



A study presented in collaboration with CCRM.

Objective
Satisfy need for a GMP compliant closed cell sorting system for enrichment of desired cell types and removal of remnant iPSCs in manufacturing

Process Developed
Utilized the CGX10 cell isolation system for enrichment of desired target cells (differentiated cardiomyocytes) and removed iPSCs using cell type specific markers

Outcomes
The CGX10 Cell Isolation System from Sony Biotechnology can be integrated into iPSC derived manufacturing processes, enabling single step multiparametric enrichment of desired cell types

Utility of the CGX10 Cell Isolation System in Manufacturing of iPSC Derived Cell Therapies

A GMP ready cell sorter for closed cell and gene therapy manufacturing

Human induced pluripotent stem cells (iPSCs) can be limitlessly expanded and differentiated into almost all cell types. iPSC derived cell therapies have the potential for treating a multitude of diseases including cardiovascular, neurological, and metabolic conditions. Apart from inconsistent differentiation efficiencies, an unsolved safety concern for clinical translation is the presence of undifferentiated iPSCs in the final product. Currently there are no GMP solutions to purify the desired differentiated cell product prior to its administration. The CGX10 is a GMP ready, closed cell sorting system from Sony, which enables enrichment of desired cell types by multi parametric selection. In this case study, using iPSC derived cardiomyocytes as a representative therapy, we studied the potential utility of the CGX10 to isolate cardiomyocytes while eliminating undifferentiated iPSCs using multi-parametric cell isolation.

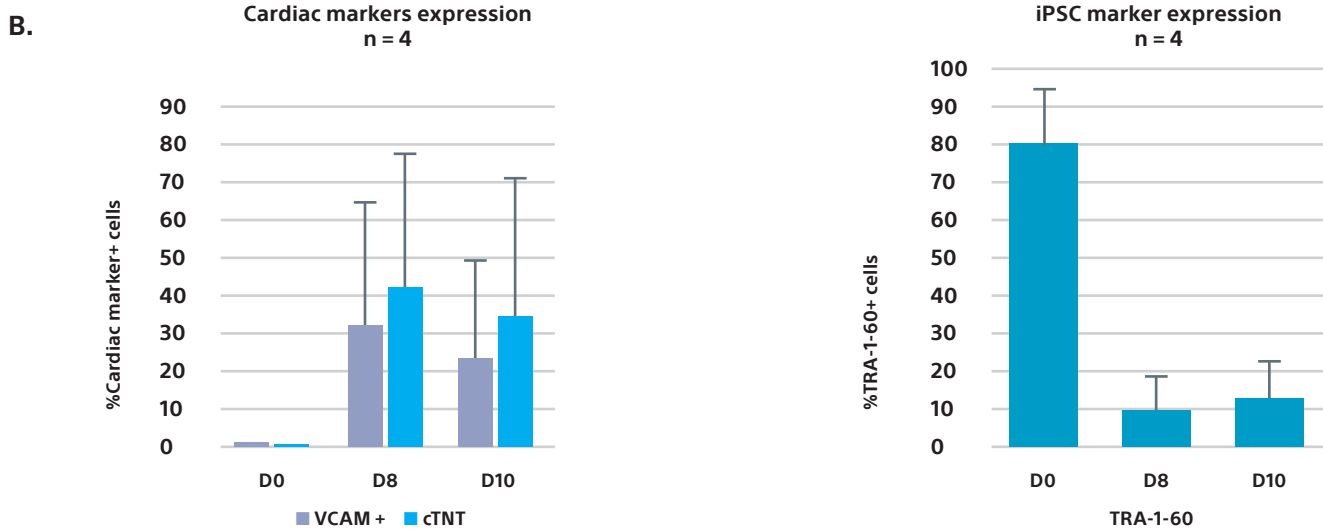
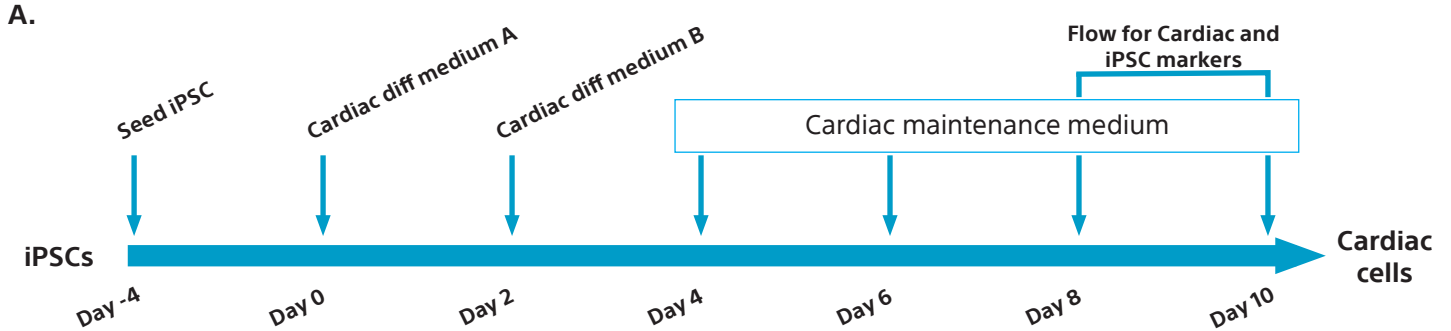
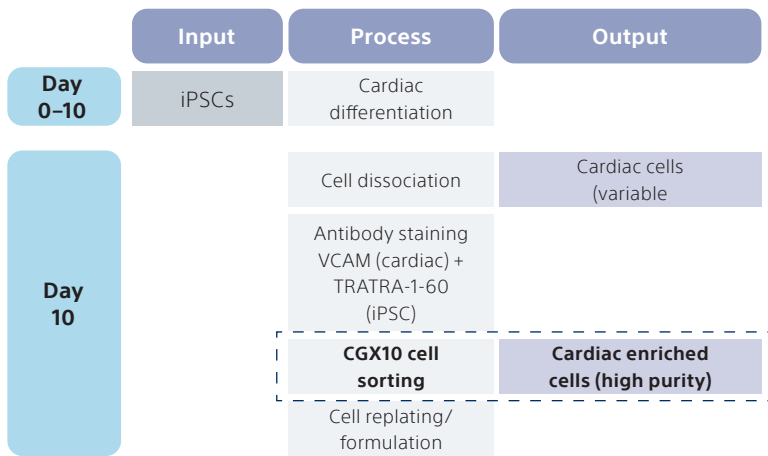


