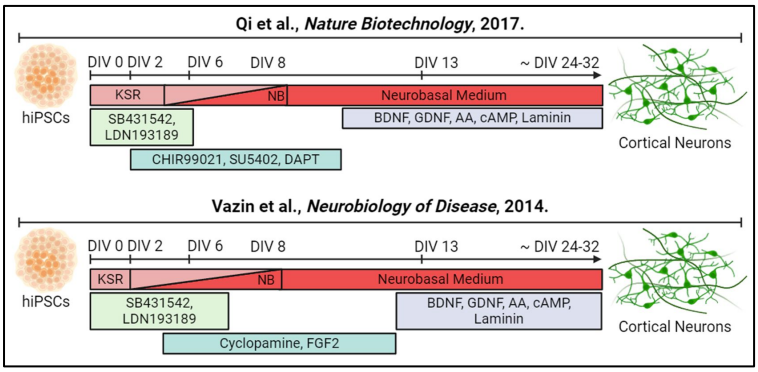


Figure 1. *Patterning protocols utilized for the generation of cortical neurons from NX One sorted iPSCs.*

At the end of the patterning protocol, the cells were lifted from the well using Versene (Thermo Fisher Scientific #15040066) and plated on glass coverslips coated with Poly-L-Ornithine (Sigma #A-004-M) and Laminin (Sigma #L2020). After seven days in culture, the patterned cortical neurons were stained with NeuroFluro™ NeuO (StemCell Technologies #01801) per the manufacturer's instructions.

Results

As demonstrated in our previous application note, the NX One (80.9%) significantly outperformed manual limiting dilution (30.2%) in isolating a single iPS cell per well of a 96-well plate.

Results

The NX One sorted iPS cells demonstrated monoclonal outgrowth of 42.4%, comparable to the 35.5% in the limiting dilution control plates. We observed that the size of the NX One sort-generated colonies was equivalent to that of the colonies obtained from manual pipetting (**Figure 2**). We picked two colonies with the highest proliferation rate to expand further into two distinct iPS cell lines (iPS_SM1 and iPS_SM2).

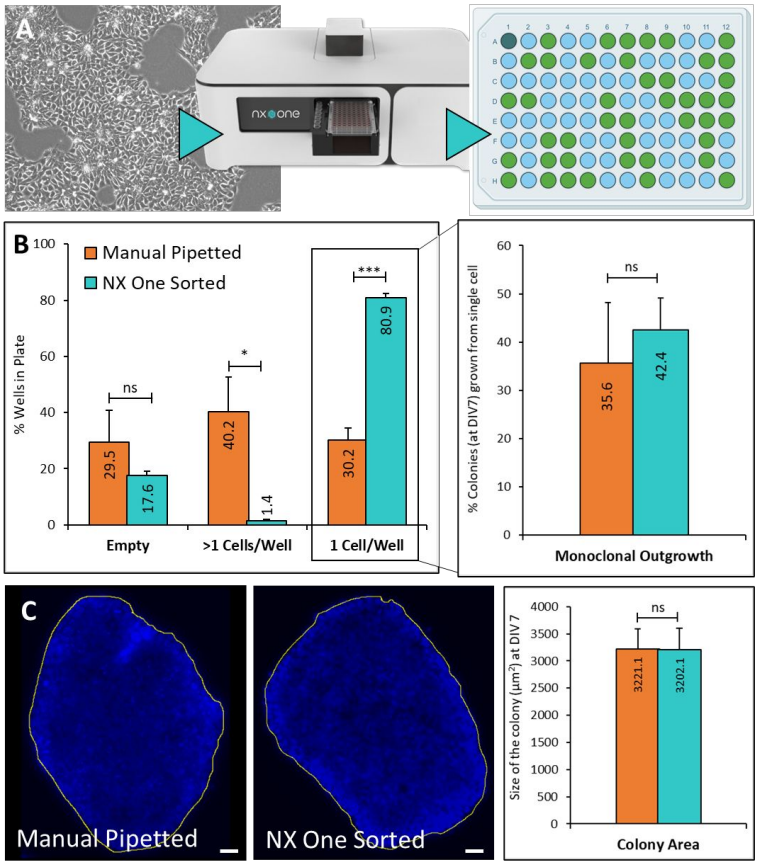


Figure 2. *(A) NX One workflow to generate colonies from a single iPS cell dispensed into one well of a 96-well plate. E.g., one such plate showed 42/95 wells containing a colony that arose from a single sorted cell. Well A1 is a spiked control (B) Quantification of numbers of wells with sorted single cell and their subsequent outgrowth (C) Nuclei were stained with Hoechst and size of the colony was measured using ImageJ. Scale bar : 100µm. Error bars indicate SD; *p < 0.05; ***p < 0.0001; ns - not significant, two-way ANOVA.*

We observed similar pluripotency potential in these isolated colonies compared to the unsorted parental iPS line after performing TRA-1-60 staining (**Figure 3**). The kit facilitated the labelling of surface marker TRA-1-60 present exclusively on pluripotent stem cells (*Lee et al., 2017*).

