British Pharmacological society - log for vacation studentship

Week 1:

- Starting my summer placement I researched selectins, CRISPR and NanoBiT technologies in order to familiarise myself with the literature and the techniques I would be using.
- I observed a PCR, electrophoresis and an assay, building up my understanding and gaining the practical experience with the techniques I will be using during the summer. I also learned how to split cells in tissue culture which is an essential skill needed when using cells.

Week 2:

- I practiced cell culture by splitting cells and plating them in eight well plates for immunolabelling, going over the mathematics needed to plate a certain number of cells in each well. I also observed an electroporation to learn the protocol, as this is a technique I will be using for CRISPR.
- I performed immunohistochemistry techniques on HUVECs to look at the expression of P- and E-selectin giving me the opportunity to have experience with a complex protocol with many steps. This practice also gave we the confidence in performing experiments independently.
- Went over primer design with my supervisor and then followed up by doing some primer design, as this will be needed when screening my cells to see if the HiBiT has been inserted and for the cutting assay and in the cutting assay. As well as this, I looked at designing the trace and guide strands of RNA for Cas9 in the CRISPR technique.
- I researched further into selectins looking at the current literature.

Week 3:

- I independently split cells maintaining the confluency of my HUVECs. Furthermore, I observed and performed the preparation, protocol and analysis of a NanoBRET assay on EGFR. This allowed me to familiarise myself with the NanoBRET technique that I could potentially use in the future to screen for P-selectins.
- I performed immunohistochemistry techniques independently for P- and E-selectin to view their expression upon the HUVECs.

Week 4:

- I conducted repeats of immunolabelling assay using HUVECs staining for E- and P-selectin perfecting my timing and technique for each step of the protocol.
- Extracted genomic DNA from HUVECs in preparation for the cutting assay which will enable the deduction into if the PAM site and guide sequence will be cut by Cas9 in the expected position.

Week 5:

- After learning the protocol earlier in the summer I performed an electrophoresis experiment to determine the optimum conditions for the amplification of the region of DNA expected to be cut by the Cas9 enzyme using the guide RNA, which can then be purified using PCR clean up protocol. This purified sample can then be used in the cutting assay.
- The multiple steps needed in order to do a CRISPR experiment highlighted the need to assess different scenarios in preparation for each stage in order to carry on.
- I learnt how cells are brought up from frozen and used this to bring up low passage cells to be used in the electroporation experiment. I made sure the ratio of cells that were put in each flask was accurate for when the electrophoresis was planned.

Week 6:

- I observed an electroporation on the low passage HUVECs to incorporate Cas9 incubated with guide RNA and tracrRNA along with the repair DNA (HiBiT dsDNA) to attempt an insertion of the HiBiT (small segment of NanoLuc luciferase) into the P-selectin genome, allowing me to learn the steps and different components needed.
- I practiced the fine motor skills needed for electroporation using a micropipette
- Having Studied and amended the protocol for a cutting assay to determine if the Cas9 enzyme cuts the DNA that is complementary to the chosen guide, I carried out the cutting assay using two positive controls (one with just DNA the other with Cas9 with no guide) and obtained results that suggests that the guide is useful.
- I learnt the protocol on how to freeze down cells, specifically HUVECs.

Week 7:

- Carried out a second attempt at inserting the HiBiT protein into the P-selectin genome using electroporation of HUVECs.
- Continued to split and freeze down cells accordingly.

Week 8:

- Attempted some bystander BRET using E-selectin HiBiT clone that has HiBiT inserted into the N terminus of E-selectin, we wanted to see if CXCR4 was closely expressed with E-selectin. To do this I came up with a protocol independently, ensuring each step was well thought out and timed correctly as we were looking at the potential kinetics, this gave me a greater understanding of how an experiment has to be thoroughly thought out, for example furimazine is quite potent so has to be left for 5 minutes for some of it to be used by the reformed NanoLuc.
- I used the Ferastar to assess the fluorescence (fluorescent ligand) and luminescence (NanoLuc) given off from the plate and found that although there was a slight indication of bystander BRET it was not sufficient to confirm that this was due to CXCR4 and E-selectin being close to each other on the cell's membrane.

Week 9:

- Observed a genomic extraction from an agarose gel run through electrophoresis and noted the differences to the protocol from that of genomic extraction from cells that I did earlier in my placement

- Did a repeat of the experiment in week 8, getting similar results.

Week 10:

- Made some adjustments to the protocol to the experiment carried out in week 8 and repeated the experiment for the third time to see if the variables I changed would make a significant difference.

Overall, over the past 10 weeks I have not only gained experience in techniques that I would have otherwise not have been able but also learned from my supervisors and people within the laboratory on when and how to adapt a protocol or try something completely different when the results are not what was expected. Furthermore, I have gained insight into interpreting results and that just because the results were not what were originally expected does not mean that nothing was revealed. This is not to mention the amazingly supportive atmosphere from all the team and I could not have imagined a more productive learning environment.