Wheat pollen single cell sequencing

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OPENPLANT FUNDED PROJECT
EARLHAM INSTITUTE AND JOHN INNES CENTRE COLLABORATION

John Innes Centre
Unlocking Nature's Diversity

Earlham Institute
Decoding Living Systems

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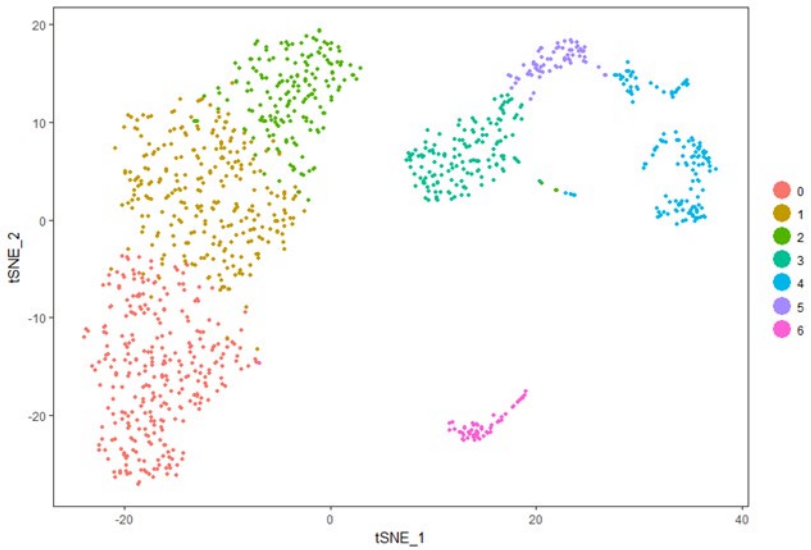
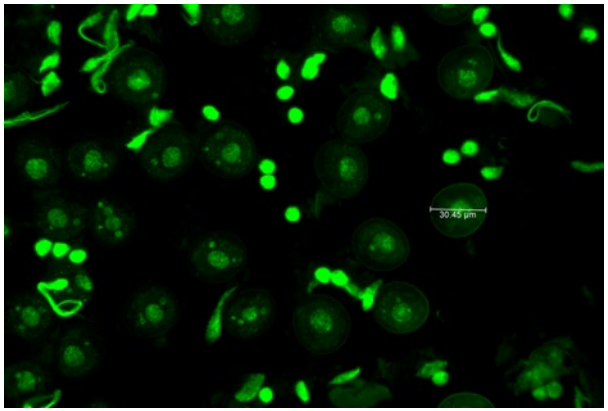
Single cell sequencing





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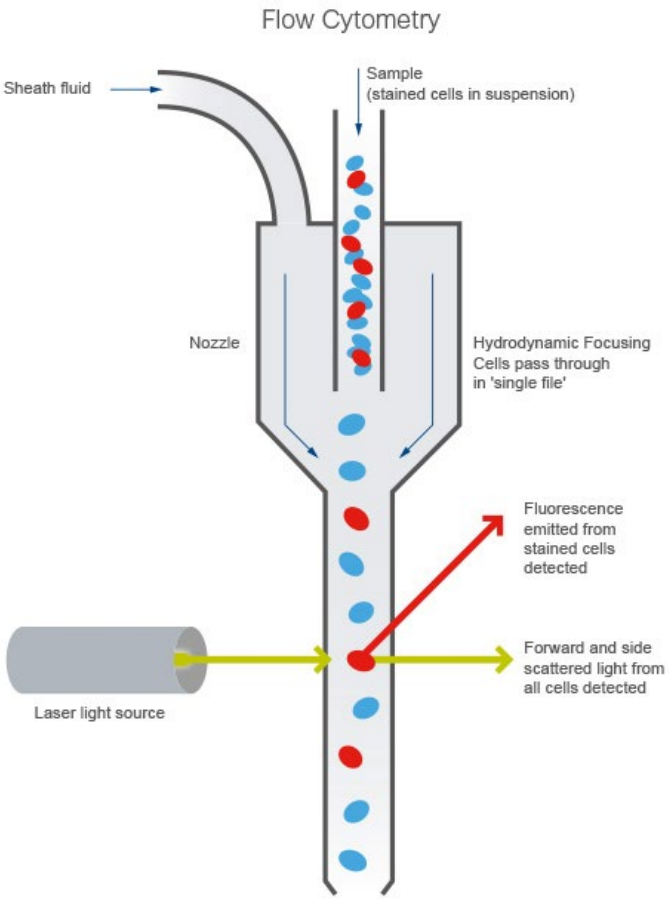
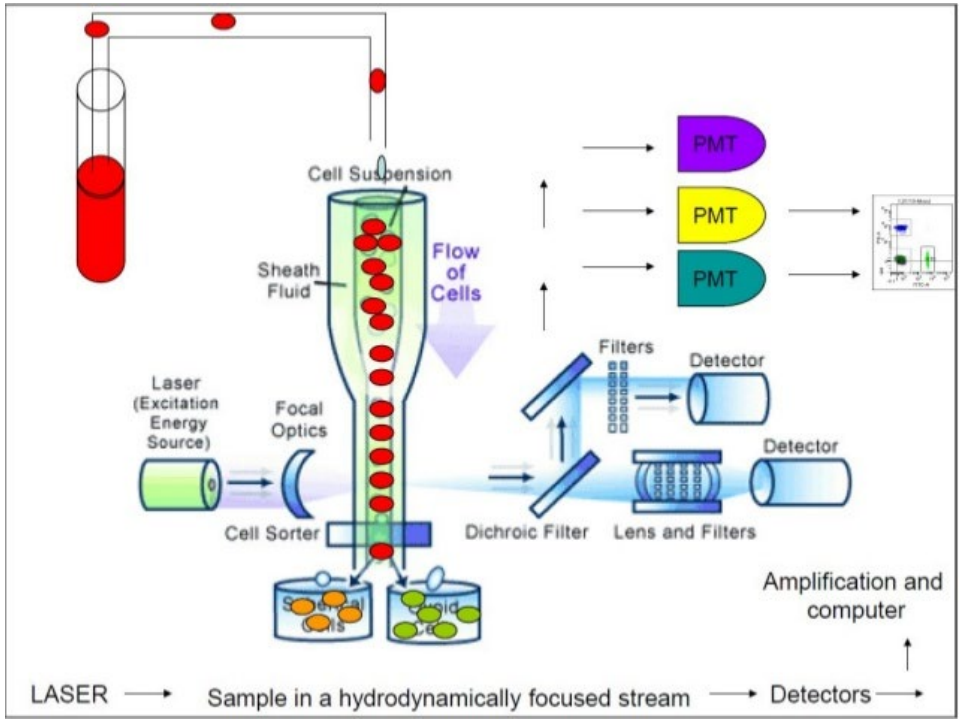
How FACS works?





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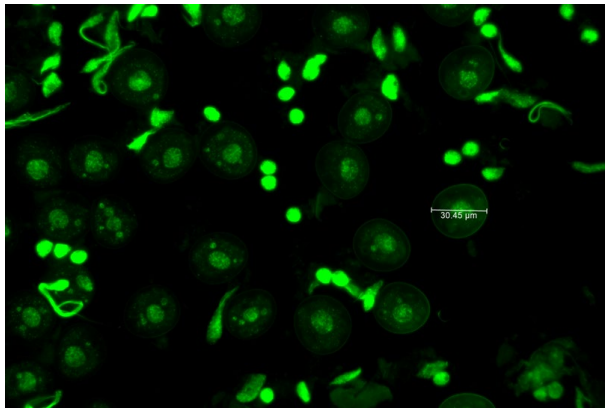
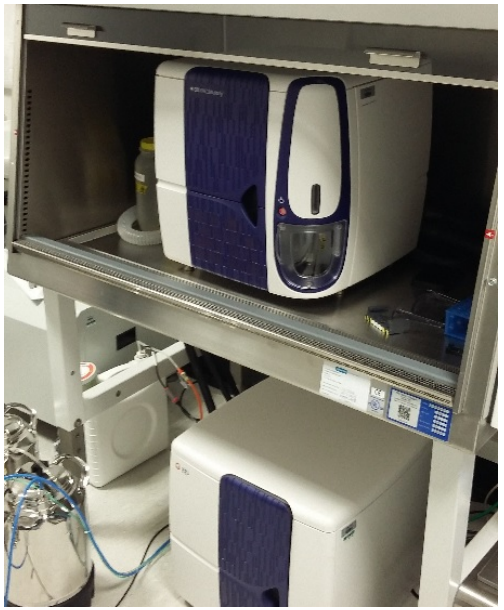
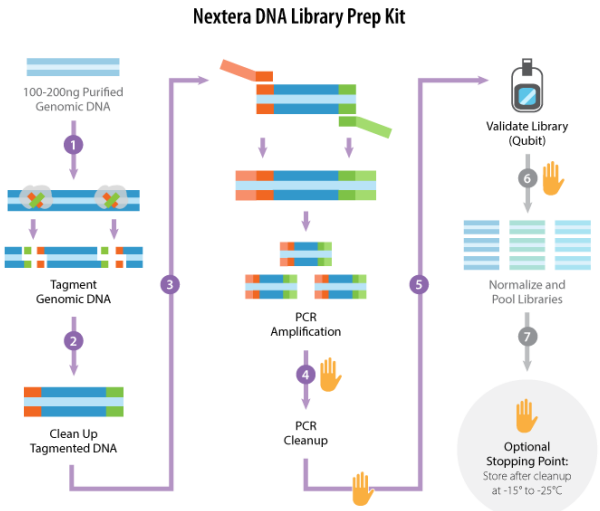
Proposal







