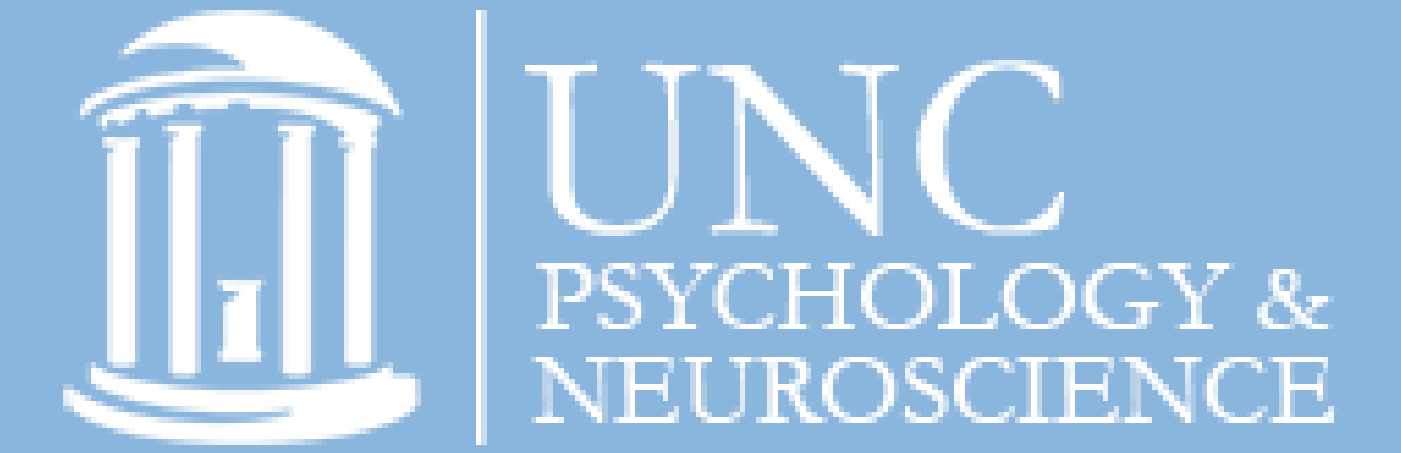


Interindividual Differences in Cell Type Proportions During Human Cortical Neurogenesis

