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?
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 ~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

Unstained pollen

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

![Graphs and charts related to transcriptome analysis]
Transcriptome analysis continued…
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
Hybrid pollen before sorting 22-01-18
Hybrid CS x C pollen 22-01-18

Unstained sample

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

Stained sample

1 x full plate sorted into PBS for REPLI-g

All samples are in -80 freezer drawer 5:5, labelled on the front.
Strange ‘corkscrew’ seen in most of the sorted pollen?
Pre-sort hybrid pollen
Sorted hybrid pollen 23-01-18

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.

www.earlham.ac.uk
Post-sort hybrid pollen 23-01-18