Title of Project

Single cell pollen meiosis screening in wheat.

Primary contact for the team

Ashleigh Lister, Research Assistant, Technical Development, Earlham Institute (EI), Ashleigh.lister@earlham.ac.uk

Team

Researchers at **EI** will provide experience in single cell genomics, FACS sorting of the wheat meiocyte samples, whole genome amplification, and screening using sequencing.

Ashleigh Lister, Research Assistant, Technical Development, EI, Ashleigh.lister@earlham.ac.uk

Dr Iain Macaulay, Group Leader, Technical Development, EI, Iain.macaulay@earlham.ac.uk

Dr Matt Clark, Head of Technology Development, EI, Matt.clark@earlham.ac.uk

Researchers at **JIC** will bring expertise on wheat meiocyte staging providing the project with dissociated meiocytes ready for FACS sorting. They are particularly interested in meiosis at the single cell level, especially in comparison to Ph1 mutant lines. The group have a biological interest in meiosis.

Prof Graham Moore, Project Leader, Crop Genetics, JIC (JIC), graham.moore@jic.ac.uk

Prof Peter Shaw, Project Leader, Cell and Developmental Biology, JIC, Peter.shaw@jic.ac.uk

Dr Azahara Martin, Post doctoral Scientist, Crop Genetics, JIC, Azahara.martinramirez@jic.ac.uk

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Summary

Recombination is a process which takes place during meiosis, a system necessary during gametogenesis in all sexual organisms. The two sets of replicated parental chromosomes undergo crossover formation followed by two rounds of segregation, to produce haploid gametes ready for fertilisation. Variation in the offspring population, caused by meiotic recombination as well as random segregation, is highly important as it creates a fitness in the event of a change in environment or pathogen attack, as well as creating genetic diversity required in agricultural plant breeding.

We would like to screen wheat meiocytes (from a given parent plant) at the single cell level because this will show the extent of meiotic recombination, and any recombination hotspots, without the need to grow hundreds, or thousands of plants. Our method would be a quick, cheap and high-throughput method of scoring new hybrids, and could be used to screen for treatments to affect meiosis. The method would identify which hybrids, or

treatments have elevated recombination events, which would be a powerful tool for breeding or programmes and for future research.

Proposal

Aims:

To develop a quick and cheap screening assay which identifies meiocytes which we want to look at in more detail (by deeper DNA sequencing). To discover the level of recombination during wheat meiosis and locate any hot spots. This will look at wheat meiosis at the single cell level which has not been done before.

Methods:

Meiocyte samples will be prepared based on the method described in Dreissig et al (2015) from wheat-rye hybrids grown at JIC.

Individual meiocytes will be FACS sorted based on scatter properties (size and granularity) and DNA content using Dye cycle fluorescent stains. Sorted populations will be analysed microscopically to ensure that the correct cell type has been isolated.

Cells will be individually sorted directly into RLT lysis buffer which releases the cell and nuclear content. DNA and RNA from the same cell, will be separated using the G&T Seq protocol (Macaulay et al, 2016), on the Beckman robotic liquid handling instrument at EI. We will aim to process 96 samples using this method.

As the initial focus will be on genomic recombination, the separated DNA will undergo whole genome amplification so that we have sufficient material to conduct Illumina Nextera library construction.

By sequencing at a low depth, we will be able to roughly screen the 96 single meiocyte DNA to select any particularly interesting samples for deeper sequencing. By processing 96 cells, and testing that the method works, we should observe a diverse representation of the recombination potential of the genotype. This will show the extent of rearrangement which has occurred during meiosis, which may differ between hybrids and genotypes). If we succeed, we would be able to implement this method to score the recombination events of different hybrids, genotypes and environmental conditions.

As a result of G&TSeq, the RNA will also be available for whole transcriptome amplification. By sequencing this material, we would gain more information about which genes are being expressed at different meiotic stages. The sequencing costs for this part of the study will be met by the EI Technical Development budget and potentially from follow on funding applications.

