Transcriptome Variation in *Arabidopsis* Under Dynamic Growth Conditions

or: How I learned to stop worrying and love RNAseq

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Project Background

- Plants experience abiotic stress in nature
- Plants exhibit natural variation in stress tolerance
- Studying natural variation can give clues to mechanism
- Match natural variation to natural conditions in study

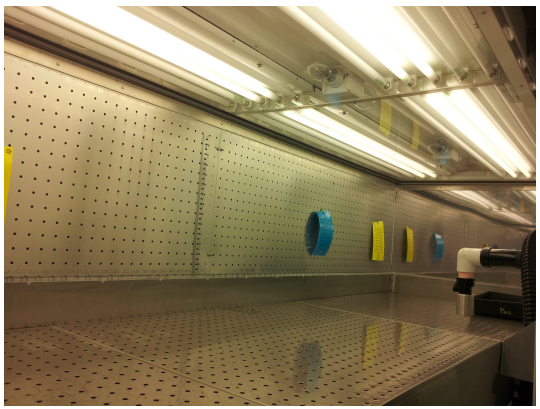
Terminology

- **Transcriptomics:** study of global gene expression
- **RNAseq:** transcriptome quantification by sequencing
- **Pipeline:** series of software which turns data into results
- **QTL Mapping:** technique to associate variation in genotype to phenotype variation

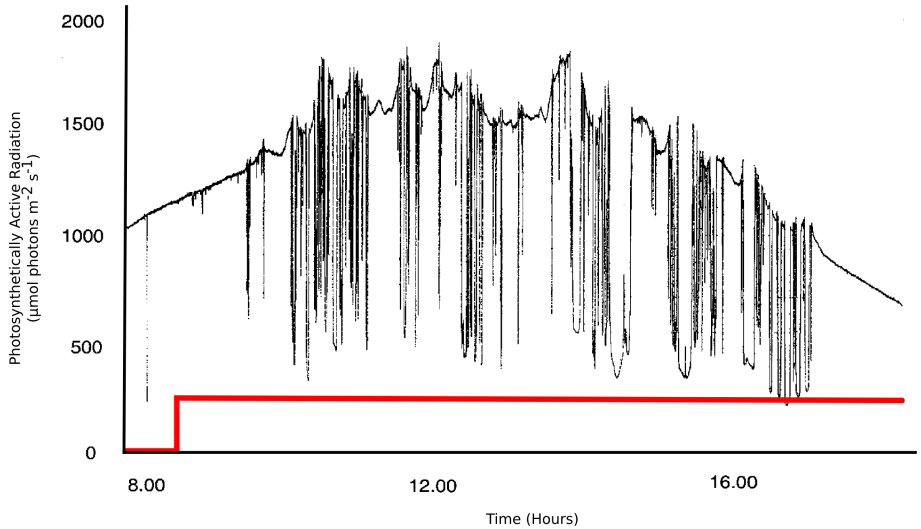
Aims

1. Design & implement dynamic growth conditions
2. Develop improved bioinformatic and molecular protocols for High-throughput RNAseq experiments
3. Determine effect of light intensity on transcriptome under dynamic light conditions

The growth condition dilemma



- Plants grow in nature
- A log of science done in labs

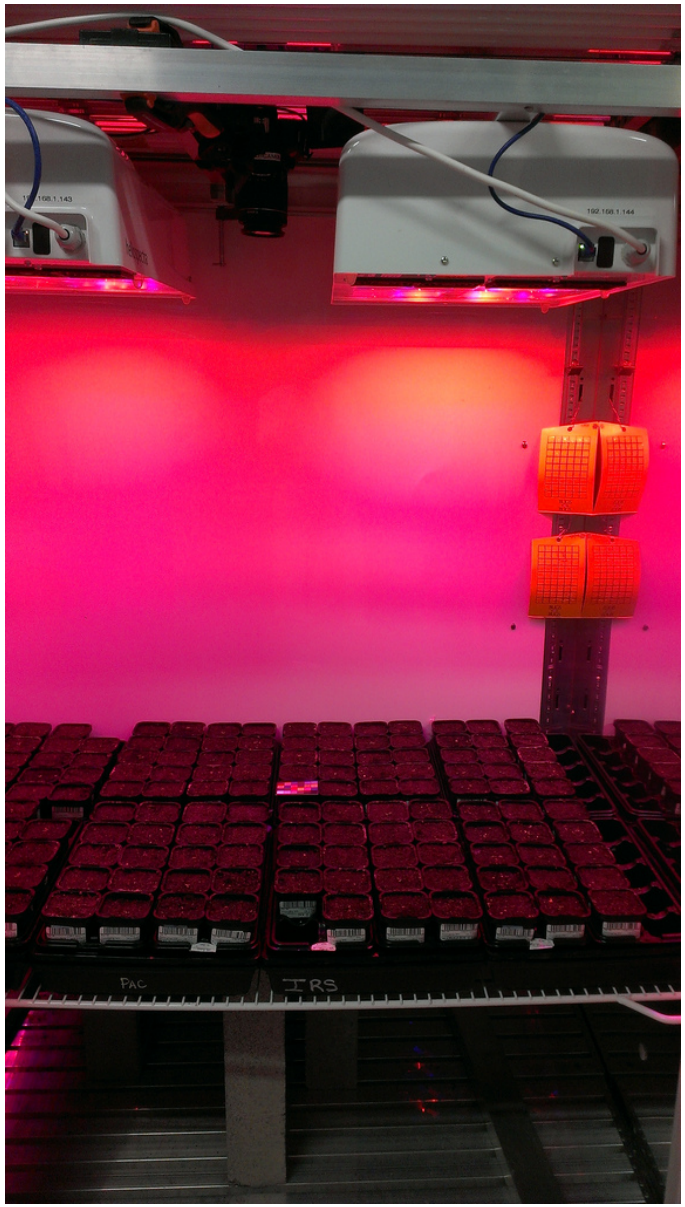


(Külheim, Ågren, and Jansson 2002)