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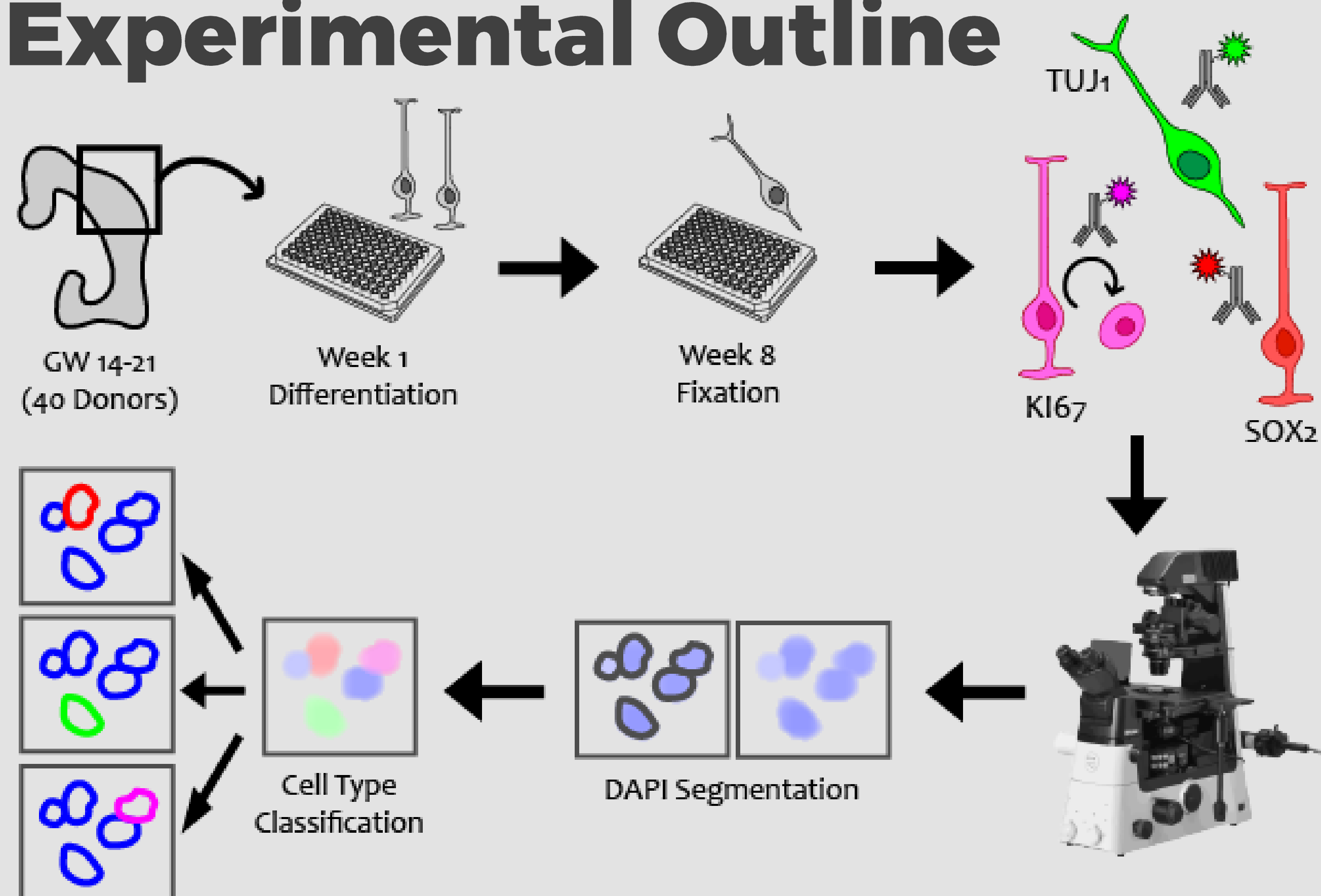
Introduction

- Cortical neurogenesis occurs during prenatal brain development and leads to the production of all of the excitatory neurons of the cortex.
- Neurogenesis may be affected by common genetic variation in the human population, leading to genetically mediated alterations in brain structure and function; research is lacking in the effect of common genetic variation on cellular phenotypes.
- Cortical development can be modelled through in vitro culture systems using human neural progenitor (HNP) cells. Progenitors are multipotent and proliferative, and can divide to produce cortical neurons that no longer have dividing potential.
- Abnormal levels of proliferation and differentiation of progenitors has been associated with diseases like Autism Spectrum Disorder and Down Syndrome.^{1,2,3}

Objective:

