Calum Connolly

Pathological Society and the Jean Shanks Foundation studentship report

My 5-week placement focussed on detecting the TERT C228T mutation in Solitary Fibrous Tumours (SFTs). Following a project briefing, I first practiced my pipetting skills which allowed me to start extracting DNA from FFPE SFT samples. This protocol spanned two days to fully lyse the fibrous tissue samples before extracting the DNA and was carried out in batches of 24 samples. Initially the practical technique was challenging, however my confidence grew as I become familiar with various pipettes and practiced my ability to accurately transfer small volumes of water onto a scale before moving onto reagents.

Once I had produced several batches of extracted DNA, the Qubit Fluorometer offered quantification of the concentration of DNA to understand how much DNA we had to use. I was not familiar with this, so I conducted background reading into Qubit Fluorometer and NanoDrop Spectrophotometer. I found Qubit and NanoDrop protocols to be straightforward to follow as the devices were intuitive to use, although I had to read the device manual to understand the significance of the results.

I progressed onto digital droplet polymerase chain reaction (ddPCR) which offered a precise way to quantify whether each sample contained the C228T mutation in TERT. There was not a pre-written protocol outlining the steps for ddPCR using the TERT C228T probe, so I wrote a step-by-step protocol for myself and others to follow which outlined 11 key steps. Of all the techniques, the ddPCR protocol was most challenging and required most preparation and attention to detail. The volumes of each reagent required to dilute the DNA, create a master-mix and the controls had to be very precise to ensure adequate droplet generation for the PCR as there were many things that could have affected the quality of results. As ddPCR incorporates water-oil emulsion droplet technology, massive sample partitioning allowed us to clearly detect the presence of the C228T mutation as our results were binary i.e., the mutation was either present or not. I also taught this protocol to a new member of the team who was conducting ddPCR with the TERT assay I was using. This improved my ability to communicate multi-step protocols to someone unfamiliar with these techniques, simultaneously improving my familiarity with the process.

Aside from learning new practical techniques, working with my supervisor and lab members gave me insight into research-orientated careers. In the future I am keen to pursue research roles alongside clinical duties which other members of the lab were able to do. Research is something I can engage in through publications or presentations which should aid my future career progression and I will be presenting at the Winter Pathology conference which should be an enjoyable experience. I am very grateful to the Pathological Society and the Jean Shanks Foundation for funding this incredible opportunity.