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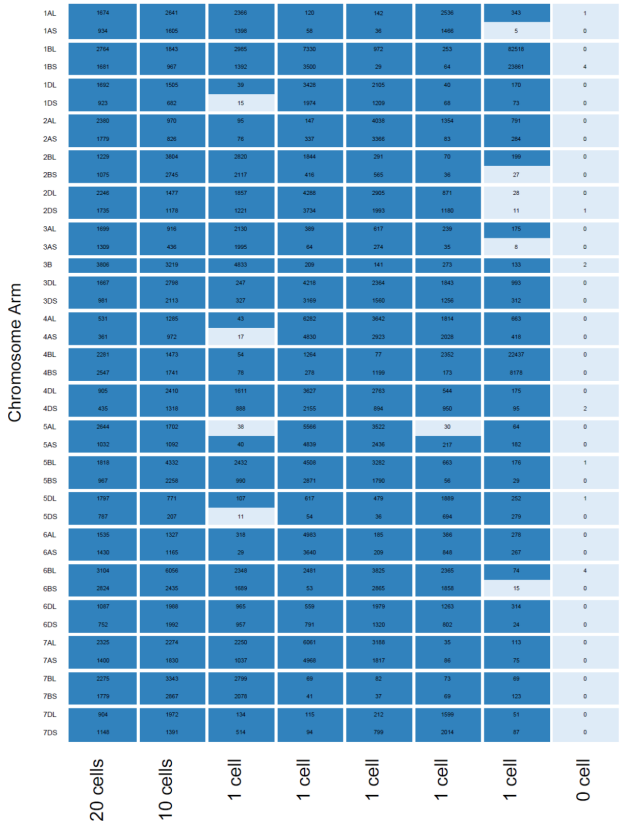
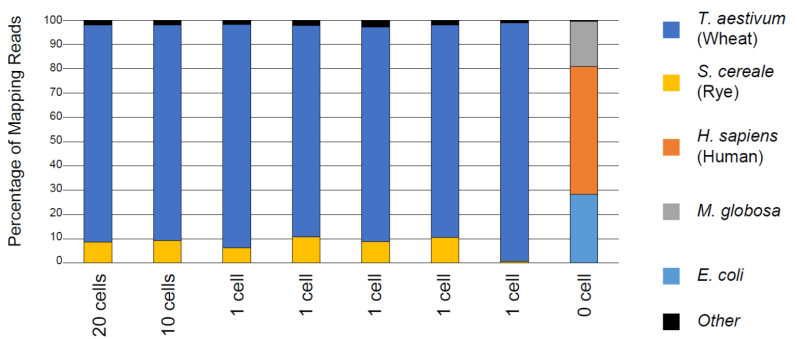
Preliminary analysis

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.





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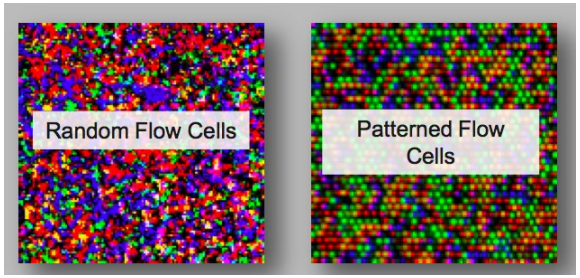
Project scales and schedules

- 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 ~X0.01



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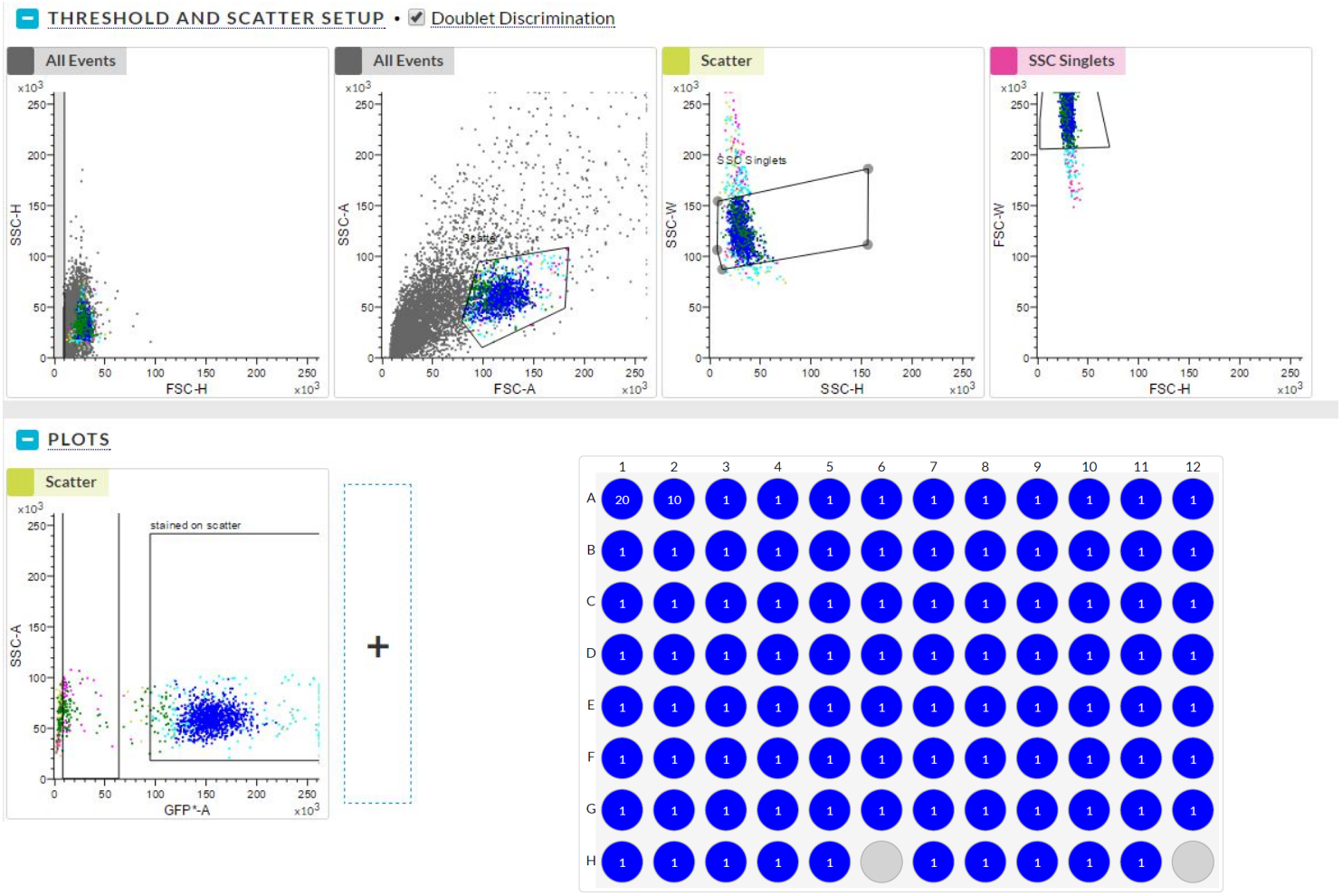
FACS cell sorting- Chinese Spring-uninucleate pollen- 27/09/17



