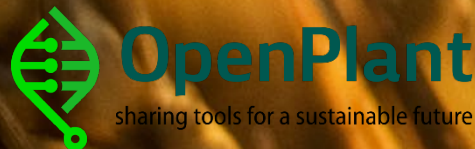


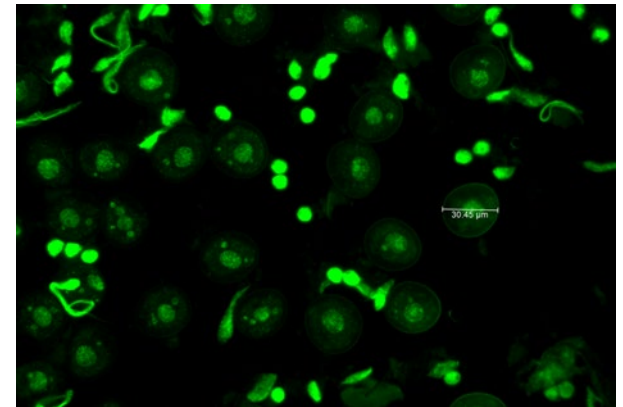
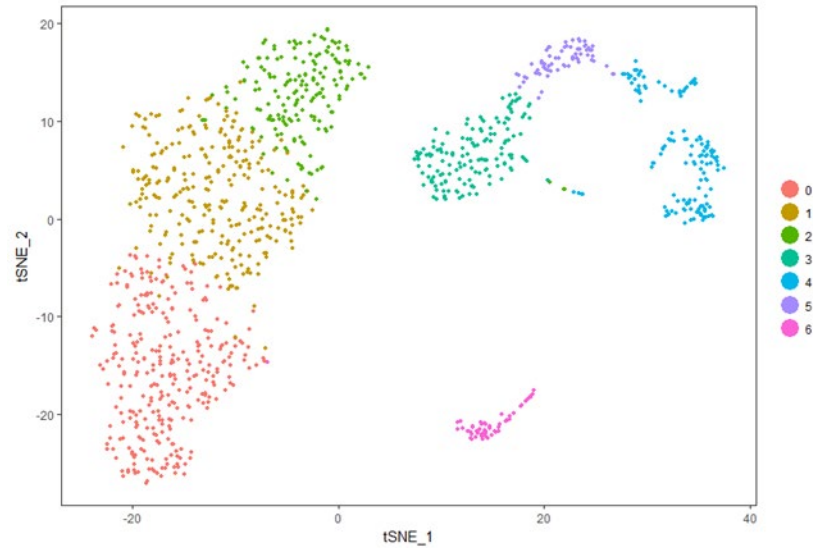
Wheat pollen single cell sequencing

Ashleigh Lister, Dr Iain Macaulay, Prof Graham Moore, Prof Peter Shaw, Dr Azahara Martin, Dr Lola Santome

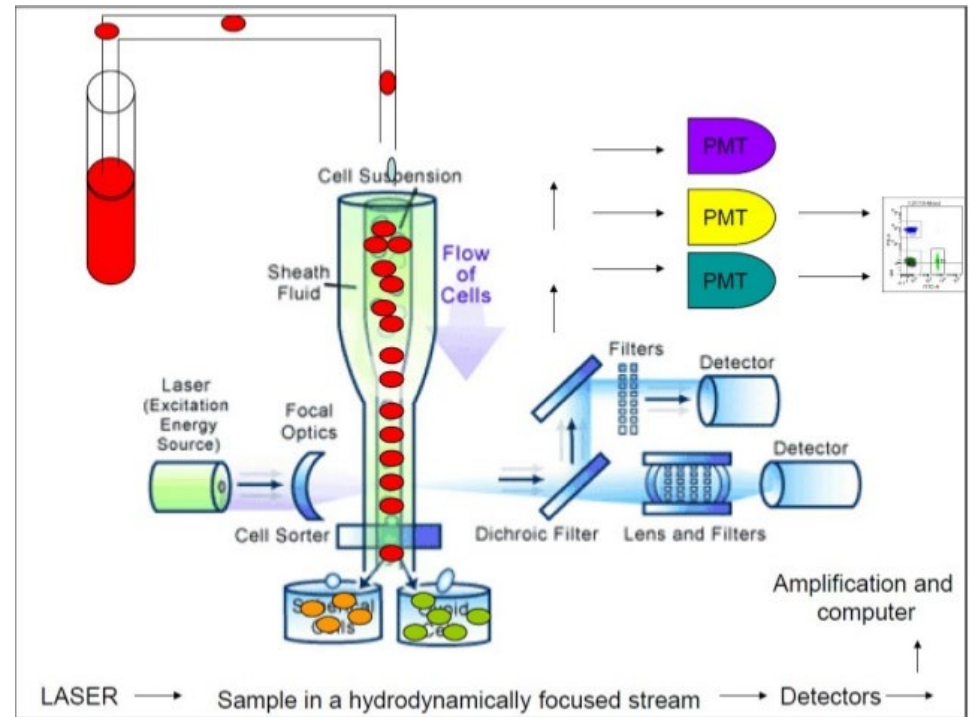
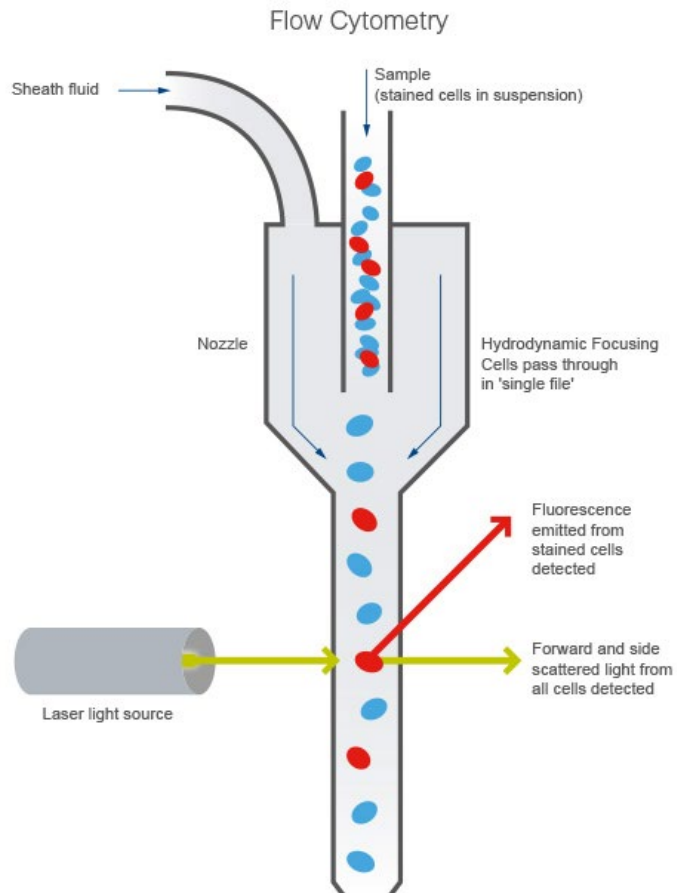
OPENPLANT FUNDED PROJECT
EARLHAM INSTITUTE AND JOHN INNES CENTRE COLLABORATION



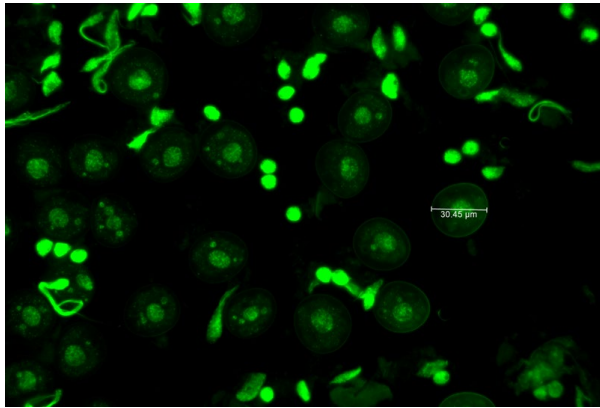
Single cell sequencing



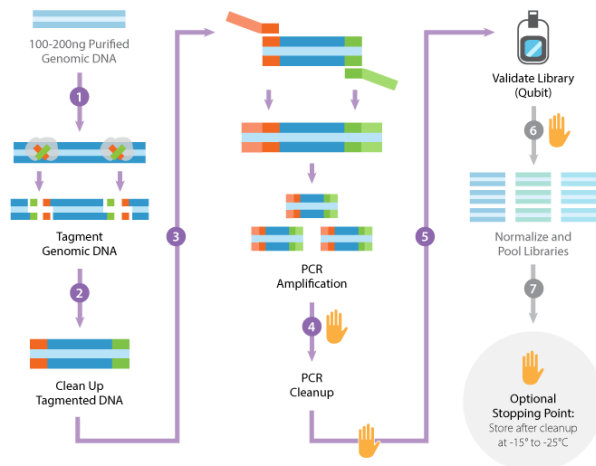
How FACS works?



