DEVELOPMENT OF AN IMPROVED FEEDER-FREE CULTURE SYSTEM FOR MOUSE PLURIPOTENT STEM CELLS

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ABSTRACT

We developed simplified new feeder free mouse ESC culture medium. In assessing and optimizing mESC culture, we focused on 3 major attributes: (1) cell growth & colony morphology, (2) maintenance of pluripotency, and (3) ability to support downstream differentiation. Incorporation of multi-parametric Design of Experiment (DOE) approaches with robust cellular assays and automated imaging & analysis enables us to test multiple components in parallel and helped identify optimal conditions through iterative experimental rounds. Taken together this work highlights both (a) our design philosophy for culture media development- identify key functional endpoints, develop or incorporate robust, scalable assays, and test a wide array of components and workflow parameters; and (b) our results to date with this new system.

INTRODUCTION

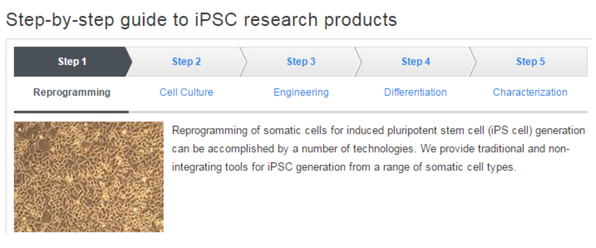
Pluripotent stem cell models provide powerful tools for the researchers to study and model wide ranging biological questions, from fundamental developmental processes to translational medicine for understanding mechanisms of disease. Although recent advances in human induced pluripotent stem cell technology have rightfully garnered much excitement and use, mouse pluripotent/embryonic stem cells (mESC) continue to be useful and complementary tools, particularly for translating complex in-vitro studies assessing genetic modifications from the cellular level to whole animal model. Over decades, several reports have introduced methods for in vitro culture of mouse pluripotent stem cells including co culture with supporting fibroblast or small molecule based feeder independent culture. Optimum culture conditions need to support multiple applications including cell line derivation from recalcitrant strains, stable cell proliferation while maintaining pluripotency, and downstream in vivo applications like chimera formation. Here we present our development approach and results in generating a new feeder free mouse ESC culture medium.

MATERIALS AND METHODS

Attachment Factor (P/N. S006100*)
Stempro® Accutase® (P/N. A1110501**)
DPBS without Ca^{2+} or Mg^{2+} (P/N. 14190144***)
SSEA1-Alexa488 (P/N.MA1-022-D488)
7AAD (P/N. S33025*)
TUJ1 and SMA antibody (P/N. A25538*)
Lipofectamine™ 3000 Transfection Reagent (P/N. L3000015*)

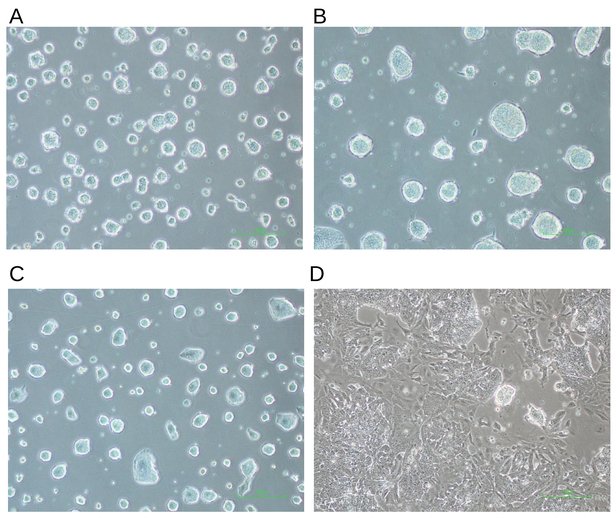
HELPFUL RESOURCE WEBPAGE

https://www.thermofisher.com/us/en/home/life-science/stem-cell-research/induced-pluripotent-stem-cells.html



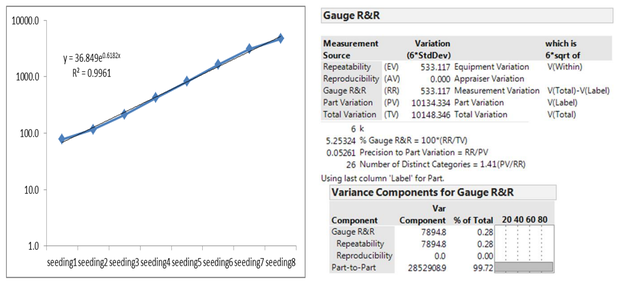
RESULTS

Figure 1. Phase image of mPSC in various feeder free systems



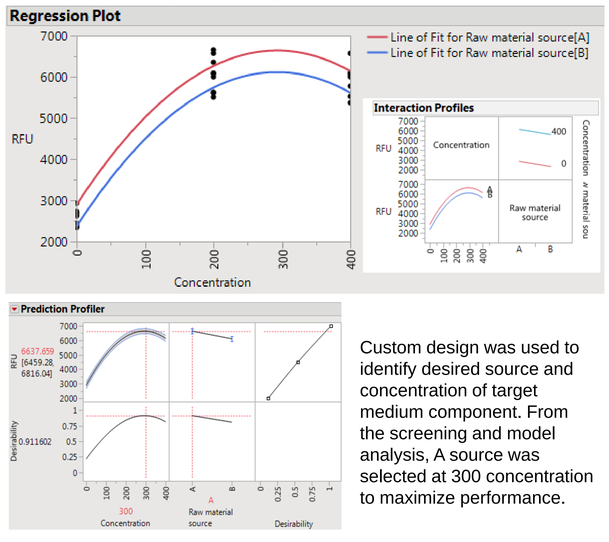
Mouse embryonic stem cells were located in existing 3 different feeder free culture systems and phase images were documented to evaluate colony morphology, colony numbers. A, B and C) 2 inhibitor based system: Cells grows as small or big 3D dome shape colony which is desired. D) LIF based system: Majority of colonies lost their round dome shape but differentiated to be flattened out and have eccentric morphology.