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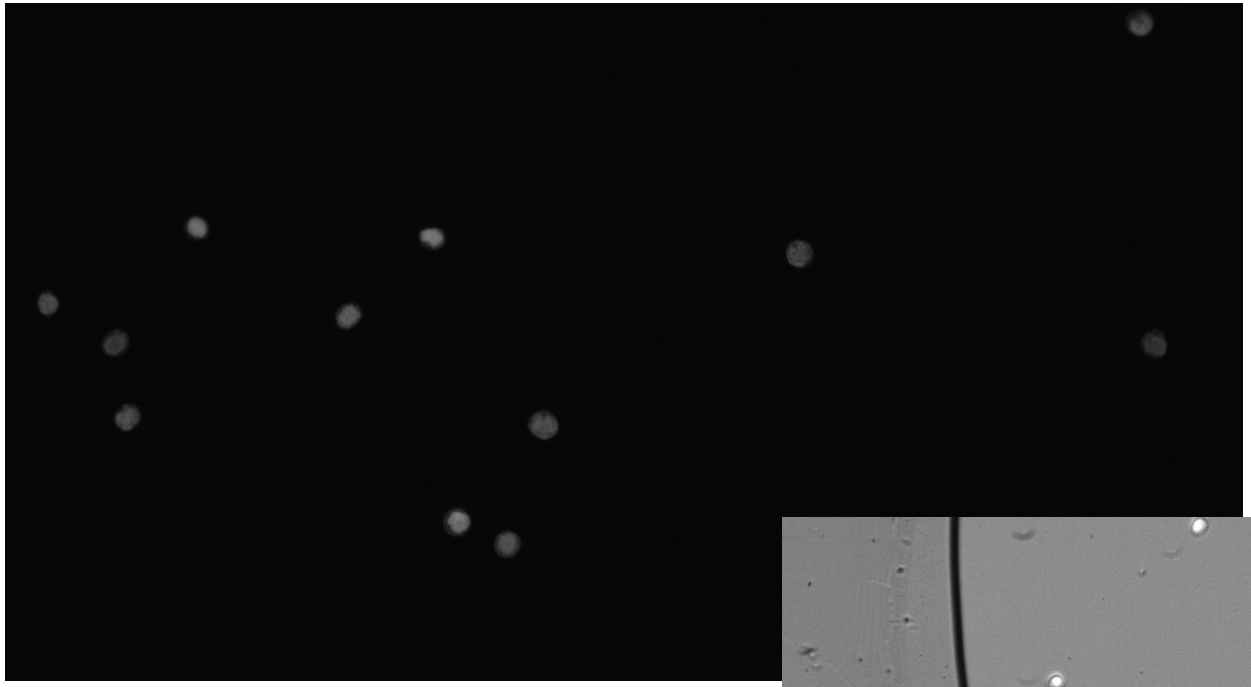
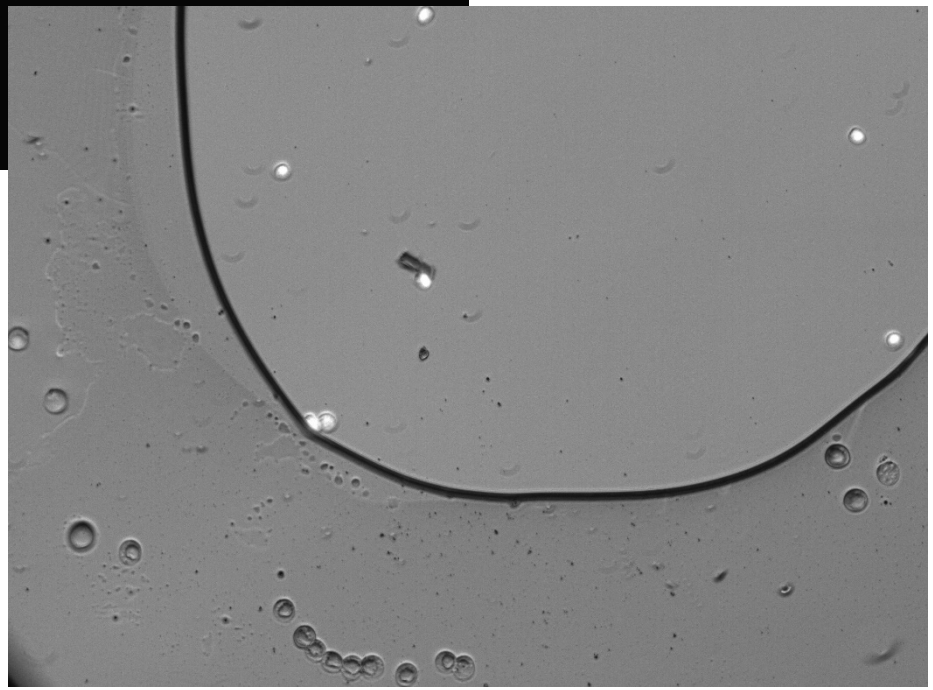
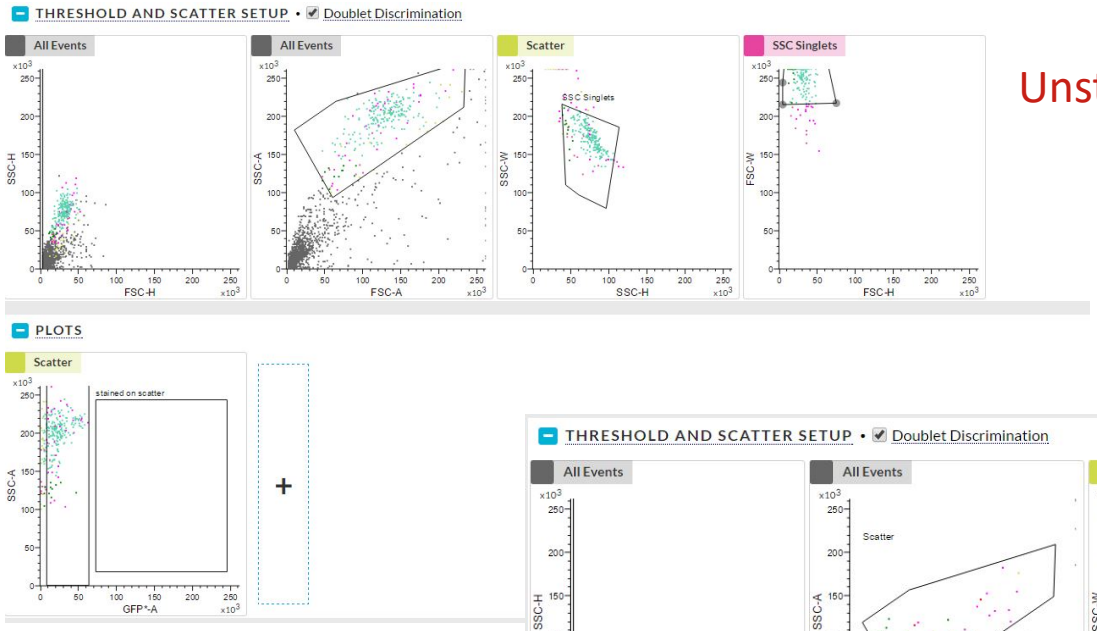


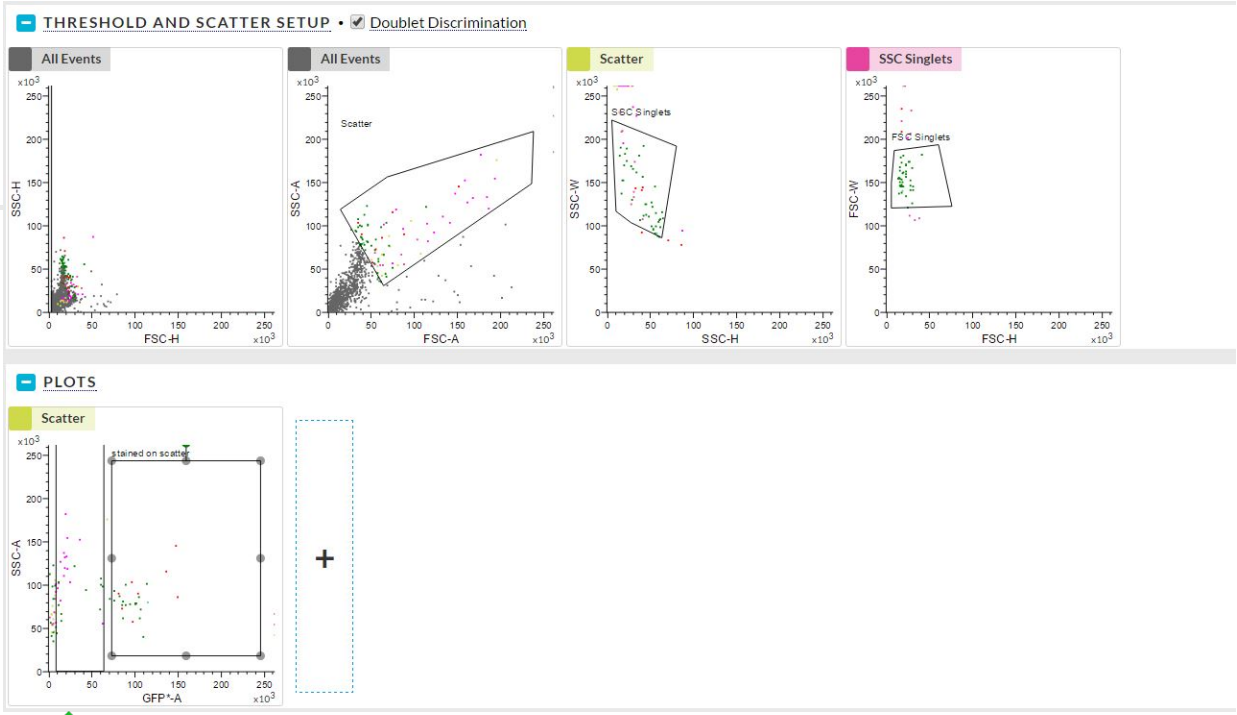


Uninucleate CS pollen(27/09/17) sorted onto microspore slides for verification of selected FACS population

