# Differentiating Human Pluripotent Stem Cells to Neurons : Approaches in Media Development

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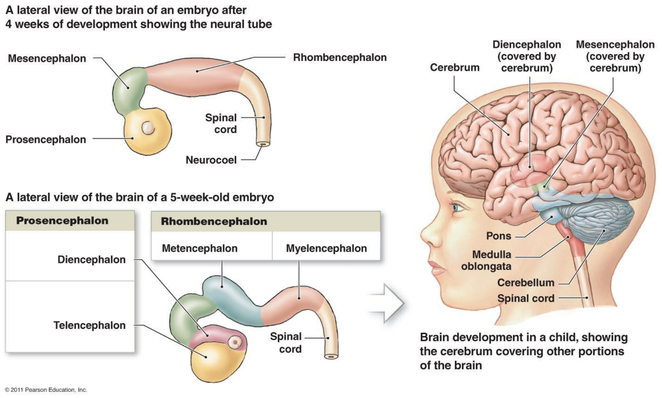
## ABSTRACT

Specialized cell culture media is a foundational tool for researchers working in diverse areas, from basic and applied research to biopharmaceutical applications. Thermo Fisher Scientific offers media systems for culture of human and rodent (primary) neural cell types and more recently has focused on identifying conditions that drive stem cell differentiation toward specific neural lineages. AIM: To develop new cell culture systems that enable robust differentiation of human pluripotent stem cells (PSCs) to distinct neuronal subtypes. METHODS: We have adopted a multifaceted approach for driving PSC to neuronal differentiation- 1.Disconnecting specification/regionalization studies from maturation and enabling parallel development activities. 2. Utilizing complex Design of Experiment (DOE) approaches and mathematical modeling paired with validated endpoint assays; 3. Incorporating small molecule chemical library screening to identify compounds with desired properties. RESULTS: We demonstrate the feasibility of distinguishing PSC specification from neuronal maturation by utilizing banks of neural stem cells (NSCs), produced in 7 days using Gibco® Neural Induction Medium. The NSCs provide a good model to screen and optimize conditions driving neural differentiation and maturation. Additional results of definitive screening DOEs as well as modeling predictions are described. CONCLUSIONS: In the last several years significant advances in stem cell biology have enabled broader adoption of these cells and provided deeper insight into the mechanisms which regulate their growth and specific cell fate determination. In this work we present our approach to harness this insight to develop next generation culture systems to create useful neuronal cell models from PSCs.

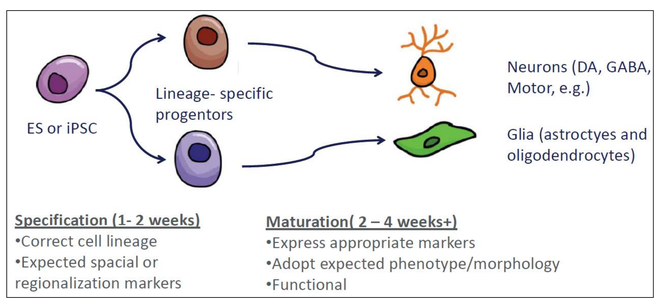
## INTRODUCTION

**Figure 1. Development of central nervous system and approach to recapitulate it with a in vitro system**

a)



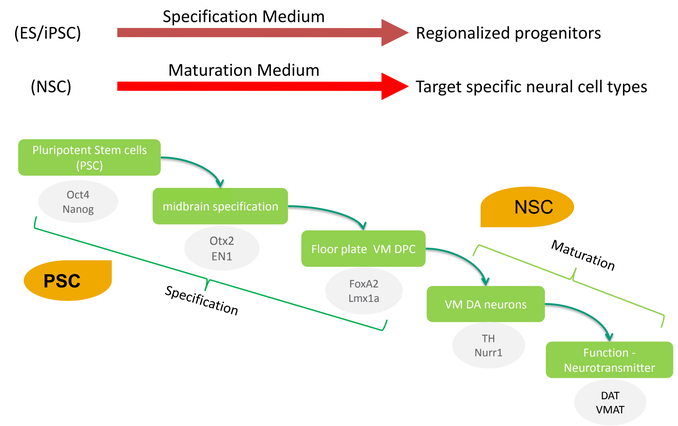

b)




Ectodermal tissue fold/wrap to create neural plate / fold to generate primary vesicle (4 weeks) which develops further to secondary vesicle (5 weeks) and adult structure (3rd Trimester)

## RESULTS

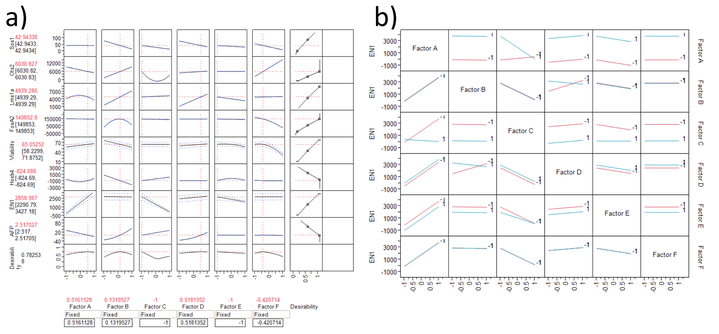
**Figure 2. Media development process**



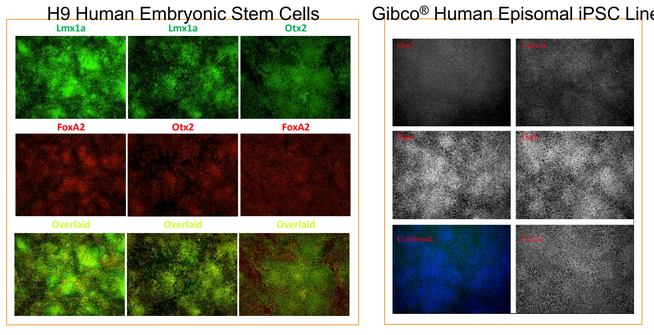

Differentiation of DA neurons: instead of staggered developmental approach, we used PSC as cell model to derive specific progenitor of midbrain floor plate cells and NSC as cell model to optimized maturation condition.