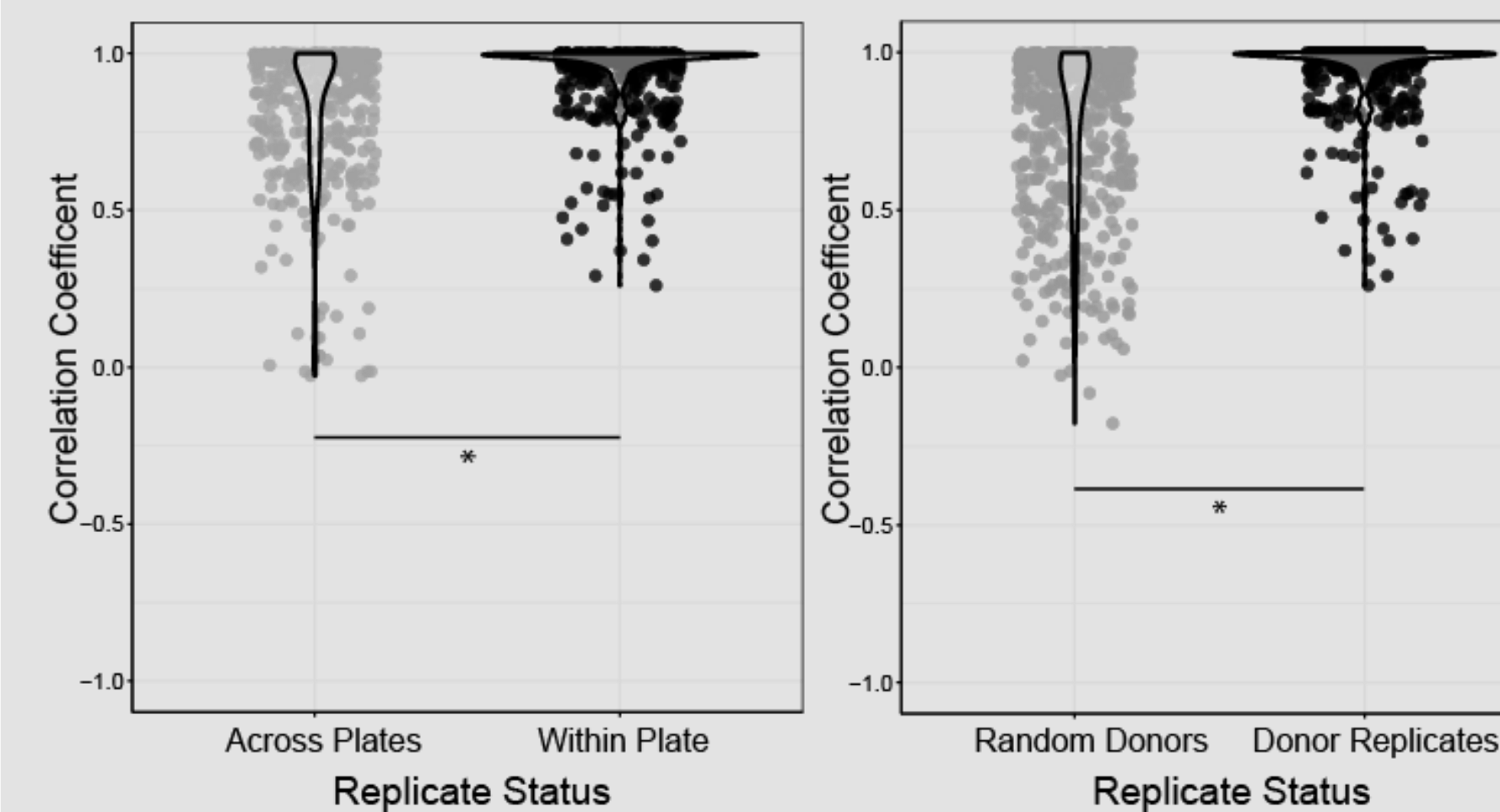
Characterize proportions of progenitors, proliferating progenitors, and neurons in cell lines from individuals with differing biological characteristics and variation in their genomes.

