Optimized HiDef-B8 Reduces Costs and Improves Accessibility for PSC Research

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Introduction

Inconsistency of ingredients and variability of animal-derived components in cell culture media remain challenges affecting research laboratories and manufacturing. Further, accessibility to tools for PSC culture is limited where cold shipping is unreliable. We assessed PSC culture performance across two facilities using data from a semi-empirical 3-factor (insulin, NRG1, and FGF2-G3) rotatable central composite DOE for complete media variants based on our commercialized frozen liquid HiDef-B8 PSC maintenance supplement with hyperstable FGF2-G3. Fit to an RSM model, our data verify HiDef-B8 is cost-optimized for functionality. Here, we follow up on our report presented at ISSCR 2022 with expanded verification data. We also highlight recent development efforts on a 'nextgen' a solid-state supplement that will increase shipping adaptability and, ultimately, advance our goal of increasing global accessibility to ingredients for cell culture.

Methods

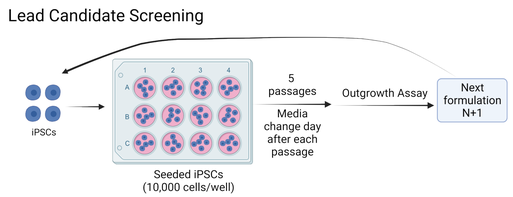

Figure 1. Lead candidate screening experimental set up. Two iPSC lines were cultured for five passages, with media changes 24 hours after passaging, before performing an outgrowth assay.

Condition	Coded Value Levels		
	NRG1	Insulin	FGF-G3
1	-1	-1	-1
2	-1	-1	+1
3	-1	+1	-1
4	-1	+1	+1
5	+1	-1	-1
6	+1	-1	+1
7	+1	+1	-1
8	+1	+1	+1
9	-1.68	0	0
10	+1.68	0	0
11	0	-1.68	0
12	0	+1.68	0
13	0	0	-1.68
14	0	0	+1.68
15	0	0	0
16	0	0	0
17	0	0	0
Uncoded	**NRG1 (ng/mL)**	**Insulin (µg/mL)**	**FGF-G3 (ng/mL)**
-1.68	0	10.00	10.00
-1	2.02	18.10	28.21
0	5.00	30.00	55.00
1	7.98	41.90	81.79
1.68	10.00	50.00	100.00

Table 1. DOE experimental setup for B8 optimization. Seventeen (17) conditions were created in a matrix according to a 3-factor rotatable central composite design (3-RCCD), with three central points (conditions 15-17). The un-coded values of each number in the matrix are shown below for every model and factor. Calculated using SigmaXL (SigmaXL, Inc., CAN).

Results

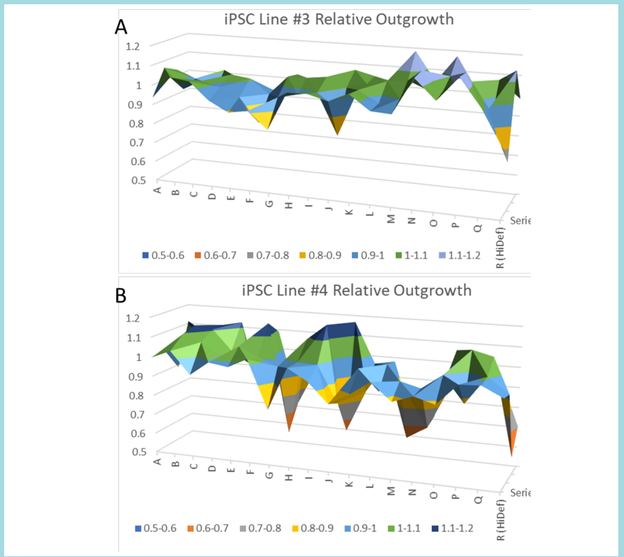

Figure 2. Outgrowth assay following five passages in two iPSC cell lines. Data is normalized to inter-plate control formulations A, H, Q.

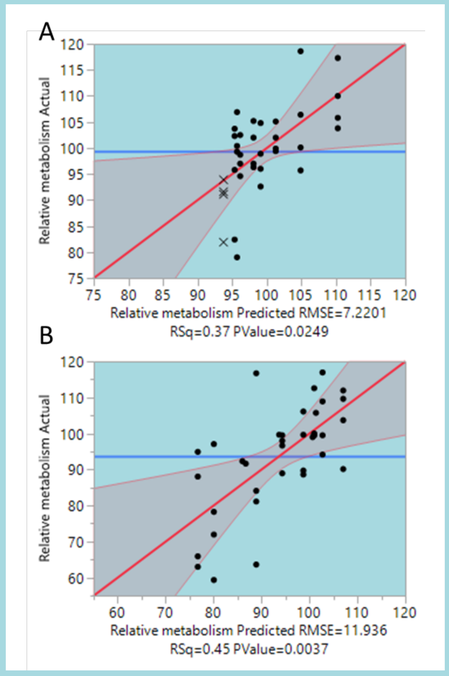

Figure 3. Actual versus predicted response in two iPSC lines. (A) iPSC line #3 and (B) iPSC line #4.

