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Methods for Rapid Establishment and Scale-up of iPSC

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ABSTRACT

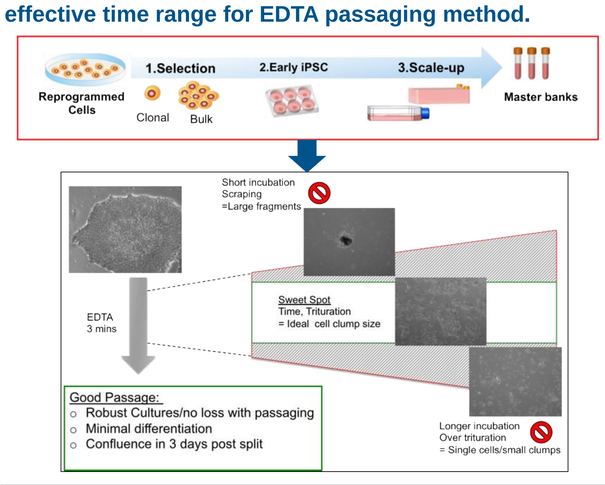
The development of reprogramming technologies for the generation of induced pluripotent stem cells (iPSCs) has catalyzed powerful possibilities in the field of stem cell research and regenerative medicine. Successful establishment of iPSCs consists of three critical steps, including initial colony isolation, early clonal expansion, and subsequent scale up of cells for use in further applications. The main challenges encountered are identifying and selecting high quality colonies, and manual passaging of early clones, both of which require a high degree of technical expertise and time commitment. Simplification and consistency of this process is necessary for rapid generation and expansion of iPSCs for use in downstream applications.

In this study we report the development of a cell passaging reagent effective for establishment of early iPSC clones. This reagent (Reagent 1) shows consistent performance across different matrices, medias and user experience levels. Cells harvested using this reagent show efficient detachment with uniform clump size. Compared to EDTA, Reagent 1 shows better attachment and faster growth rates when reseeded for further culture. Additionally, very early passage clones, that often need to be manually passaged using mechanical methods, can be subjected to bulk harvesting using this reagent without compromising quality or survival. Unlike EDTA, a commonly used passaging solution for iPSCs, this reagent can be used to passage cells across a broader incubation time window and for varying densities of cells. These features collectively enable the use of this cell harvesting reagent across the entire iPSC workflow from early iPSC clonal establishment to scale up in cell factories. The ability to successfully scale up early iPSC clones with minimal manual manipulations streamlines the bioprocessing workflow for both basic and translational research.

INTRODUCTION

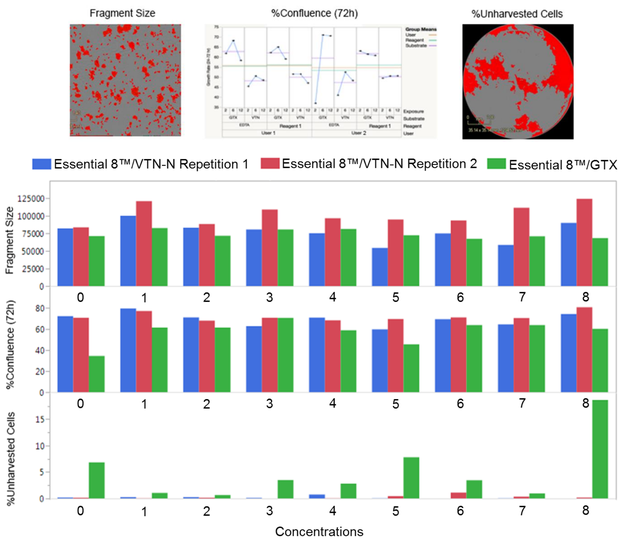
Critical parts of the reprogramming workflow are the clonal selection, early culture towards iPSC establishment and passaging in large non-traditional dish formats such as Cell Factories. These steps often requires manual manipulation or user expertise to gently handle the wide range in number of cells to minimize exposure to facilitate passage of the cells as clumps. Common non-enzymatic methods such as EDTA, although robust for routine passaging, can be variable in the hands of new users here. Here we report the development of a new alternate solution that overcomes current constraints and effective across a range of incubation times and applications.

Scheme 1: Key step in establishment of iPSC & effective time range for EDTA passaging method.



