unPAK: Phenotyping single gene knockout mutants in *Arabidopsis thaliana*

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BACKGROUND:

- unPAK: undergraduates Phenotyping Arabidopsis Knockouts
- Most studies of *Arabidopsis thaliana* knockouts mutants find no phenotype in the majority of lines (e.g. Kuromori *et al.* 2006)
- Explanations include: genetic redundancy, assay environment / trait / replication levels (Lloyd and Meinke 2012)

Fitness-related traits including:

- germination success
- days to bolting
- rosette and inflorescence size
- fruit (silique) production

seedling

shortly after bolting

with maturing siliques

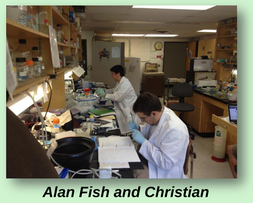
- We have developed a large scale undergraduate-centered workflow to both genotype and assay fitness-related phenotypes in many *A. thaliana* knockout lines (SALK insertion lines)
- Fitness measures for single gene knockouts in other organisms have been critical in examining problems of evolutionary genomics (e.g. in yeast: Hirsh and Fraser 2001)
- More information is available on our website:

FARMING:

- ✓ **Thus far:** More than **8000** plants grown at College of Charleston and Barnard College for development of seed stocks and leaf tissue for genotyping
  - ✓ Over 1000 Salk T-DNA lines
  - ✓ Over 100 phytometer lines
  - ✓ Goals: 3000+ Salk T-DNA lines

Farming in the new greenhouse at CofC

GENOTYPING: *a two pronged approach*

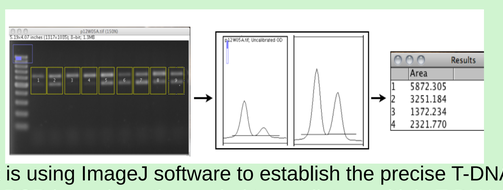


Alan Fish and Christian Lehman at HSC

CofC students helping with DNA extractions

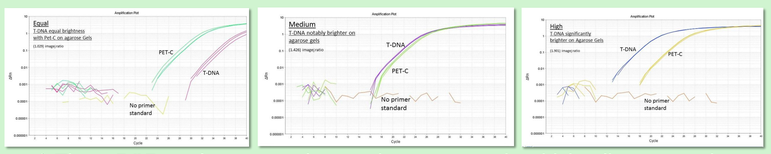
Hanna Vann at CofC

(1) DUAL-TARGET PCR:



✓ Current work is using ImageJ software to establish the precise T-DNA:PetC conventional PCR intensity ratio needed to predict a multiple T-DNA insert SALK line.

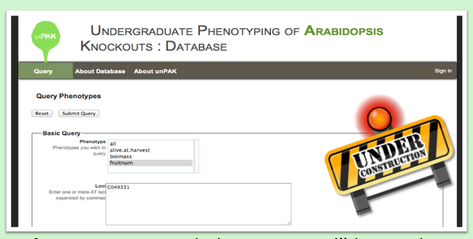
(2) Q-PCR



✓ The q-PCR analyses reveal a consistent pattern in which a line cannot be confirmed as a true multiple insert line unless there is a marked difference between the intensity of the bands on a conventional PCR gel.

Database:

Thomas Fussell CofC class of '12



Data and metadata for genotypes and phenotypes will be made available in a public database

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SALK T-DNA Lines
1000s of T-DNA insertion mutants at known loci (O'Malley and Ecker 2010)
Source: ABRC

Natural Accessions & Common Lab Lines
- From ABRC
- All in the 1001 genome project

Farming
Generate leaf tissue for genotyping and bulk seed

Genotyping
Screen SALK T-DNA lines for single vs. multiple inserts

Phenotyping
Building a populated database of fitness-related phenotypes for *all* available single insert SALK T-DNA lines grown with phytometer controls

With phytometers
Calibrate environmental variation in farming and across experiments with control line and natural accession variation

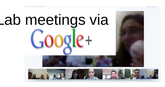
Across environments
Are mutant phenotypes uncovered by varying environments?

Database
Public dissemination of data

Education

- Studying student research networks
- Bring research experience into the undergraduate classroom
- Podcasts of methods to share across institutions
- Cross-campus connections – e-lab meetings via web2.0
- Undergraduate research apprenticeships
- Undergraduate co-authors denoted by †.

Lab meetings via Google+

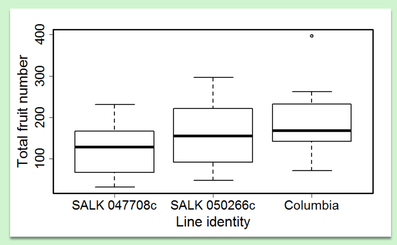


PHENOTYPING:

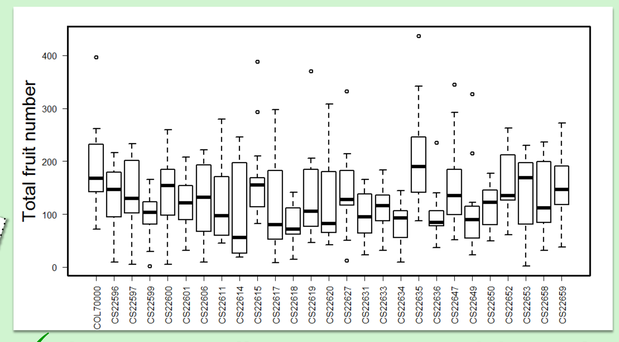
Barnard College lab

Barnard students phenotyping plants

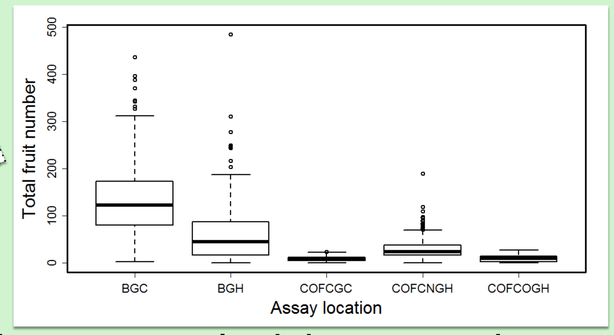
College of Charleston labs



✓**We can identify mutant lines with phenotypes for germination, life history, morphology and reproduction.**



✓**Phytometers allow assessment of the scale of natural variation within environments**



Phytometers reveal variation among environments:
Location key: B = Barnard, COFC = College of Charleston, GC = Growth chamber, GH = Greenhouse (ground OGH and rooftop NGH at CofC)

References:

- Hirsh AE and Fraser HB. 2001. Protein dispensability and the rate of evolution. Nature 411:1046-1049.
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- Lloyd J and Meinke D. 2012. A comprehensive dataset of genes with a loss-of function mutant phenotype in *Arabidopsis*. Plant Phys. 158:1115-1129.
- O'Malley RC and Ecker JR. 2010. Linking genotype to phenotype using the Arabidopsis unimutant collection. Plant J. 61: 928-940.