Proposal



Nextera DNA Library Prep Kit

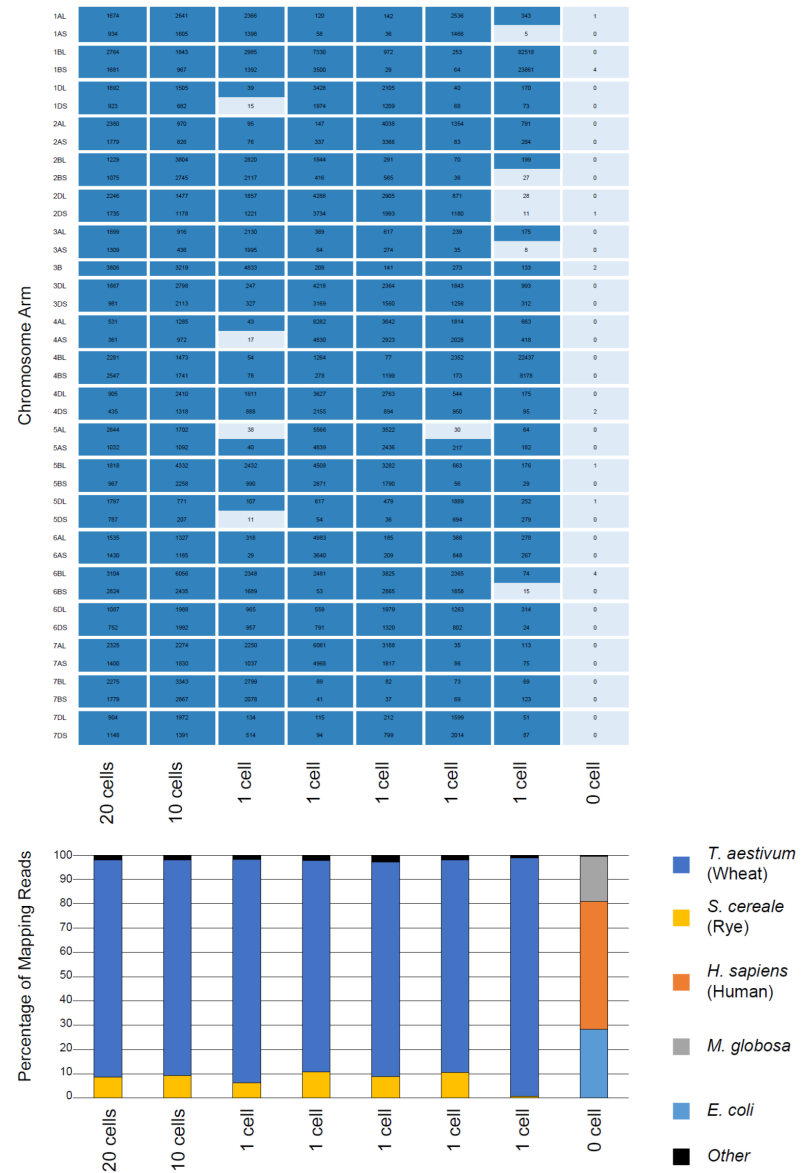


Preliminary analysis

Chinese Spring vs Rye
hybrid pollen FACS sorted.

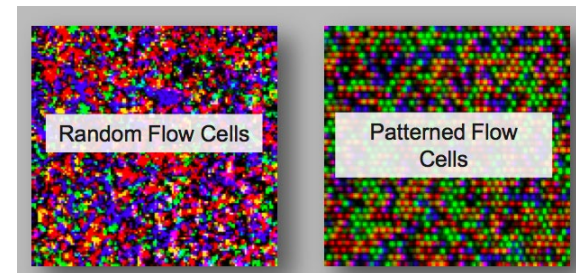
8 libraries pooled and very
shallow sequenced using
an Illumina MiSeq Nano
run.

Mapped against both
genomes and checked for
contamination.