- Aim to merge elements of these two scenarios

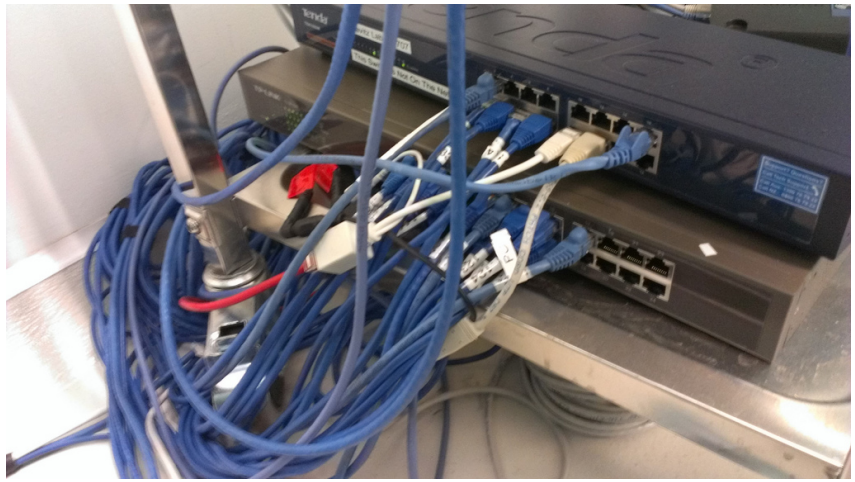
Introducing the SpectralPhenoClimatron

- Several new technologies
  - Growth Cabinets
  - LED Arrays
  - Imaging hardware
- Simulate regional climates
- Model diurnal and circannual trends of climate
- Use model simulation to drive actual growth cabinet conditions