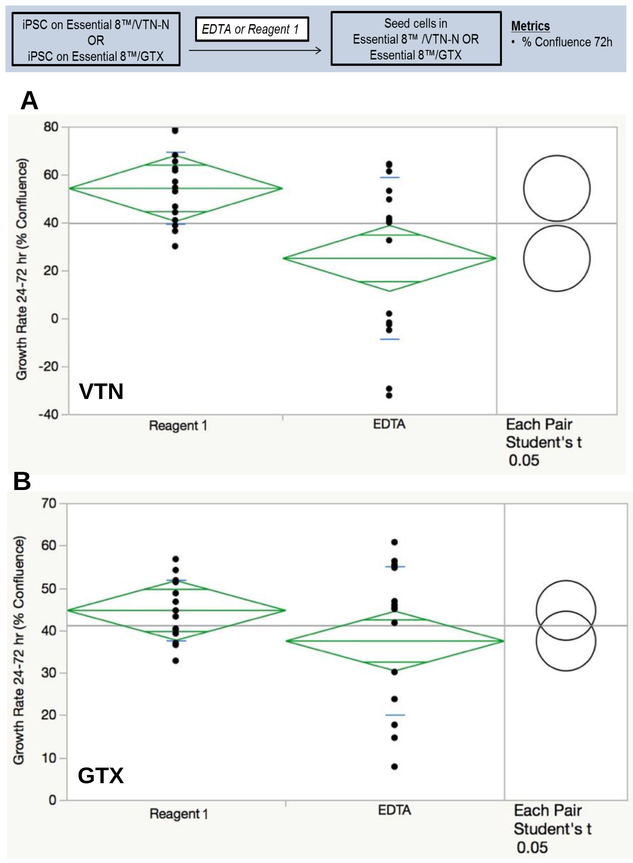
RESULTS

Figure 1. DOE analysis to identify optimal solution



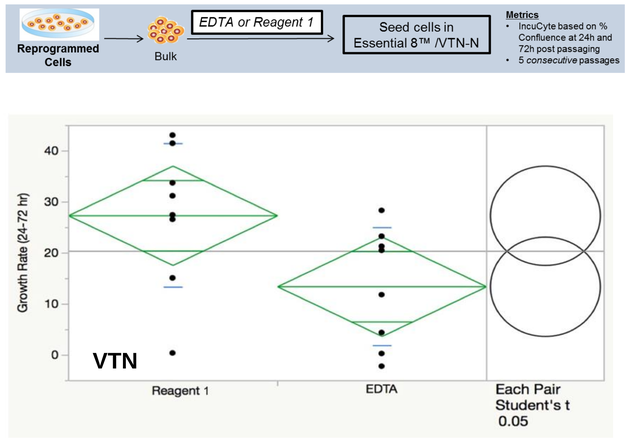
Design of Experiments (DOE) was carried out with solutions (0-8) comprising of various concentrations of cationic chelators to identify the ideal solution that resulted in maximal growth post harvesting and seeding. Three key parameters measured were the size of the harvested clumps (fragment size), Growth (%confluence at 72h) and efficiency of harvesting (%unharvested cells) of iPSC cultured on Geltrex (GTX) or Vitronectin (VTN-N).

Figure 2. Reagent 1 is optimal for routine passaging of iPSC culture



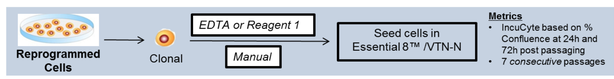
An established iPSC line was routinely harvested on either GTX or VTN-N, where the percent confluence was monitored every twelve hours with IncuCyte® live imaging. Growth rates were calculated for each passage using the 24th and 72nd hour time points. **(A)** Results indicated a significant increase of growth rates for cells on GTX treated with Reagent 1 (μ= 52.42, σ= *14.92*) over EDTA (μ = 25.23, σ = 33.53), $p < 0.05$. **(B)** There was no significant difference between the reagent groups on VTN-N; Solution A (μ = 44.81, σ = *7.09*) and EDTA (μ = 37.59, σ = 17.41), $p > 0.05$. Reagent 1 showed less variation on both matrices; as indicated by the standard deviations.

Figure 3. Reagent 1 can be used to generate pooled iPSC clones



Early passage pooled iPSC clone on VTN-N, indicated a significant increase in growth rates for cells treated with Reagent 1(μ= 27.3, σ= *14.0*) over EDTA (μ = 13.4, σ = 11.5), p=0.05.