Figure 2. Robust cellular assay development



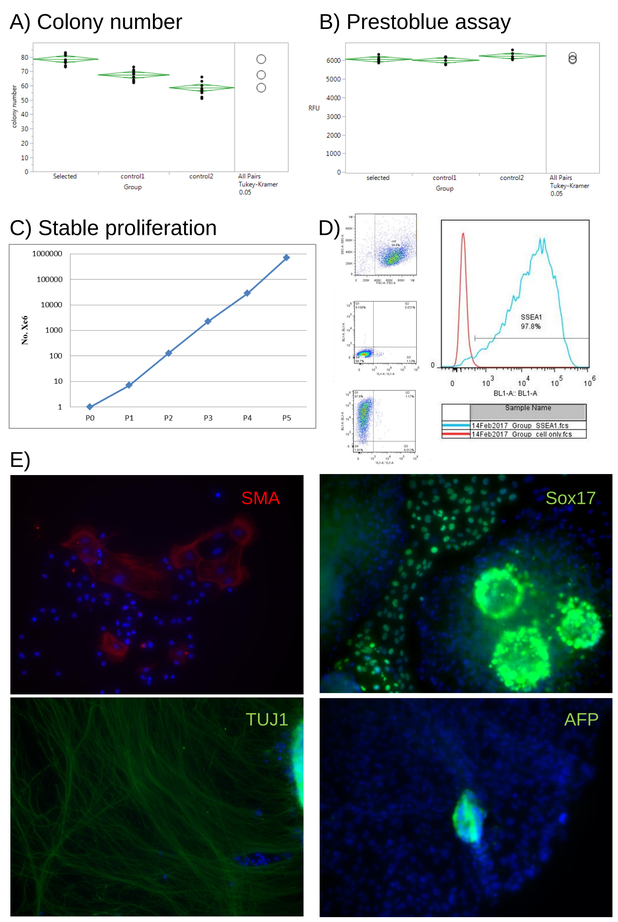
Among the tested parameters (Cell count by cell counter, Automated confluency test, ICC, FACS, qPCR), cell number seems to be most sensitive to discriminate culture condition tested. Prestoblue assay was developed with desired sensitivity and capability of high throughput assay. mPSC was plated in 8 seeding densities and after 3 days in culture, metabolites were analyzed to have high resolution (26 categories) and power. (99.72% variation from part to part)

Figure 3. Example of Design of Experiment (Raw material selection & con. optimization)



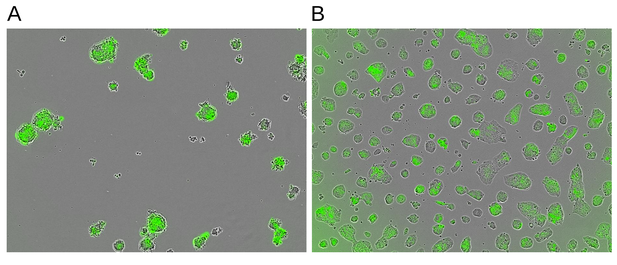
Custom design was used to identify desired source and concentration of target medium component. From the screening and model analysis, A source was selected at 300 concentration to maximize performance.

Figure 4. Performance of finalized formulation



Kit performance (Selected) was compared to published or commercialized solutions (control 1 and 2). Cells in selected formulation grow as homogenous colony to have improved colony number A) without the sacrifice in cell proliferation B). Upon stable proliferation C) in selected formulation, SSEA1 expression was monitored by flow cytometry D). Over 97% cells maintained target pluripotent marker of SSEA 1 antibody. To measure differentiation potential, expanded cells were differentiated for 7-10 days and population was examined with antibody specific to ectoderm (TUJ1), Endoderm (Sox17 and AFP) and Mesoderm (SMA) E).

Figure 5. Transfection of DNA or mRNA



Delivery of Cas9 mRNA with gRNAs into mESC has been used effectively to generate new knock-out mouse models. Mouse embryonic stem cells were transfected with Lipofectamine™ 3000 to deliver either an EF1a-GFP plasmid or eGFP mRNA during re-plating in feeder-free conditions. MESC reattached overnight and GFP expression was detected within the majority of mES cells by 24 hours after transfection with DNA A) or mRNA B).

CONCLUSIONS

1. Multi-parametric Design of Experiment (DOE) was applied to test multiple components in parallel and identify optimal conditions.
2. Mouse Pluripotent stem cells were expanded and maintained in developed formulation for multiple passages.
3. Cells expanded in dome shape and maintained pluripotent phenotypes marker of SSEA1.
4. Pluripotency was further evaluated to confirm differentiation potential. Through EB formation, population was differentiated to result in 3 germ layers confirmed with phenotype markers.
5. Mouse ES cells could readily be transfected with DNA or mRNA in feeder-free conditions, enabling gene editing studies without contaminating wild type MEFs.
6. Interested in early access & testing?
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TRADEMARKS/LICENSING