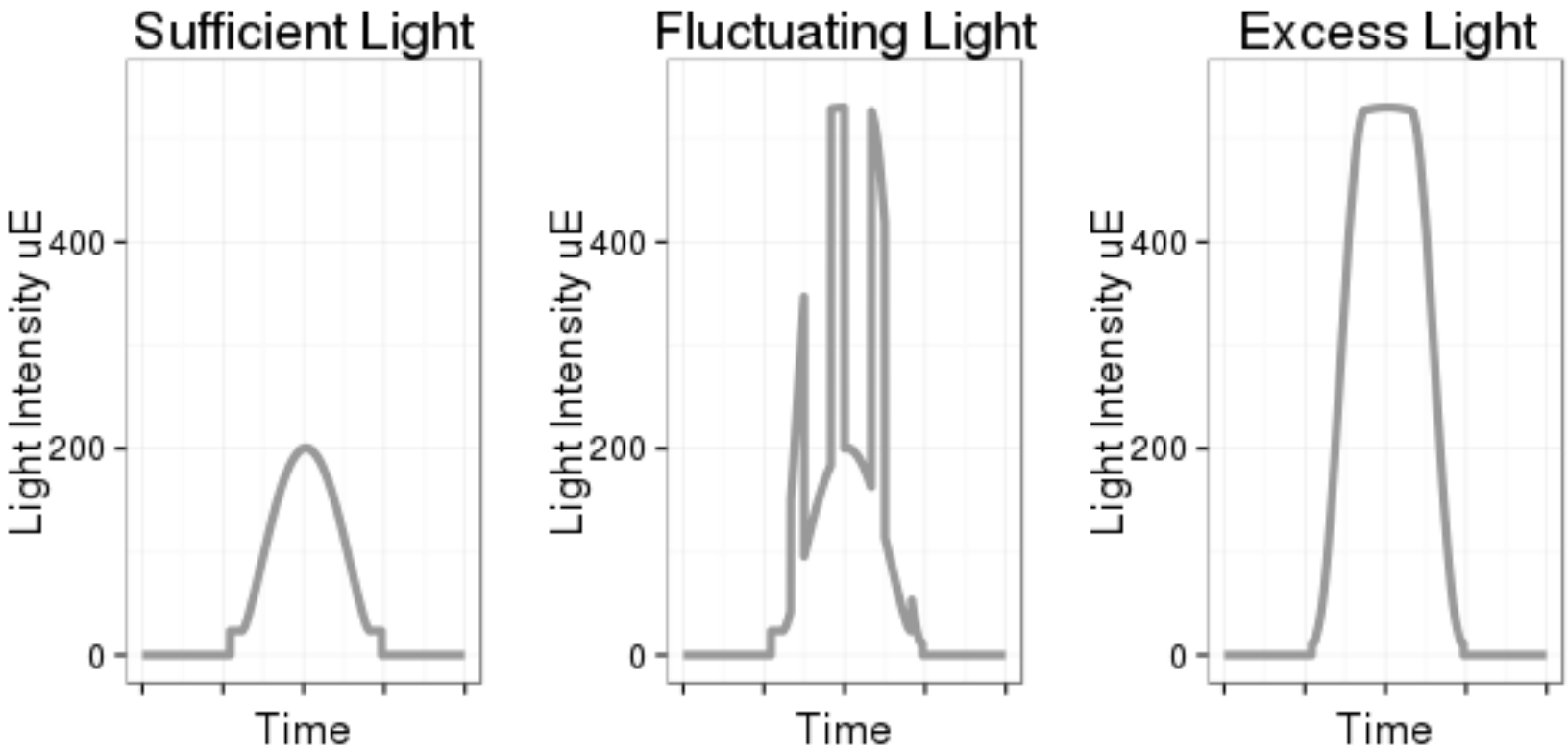
Controlling the SpectralPhenoClimatron

- Disparate pieces of technology
- Need software "glue" to stick bits together
- Wrote `spcControl` Python3 module
  - 750 lines
  - 134 minor, 16 major versions
  - Open source, on `github.com`



Investigating altered light intensity

- Within a simulated climate, modify light intensity
- Create 3 new conditions:
  - Sufficient light
  - Fluctuating light
  - Excess light

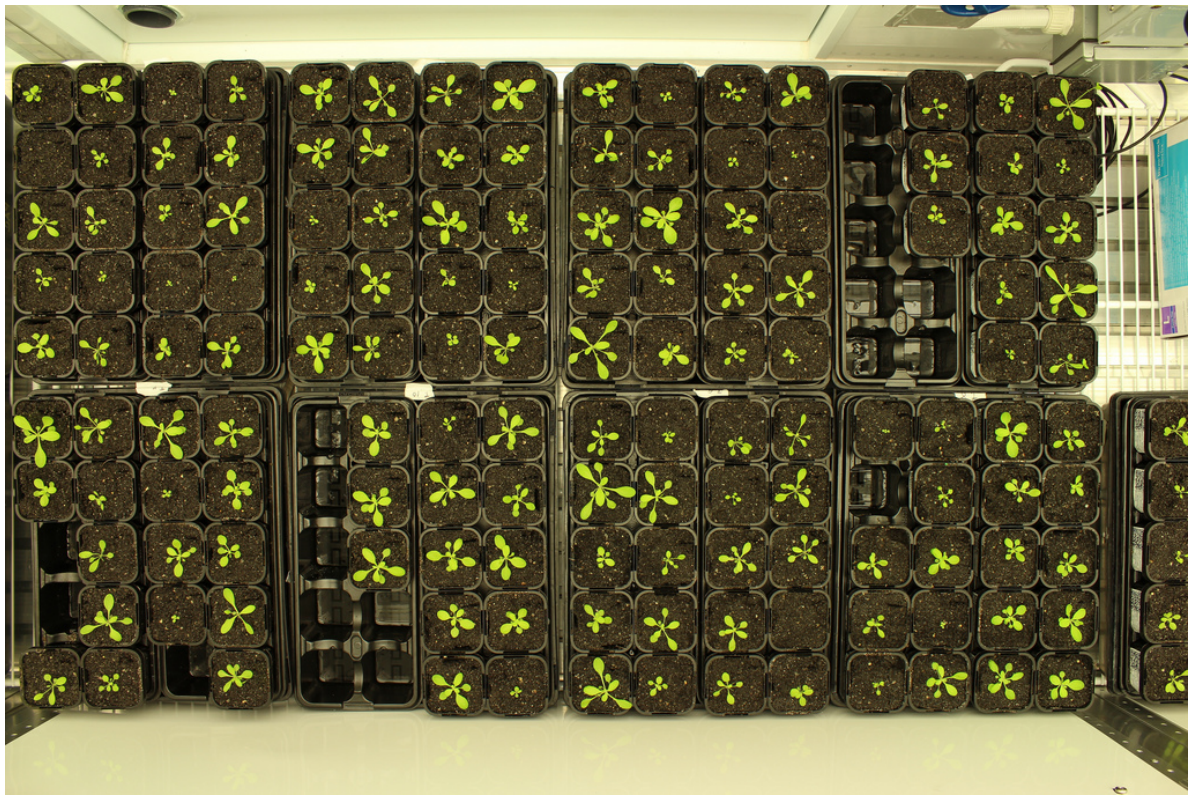


Investigating altered light intensity

- Hypothesised "hardening" of plants to harsher conditions
  - Increased steady state expression of stress genes
  - Decreased induction of stress genes after stress
- Hypothesised a relative order of "hardening"
  1. Fluctuating light
  2. Excess light
  3. Sufficient light
  4. Standard growth conditions

Plant Growth Under Dynamic Growth Conditions

- A QTL mapping set; Col, Cvi, Ler Ecotypes
- Over 1200 plants planted
- Grown for 3 weeks dynamic growth conditions
- Assay expression before and after high light pulse treatment