Figure 1: Outline of iPSC to cardiac differentiation (A) and flow cytometry analysis (B)

Cardiomyocytes are a promising cell type for cell replacement therapy. In this study, we differentiated a commercially available iPSC line into cardiomyocytes using a commercially available kit (Figure 1A). To monitor the progression of differentiation, we used VCAM and cardiac troponin (cTNT) as cardiomyocyte markers that are known to peak at approximately 8-10 days into differentiation. For staining undifferentiated iPSCs, we used the iPSC surface marker TRA-1-60. Although there was a notable increase in cardiac

markers over time and a decrease in TRA-1-60+ iPSCs at the end of the protocol (Figure 1B), the differentiation efficiency was highly variable, and a considerable percentage of non-cardiac cells and iPSCs remained in the final product, warranting purification of the cardiac cells before formulation.

Methods and results



CGX10 Cell Isolation System

Figure 2: Process flowchart for utilizing the CGX10 to purify iPSC derived cardiac cells before formulation

iPSC derived cardiac cells were stained with VCAM (cardiac) and TRA-1-60 (iPSC) antibodies on Day 10 of the differentiation and subjected to low pressure sorting using CGX10 purity mode (Figure 2). The CGX10 was configured to isolate VCAM+, TRA-1-60- cells, and pre- and post-sort purity was assessed using the CytoFLEX flow cytometer. As shown in Figure 3A, CGX10 cell sorting resulted in enrichment of cardiac cells and a significant removal of iPSCs,

rendering a uniform final cell product. Sorting did not impact the cell viability of the cardiac cells (Figure 3B). The CGX10 can be utilized to isolate specific sub-types within the differentiated cell product using combinations of cell-type specific markers, thereby enabling integration into varied iPSC derived cell therapy manufacturing processes.