Outcomes:

This pilot project will facilitate the expertise and capabilities of groups at JIC and EI in wheat meiosis and single cell genomics. We will generate a unique dataset of genotypes from individual post-meiotic pollen grains, linked with transcriptional information from the same cells. During the course of the project we will refine methods for cell isolation, sorting and

processing for single cell genomics analysis, with the aim of developing robust methods that can be applied in both fundamental and applied plant research.

Methods for high-throughput genotyping/sequencing of individual pollen cells will be of benefit to academic and industrial researchers with an interest in plant breeding. Our aim is to use preliminary data generated during this study of 96 samples, to support larger grant applications which will scale up and further refine these approaches.

We are currently discussing the potential of these methods the breeding company KWS (Cambridge), who have expressed an interest in using this single pollen genotyping as a breeding platform and in working with us as an industrial collaborator in future funding applications.

The EI holds a BBSRC National Capability in Genomics, which means that our robotic platforms and expertise could develop this into a service for customers, whether this is for plant pollen or gametes from other organisms.

Who is involved: The Technical Development team at EI and the Moore group at JIC.

Benefits and outcomes

This project will develop novel methods that could potentially be applied across the spectrum of plant reproductive research and breeding. This will be of benefit to researchers studying the basis of plant meiosis (e.g. the Moore Group at JIC) as well as breeders aiming to harness the power of meiotic recombination for trait selection.

The funding would also strengthen a newly established collaboration between JIC and EI which aims to establish tools and techniques for the single cell analysis of pollen and other plant cells. This collaboration is interdisciplinary within the biology umbrella - the Moore/Shaw groups are cytogeneticists and cell biologists, and members at the EI are molecular geneticists with extensive technological development and bioinformatics experience.

Furthermore, it will allow the primary contact and project lead, Ashleigh Lister, to develop research interests and leadership skills, with a view to being lead author on manuscripts arising from the work.

Both JIC and EI have dedicated ‘outreach’ teams which allows showcasing of the projects at multiple events, institutional visits, as well as posters and talks on sites and externally at international conferences. All protocols and publications arising from the project will be open access, in line with EI policy.

Sponsor for the research and cost centre

Dr Iain Macaulay, EI, Technical Development, iain.macaulay@earlham.ac.uk
Cost code: GG005-CO1-A

I confirm that I have the full support of the sponsor listed above and that they can be added to the OpenPlant Fund mailing list to receive project updates (to which they can unsubscribe at any time).

Budget

Budget is requested for the isolation and analysis of 96 cells in total. The extra funding will be supplemented using the EI Technical Development budget. With additional BBSRC National Capability Core funding, and future grant applications, we will aim to perform next generation sequencing the DNA and RNA of these cells.

Item	**Description**	**Cost (£)**
Cell Isolation and Sorting	Cell sorting will be performed free of charge at EI	n/a
G&T Seq separation	Includes various consumables and reagents to process 96 cells	289
Whole Genome amplification	Qiagen REPLI-g Single Cell Kit to process 96 cells	1201
Library construction	Low volume Nextera protocol	203.32
DNA sequencing	In-house sequencing using Illumina HiSeq4000	2764.74
Total requested		**4458.06**

With additional BBSRC National Capability Core funding, and future grant applications, we would like to sequence the RNA. This should give copy number variation and transcriptional information for each meiocyte.

References:

Dreissig S, Fuchs J, Cápal P, Kettles N, Byrne E, Houben A (2015) Measuring Meiotic Crossovers via Multi-Locus Genotyping of Single Pollen Grains in Barley. PLoS ONE 10(9): e0137677. doi:10.1371/journal.pone.0137677

Iain C Macaulay, Mabel J Teng**,** Wilfried Haerty**,** Parveen Kumar**,** Chris P Ponting**,** & Thierry Voet, (2016) Separation and parallel sequencing of the genomes and transcriptomes of single cells using G&T-seq, Nature protocols, 11(11) p2081- 2103 doi:10.1038/nprot.2016.138