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



Unstained pollen



Stained pollen

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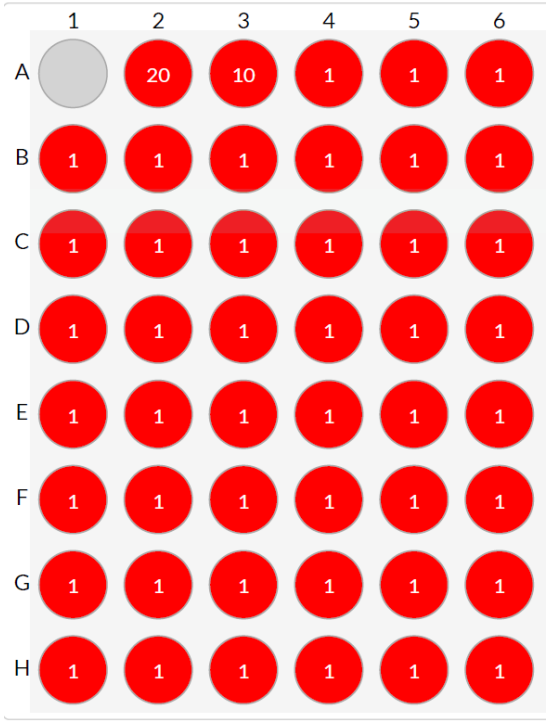
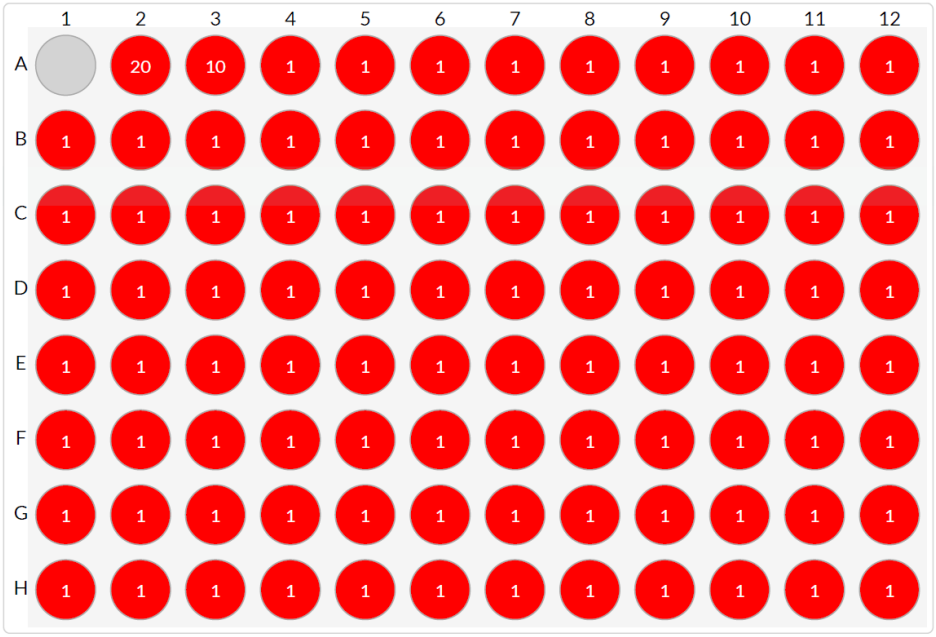
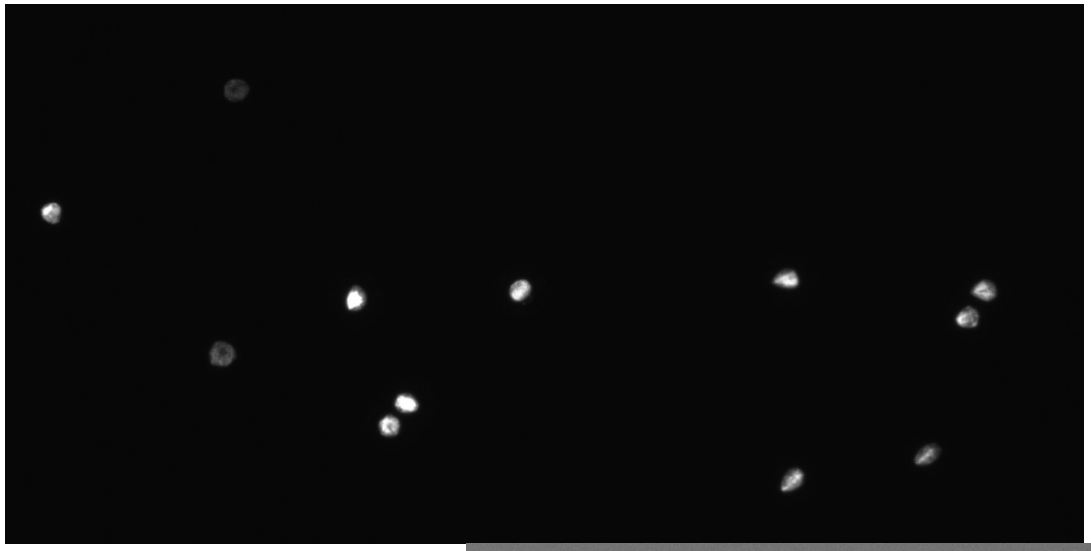
Cadenza Plate layouts 2/10/17

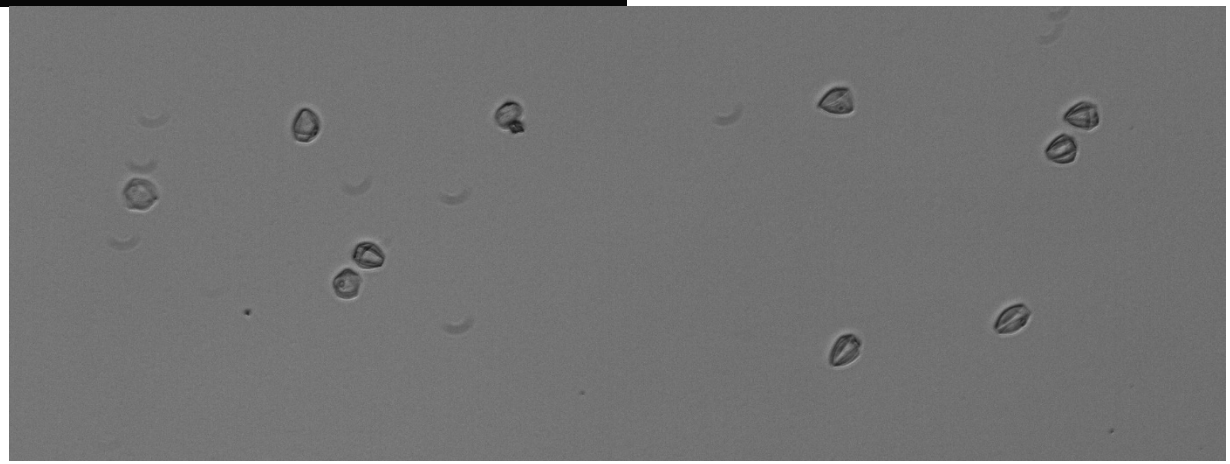


Pollen sort into 2ul PBS read for MDA



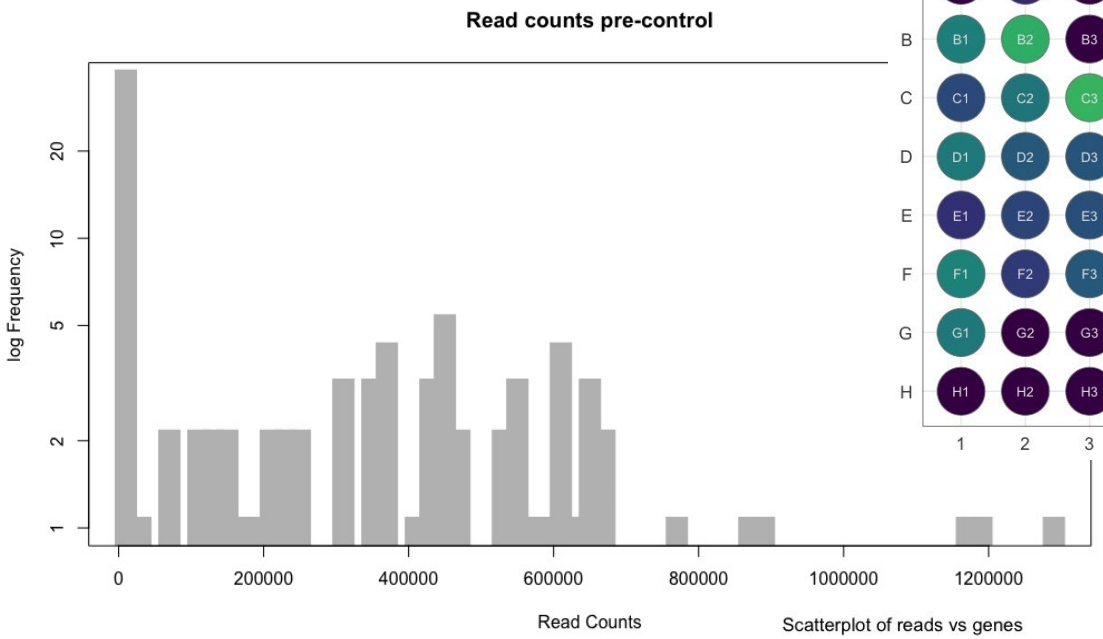
Pollen sort into 2.4ul RLT ready for G&Tseq, would like to have sorted x2 plates but the sorted errored (USB fault)

