SEED & GO: An Improved Cryopreservation Method for Enhanced iPSC Reproducibility

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Stem cell services and QC

ABSTRACT

Induced pluripotent stem cells (iPSCs) have emerged as a promising tool for regenerative medicine and drug discovery. However, as highlighted by the publication of the ISSCR standards guidance last year, challenges related to reproducibility, reliability, and consistency in iPSC culture and assays have hindered research. The SEED & GO method aims to address these issues by providing a bespoke cryopreservation approach that significantly improves reproducibility and reduces assay timelines.

Using highly optimised protocols to cryopreserve iPSCs as single cells, SEED & GO minimizes variability introduced by traditional clump passaging. This results in highly uniform cell banks with minimal vial-to-vial differences. Additionally, SEED & GO minimises the time between thaw and differentiation, reducing the overall protocol by 5 days. Cryopreserved cells maintain high viability and express key undifferentiated state markers.

SEED & GO offers a significant advantage for drug discovery by improving iPSC reproducibility, reducing assay timelines, and enabling more efficient and reliable screening assays.

METHOD & RESULTS

Cell viability and adherence:

To develop robust protocols for single-cell cryopreservation we employed a panel of cell lines with differing growth properties, sensitivity to single-cell handling and cryopreservation. Four cell lines were used for the initial assessment (1-4) and were subjected to four freezing and handling protocols (A-D). Cell viability on revival was assessed and all conditions demonstrated greater than 86% viability (range 86-97%). (Figure 1a). While cell viability is a key metric, cells in poor condition can still be viable on thaw but demonstrate poor adherence 24 hours post-thaw. To assess the adherence of the revived cells, we seeded the cell lines at a range of confluence (low, medium, and high) and utilised the whole well imaging, AI-based iPSC confluence app on the Cell Metric® X platform to compare the adherence and confluence 24hrs post revival for three of the protocols (A, B, and D). Cells frozen using protocol D demonstrated significantly greater attachment and adherence post-thaw (Figure 1b).

a)

	Cell line 1	Cell line 2	Cell line 3	Cell line 4
Protocol A	94	94	97	92
Protocol B	86	90	94	93
Protocol C	88	92	95	90
Protocol D	88	91	97	92

b)

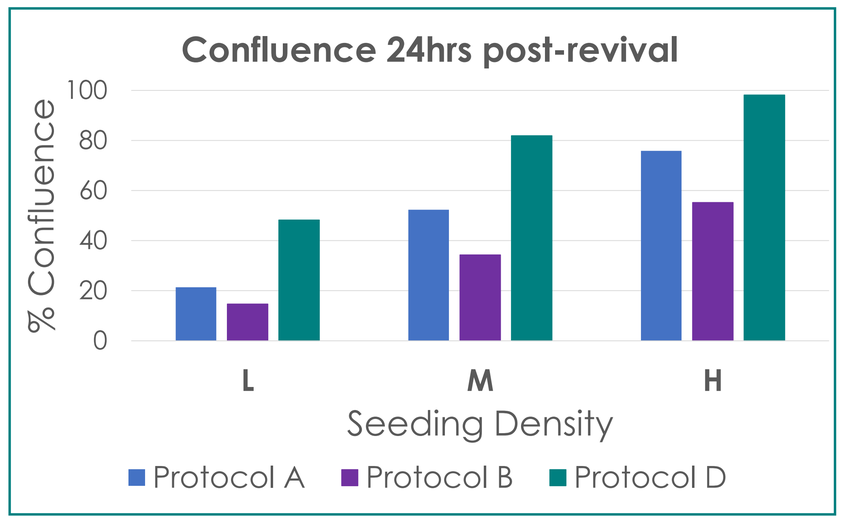

Figure 1 a) Cell viability (%) at thaw for the 4 cell lines frozen using protocols A-D. a) Cell confluence at 24hrs post-thaw was assessed using the Cell Metric X iPSC confluence app for cells frozen using protocols, A, B and D

Functional Assays:

To validate that the SEED & GO cryopreserved cells retained expression of markers of the undifferentiated state, we performed ICC/IF on both SEED & GO cryopreserved cells and traditional clump banked cells as a control (Figure 2).

To demonstrate that the SEED & GO cryopreserved cells are pluripotent, we compared the differentiation capability of SEED & GO cryopreserved cells vs traditional clump banked cells, using the STEMdiff™ Ventricular Cardiomyocyte Differentiation Kit from StemCell Technologies. All conditions were evaluated in triplicate. Cells cryopreserved using clumping passaging required an extra 5 days post-revival and a passage before differentiation was initiated, whereas SEED & GO cryopreserved cells were ready for differentiation only 2 days post-revival after seeding at a range of densities. The SEED & GO cryopreserved cells demonstrated coordinated beating from day 8 onwards. Figure 3 outlines the workflow time savings using the SEED & GO process and videos of the beating cardiomyocytes 14 days after seeding. RT-qPCR analysis of TNNT2 expression in cardiomyocytes derived from SEED & GO cryopreserved cells demonstrated similar expression levels as cardiomyocytes derived from traditionally cryopreserved cells (Figure 4).

