# Investigating the Underlying Mechanisms of Cortical Surface Area Overgrowth in Autism Spectrum Disorder Through Induced Pluripotent Stem Cells

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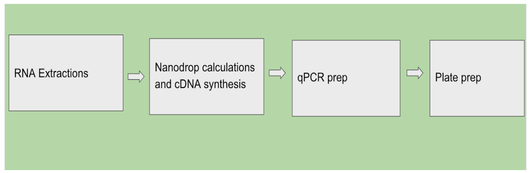
## Introduction

- Many individuals who have Autism Spectrum Disorder (ASD) exhibit larger brain volumes earlier in life (Hazlett and Poe, 2011)
  - The cortex is hyperexpanded prior to the onset of behavioral symptoms
- The cellular and molecular mechanisms behind cortical surface area overgrowth are not well known (Courchesne, 2019)
- Advances in the understanding of in-vitro protocols for the differentiation of pluripotent stem cells such as iPSCs has led to the development of brain organoid technologies (Courchesne, 2019)
- Naïve = pre-implantation cells
- Primed = post-implantation cells
- Aim: Address whether differences in pluripotency potential of reprogrammed cells influences downstream differentiation
- **Hypothesis:** The initial pluripotency state of the iPSCs is not associated with differentiation potential. There is no statistically significant correlation between stem cell state and differentiation outcome or progenitor proliferation rates, suggesting other in-vivo in-vitro correlations are not impacted by this potential technical confound.

## Experimental Design




| Group: | Gene: |
|---|---|
| Control | EIF4A2 |
| Pluripotency | OCT4, SOX2 |
| Primed | ZIC2, DUSP6 |
| Naive | TFAP2C |

## Methodology

- The qPCR protocol is based on 4 groups: control, pluripotency, primed, and naive marker genes.
- The control is the baseline expression for living cells. For functioning mitochondria and ribosomes, this housekeeping gene (EIF4A2) should be present at all times. All of the other markers will have expression relative to the control gene.
- The pluripotency group is also a control group since all cell lines should be pluripotent.
- The naive marker is our experimental gene. Ground state naive pluripotency is established in the epiblast of the mature blastocyst and may be captured in-vitro in the form of embryonic stem cells (Nichols 2009)
- Primed markers are also another experimental group and are representative of the post-implantation epiblast cell (Nichols 2009)
- Ideally, the primed and naive state should have no relation to cortical surface area overgrowth.

## Analyzing the CT Mean and Log Fold Change For Each Sample Within the Target Genes

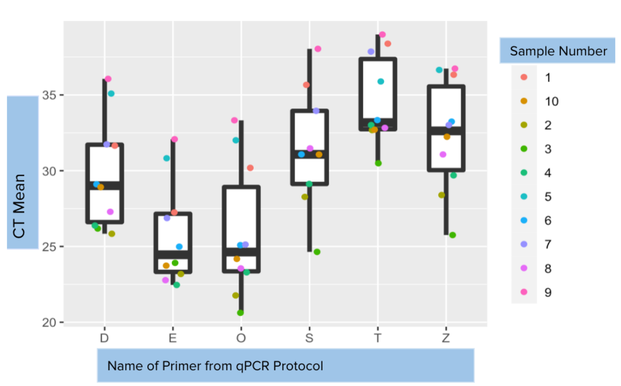



**Figure 1. An Analysis of the CT Mean for All Samples within Each Target Gene**

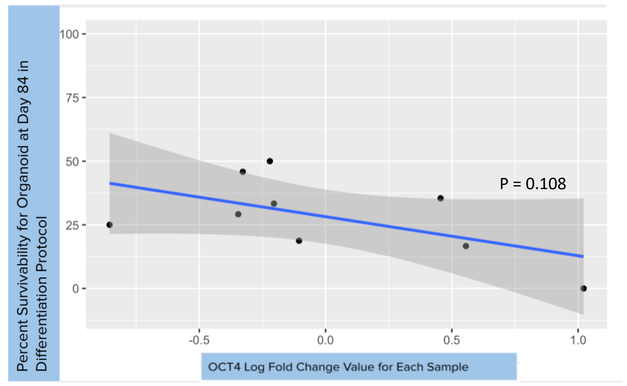



**Figure 2. Plot Correlating Day 84 Survivability and Log Fold Change for OCT 4 Gene (Pluripotency)**

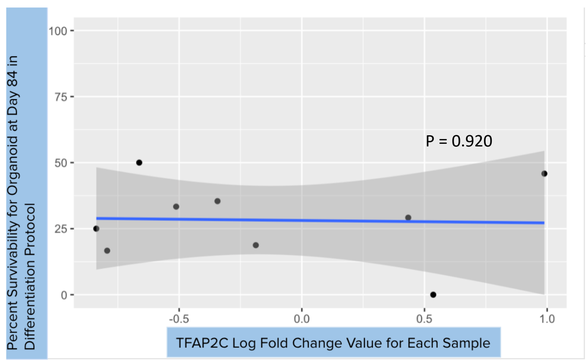



**Figure 3. Plot Correlating Day 84 Survivability and Log Fold Change for TFAP2C Gene (Naive)**

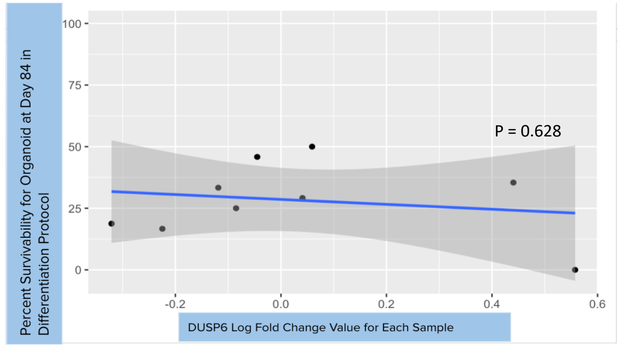



**Figure 4. Plot Correlating Day 84 Survivability and Log Fold Change for DUSP6 Gene (Primed)**

## Results

- All of the p values calculated for each correlation plot are above 0.05, which is an indication that we did not detect a statistically significant correlation between stem cell state and differentiation outcome or progenitor proliferation rates.
- This suggests that this technical confound of the pluripotency state may not be associated with differentiation outcome, though we aim to replicate this result in a larger sample size.
- If there was a potential correlation between stem cell expression and differentiation outcome, then it would have made it difficult to correlate organoid phenotype to participant outcomes. Because there is no evidence of a statistically significant correlation, we do not have evidence to support that cell state explains differentiation outcomes.

## Discussion

- Potentials
  - Organoids are ideal in-vitro models of development, disease pathogenesis, and platforms for drug screening (Di Lullo, 2017)
  - Understanding the developmental origins of early brain overgrowth provides insights into ASD risk
- Limitations
  - Differentiations are long and there is known variability in outcomes of the differentiations.
    - This variability may confound biological signals and impact the differentiation, which can affect how well an organoid grows in an experiment.
- Future Directions
  - Continuing this experiment in other labs can help understand the correlation with cell composition in the organoid, which can yield a better understanding of how cell state impacts differentiation outcomes
  - Continuing this qPCR design with more primers would help to better understand the pluripotency involved in iPSC lineage
  - Continuing qPCR testing of iPSC cell state for remaining iPSCs reprogrammed from our study of autism

## Acknowledgements

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