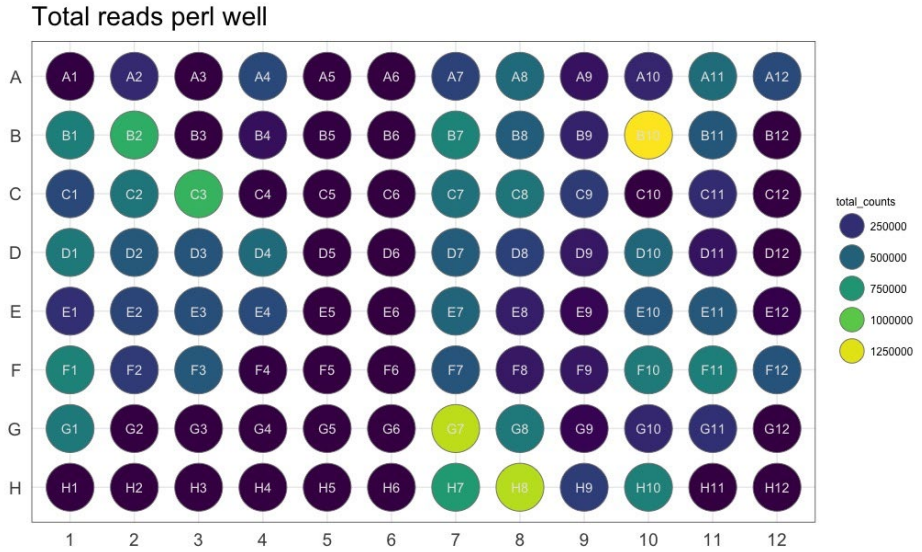


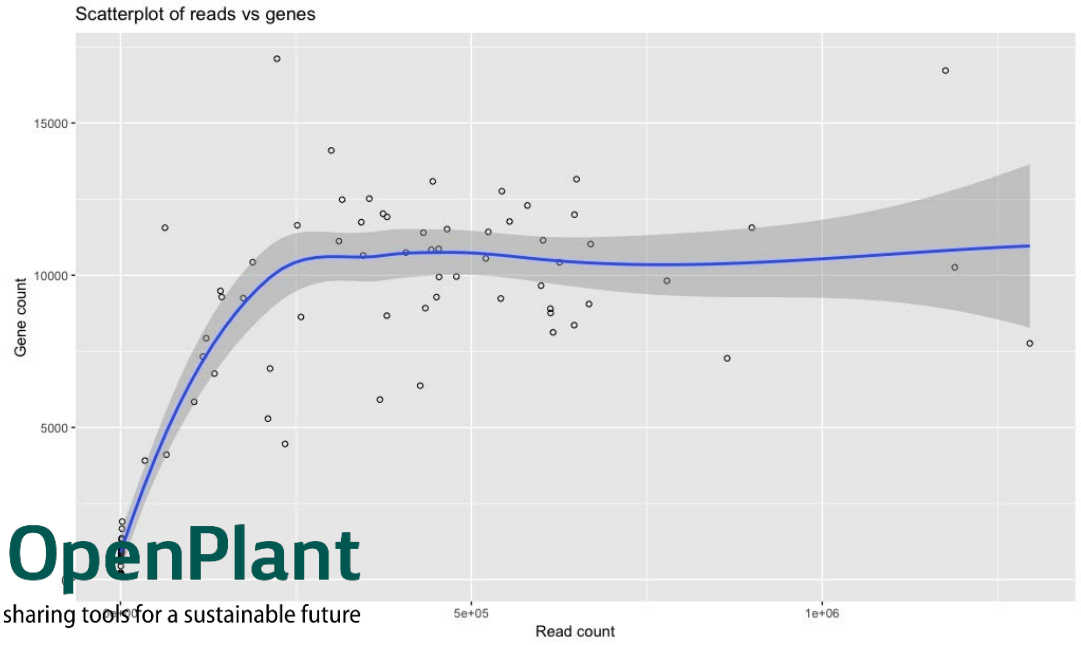


Uninucleate Cadenza pollen (2/10/17) sorted onto microspore slides for verification of selected FACS population

Transcriptome analysis







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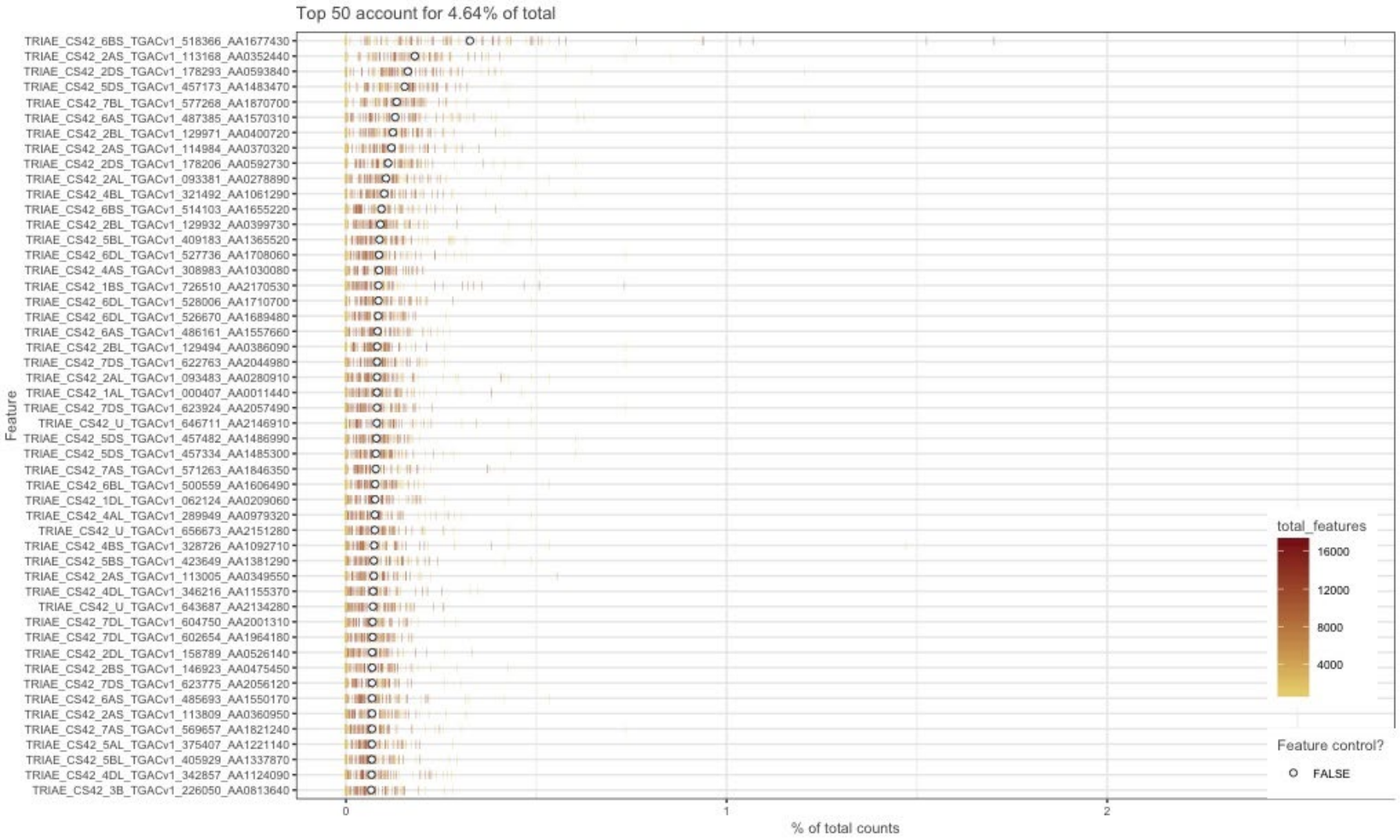
Transcriptome analysis continued…



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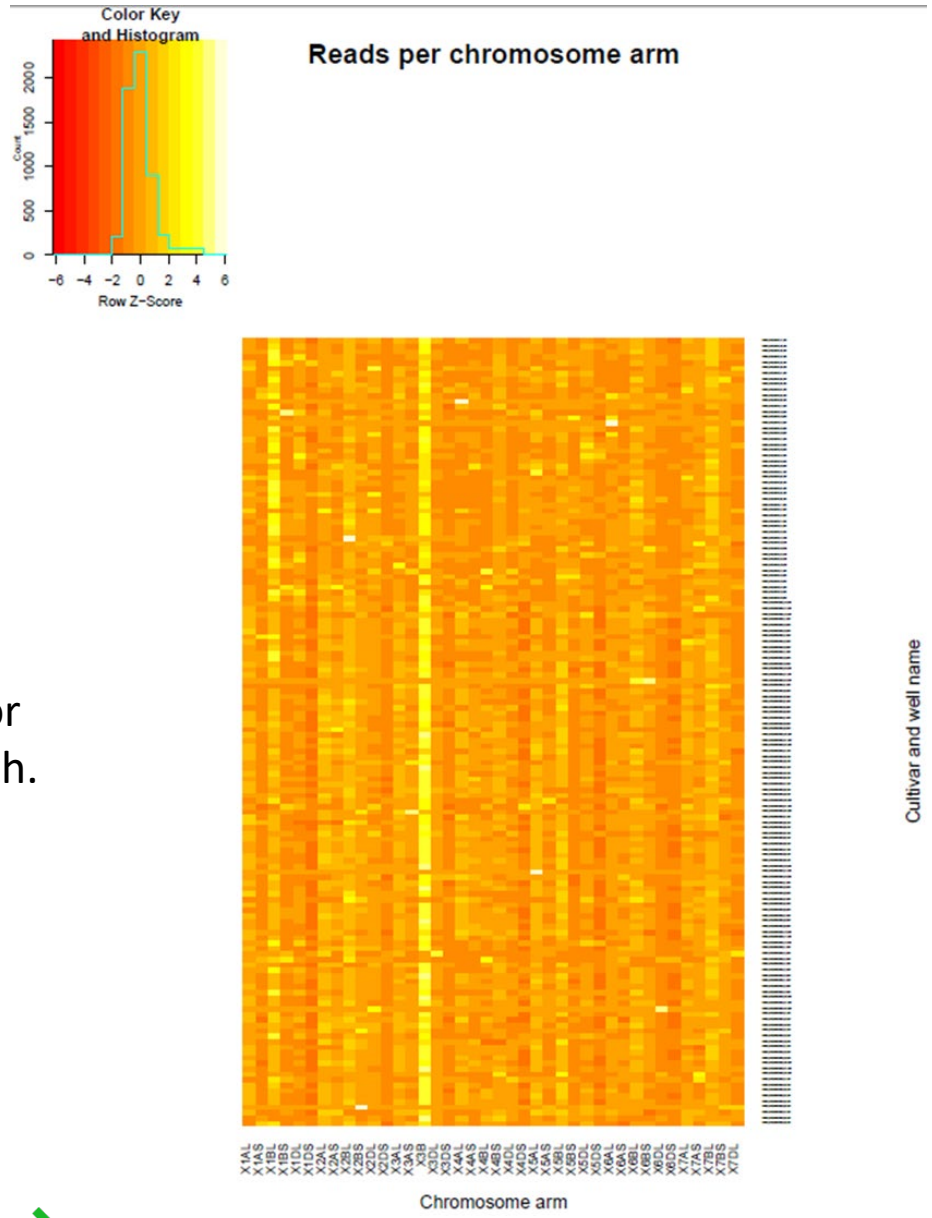
Genome analysis



Not normalised for chromosome length.