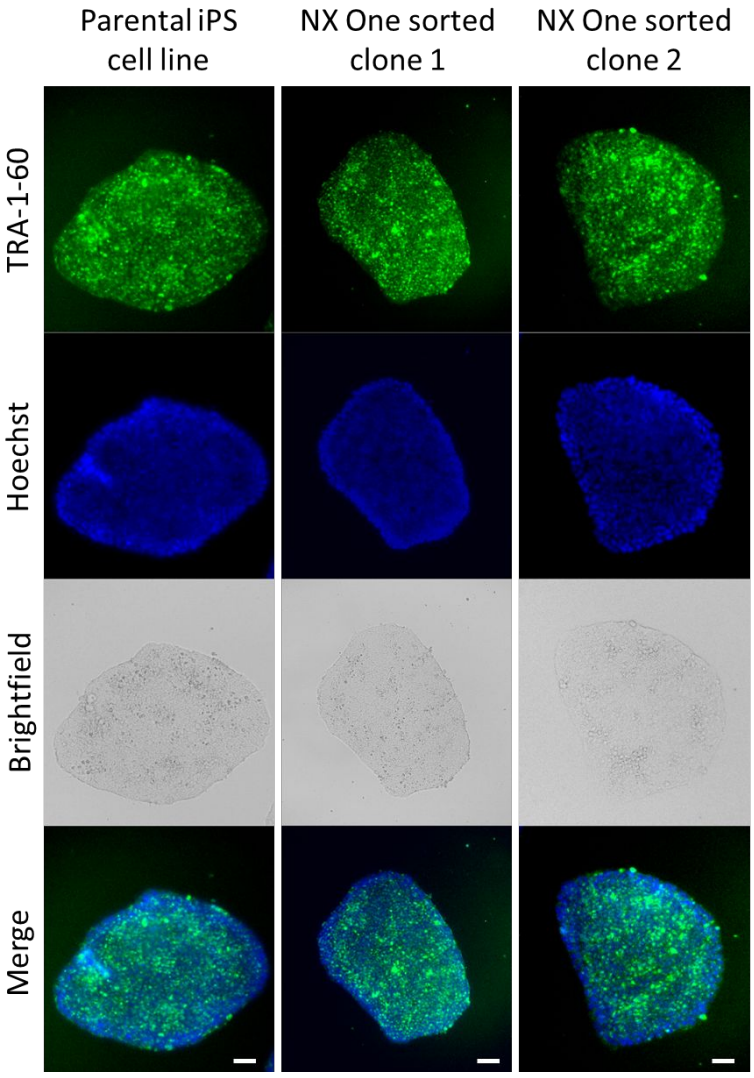


Figure 3. *Representative images of pluripotency marker (TRA-1-60) staining on NX One sorted iPS colonies and parental iPS cell line. Nuclei were stained using Hoechst. Scale bar : 100µm.*

Following two small molecule-induced patterning protocols, we generated cortical neurons from two sorted clones and the parental iPS cell line. In brief, the small molecules used in these protocols pattern the iPSCs by inhibiting signaling pathways like BMP/SMAD (*Chambers et al., 2009*), WNT, TGF-β, MEK, GSK-3β, VEGFR-2, Notch (*Qi et al., 2017*) and the Hedgehog pathway (*Vazin et al., 2014*). Once at the progenitor stage, these neural progenitor cells (NPCs) are lifted and plated in a media containing growth factors like BDNF, GDNF, Ascorbic Acid, Laminin, and cyclic AMP to facilitate the maturation of newly generated neurons (*Mahajani et al., 2019*).

The neural progenitors were plated on Poly-L-Ornithine and Laminin-coated coverslips to form cells with neuronal-like morphology.

Results

We used a live neuronal marker to stain for neurons and Hoechst to label the nuclei (**Figure 4**).