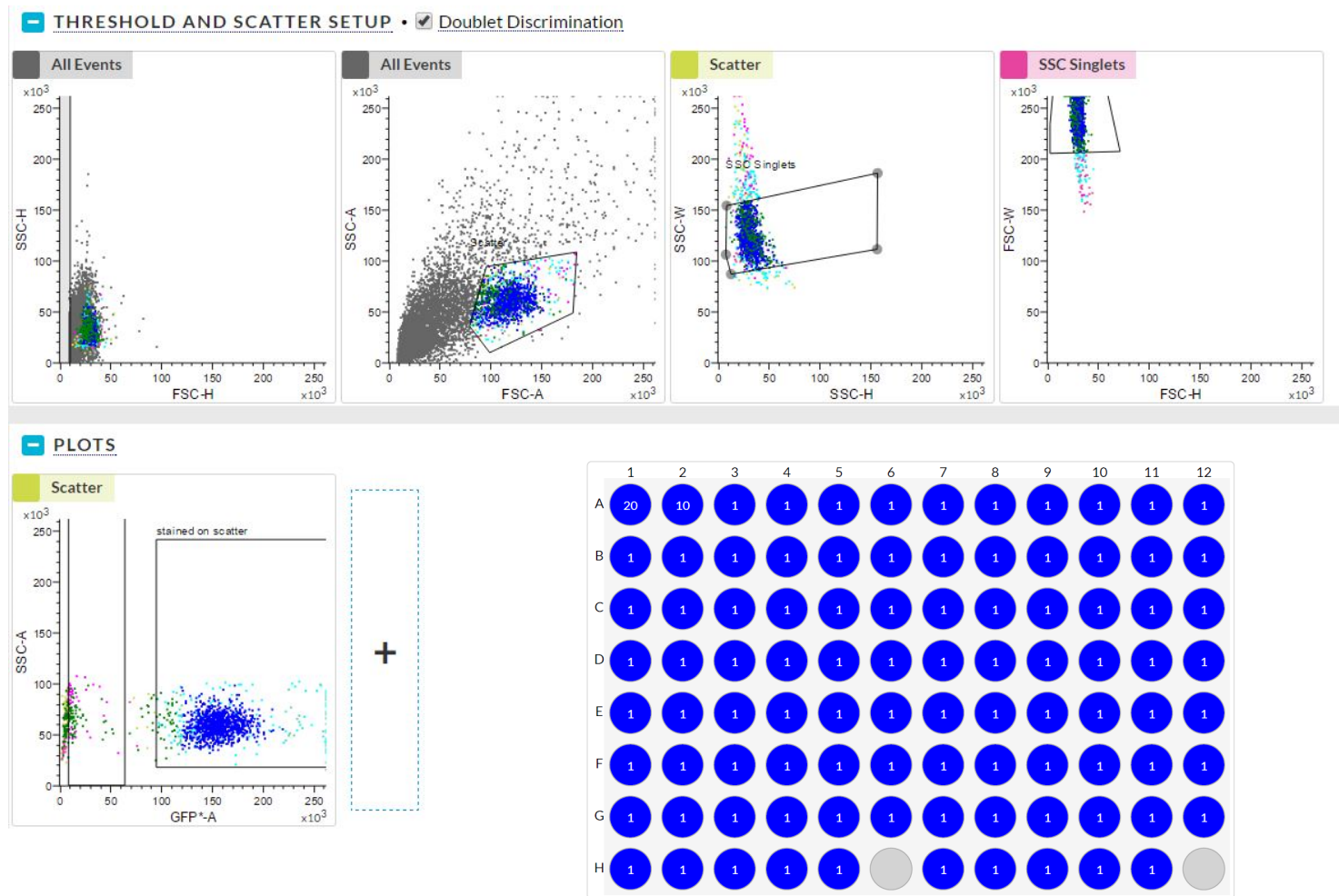
Project scales and schedules

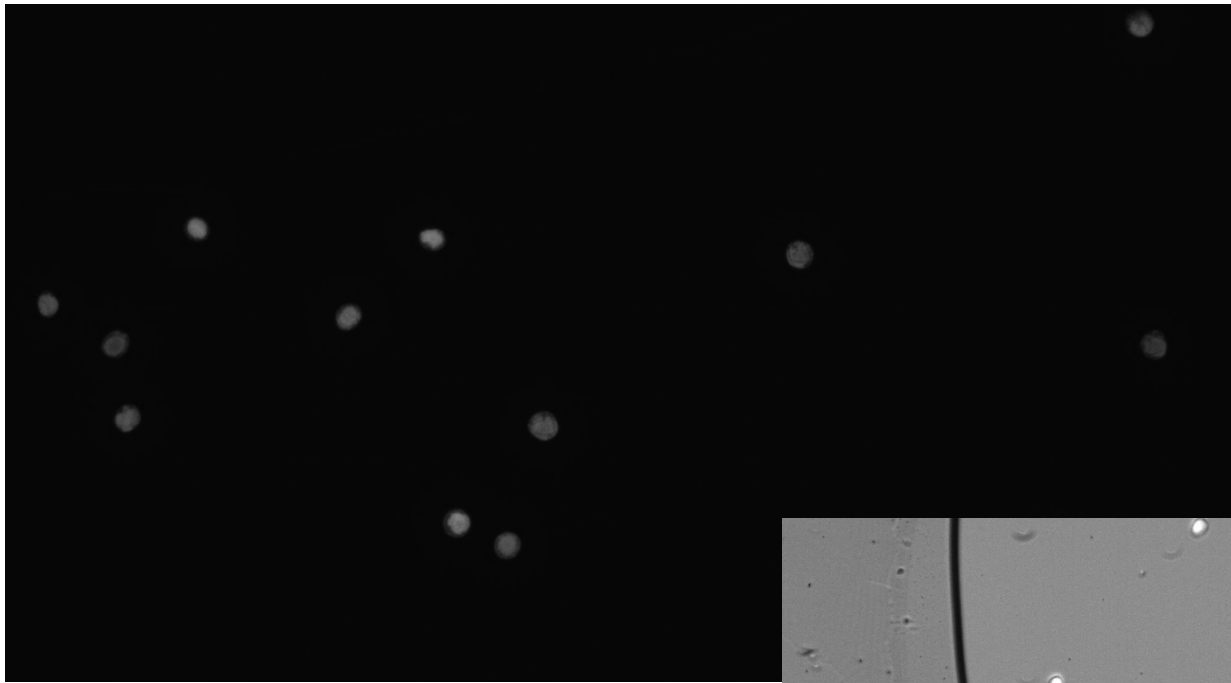
- 48 wells of single cell Chinese Spring pollen- control- Samples already sorted, libraries constructed and sequenced, data being analysed
- 48 wells of single cell Cadenza pollen- control- Samples already sorted, libraries constructed and sequenced, data being analysed
- 1 plate of Chinese Spring pollen processed using G&TSeq- Samples already sorted, libraries constructed and sequenced, data being analysed
- 1 plate of single cell Chinese Spring vs Cadenza hybrid pollen- pollen ready for collection in Feb
- Multicell and empty well controls in each plate
- Pool all and run on multiple lanes of a HiSeq4000 flow cell at a depth of $\sim X0.01$



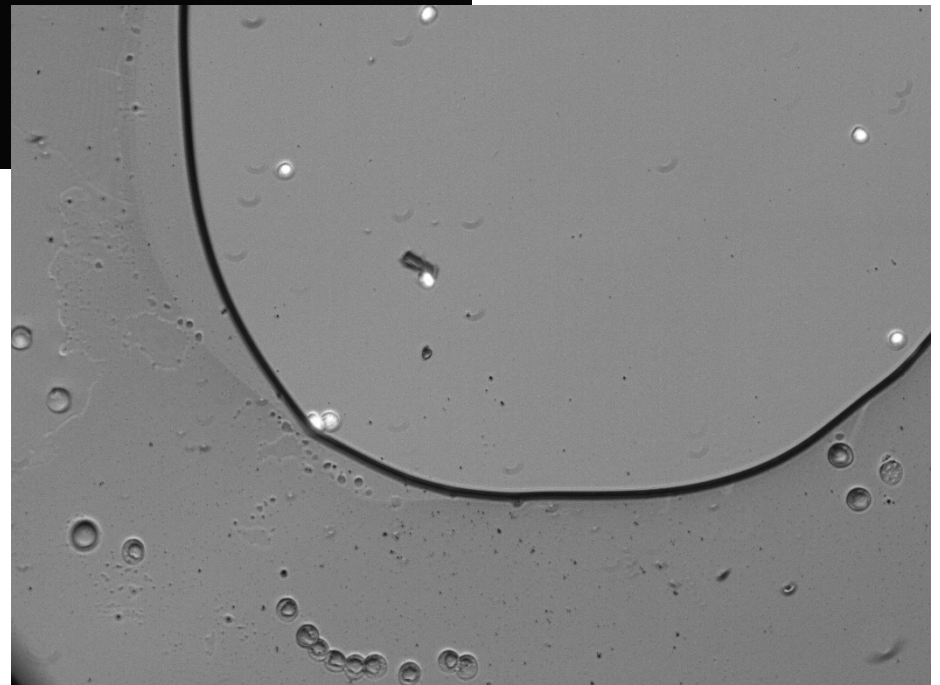
FACS cell sorting- Chinese Spring-uninucleate pollen-

27/09/17



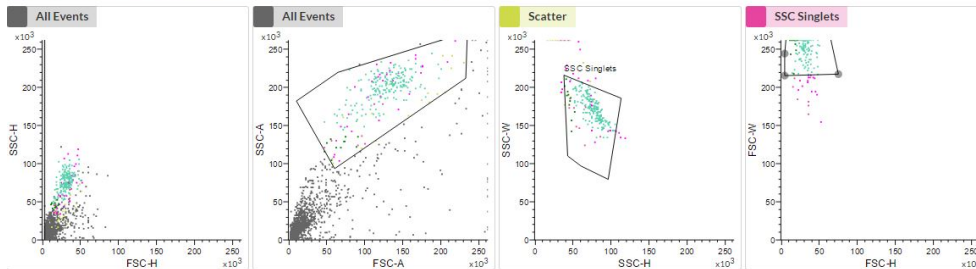


Uninucleate CS
pollen(27/09/17) sorted
onto microspore slides for
verification of selected
FACS population



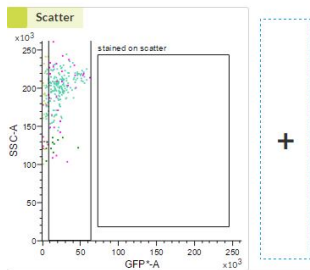
FACS sorting- Cadenza uninucleate pollen- 2/10/2017