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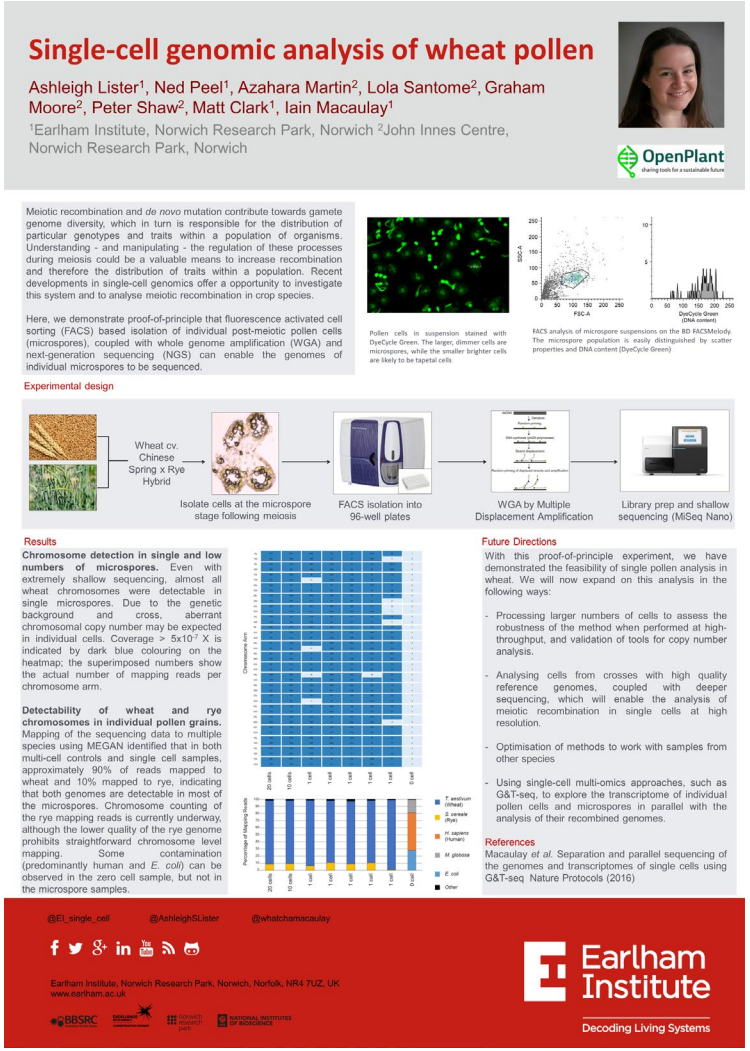
Findings based on transcriptome analysis of single cell pollen

- 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



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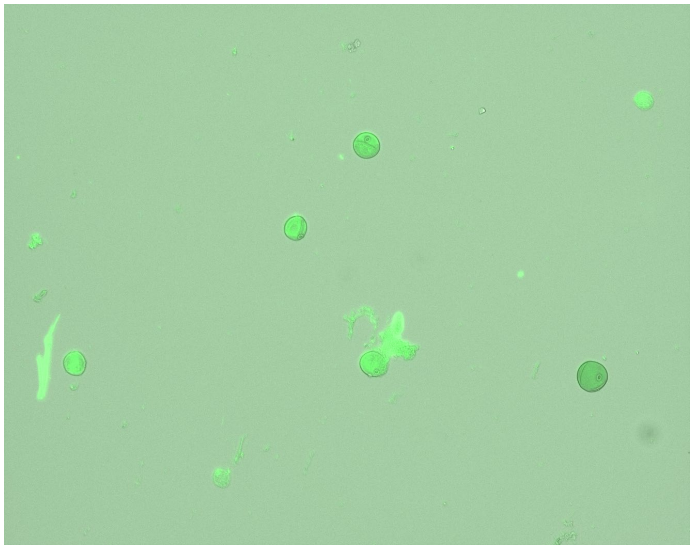
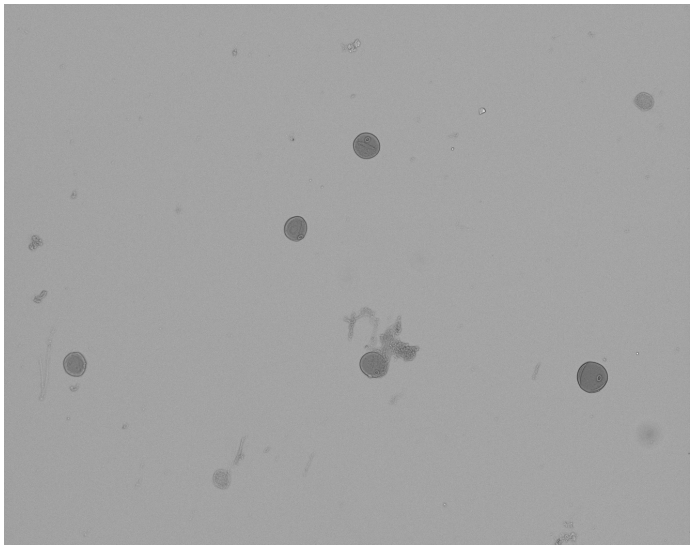
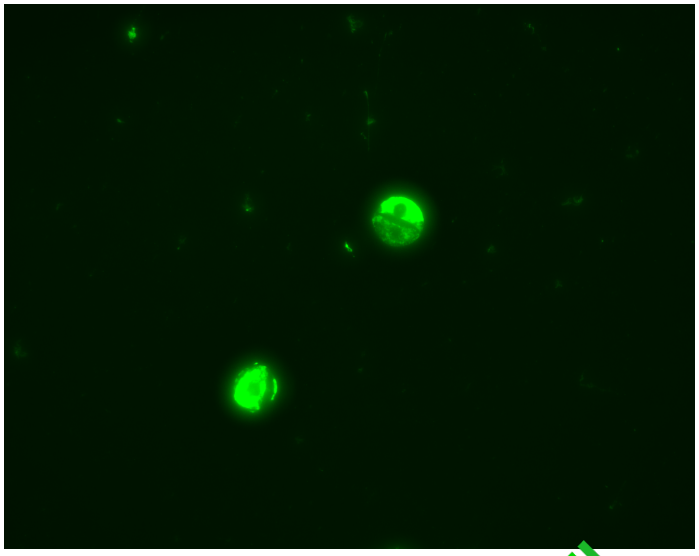
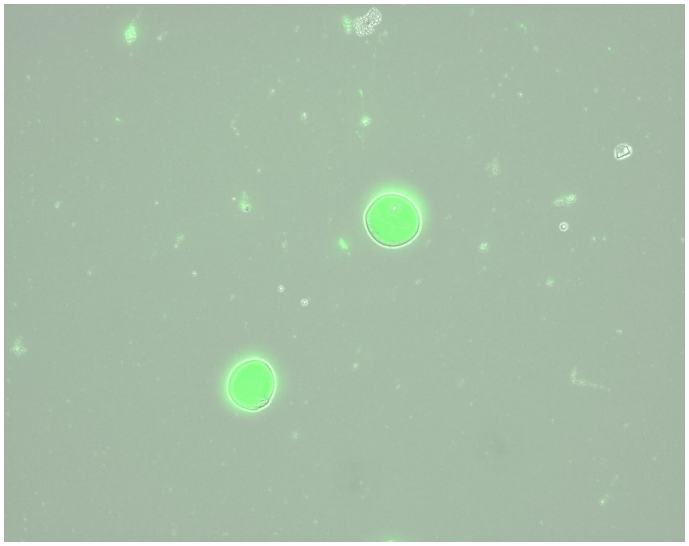
Hybrid pollen before sorting 22-01-18