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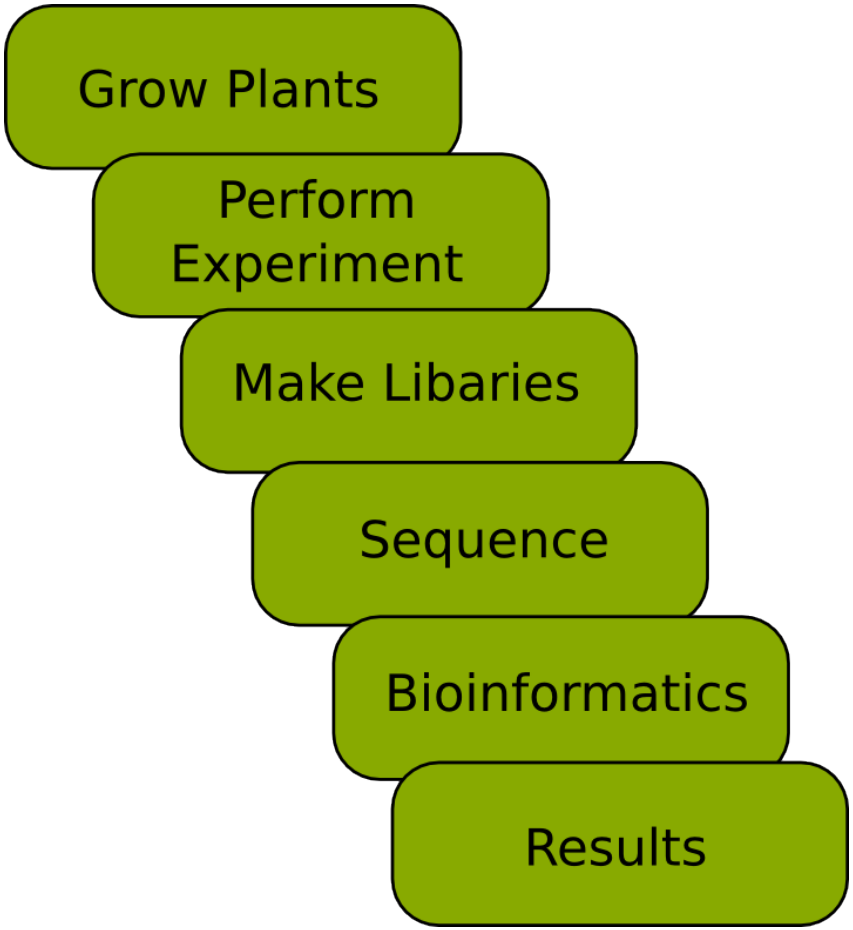
How does RNAseq work?



- Assay **ALL** expression in your tissue
- Unbiased, as quantitative as qPCR
- Becoming cheaper and easier