THRESHOLD AND SCATTER SETUP • ☒ Doublet Discrimination

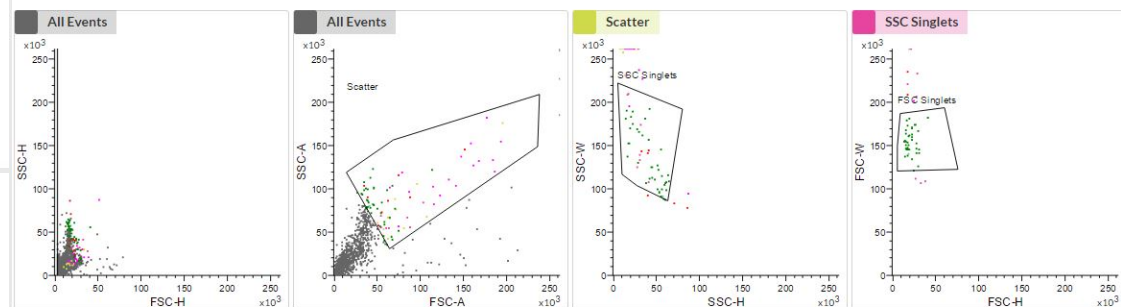


Unstained pollen

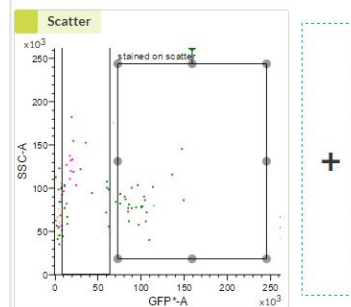
PLOTS



THRESHOLD AND SCATTER SETUP • ☒ Doublet Discrimination

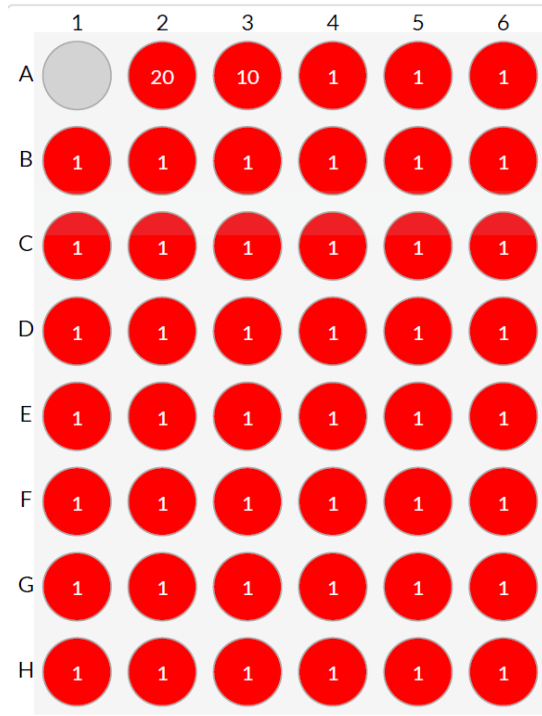


PLOTS

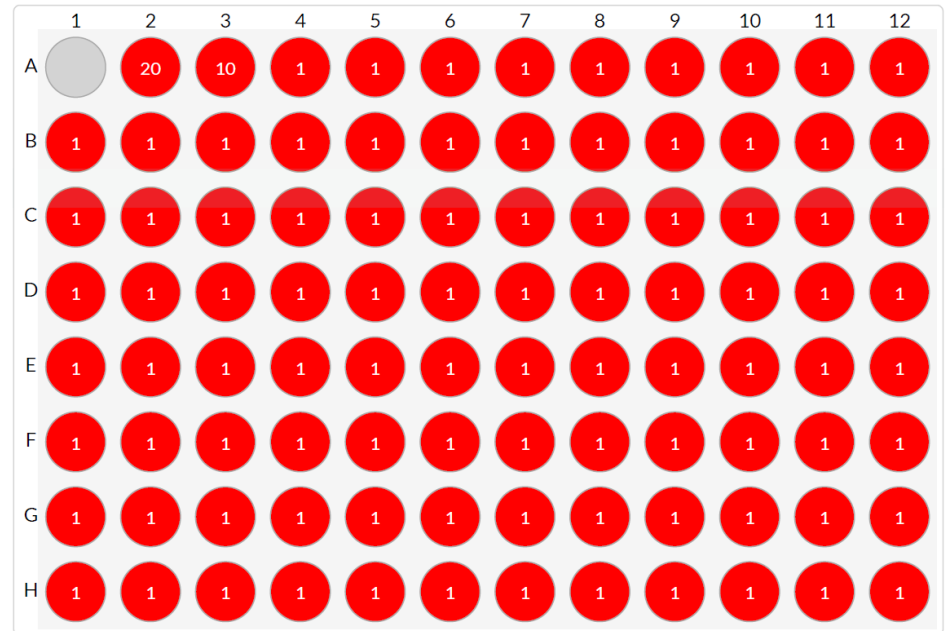


Stained pollen

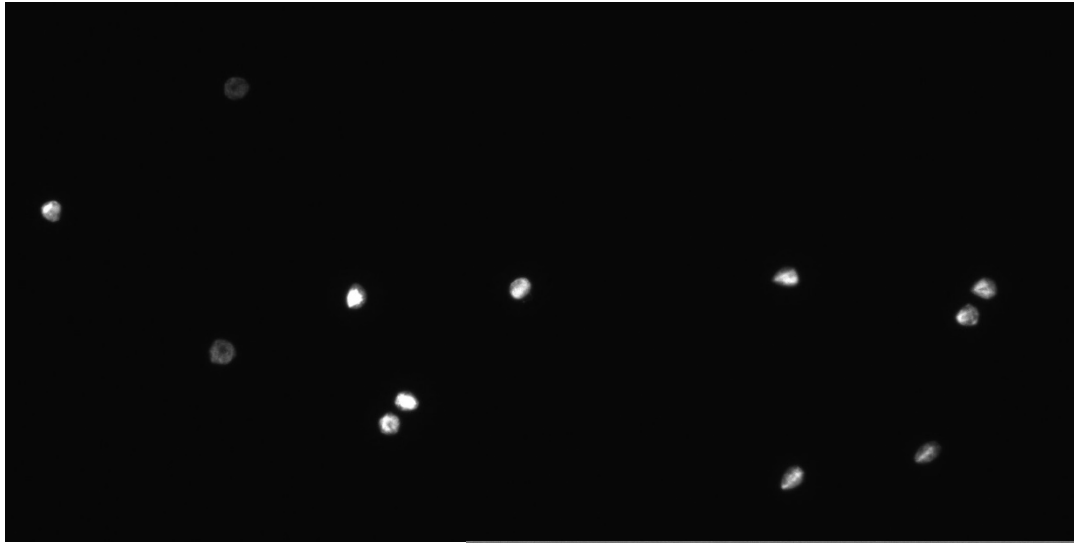
Cadenza Plate layouts 2/10/17



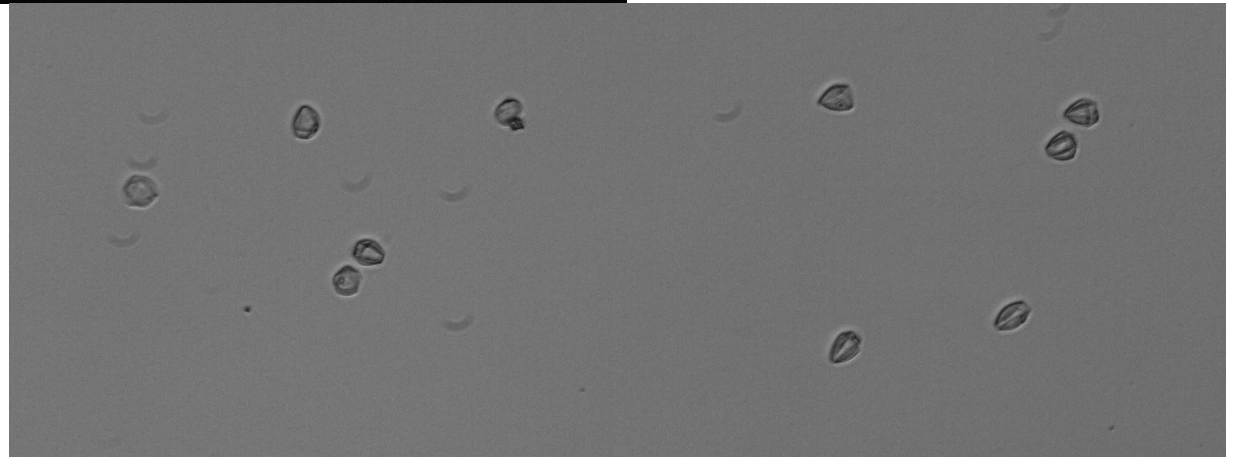
Pollen sort into 2ul PBS read
for MDA



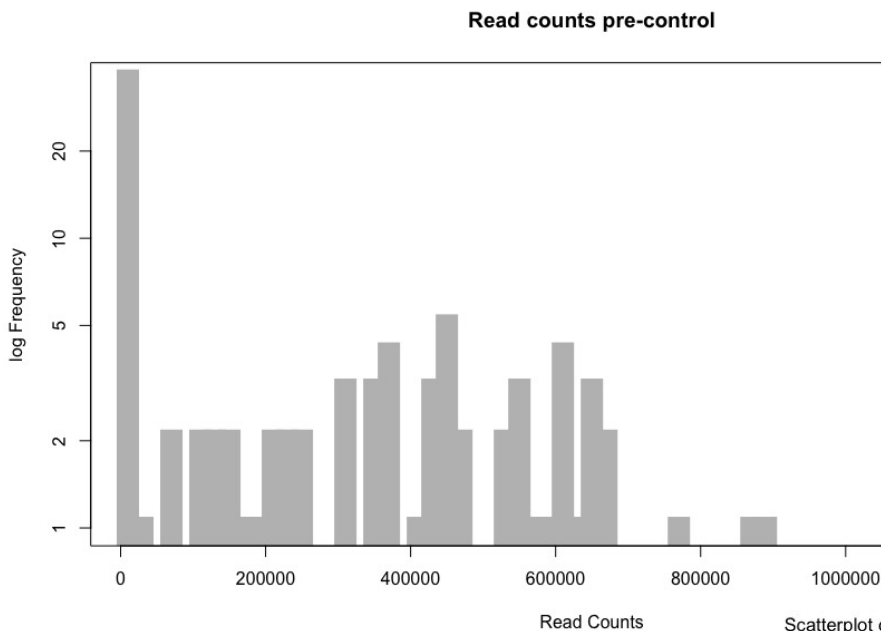
Pollen sort into 2.4ul RLT
ready for G&Tseq, would like
to have sorted x2 plates but
the sorted errored (USB fault)



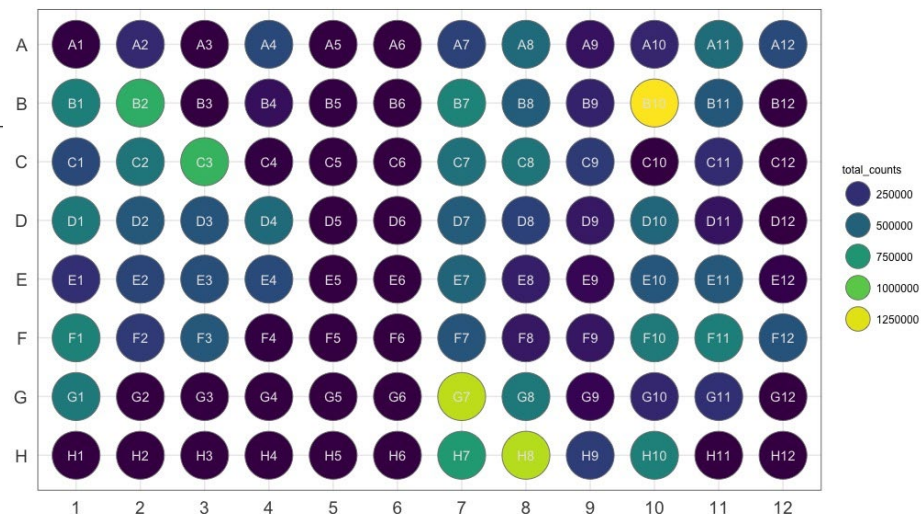
Uninucleate Cadenza pollen
(2/10/17) sorted onto
microspore slides for
verification of selected FACS
population