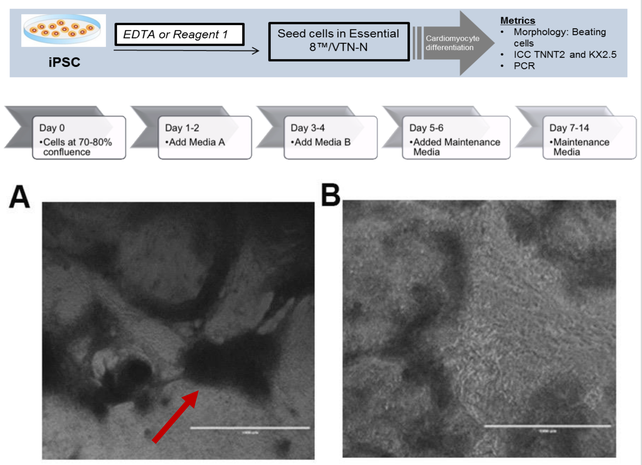
Figure 4. Reagent 1 is effective in early clonal passaging for successful iPSC establishment



CytoTune 2.0 Essential 8/GTX		Passaging Method														
		Manual					EDTA					Reagent 1				
Users	Clones	P3	P4	P5	P6	P7	P3	P4	P5	P6	P7	P3	P4	P5	P6	P7
A	1															
	2															
B	1															
	2															
	3															
	5															

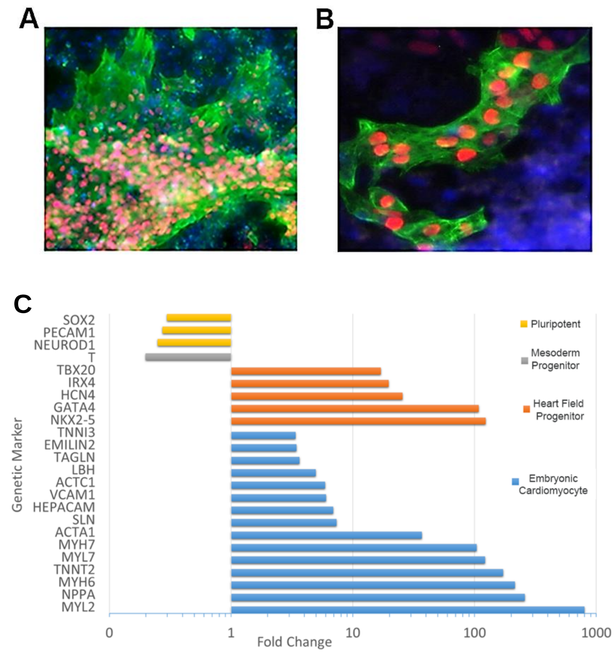
HDFa cells were reprogrammed with CytoTune™ 2.0. At the end of reprogramming, iPSC clones were picked and passaged manually. At passage three, each clone was plated in triplicate and passaged in parallel using each of the three different methods; manual, EDTA, and Reagent for up to seven passages. Green boxes indicate survival, and red boxes indicate that cells did not survive that passage. User A had less than 1 year of experience, while User B had more than 5 years of cell culture experience.

Figure 5. iPSC cultured with harvested Reagent 1 can be used for downstream differentiation.



iPSCs were passaged with either Reagent 1 **(A)** or EDTA **(B)** and seeded at 40K cells per well. They were differentiated with Gibco® PSC Cardiomyocyte Differentiation Kit. On Day 14 of differentiation, cells were imaged and monitored for beating cells. Cells passaged with Reagent 1 had higher density of cells and several clusters of beating cells (as indicated by the red arrow) suggesting that seeding cells at required cell density was enabled by the milder reagent relative to the EDTA harvested cells.

Figure 6. iPSC culture harvested with Reagent 1 shows higher cardiomyocyte marker expression



Day14 differentiated cardiomyocytes were also imaged and relative gene expression analysis was preformed using a cardiomyocyte-specific Custom Taqman® Array Plate. Panels **(A)** and **(B)** show 10X magnification and 20X magnification of cells stained with cardiomyocytes-specific markers, TNNT2 (green) and NKX2.5 (red). Reagent 1 treated cells had higher cardiomyocyte gene expression than cells treated with EDTA **(C).** Data is represented as normalized mRNA fold change expression of cells harvested with Reagent 1 relative to cells harvested with EDTA. GAPDH was used to normalize gene expression. Genes that were differentially expressed 3 fold or greater, were plotted for clarity.

CONCLUSIONS

Reagent 1 offers better cell growth rates for routine passaging of established cell lines on GTX.

Early passage, pooled clones passaged routinely with Reagent 1 also show increased growth rates.

While manual passaging is considered the method of choice for early clonal establishment, bulk expansion with Reagent 1 is quick, not labor intensive, consistent across experience levels and gentler than EDTA.

In a differentiation workflow, Reagent 1 can be used to rapidly form more mature cardiomyocytes than EDTA.

ACKNOWLEDGEMENTS

This study was funded by the California State University-San Marcos CIRM Bridges to Stem Cell Research Training Grant.

TRADEMARKS/LICENSING