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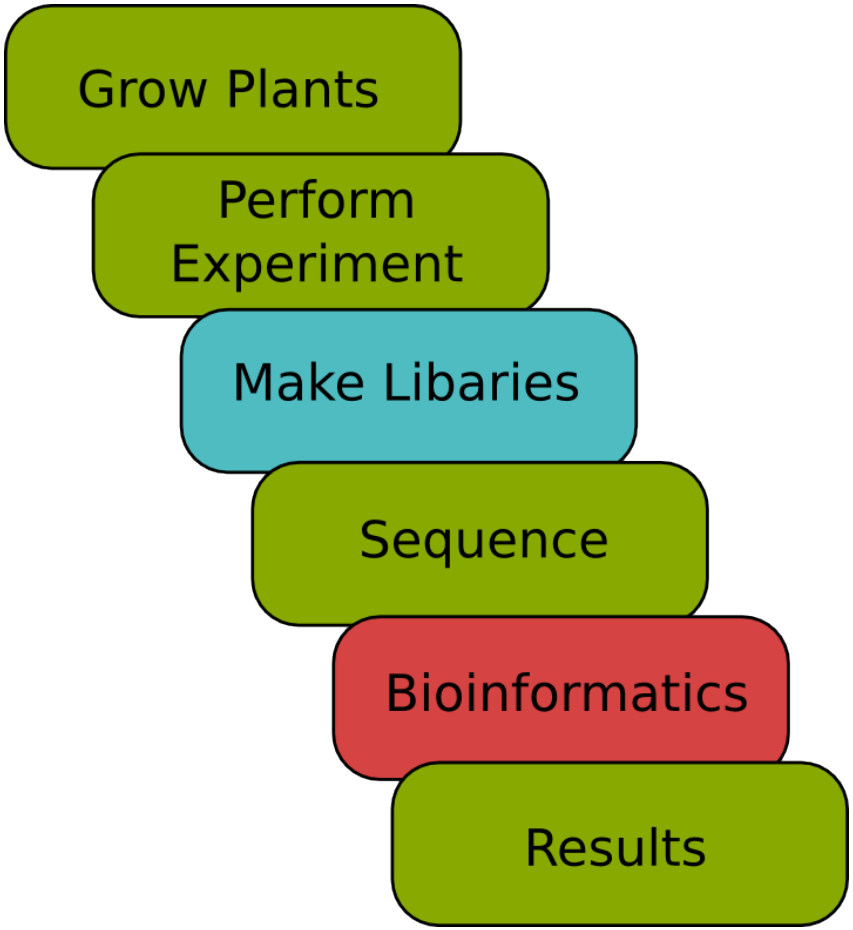
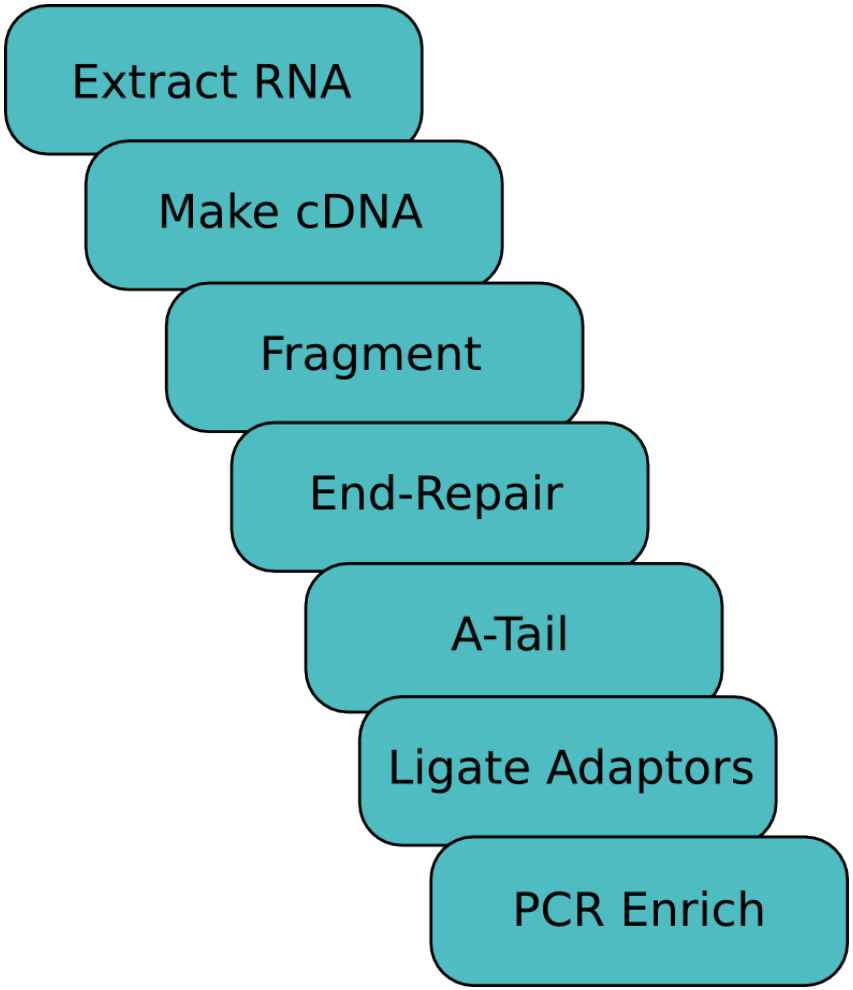


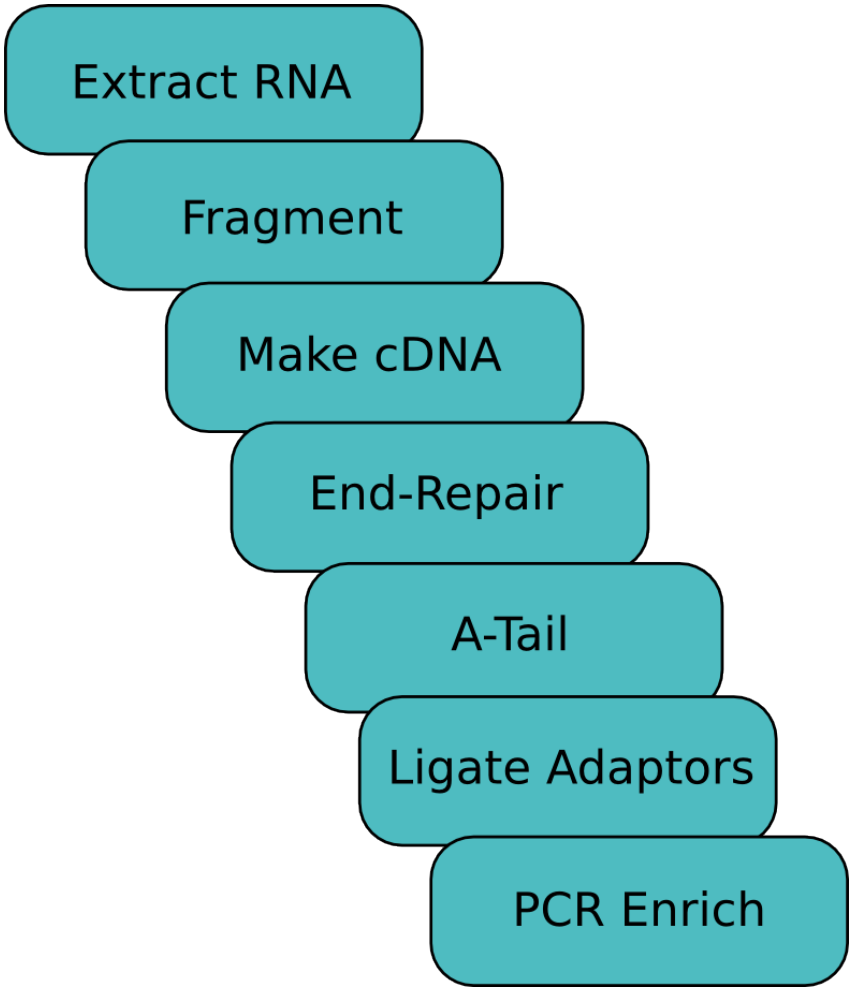
- Will focus on two areas of improvement
  - Making RNAseq library prep. cheaper & higher throughput
  - Making RNAseq data analysis easier & faster