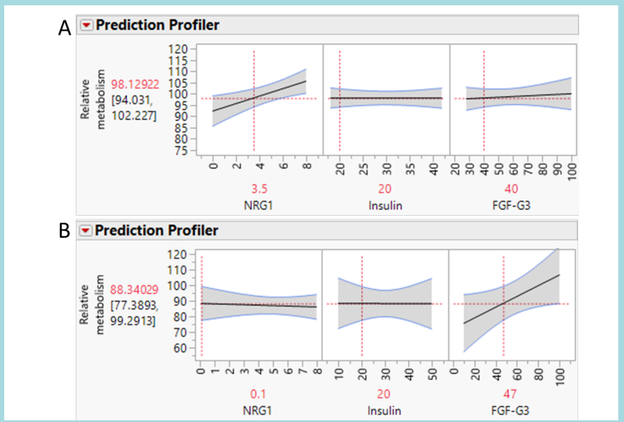

Figure 4. Combinatorial insulin response stability optimized by NRG1 concentration for (A) iPSC line #3; and FGF2G3 concentration for (B) iPSC line #4.

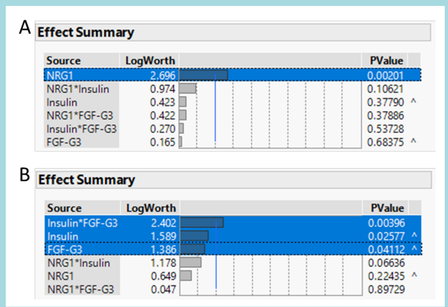

Figure 5. Individual and combinatorial component concentration significance from DOE model in two iPSC cell lines. (A) iPSC line #3 and (B) iPSC line #4.

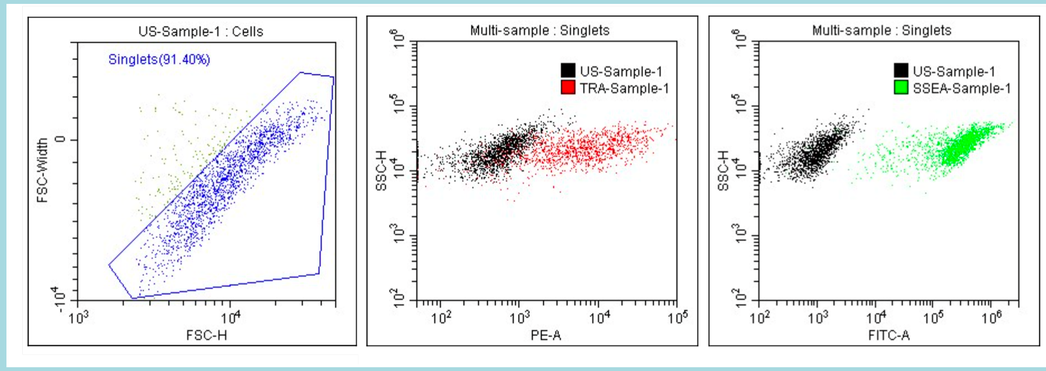

Figure 6. Flow cytometry of single cell iPSC for surface markers SSEA-4 and TRA-1-60-R. (Left) Single cells recorded. (Center) Single staining for TRA-1-60-R. (R) Single staining for SSEA-4.

Future Directions

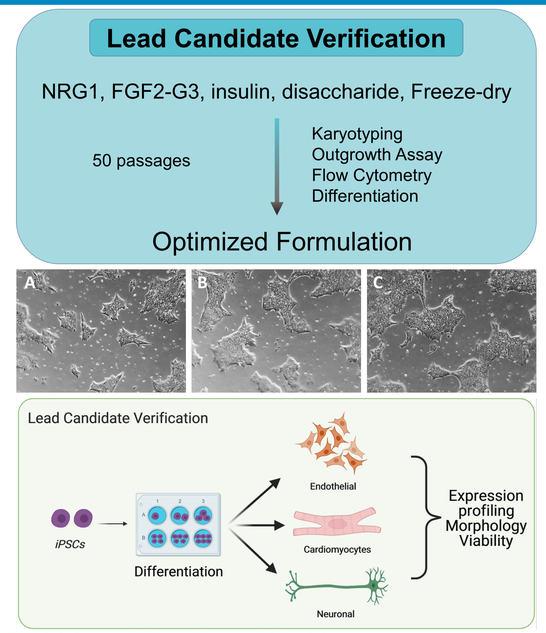

Figure 7. iPSCs will be cultured for 50 passages in media from top supplement candidates. Liquid control (A) will be compared to freeze-dried counterpart without cryopreservative (B) and with (C), then iPSC will be characterized for karyotype and proliferation rate. Differentiation potential will be verified via three lineages: endothelial, cardiomyocytes, and neuronal. Differentiated cell fate will be confirmed using expression profiling by flow cytometry and morphology.

References

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