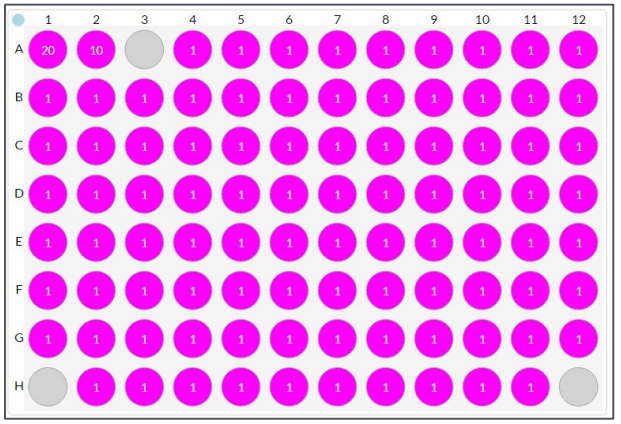


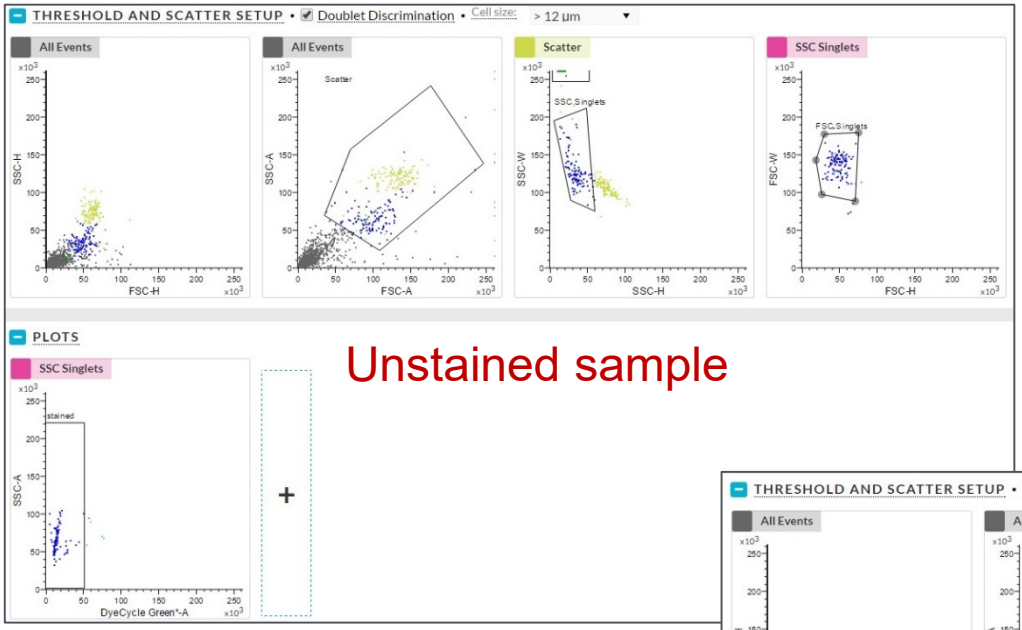




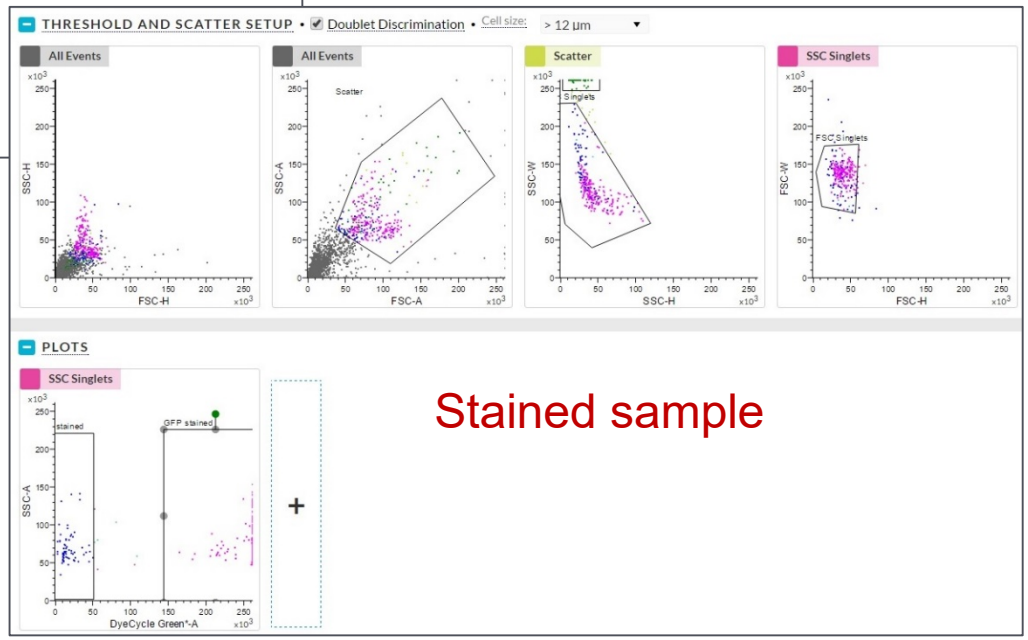
Hybrid CS x C pollen 22-01-18

Plate plan





Unstained sample



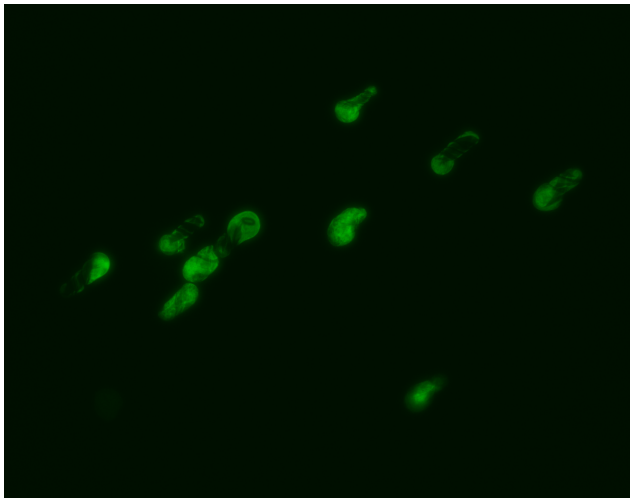
Stained sample

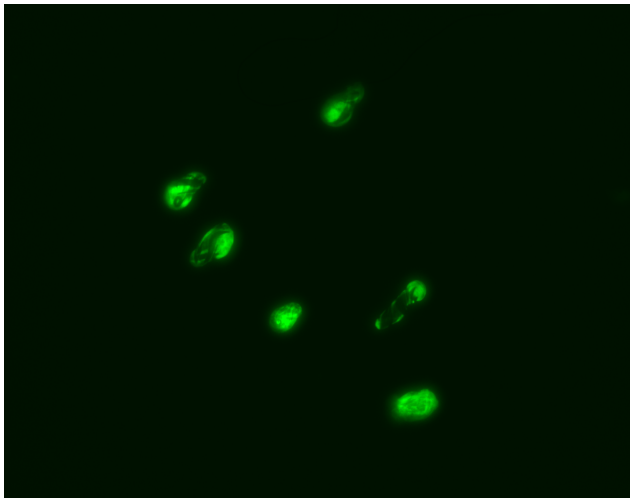
3 x full plates sorted into RLT read for G&T Seq

1 x full plate sorted into PBS for REPLI-g

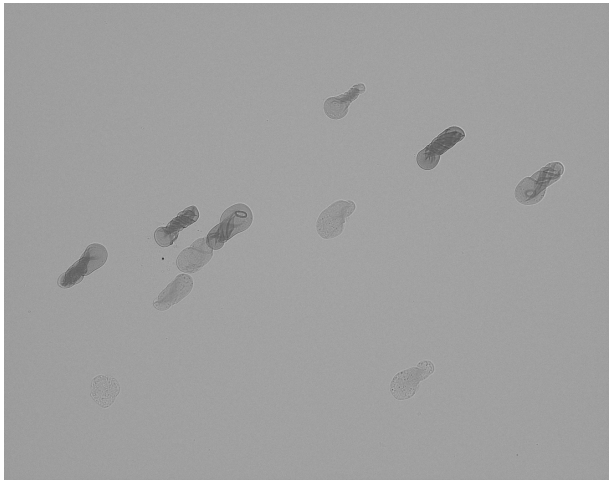
All samples are in -80 freezer drawer 5:5, labelled on the front.