**Figure 3. Example of Definitive Screening Design (DOE) to differentiate hPSC into FP/vmDPC**

a) b)

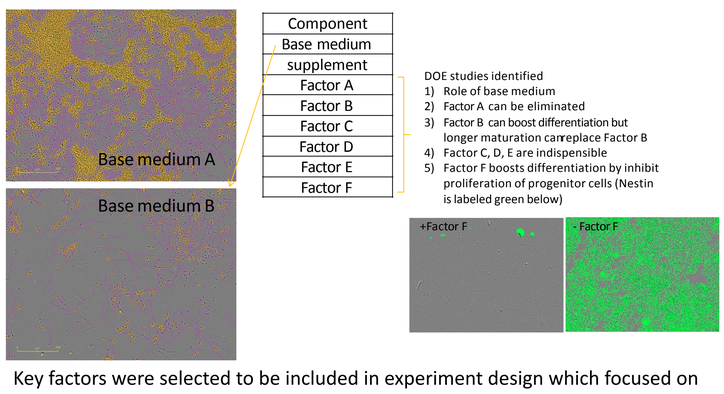



c)



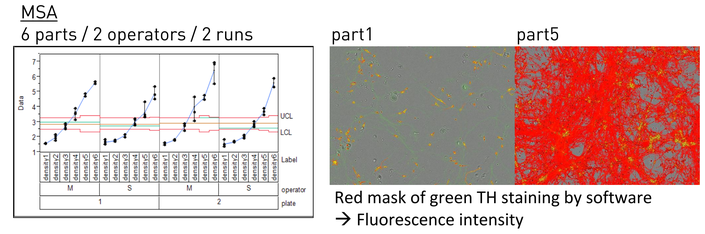

Following literature review, key factors were selected to be included in experiment design. A Definitive Screening model was used to identify the role of factors (a) and interactions (b) among the other pathway molecules and resulted in candidate formulation where denoted parameters were maximized (c).

**Figure 4. Development of maturation medium**



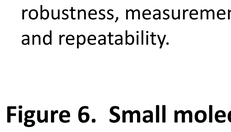

Key factors were selected to be included in experiment design which focused on identifying indispensible and dispensable factors as well as optimal concentrations , leading to candidate formulation(s).

**Figure 5. Measurement system analysis / Assay development for Non Hypothesis driven screening study**




For screening purpose, a 96 well assay format was adapted for maturation medium (shown above) and specification medium (data not shown) screening. To verify assay robustness, measurement system analysis (MSA) was performed to gauge variability and repeatability.

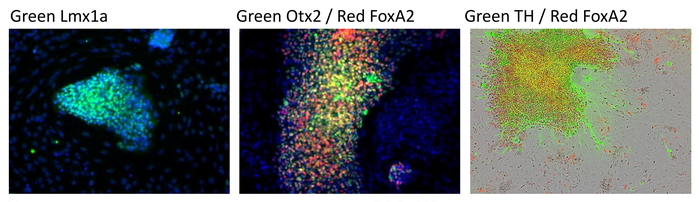
**Figure 6. Small molecule library screening**




| Compound | Setup Date | TH Expression (% of Positive Control) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Conc 6 | Conc 5 | Conc 4 | Conc 3 | Conc 2 | Conc 1 |
| Hit #1 | Setup 1 | 251.915 | 205.356 | 146.335 | 140.376 | 106.037 | 83.0577 |
| | Setup 2 | 255.462 | 206.208 | 83.1177 | 88.104 | 79.9014 | 43.6069 |
| Hit #2 | Setup 1 | 194.866 | 109.387 | 105.849 | 49.9931 | 41.9249 | 34.0141 |
| | Setup 2 | 198.328 | 113.638 | 47.0686 | 34.8689 | 21.3554 | 23.5376 |
| Hit #3 | Setup 1 | 177.501 | 150.598 | 171.236 | 155.519 | 134.547 | 165.16 |
| | Setup 2 | 220.932 | 124.684 | 106.016 | 127.375 | 132.353 | 60.3306 |

To improve the formulation, we screening small molecule compound library, ultimately identifying 3 compounds which positively impact TH expression

**Figure 7. Bridging Specification and Maturation medium**




New formulations were serially applied on PSC (H9) and the differentiated cells were characterized with phenotype marker related to midbrain DA neurons.

## CONCLUSIONS

1.To dissect and mimic complicated and sophisticated development process, we identified candidate pathway molecule and utilized design of excellence tools
2.Candidate floor plate population has been obtained which can be matured further to midbrain dopaminergic neurons.
3.Further development is under way to

- Replace unstable or expensive pathway molecule with stable / inexpensive alternatives (Compound library screening)
- Formulation and process optimization – simplify process and shorten differentiation duration and reduce variability

## REFERENCES

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## ACKNOWLEDGEMENTS

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