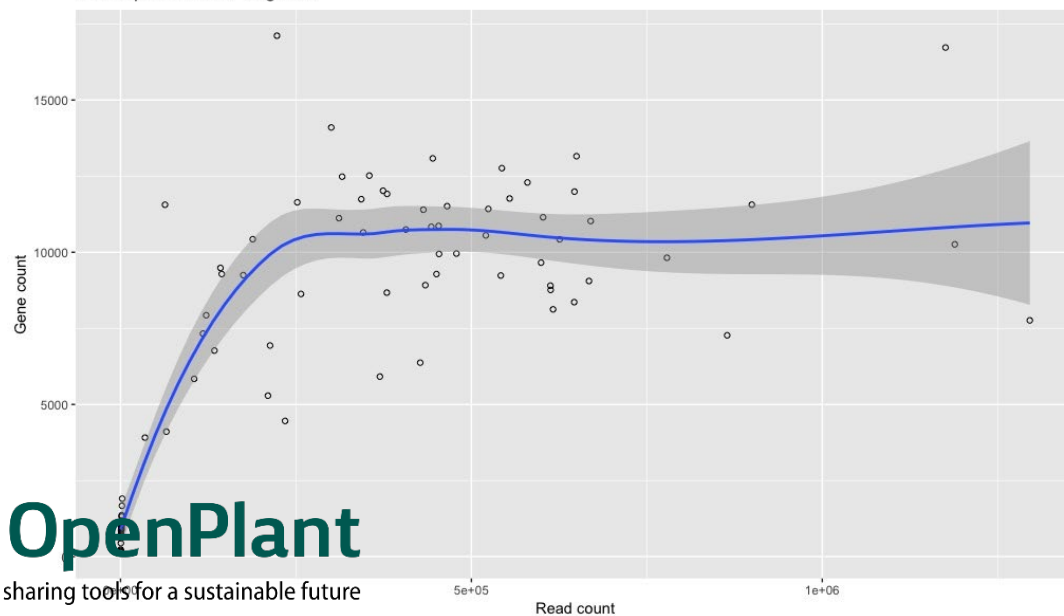
Transcriptome analysis



Total reads per well

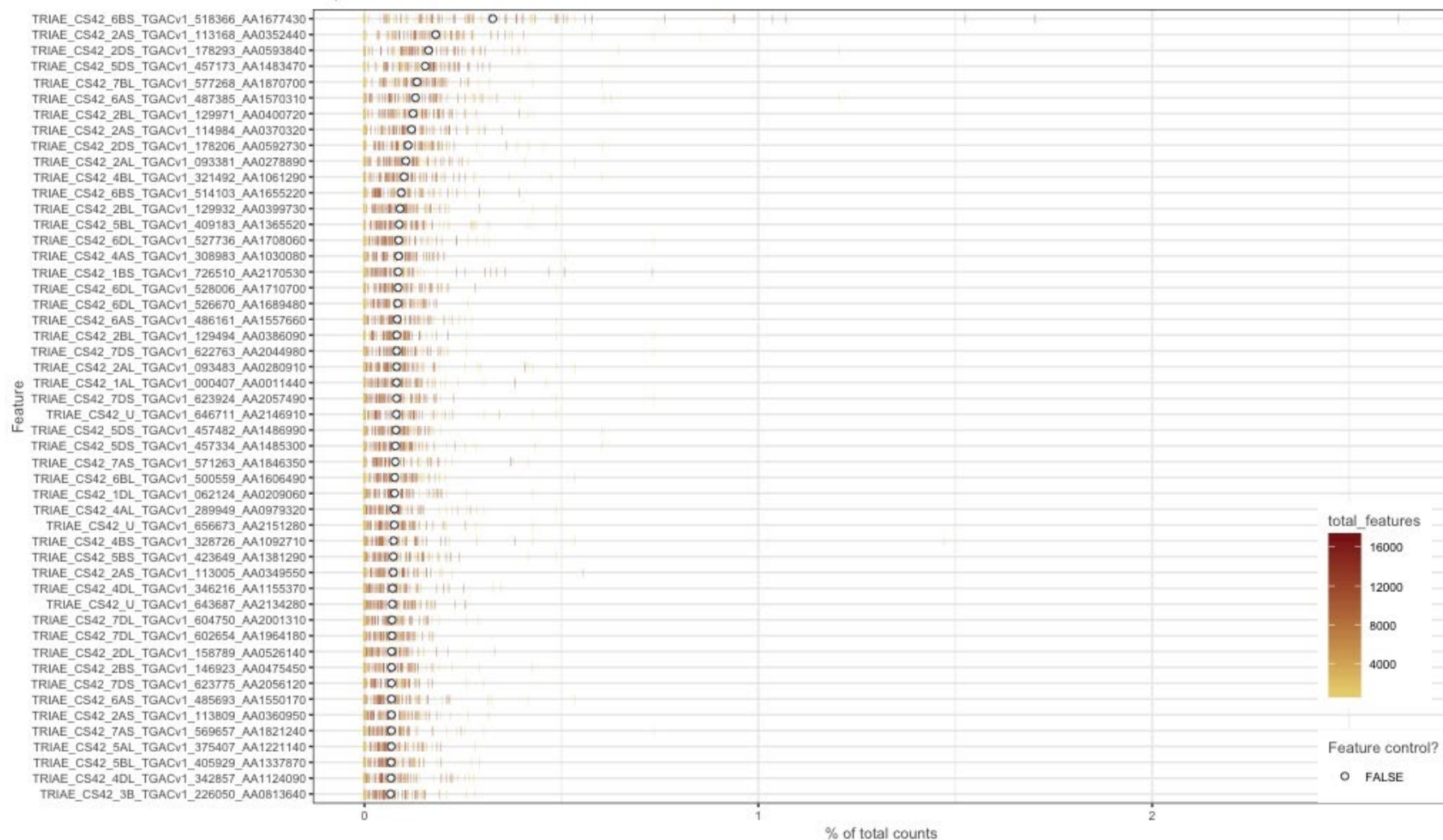


Scatterplot of reads vs genes

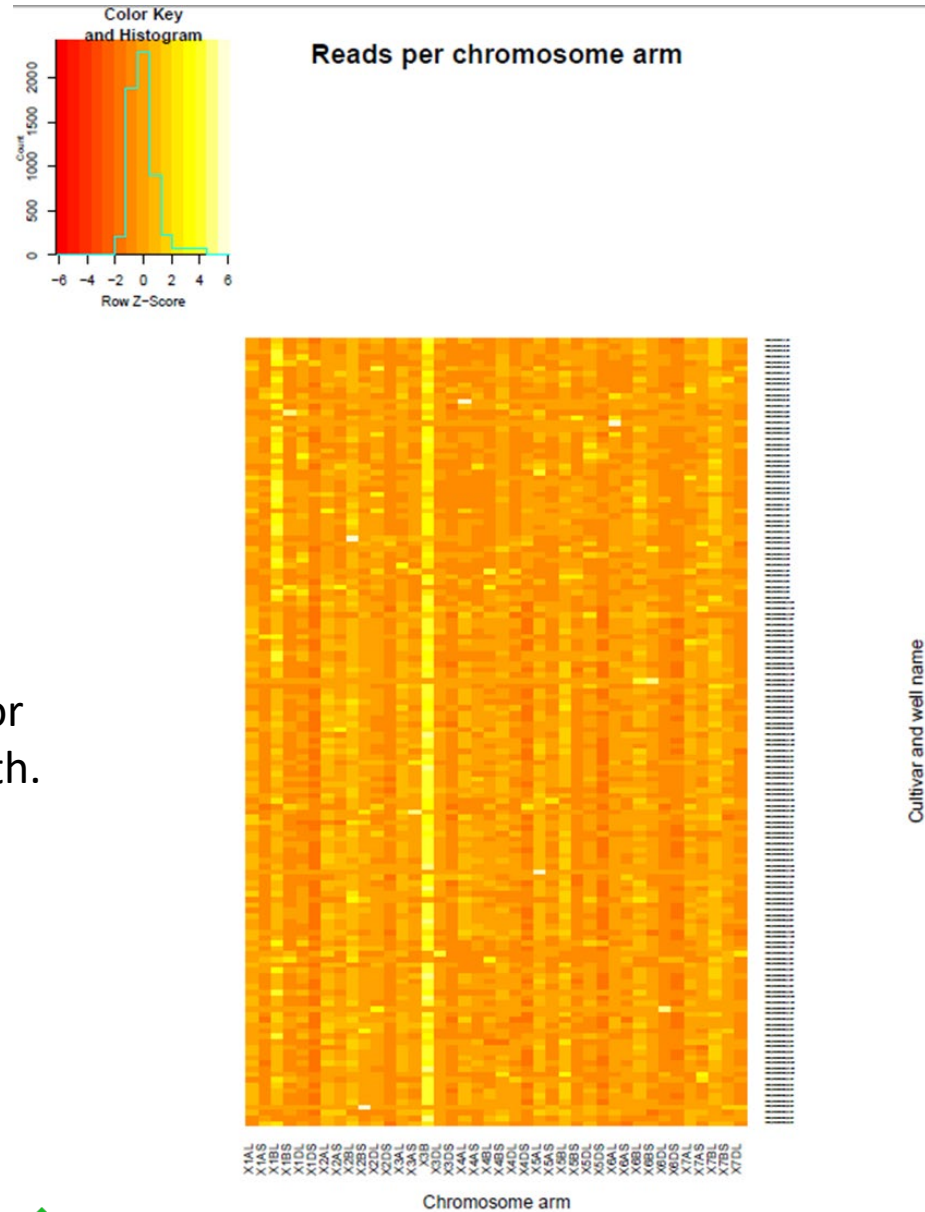


Transcriptome analysis continued...