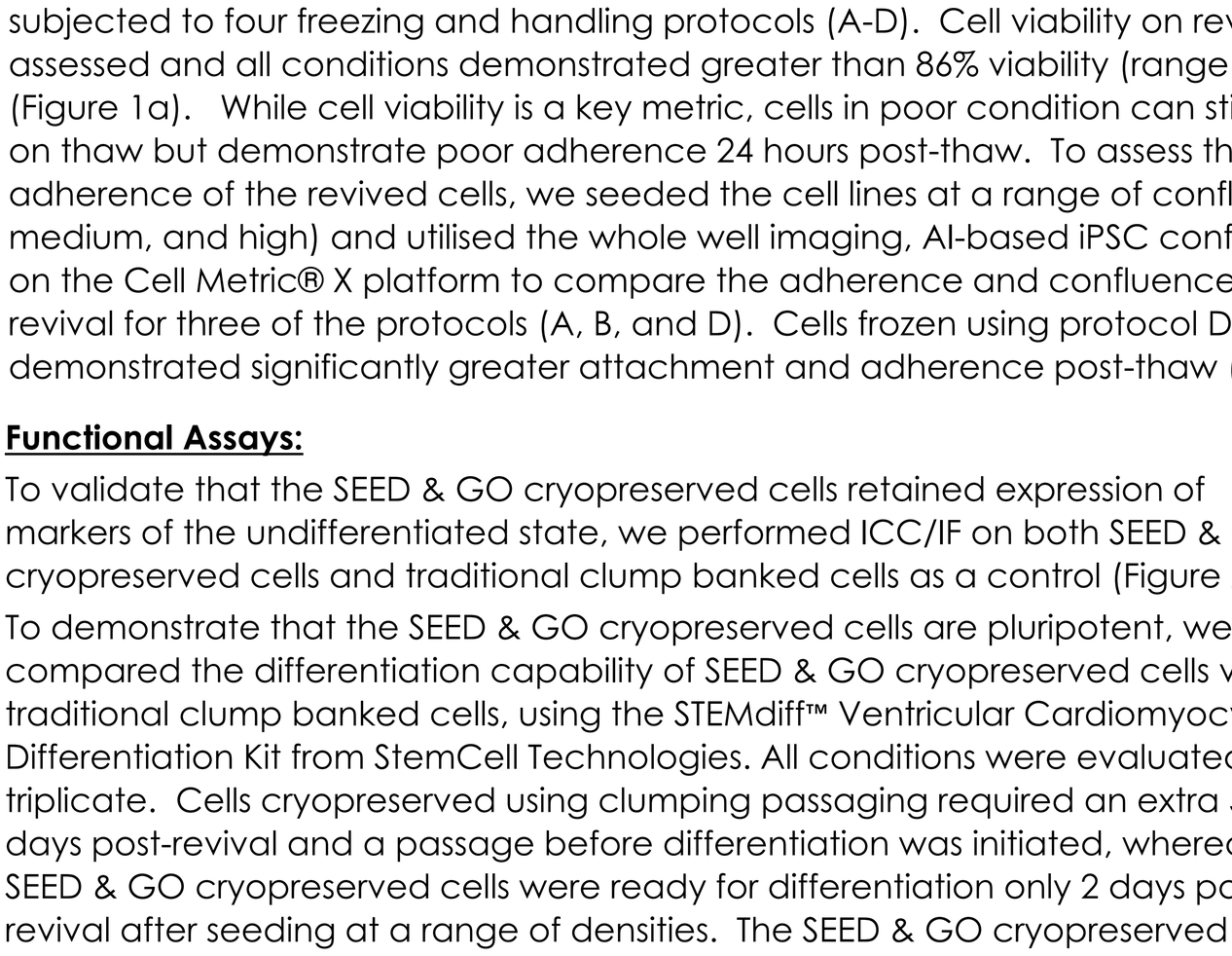

Figure 2 ICC/IF staining (DAPI/TRA-1-60/SOX2) for a) cells cryopreserved using traditional clump methods and b) cells frozen using the optimised SEED & GO protocols