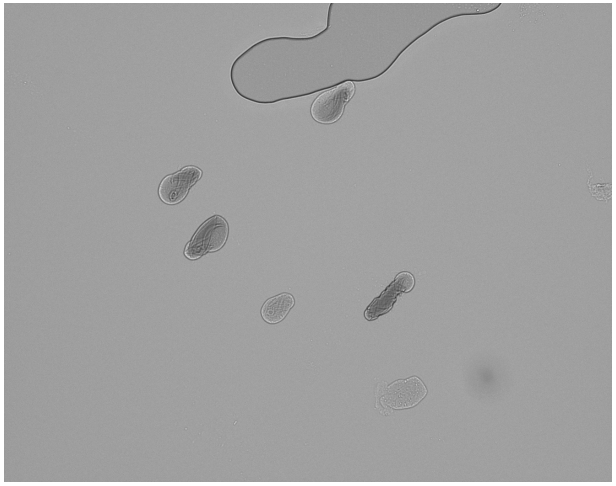
Sorted hybrid pollen 22-01-18



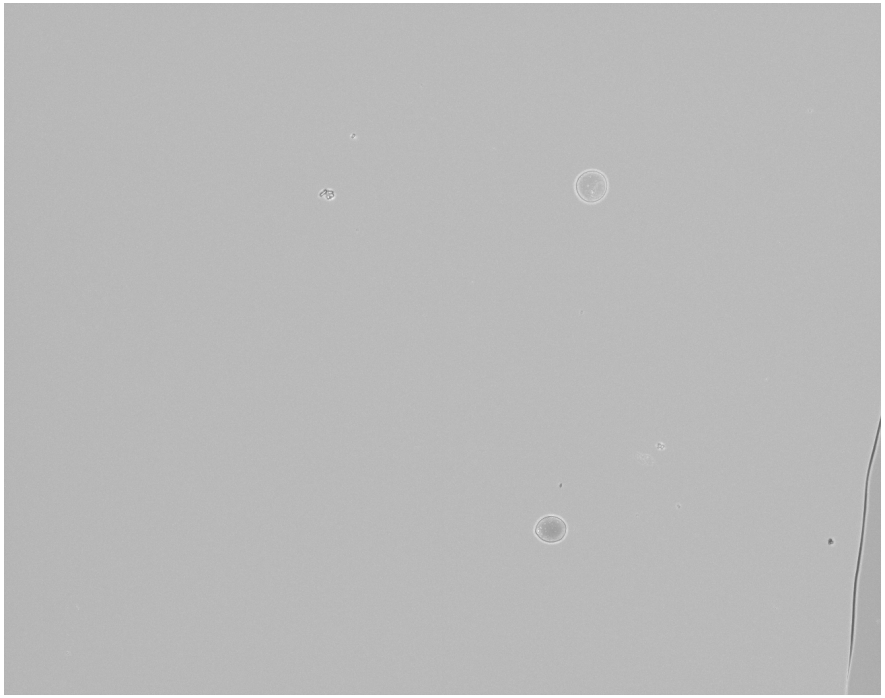


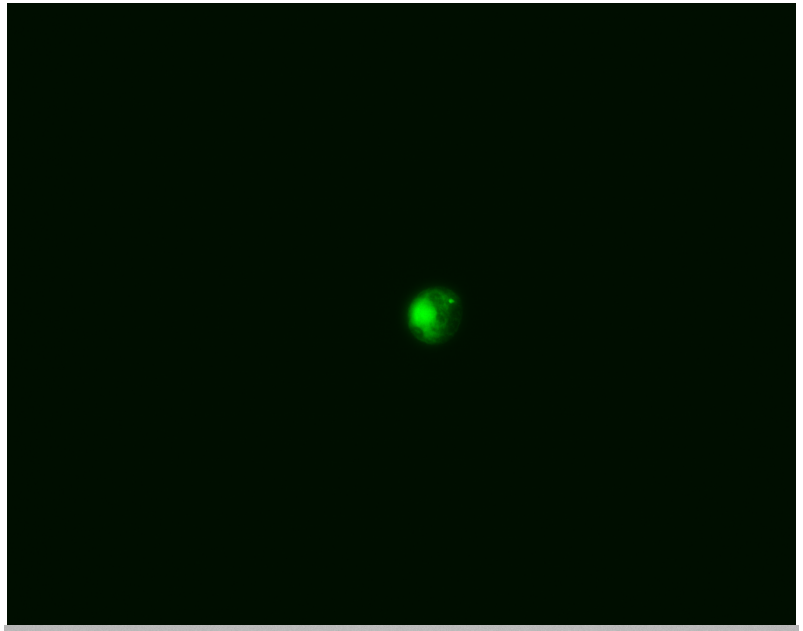
Strange ‘corkscrew’ seen in most of the sorted pollen?

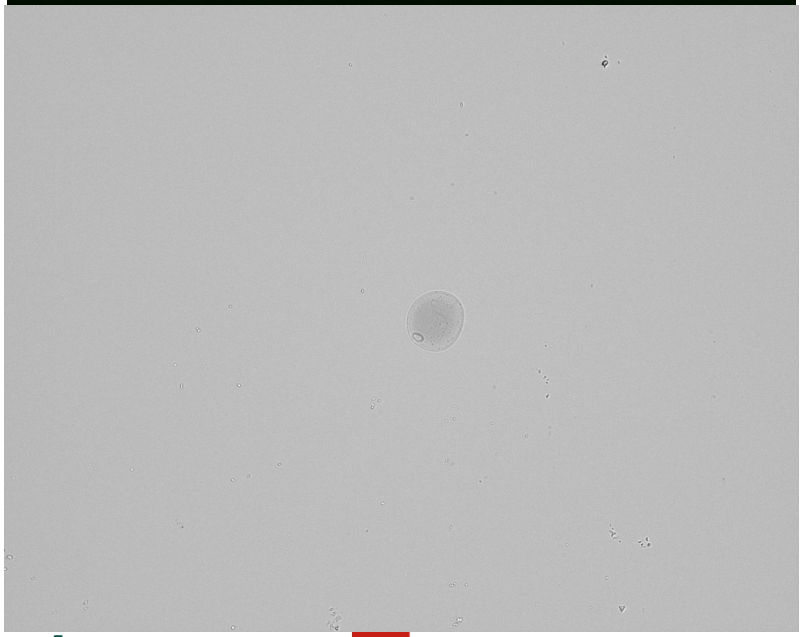




Pre-sort hybrid pollen







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Sorted hybrid pollen 23-01-18

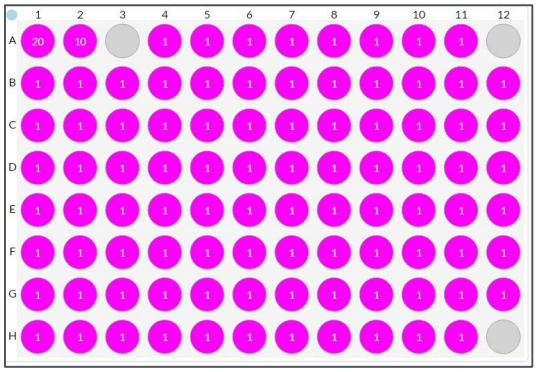
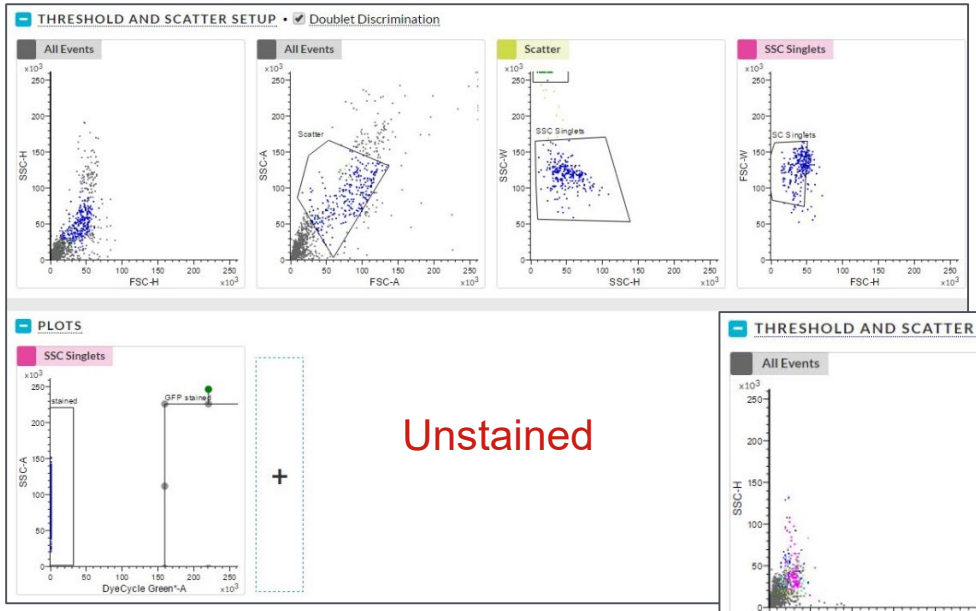


Plate plan



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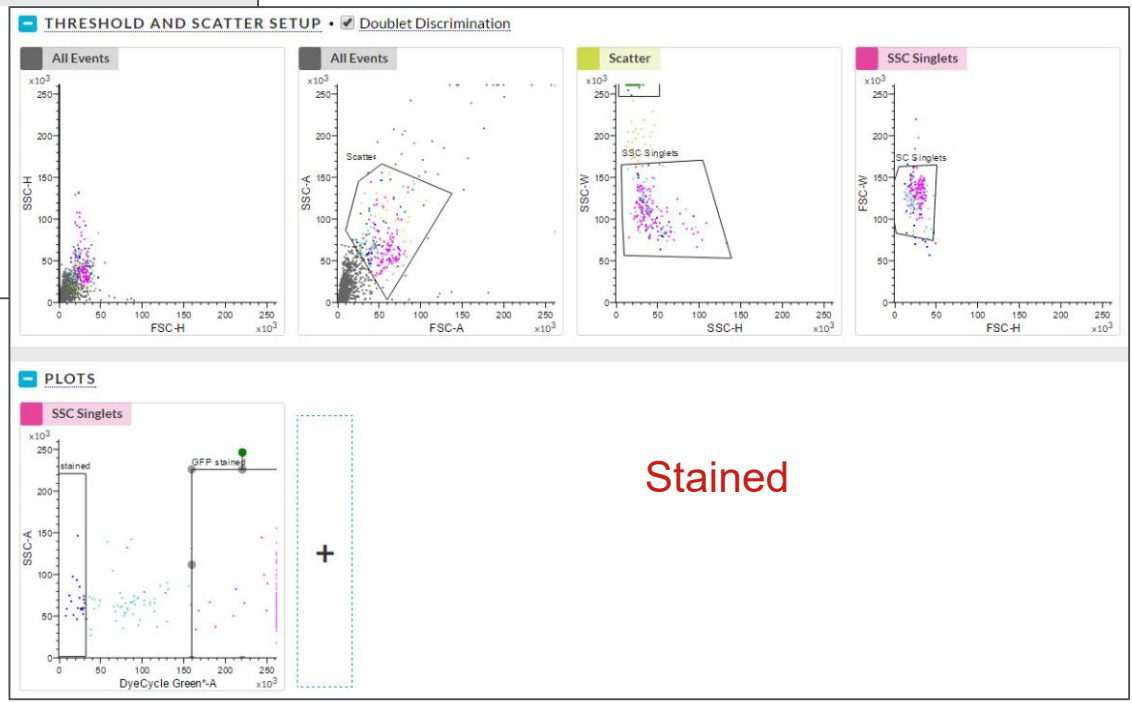

Stained

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.

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Post-sort hybrid pollen 23-01-18