Defining Culture: Cost-effective Medium to Improve Stem Cell Reproducibility

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Introduction

Inconsistency of growth media for cell culture and variation in animal-derived products is a significant problem in both industry and academia that affects research laboratories and manufacturing. We formulated HiDef-B8 with hyperstable FGF2-G3 for commercialization using empiricle methods to improve stem cell culture and performance *vs.* cost. Here, we apply a semi-empirical 3-factor rotatable central composite design of experiments (DOE), to fit a response surface methodology (RSM) model verifying our formulation with potential to further optimize combinatorial concentrations of neuregulin 1 (NRG1), insulin and fibroblast growth factor 2 (FGF2) for added cost savings

Methods

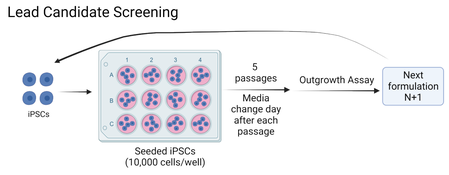

Figure 1. Lead candidate screening experimental set up. Two induced pluripotent stem cell (iPSC) lines were cultured for five passages, with media changes 24 hours after passaging, before performing an outgrowth assay.

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

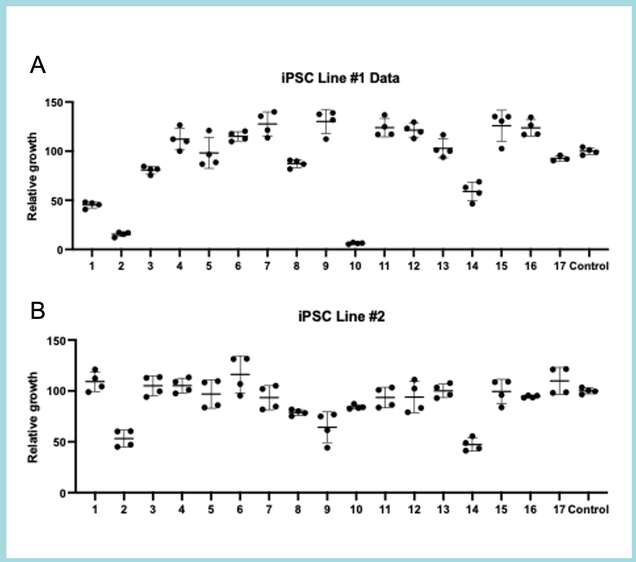

Figure 2. Outgrowth assay following five passages in two iPSC cell lines. Data is reported as mean ± SD.

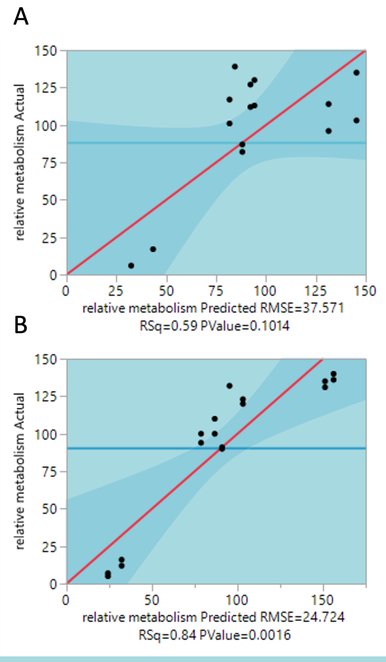

Figure 3. Actual versus predicted response in two iPSC cell lines. (A) iPSC line #1 and (B) iPSC line #2.

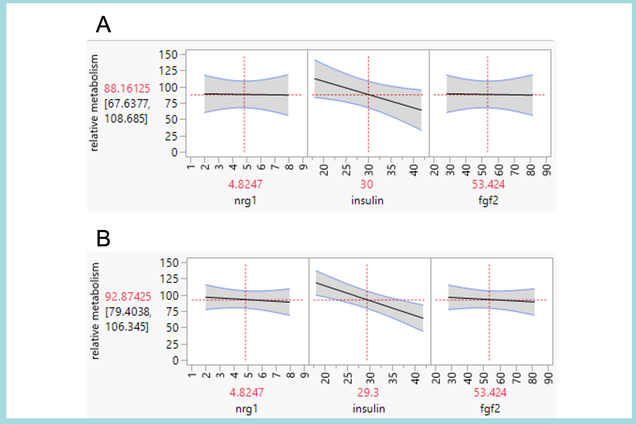

Figure 4. Individual component response in two iPSC cell lines. (A) iPSC line #1 and (B) iPSC line #2.