Top 50 account for 4.64% of total



Genome analysis



Not normalised for chromosome length.

Findings based on transcriptome analysis of single cell pollen

- The wheat transcriptome is not good enough, it may be better to relate it to the arabidopsis pollen transcriptome
- This needs to be repeated for each stage of meiosis to capture differences in expression
- Still requires data from hybrid pollen, hopefully the transcriptome annotation is good enough to pick up on the meiotic rearrangements

Outreach/ outcomes of project

- Presented poster using preliminary MiSeq data at Genome10K and Genome Science conference
<http://www.earlham.ac.uk/genome-10k-and-genome-science-conference>
- Presented poster at SAB.
- Iain will present project at AGBT conference in Feb 2018, <http://www.agbt.org/gm-agenda/>
- Led to other similar projects, single cell Zebrafish sperm and mouse sperm sorting looking also at meiosis and recombination
- Led to a meiosis conference 'Meiosis and Beyond' <http://www.earlham.ac.uk/meiosis-and-beyond>, to be held 5th March 2018

Single-cell genomic analysis of wheat pollen

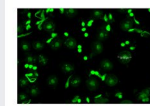
Ashleigh Lister¹, Ned Peel¹, Azahara Martin², Lola Santome², Graham Moore², Peter Shaw², Matt Clark¹, Iain Macaulay¹

¹Earlham Institute, Norwich Research Park, Norwich ²John Innes Centre, Norwich Research Park, Norwich

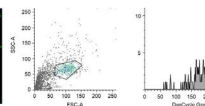


Meiotic recombination and *de novo* mutation contribute towards gamete genome diversity, which in turn is responsible for the distribution of particular genotypes and traits within a population of organisms. Understanding - and manipulating - the regulation of these processes during meiosis could be a valuable means to increase recombination and therefore the distribution of traits within a population. Recent developments in single-cell genomics offer a opportunity to investigate this system and to analyse meiotic recombination in crop species.

Here, we demonstrate proof-of-principle that fluorescence activated cell sorting (FACS) based isolation of individual post-meiotic pollen cells (microspores), coupled with whole genome amplification (WGA) and next-generation sequencing (NGS) can enable the genomes of individual microspores to be sequenced.

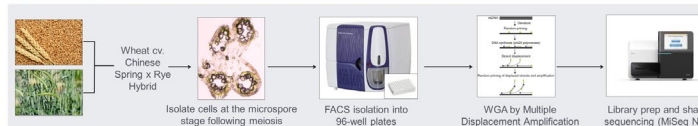


Pollen cells in suspension stained with DAPI. The larger, dimmer cells are microspores, while the smaller, brighter cells are likely to be tapetal cells.



FACS analysis of microspore suspensions on the BD FACSMelody. The microspore population is easily distinguished by scatter properties and DNA content (DyCyt Green).

Experimental design

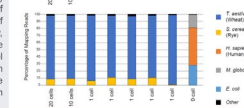


Results

Chromosome detection in single and low numbers of microspores. Even with extremely shallow sequencing, almost all wheat chromosomes were detectable in single microspores. Due to the genetic background and cross, aberrant chromosomal copy number may be expected in individual cells. Coverage $> 5 \times 10^4 \times$ is indicated by dark blue colouring on the heatmap; the superimposed numbers show the actual number of mapping reads per chromosome arm.



Detectability of wheat and rye chromosomes in individual pollen grains. Mapping of the sequencing data to multiple species using MEGAN identified that in both multi-cell controls and single cell samples, approximately 90% of reads mapped to wheat and 10% mapped to rye, indicating that both genomes are detectable in most of the microspores. Chromosome counting of the rye mapping reads is currently underway, although the lower quality of the rye genome prohibits straightforward chromosome level mapping. Some contamination (predominantly human and E. coli) can be observed in the zero cell sample, but not in the microspore samples.



Future Directions

With this proof-of-principle experiment, we have demonstrated the feasibility of single pollen analysis in wheat. We will now expand on this analysis in the following ways:

- Processing larger numbers of cells to assess the robustness of the method when performed at high-throughput, and validation of tools for copy number analysis.
- Analysing cells from crosses with high quality reference genomes, coupled with deep sequencing, which will enable the analysis of meiotic recombination in single cells at high resolution.
- Optimisation of methods to work with samples from other species.
- Using single-cell multi-omics approaches, such as G&T-seq, to explore the transcriptome of individual pollen cells and microspores in parallel with the analysis of their recombined genomes.

References

Macaulay et al. Separation and parallel sequencing of the genomes and transcriptomes of single cells using G&T-seq. Nature Protocols (2016)

@E1_single_cell @AshleighSLister @whatchamacaulay



Earlham Institute, Norwich Research Park, Norwich, Norfolk, NR4 7UZ, UK
www.earlham.ac.uk