Experimental Outline



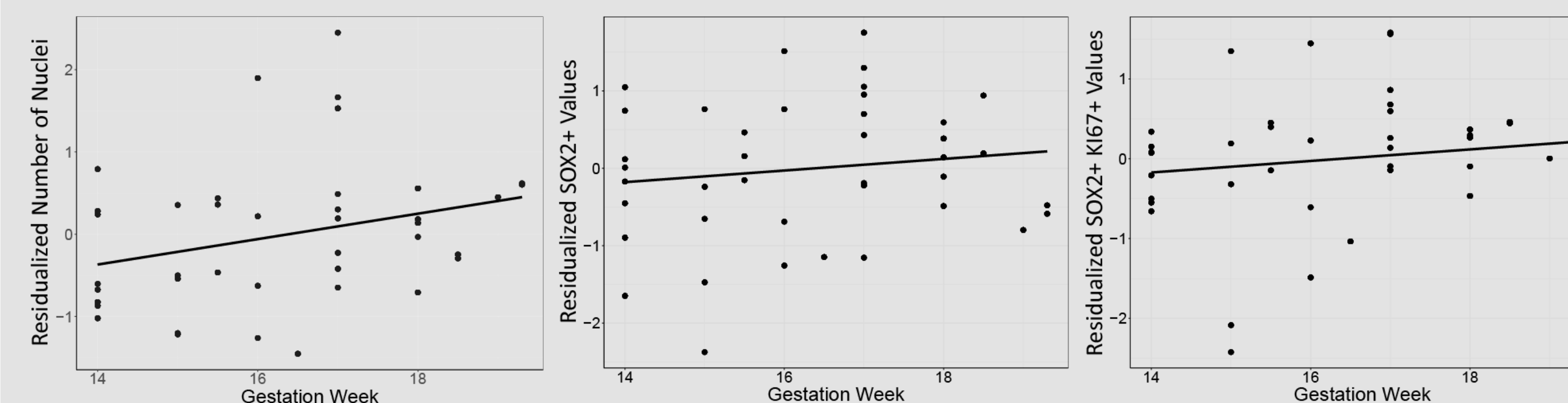
- Tissue from gestation weeks 14-21 was acquired through the UCLA Gene and Cell Therapy Core
- HNPs were plated in 96 well plates and differentiated in multiple rounds for 8 weeks
- Using immunocytochemistry, DAPI was used as a nuclear stain, and anti-SOX2, anti-KI67, and anti-TUJ1 antibodies were used to label progenitors, proliferation, and neurons, respectively
- The program Cellpose was used to segment nuclei before image analysis
- CellProfiler was used to class each nucleus as positive or negative for each marker, and percentages of each cell type were calculated

Donor Replicability

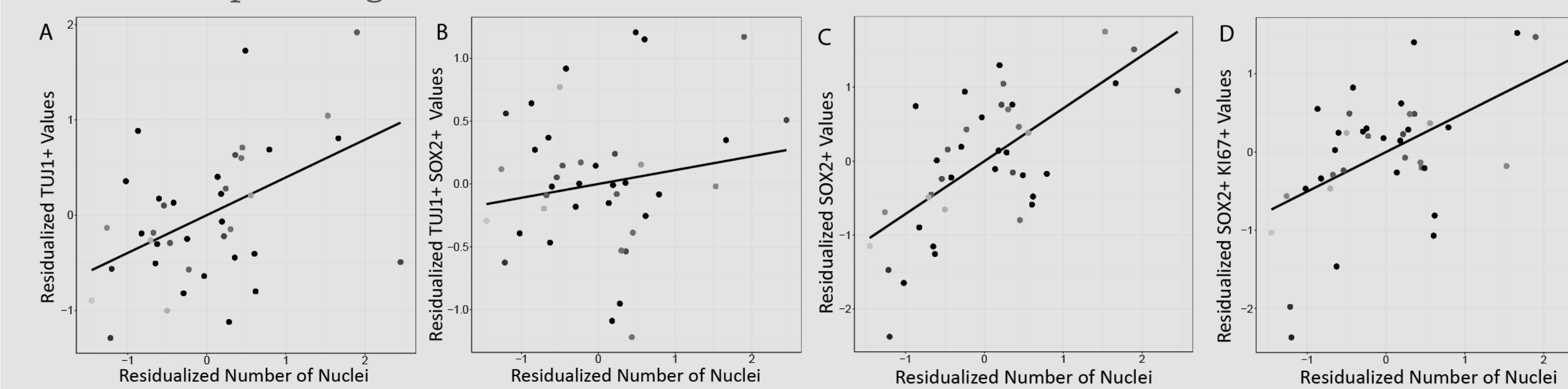


The average correlation coefficient for replicates within a plate ($r=0.945$) is significantly higher than that of replicates across plates ($r=0.820$, $t(505.81)=-9.69$, $p<2.2*10^{-16}$), and the correlation coefficient for replicates of the same donor ($r=0.945$) is significantly higher than that of random donors ($r=0.764$, $t(715.99)=14.8$, $p<2.2*10^{-16}$).