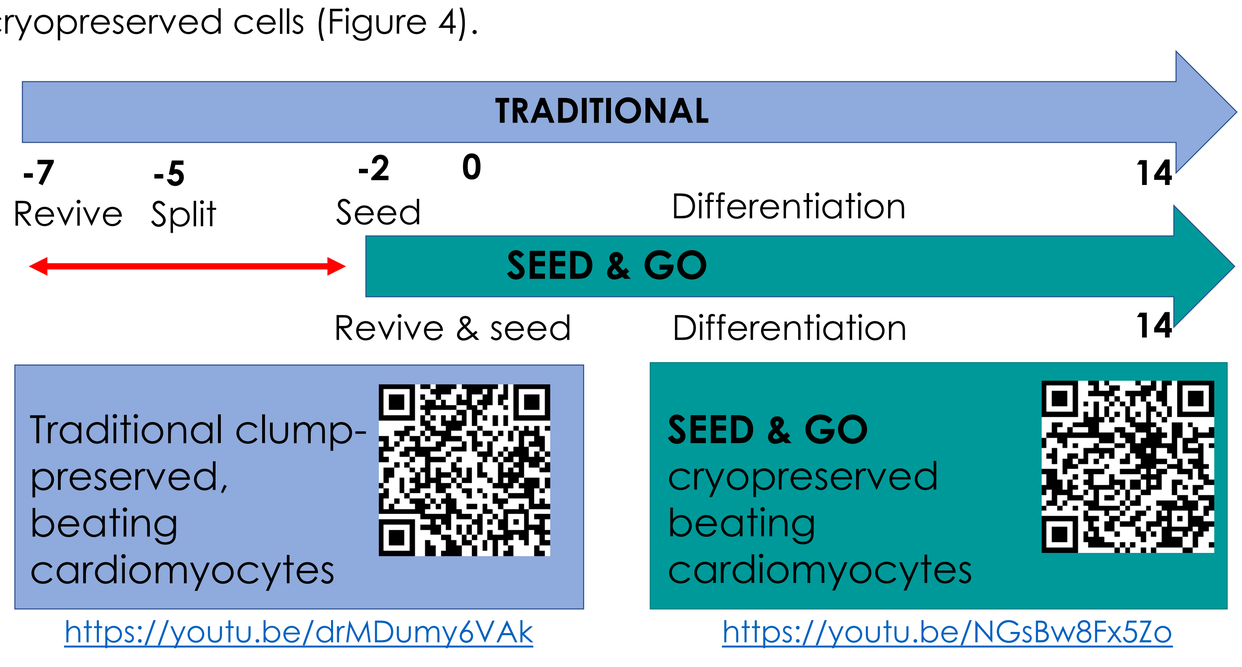

Figure 3: Workflow overview and cardiomyocyte differentiation videos for iPSCs cryopreserved using traditional clump methods or SEED & GO.

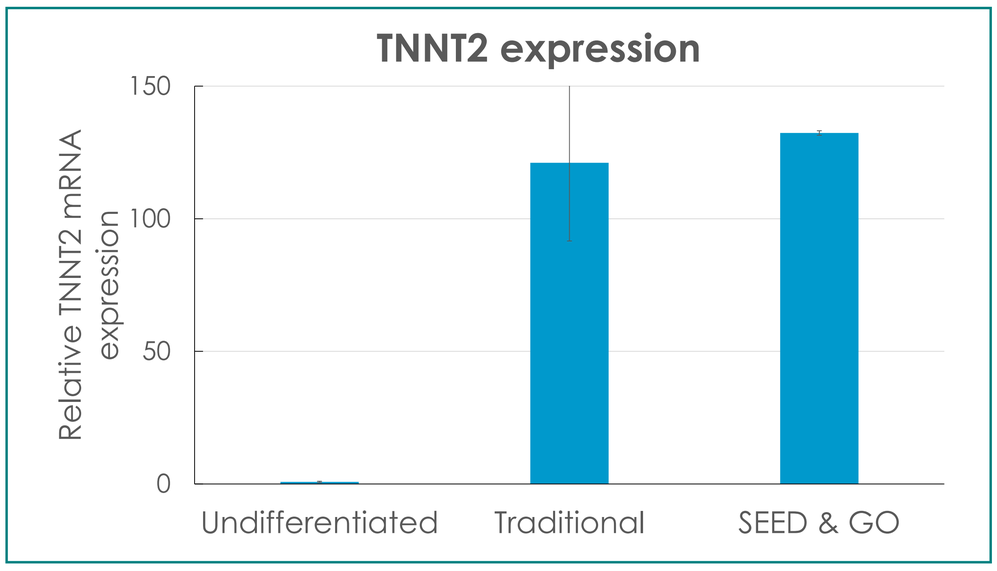

Figure 4: RT-qPCR analysis for TNNT2 expression relative to GAPDH

CONCLUSIONS

In this study we have optimised and validated protocols to achieve single-cell cryopreserved cell banks that can be rapidly deployed for differentiation assays. This saves significant time and costs associated with extended cell culture and improves reproducibility through high uniformity between vials.

Assay reproducibility is a challenge for many laboratories as highlighted by the recent ISSCR standards publication (https://www.isscr.org/standards-document) . SEED & GO is designed to support stem cell scientists when combined with regular QC and characterisation by a) improving the reproducibility of assays and cell cultures and b) reducing costs and time associated with repetition of work.