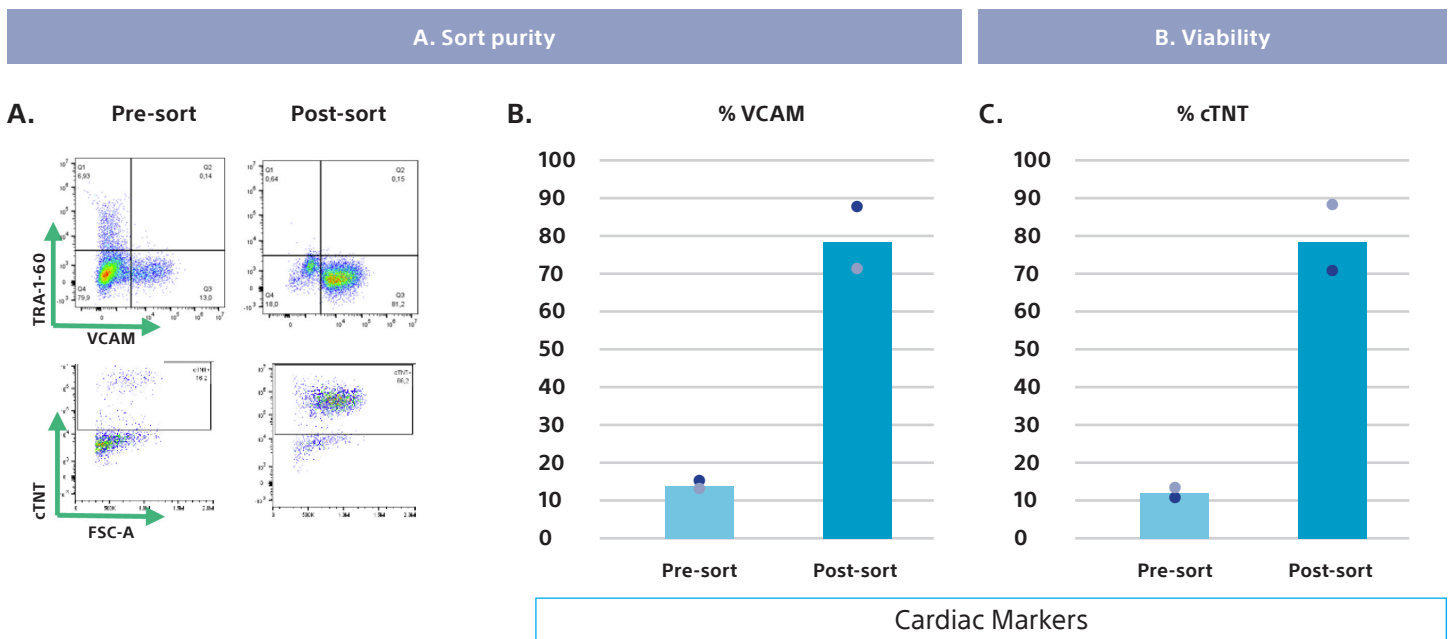


Figure 3: Assessment of pre-and post-sort purity and viability

■ Average n=2 ● Exp 001 ● Exp 002

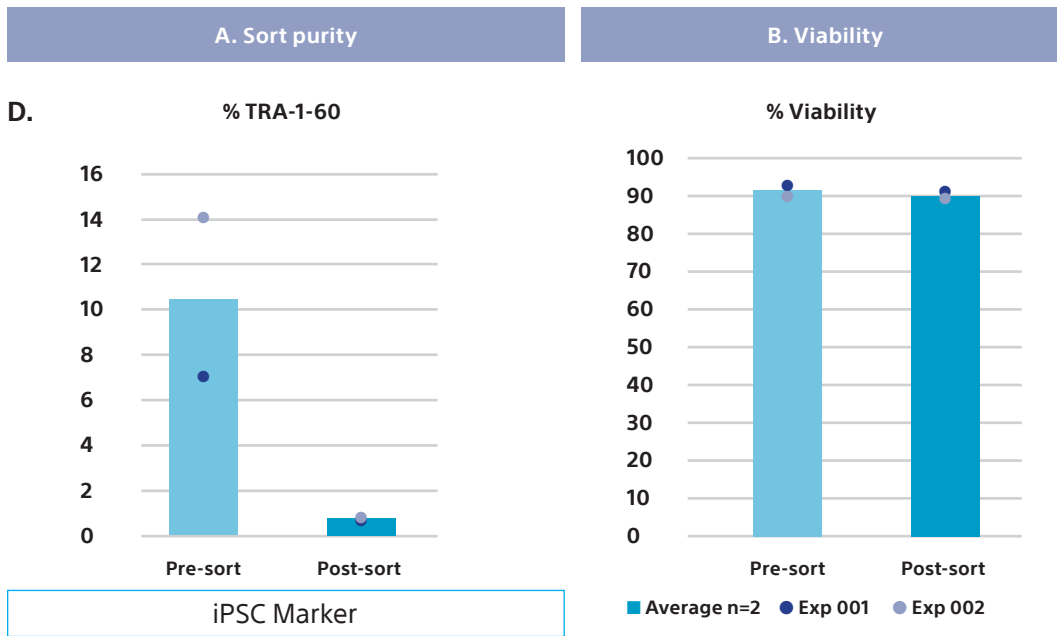
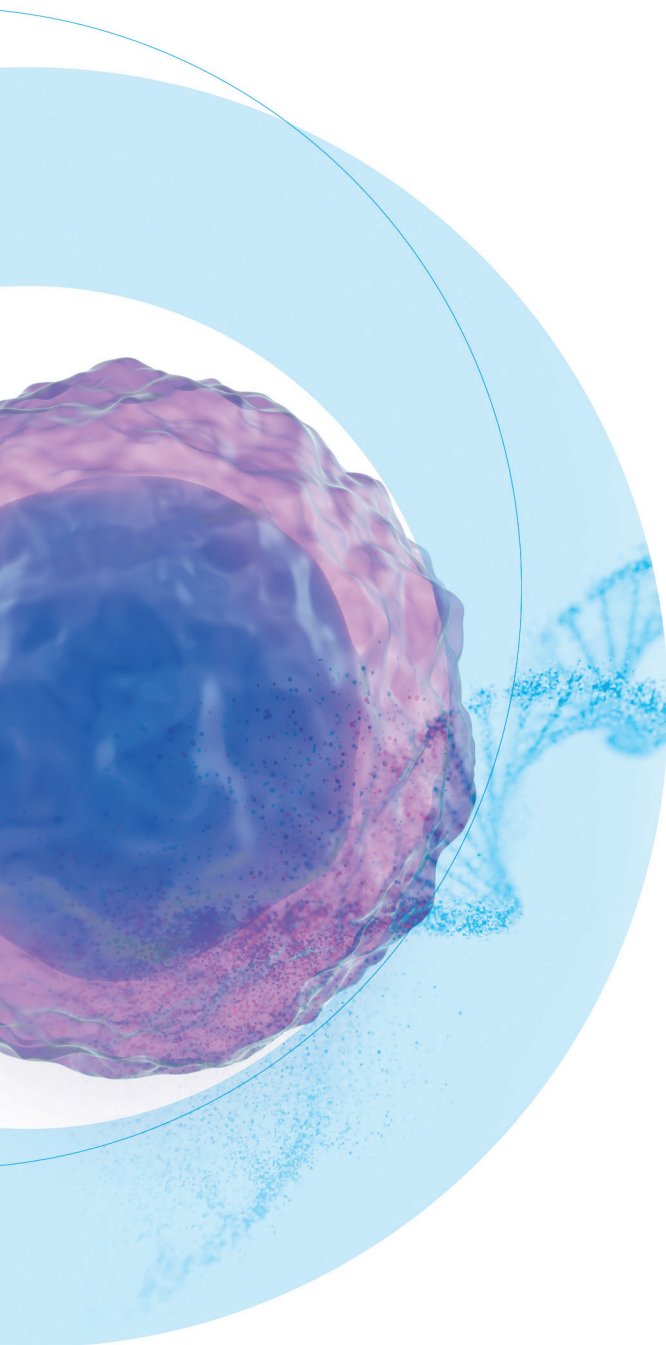


Figure 3: Assessment of pre-and post-sort purity and viability

Conclusion

- The CGX10 cell isolation system is a unique “closed” cell sorting instrument with customized single unit disposables and a GMP compatible user interface.
- The CGX10 has the ability to isolate desired iPSC derived differentiated cell populations using customized markers, without impacting the cell viability.
- The CGX10 cell isolation system has potential to be integrated into varied iPSC derived cell therapy and immunotherapy manufacturing pipelines.



Customer support

Sony provides the highest quality of customer service, scientific support, and field service resources to ensure the best customer experience.

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