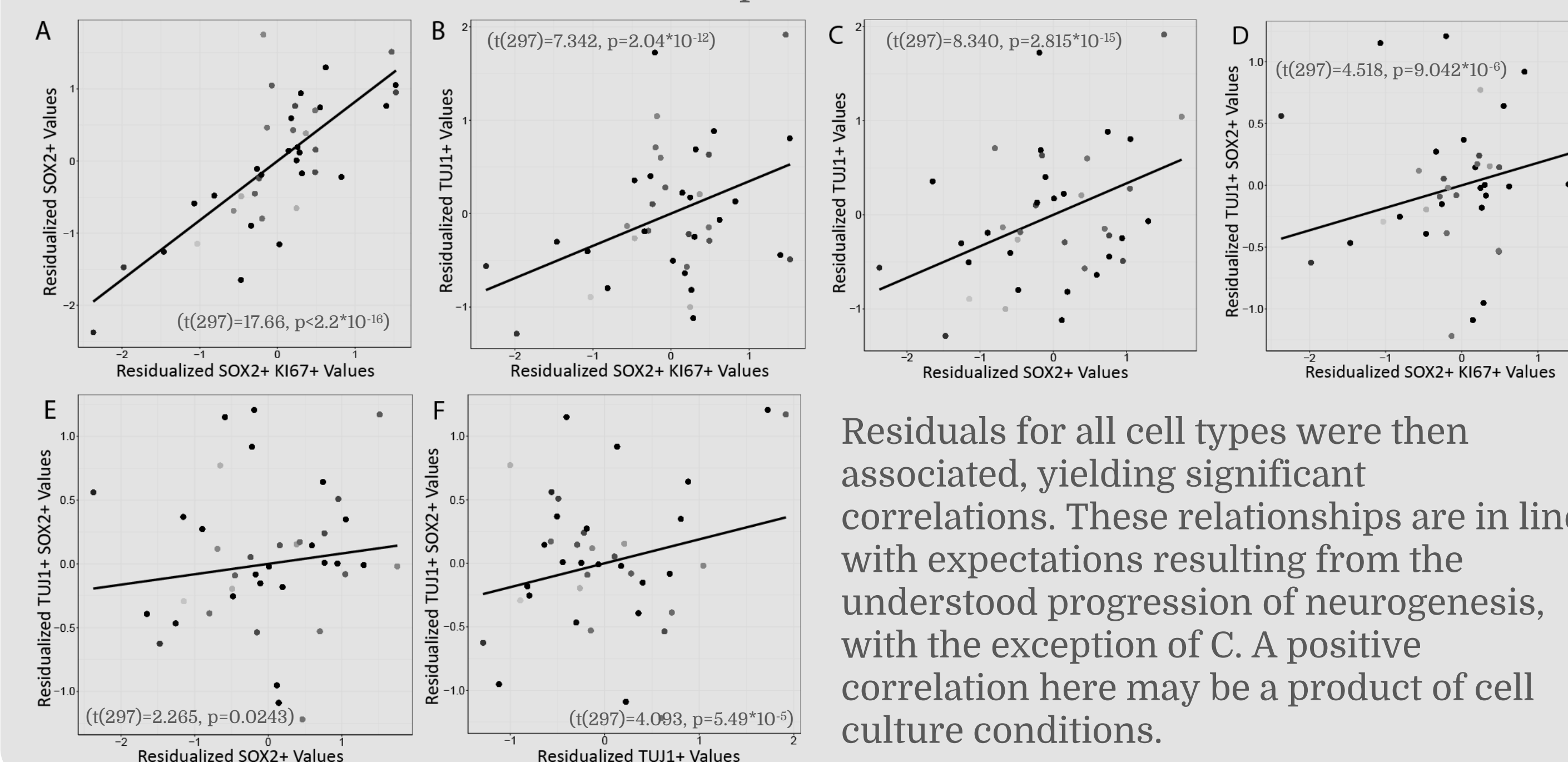
Cell Type Correlations



After normalizing the data and controlling for the round of culture, the residualized values for number of nuclei ($t(297)=5.399$, $p=1.38*10^{-7}$), progenitors ($t(297)=2.451$, $p=0.0148$), and proliferating progenitors ($t(297)=2.679$, $p=7.81*10^{-3}$) all showed significant positive relationships with gestation week.



Residualized values for neurons ($t(297)=9.949$, $p<2.2*10^{-16}$), newly generated neurons (TUJ1+ SOX2+) ($t(297)=2.983$, $p=3.088*10^{-3}$), progenitors ($t(297)=16.56$, $p<2.2*10^{-16}$) and proliferating progenitors ($t(297)=11.72$, $p<2.2*10^{-16}$) also showed a significant, positive correlation with the total number of nuclei. These relationships could result from robust vs. weak cultures.

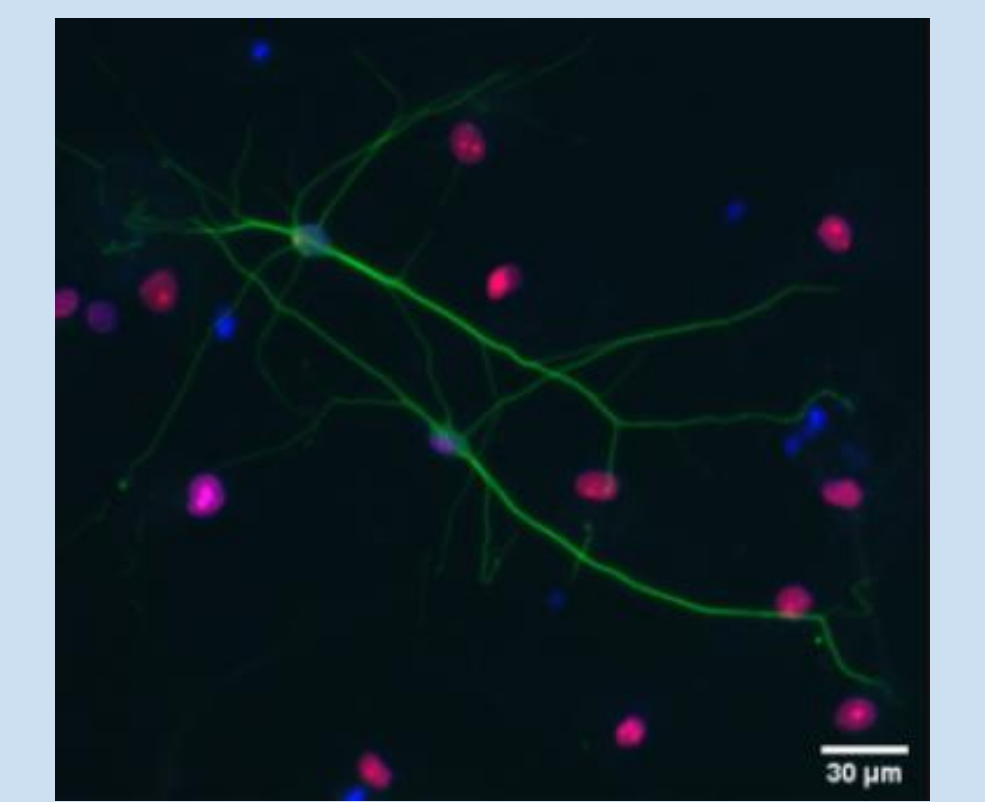


Residuals for all cell types were then associated, yielding significant correlations. These relationships are in line with expectations resulting from the understood progression of neurogenesis, with the exception of C. A positive correlation here may be a product of cell culture conditions.