Hybrid pollen before sorting 22-01-18

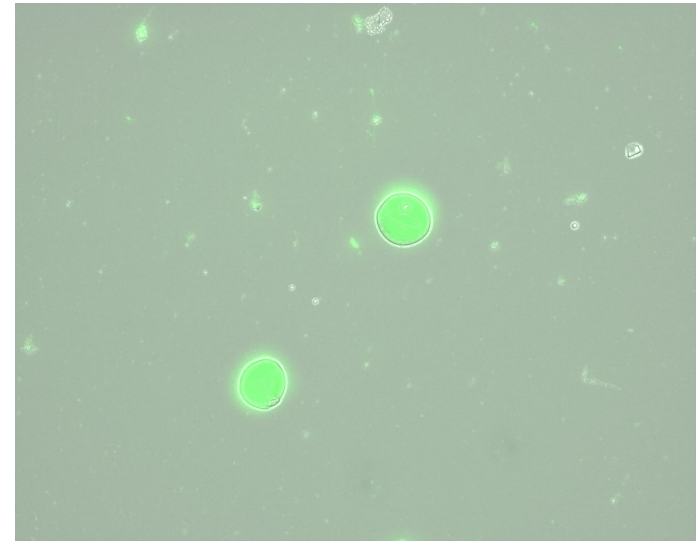
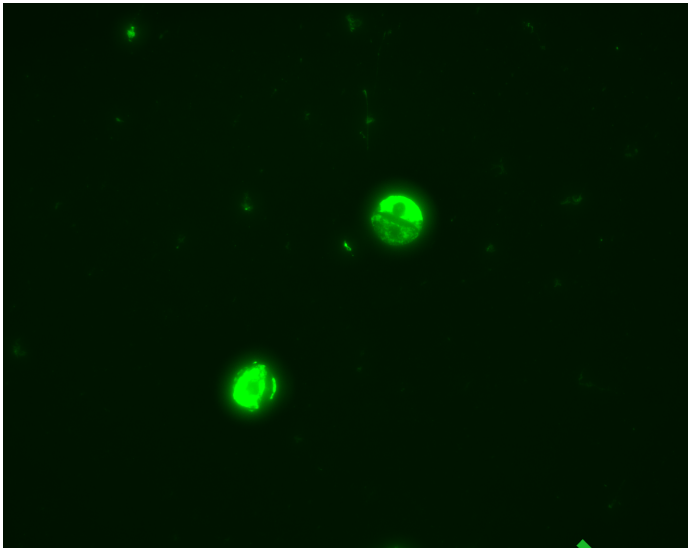
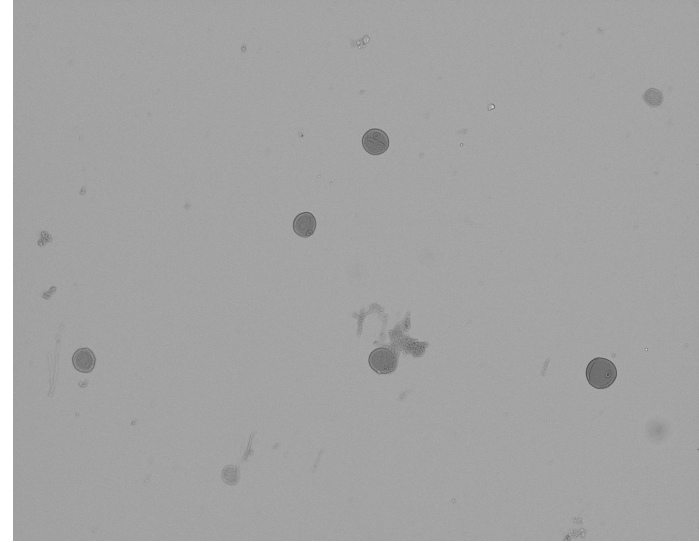
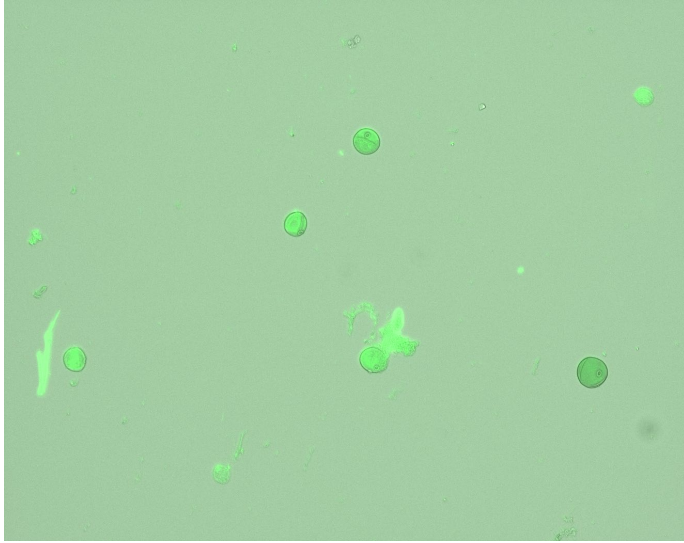
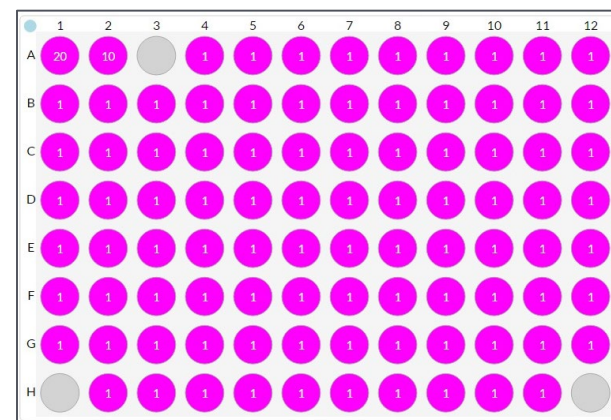
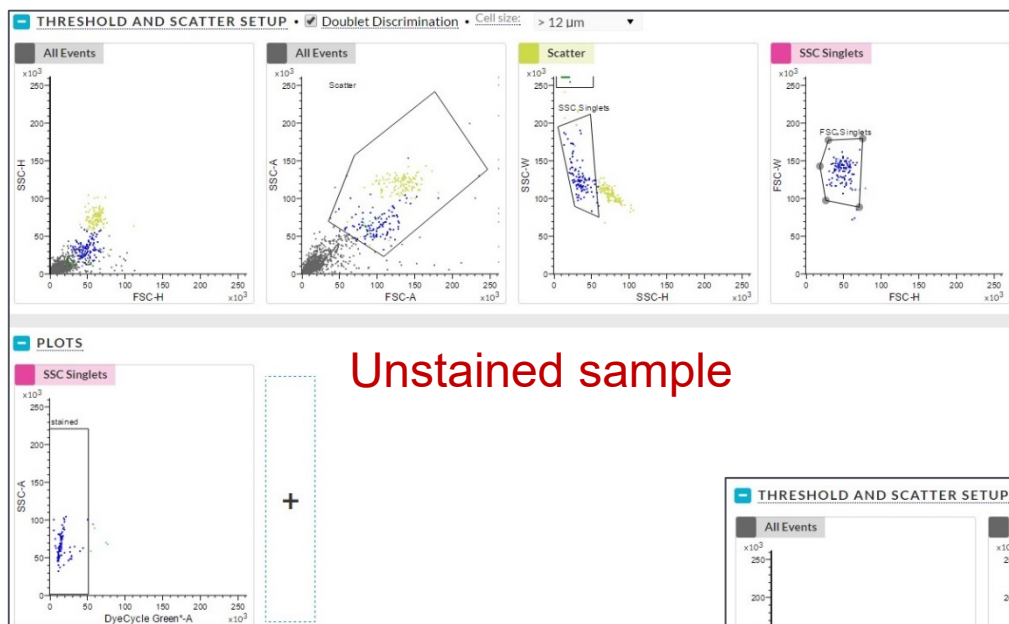


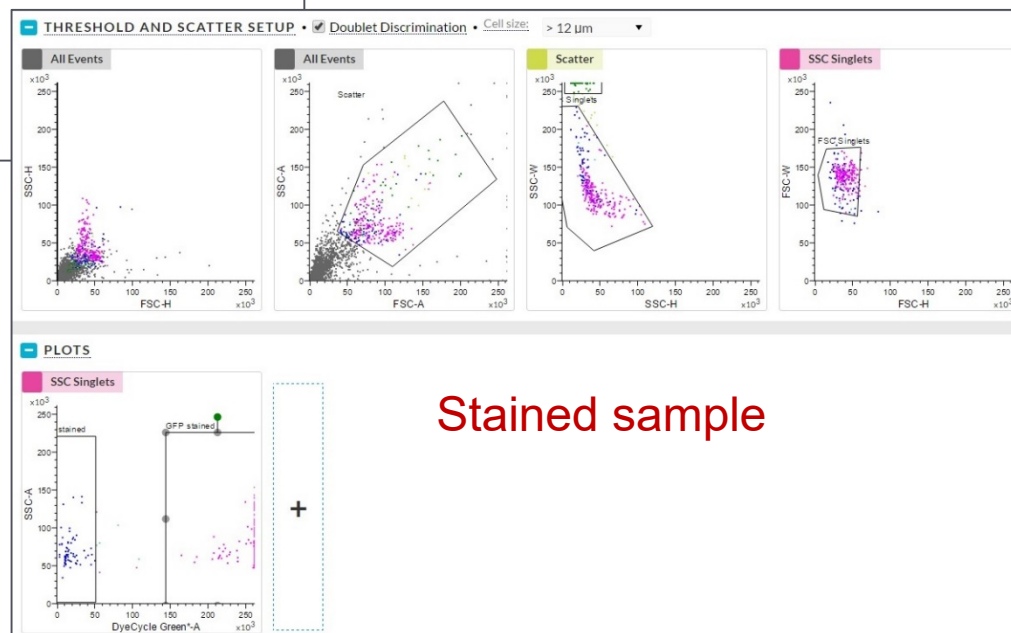
Plate plan



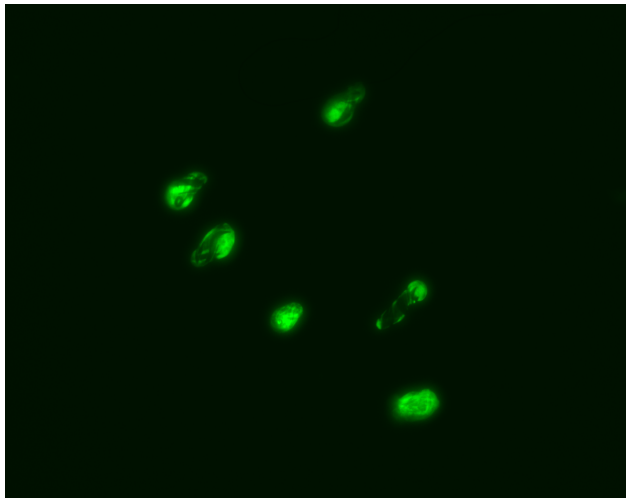
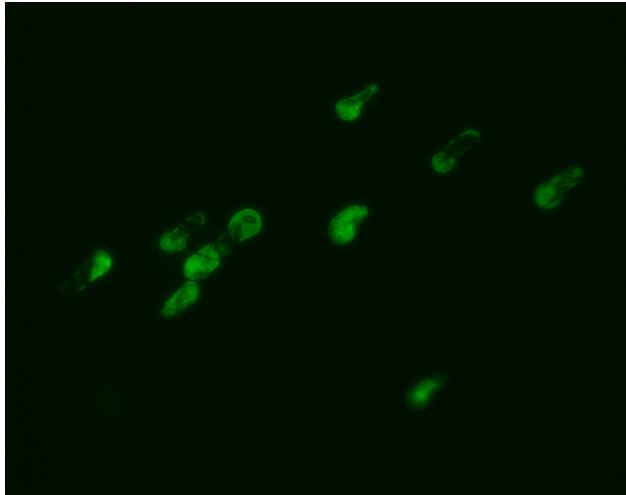
3 x full plates sorted into RLT read for G&T
Seq

1 x full plate sorted into PBS for REPLI-g

All samples are in -80 freezer drawer 5:5, labelled on the front.



