

Summer project report 2019 – Willow Rolls

This summer I undertook a research project in the Whitby lab in the Oxford Biochemistry department investigating the molecular mechanisms responsible for generating complex genome rearrangements (CGRs), under the supervision of Judith Oehler. Complex genome rearrangements (CGRs), which consist of two or more breakpoint junctions are associated with many genetic disorders and are a common feature of cancers. The type of CGRs investigated in the project I was involved with are homology catalysed reinsertion and investigating how different factors affected the recombination frequency. These variables were the orientation of the KanMX gene, the amount of homology either side of the gene, the location of the gene upstream or downstream of the stalled replication fork and the size of the package to be relocated. This involved the creation of plasmids in *E. coli*, amplification and verification of the plasmid and then transformation into the model organism *S. pombe* (fission yeast) to investigate the effect.

The beginning stages of my project involved the creation of plasmids in *E. coli*, a simpler organism to work with and genetically manipulate. Restriction enzymes were involved to switch the marker of the plasmid, enabling easier selection once transformed into *S. pombe* (fission yeast) as allowing for Leu⁺ screening and ensuring that the marker was different to the region of homology to be relocated. Further plasmid creation stages were followed by a colony PCR screen to determine the success of the process. The initial screen showed false positives and after looking at the gene maps realised the reason behind this, so adjusted the screening method that had reduced efficiency but increased precision. This taught me the importance of in research balancing time and cost saving measures against the accuracy and precision of the results. Despite not everything being successful the first time around, I enjoyed the troubleshooting that resulted. Having to use my knowledge of the techniques and researching to find out more to understand what has gone wrong and the required steps to resolve it. It was important to understand the vital steps in the purification processes that significantly affected the yield, to ensure that there was the greatest amount of end product possible.

Throughout the project I have gained experience in a variety of molecular biological techniques and traditional cloning mechanisms. I found it interesting to be able to practice many of the techniques that have been learnt about theoretically in the lecture courses of my first year and to be able to see them in practice and understand their use.

Overall, the project has given me invaluable insight into what is involved in a research lab environment. It has reaffirmed my desire to pursue research after my undergraduate degree. I am grateful for the experience and opportunity allowed to me by the Whitby lab and the funding from Merton college.