Cheaper, Higher Throughput RNAseq

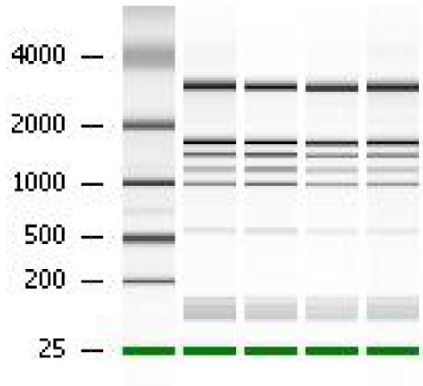


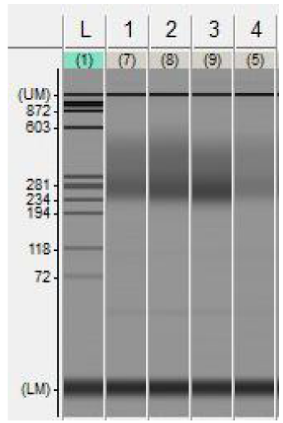
- Adapted from Kumar et al. (2012)
- On-bead SPRI protocol
- Performed in 96 well plate
- $\approx$ \$50 per sample, 96 samples per lane
- Successful until final step
- Sidelined due to lack of time

Illumina RNAseq Library Prep.

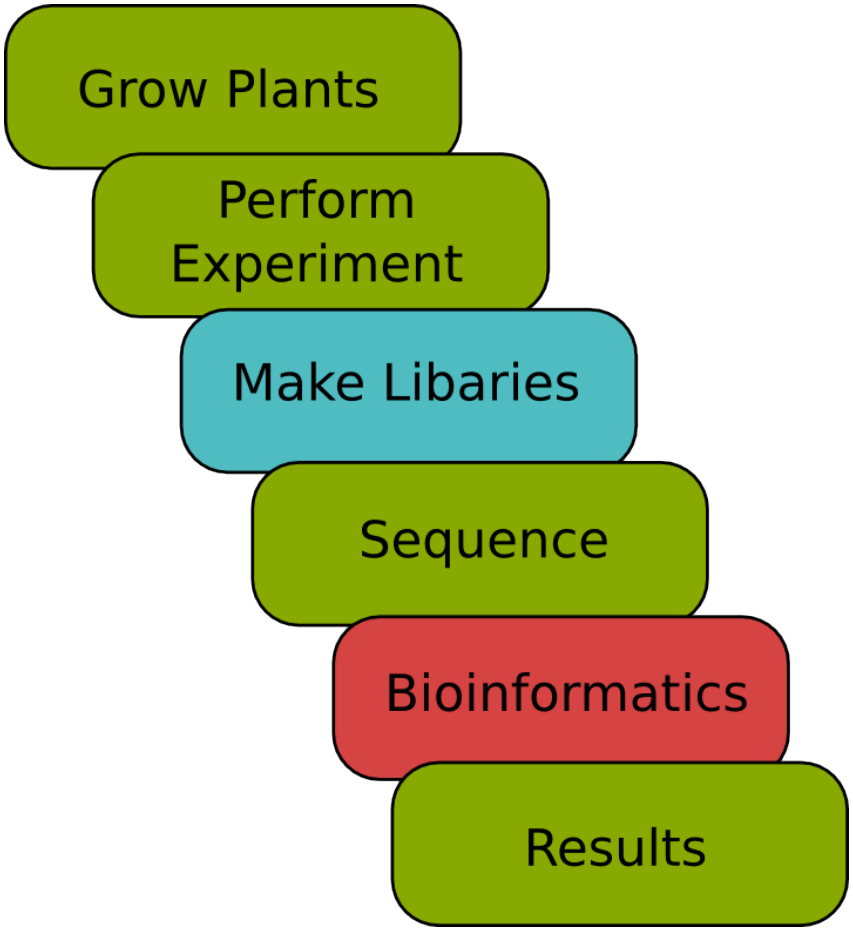


- 3-5 day protocol
- Up to 12 samples per lane
- $240-400 per sample





RNAseq Analysis Made Easy!