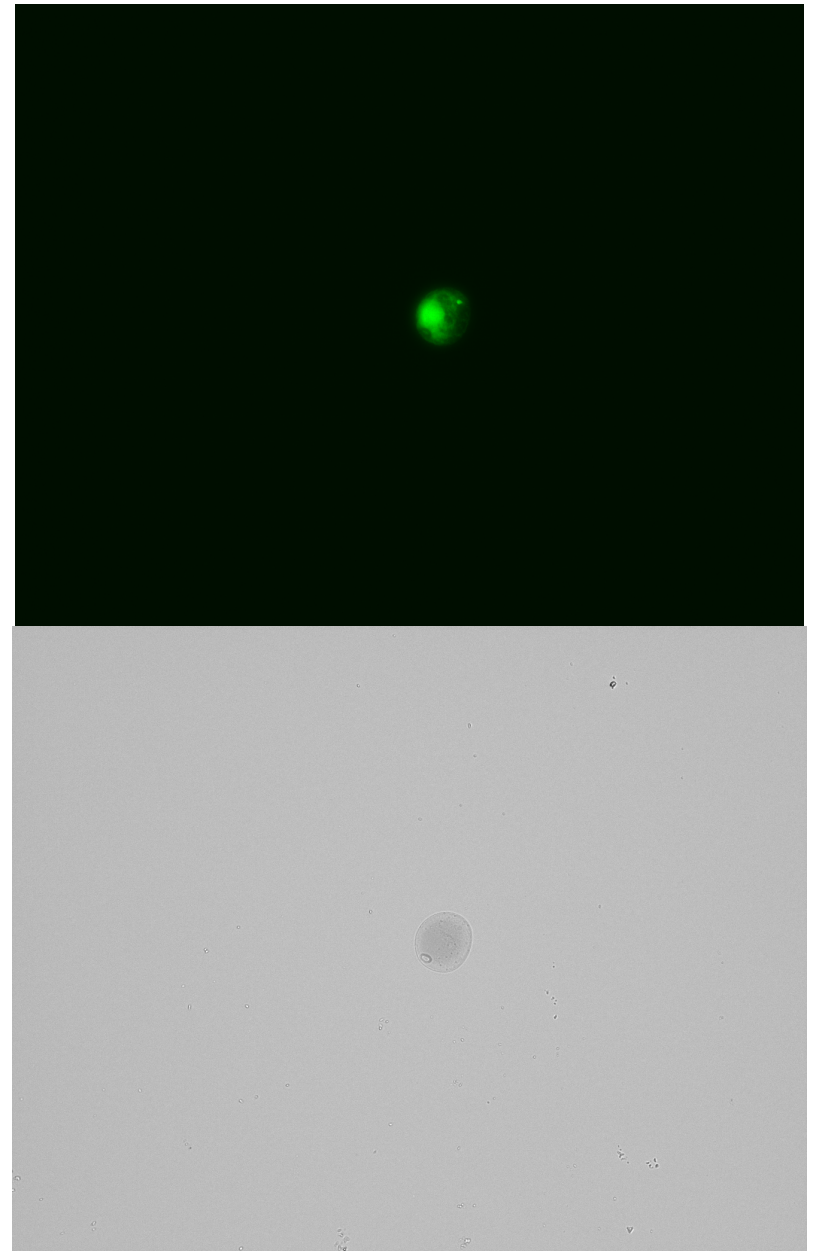
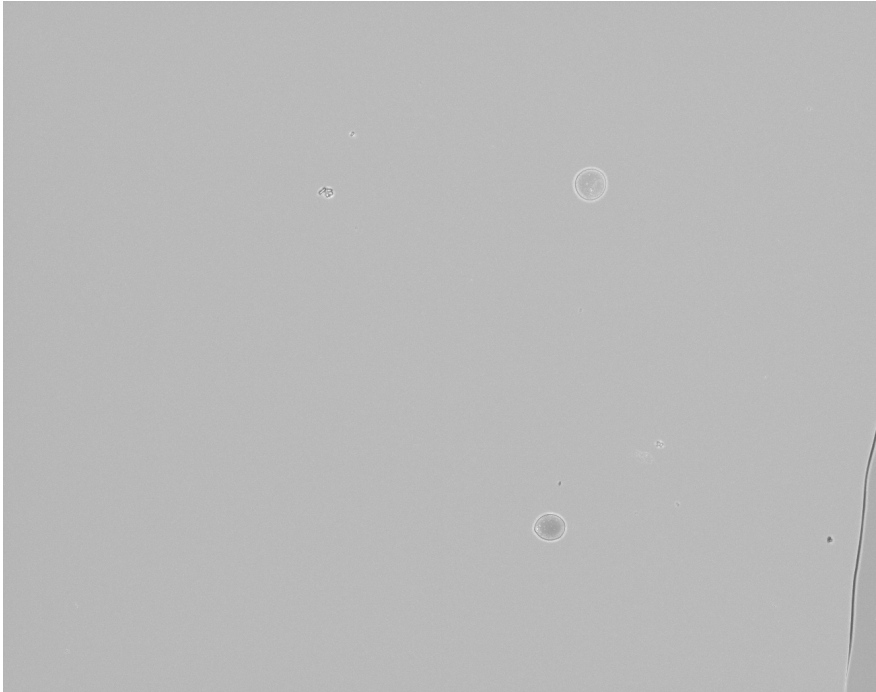
Sorted hybrid pollen 22-01-18



Strange
'corkscrew'
seen in
most of the
sorted
pollen?



Pre-sort hybrid pollen



Sorted hybrid pollen 23-01-18

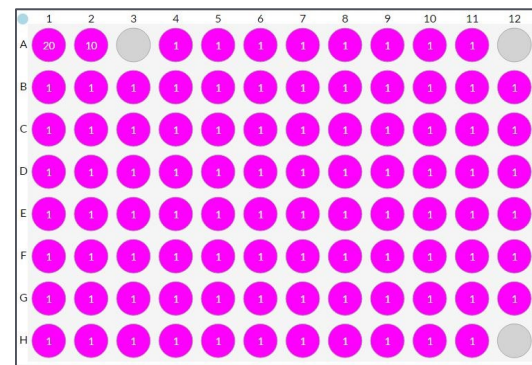
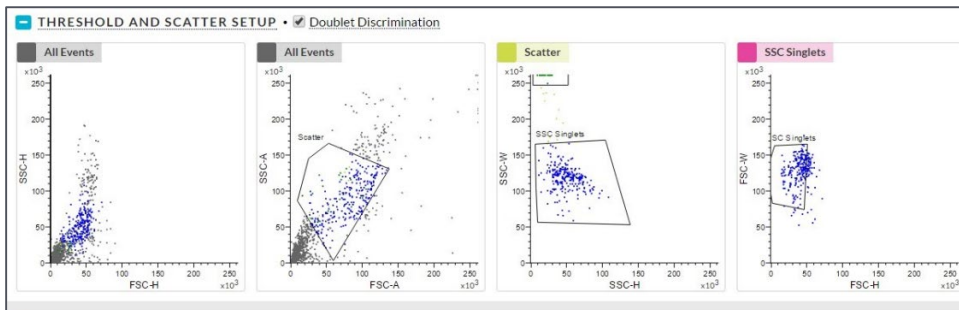
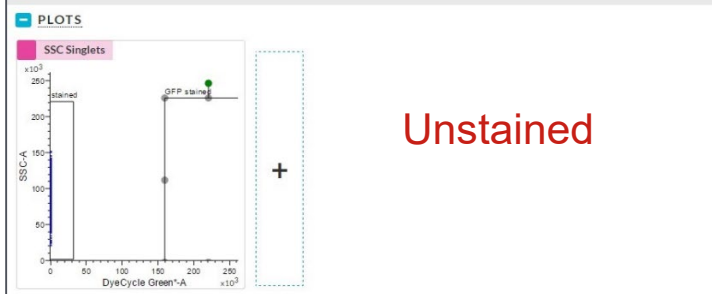
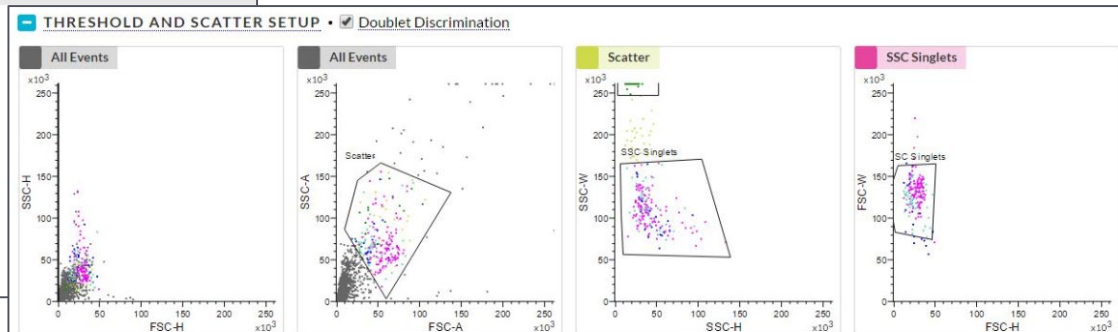


Plate plan



Unstained



Stained

3 x plates of pollen sorted into RLT ready for G&Tseq

1 x plate sorted into PBS ready for REPLI-g DNA Seq

All samples are in -80 freezer drawer 5:5, labelled on the front.

Post-sort hybrid pollen 23-01-18

