

EASTBIO REP Report

By Julia Oh

My EASTBIO Rep project was to attempt to investigate the role of PAX6 in neural connectivity, and particularly if pleiotrophin can rescue the changes from PAX6 knockout. This is built upon previous work that found that PAX6 KO favours the differentiation to inhibitory neurons rather than excitatory neurons, and the pathways that were most affected were pleiotrophin and MAPK. The project fits with the wider thematic area of the role of PAX6 in developmental biology.

My main goals during this placement were to experience a working research environment and to improve my understanding of organoids which I had previously done a presentation on. My main disciplinary is immunology and although I have some background in genetics, I did not have experience with neurodevelopment thus I wanted to fill this gap in my knowledge.

My first week of the project was guided by my supervisor in order to get used to the lab environment, and have the training necessary for the latter half of the project. I was given the opportunity to talk to the lab masters student and members of other labs on their projects. I became familiarised with organoid protocols and cell culture sterility. The project utilised 20 day old organoids of both wild type and PAX6 KO that were treated with pleiotrophin at 2 different concentrations. My role was to fix and freeze the organoids before cryosectioning them onto slides for immunohistochemical staining. After obtaining the images using fluorescent microscopy, we then used a software QuPath to estimate the number of excitatory and inhibitory neurons present on an organoid.

The most challenging part of the project was definitely working with organoids as with working with cells it can be quite delicate. Organoids which were developed earlier were not able to survive the extensive freezing process causing the slides from those blocks to be unusable. I also found that using fluorescent antibodies and the staining protocol were quite difficult as we had to ensure we worked fast enough to ensure cells did not dry out and also that when we mounted the slides there were no air bubbles to disrupt imaging. Despite this, the project was quite fruitful and I was guided thoroughly and definitely improved while working there. While we had not done statistical analysis on our findings yet, what was surmised was that pleiotrophin may lead to a shift in the Waddington landscape favouring excitatory neurons but not necessarily rescuing the to the excitatory cell fate.

The most beneficial part of the project to me was definitely working in the lab. I found that I really leaned into my organisational strengths in terms of keeping track of tasks to ensure project completion, ensuring lab reagents and materials are full and optimising QuPath to use for cell counting as it had not been previously used in our lab. Due to time constraints I was not able to obtain cell culture safety training, even so my

supervisor allowed me to observe and demonstrated proper cell culture techniques and organoid formation for future reference. Interacting with lab members and other labs in the building was definitely fruitful in terms of furthering my interest in research. My supervisors nurtured and trusted me a lot in my development during the project which I definitely appreciated. I had a fixed schedule during the week and had frequent meetings with my supervisor to discuss the project and learn more of the topic as well. I also attended symposiums hosted by the building, other lab meetings as well as the development biology

At the end of the project, I definitely achieved my goals of my personal development and lab skills. I appreciated the work environment and the only issue was due to time constraints I was not able to complete the whole project and unable to finish the statistical analysis, I look forward to hear back from the group on the fruition of the project. Although, the topic was not necessarily of interest for me, the skills I gained in the lab were definitely applicable in other research areas and I will find them useful in my final year project.

In conclusion, my EASTBIO REP project was a valuable experience that allowed me to work with an amazing group of professionals who guided me in the hands-on lab experience as well as furthered my knowledge on neurodevelopment and organoids.