**University of Leicester**

**BBSRC MIBTP Studentship Project 2024-5 entry.**

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| **Project Reference** |  |

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**Section 2 – *Project Information***

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| **Project Title** | Unlocking the genetic potential of barley by modulating recombination  |
| **Project Summary**  |
| Barley is a major worldwide crop used for malting and animal feed. However, breeding new varieties is constrained by the frequency of genetic crossovers (1-3 crossovers per chromosome pair) and their distribution (biased towards the chromosome ends) that underpin crop improvement1,2. Genetic crossovers form during meiosis, a specialised cell division that is required to halve the number of chromosomes for gamete production. This project aims to utilise the phenotype of barley *pachytene checkpoint 2* (*pch2*)3 CRISPR/Cas mutants (that we have generated) that appear to delay the timing of meiotic recombination, thus enabling crossovers to form in chromosomal regions that rarely or never recombine.   In addition to the wider aim of crop improvement, this project will also investigate fundamental biological questions regarding crossover control in eukaryotes by utilising cutting edge microscopy techniques. This will include structured illumination super-resolution microscopy in conjunction with a panel of antibodies that we have generated in the lab that specifically bind to meiotic proteins that control crossover number and position1,2. By perturbing recombination dynamics in the *pch2*3 mutants we will be able to monitor each step of the recombination pathway and determine which sites are selected for crossover designation. We will also develop KASP molecular markers to genetically map recombination in the mutant and test how this compares to the cytological data. Barley is an excellent model for investigating meiotic recombination in species with large chromosomes when compared to the more extensively studied small chromosomes of *Arabidopsis thaliana*, thus providing a more complete picture of how these conserved processes adapt and function in sexually reproducing eukaryotes.  **Objectives:** 1. Determine crossover positions on wild type and *pch2* mutant barley chromosomes utilising ZYP1 and HEI10/MLH3 antibodies
2. Determine the relationship between crossover position and epigenetics utilising antibodies raised to specific chromatin modifications and DNA methylation
3. Determine the role of PCH2 and ZYP1 in crossover control by assessing recombination dynamics in the *pch2* mutant and *ZYP1* RNAi knockdown lines
4. Determine timing of meiosis in *pch2* mutants by incorporating DNA base analogs during premeiotic replication
5. Develop KASP markers to measure recombination in wild type and pch2 mutant

Techniques that will be undertaken during the projectSuper-resolution structured illumination microscopy, immunolocalization, fluorescence in situ hybridisation, PCR, cloning, DNA sequencing.  |
| **References** |
| 1.  Higgins JD, Perry RM, Barakate A, Ramsay L, Waugh R, Halpin C, Armstrong SJ, Franklin FC. (2012) [Spatiotemporal asymmetry of the meiotic program underlies the predominantly distal distribution of meiotic crossovers in barley.](https://pubmed.ncbi.nlm.nih.gov/23104831/) Plant Cell. 2012 24(10):4096-109. doi: 10.1105/tpc.112.102483.   [2. Barakate A, Higgins JD, Vivera S, Stephens J, Perry RM, Ramsay L, Colas I, Oakey H, Waugh R, Franklin FC, Armstrong SJ, Halpin C. (2014). The synaptonemal complex protein ZYP1 is required for imposition of meiotic crossovers in barley.](https://pubmed.ncbi.nlm.nih.gov/24563202/) *Plant Cell*. 26(2):729-40. doi: 10.1105/tpc.113.121269.   3. Lambing C, Osman K, Nuntasoontorn K, West A, Higgins JD, Copenhaver GP, Yang J, Armstrong SJ, Mechtler K, Roitinger E, Franklin FC (2015). [Arabidopsis PCH2 Mediates Meiotic Chromosome Remodeling and Maturation of Crossovers.](https://pubmed.ncbi.nlm.nih.gov/26182244/) *PLoS Genetics*, 11(7):e1005372. doi: 10.1371/journal.pgen.1005372.   |

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