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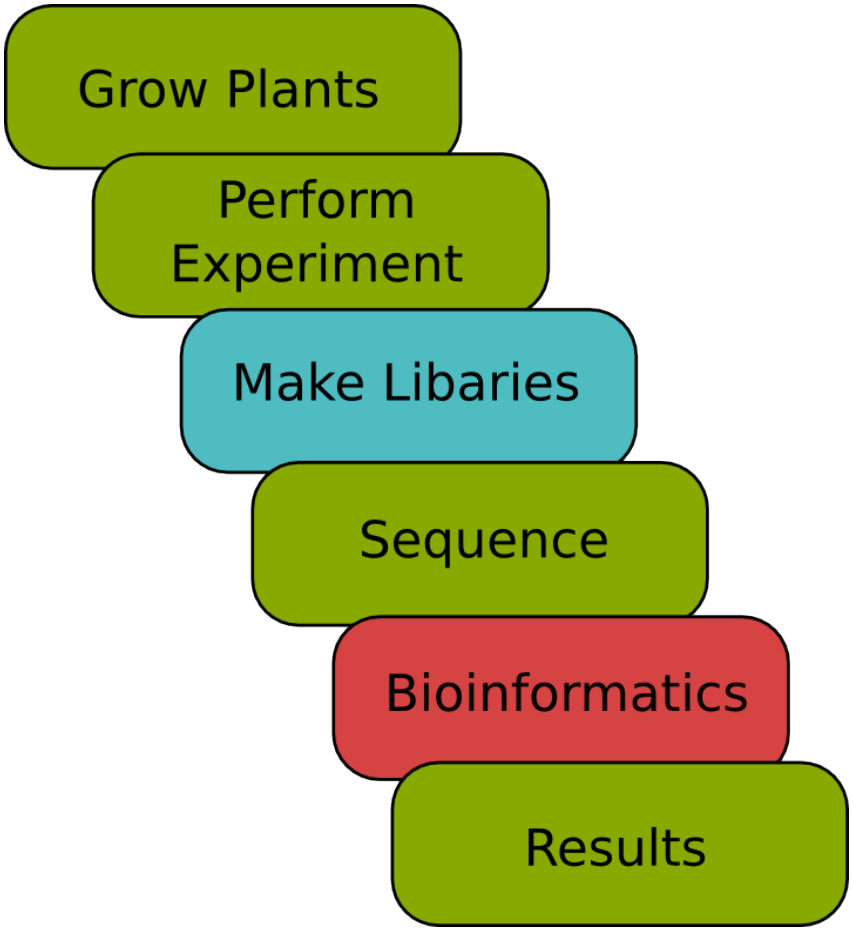

"Can't there just be a 'do my bioinformatics' button?"

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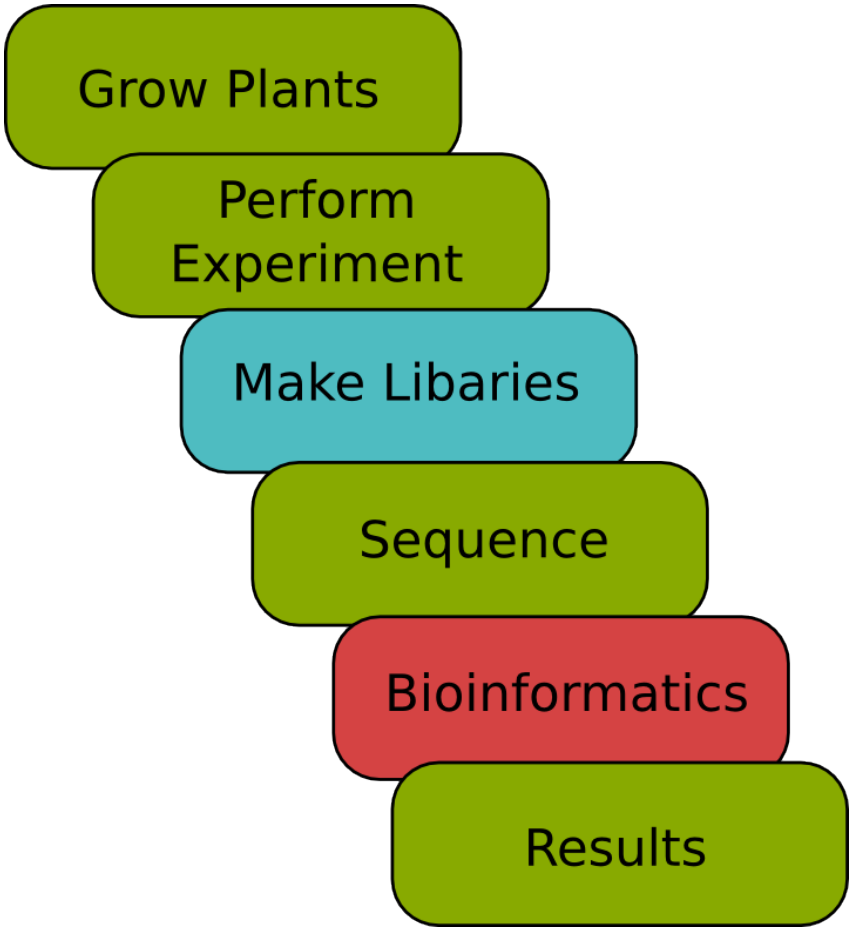