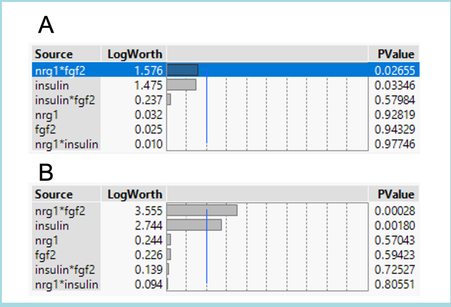

Figure 5. Statistics from DOE model in two iPSC cell lines. (A) iPSC line #1 and (B) iPSC line #2.

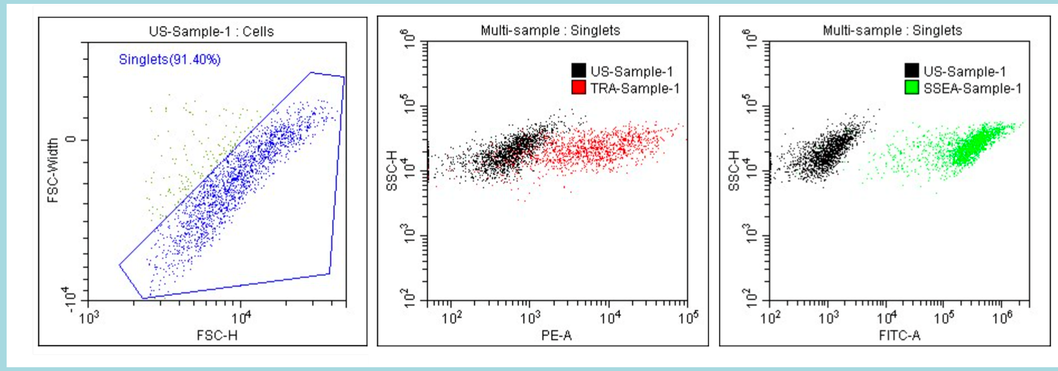

Figure 6. Flow cytometry of single cell iPSC for surface markers SSEA-4 and TRA-1-60-R. (Left) Single cells recorded. (Center) Single staining for TRA-1-60-R. (R) Single staining for SSEA-4.

Future Directions

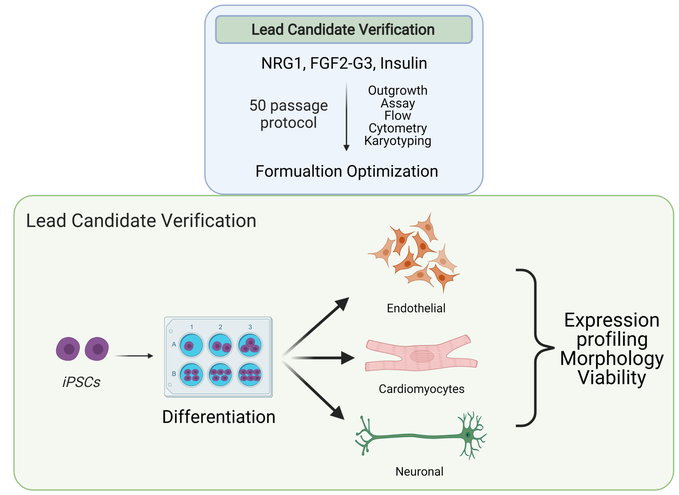

Figure 7. iPSCs will be cultured for 50 passages once the lead candidate has been identified to verify. Cells will be differentiatied into three lineages; endothelial, cardiomyocytes, and neuronal. Cell fate will then be confirmed using expression profiling by flow cytometry, morphology and viability in an outgrowth assay.

References

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Acknowledgements

Burridge Lab, Northwestern Feinberg School of Medicine

Moutri Lab, University of California San Diego School of Medicine

NIH NIGMS Award No. 1R44GM140750