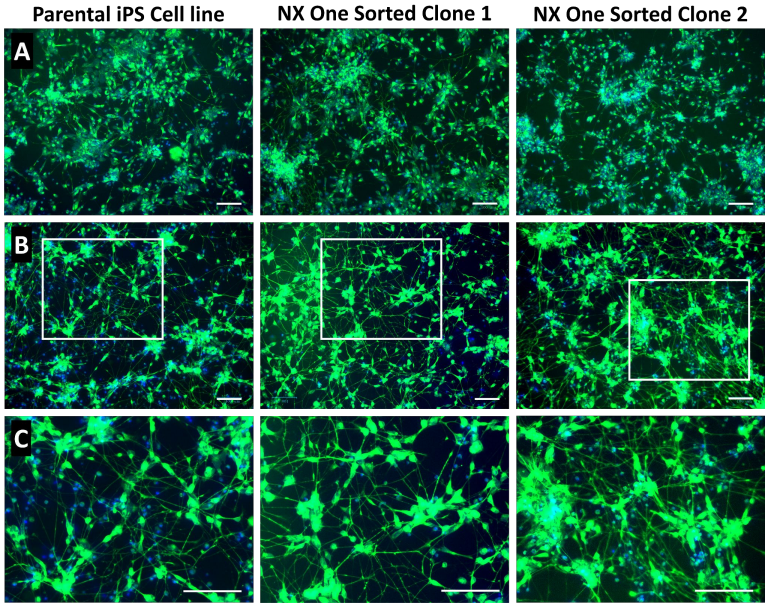


Figure 4. *Representative live neuronal marker (*NeuroFluro™ NeuO*) stained images with magnified insets (C) of patterned neurons generated from NX One sorted iPS colonies and parental iPS cell line using the Qi et al., 2019 (A) and the Vazin et al., 2014 (B) protocol.. Nuclei were stained using Hoechst. Scale bar : 100µm.*

Upon quantification, we observed similar percentages of neurons from the NX One sorted progenies and parental iPS cell line with both patterning protocols (**Figure 5**). Three independent patternings were performed with quantification on six images. We observed a higher percentage of neurons using the *Vazin et al., 2019* protocol compared to the *Qi et al., 2014* protocol, which, interestingly, is also in accordance with the percentage of neurons that these two patterning protocols report.

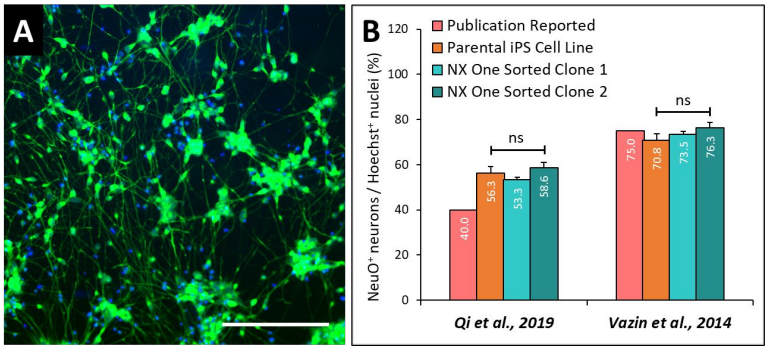


Figure 5. *Representative 10x image (A) of patterned neurons stained with live neuronal marker (*NeuroFluro™ NeuO*). Nuclei were stained using Hoechst. Quantification of percent NeuO⁺ neurons over all the cells in culture (B). Scale bar : 100µm. Error bars indicate SD; ns - not significant, one-way ANOVA.*

We observed a higher percentage of neurons patterned from the parental iPS cell line and both progeny clones using the *Qi et al., 2019* protocol when compared to their reported percentage, but this could be attributed to the difference in the iPS cell line used (*Mahajani et al., 2019*).

Conclusion

This application note highlights the efficiency of the NX One system in sorting single iPSCs with high clonogenic ability, leading to the generation of monoclonal iPSC colonies. These sorted colonies demonstrated robust pluripotency and were able to differentiate into cortical neurons using established small molecule-induced patterning protocols. The similarity in pluripotency and neuronal differentiation potential between single-cell sorted progenies and the parental iPS cell line highlights the gentleness and reliability of the NX One in maintaining key iPSC characteristics. Overall, these results support the utility of the NX One in facilitating efficient and reliable iPSC-based research.

References

1. *Chambers et al., Nature Biotechnology, 2009*
2. *Lee et al., Cell Reports, 2017*
3. *Mahajani et al., Cell Death & Disease, 2019*
4. *Qi et al., Nature Biotechnology, 2019*
5. *Vazin et al., Neurobiology of Disease, 2014*

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