Conclusion

- The interindividual variation in cell type proportions was significantly higher than the variation within donors, which preliminarily shows within-donor reproducibility and cross-donor phenotypic differences
- Gestation week is a biological variable that has an impact on outcome measures, where round of culture is a technical variable that also has a significant effect on cell type percentages.

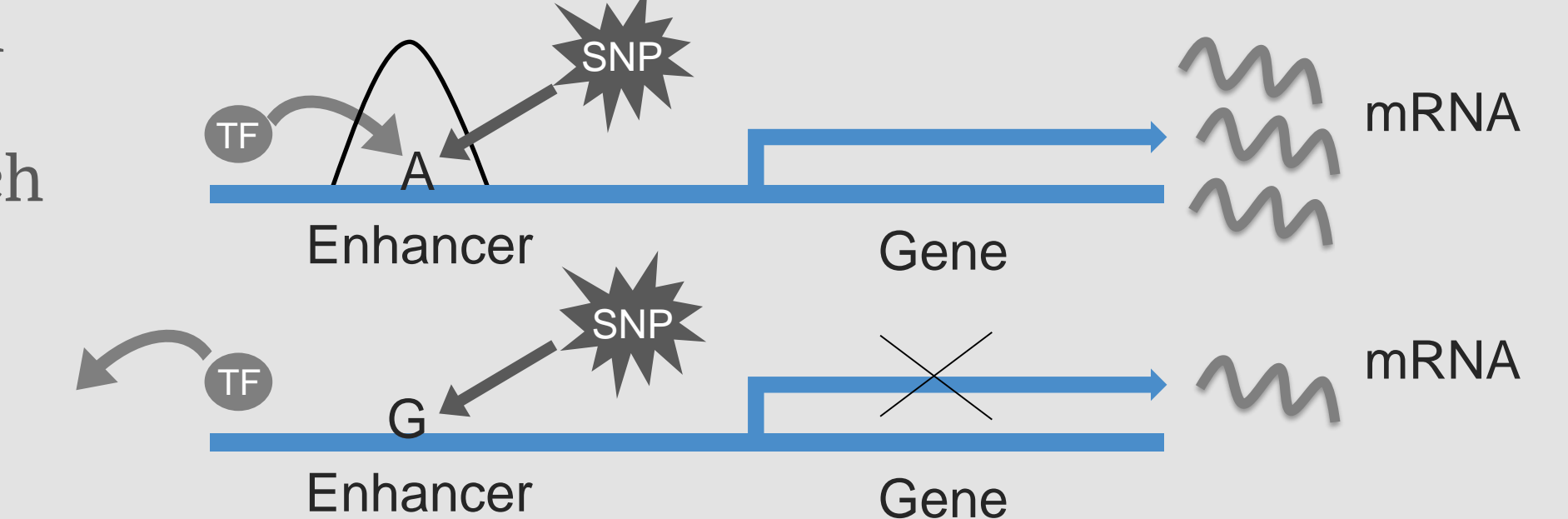
Our results demonstrate that the in vitro culture system represents a reproducible and biologically valid system, which can later be used to perform genetic association studies to further investigate the relationship between common genetic variation and cellular phenotypes during neurogenesis.



SOX2 (red), KI67 (magenta), TUJ1 (green)
Magnified image of cells after immunostaining

Next Steps

- Perform a genome-wide association study (GWAS) to identify variants that are associated with cell type proportions during neurogenesis
- Use RNA-seq and ATAC-seq data (measuring gene expression and chromatin accessibility, respectively) to gain insight into the mechanism by which SNPs alter cell type proportions



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