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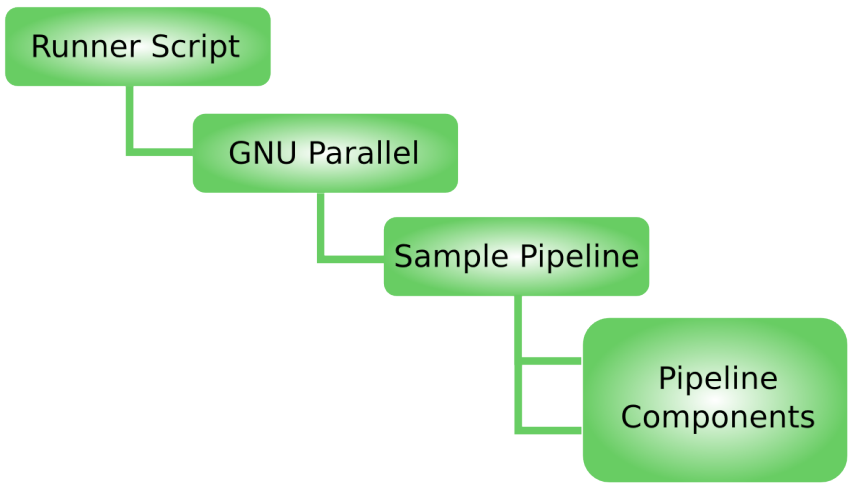
How To Run a Pipeline

Command:

```
bash runner.sh keyfile.key
```

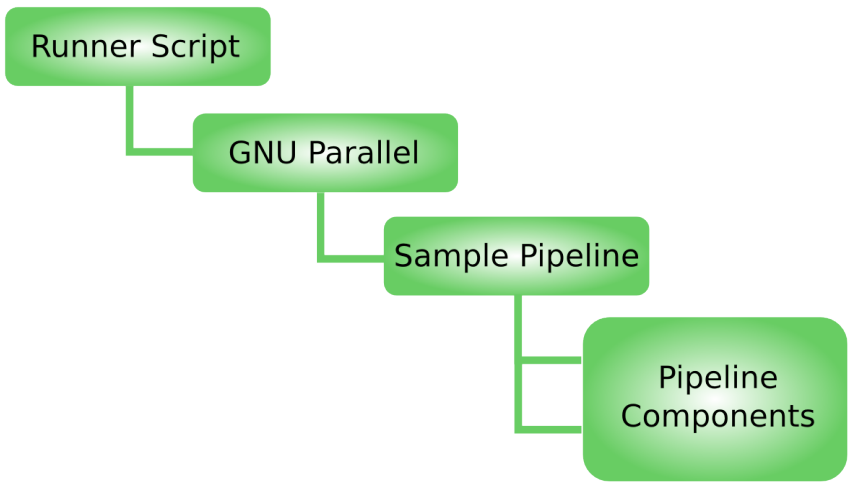


- Let's dissect that:
  - `bash runner.sh`

    Call the runner script
  - `keyfile.key`

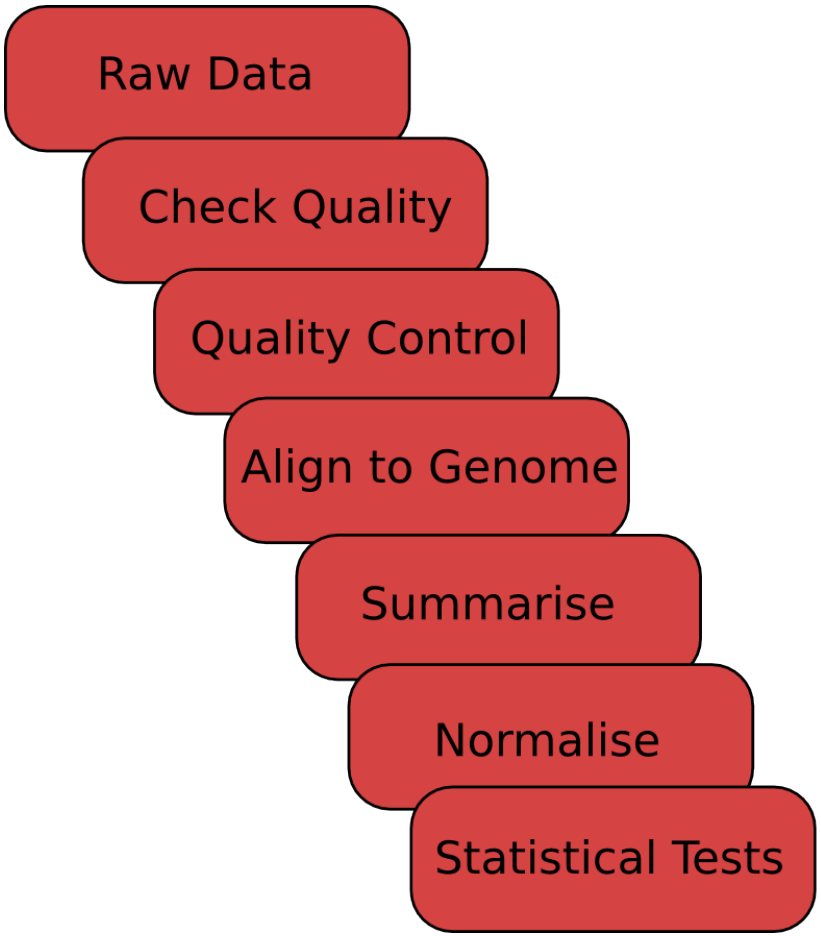
    Give it the "keyfile"
- No need to run each component separately

How does that work?



- More than 1300 lines of code
- Written in bash, python and R
- 144 minor versions
- Code is on `github.com`
- Open source (GPL v3)
- You should use it!

RNAseq Pipeline Components



`fastqc`

`scythe, seqtk`

`subread (tohat, subjunc)`

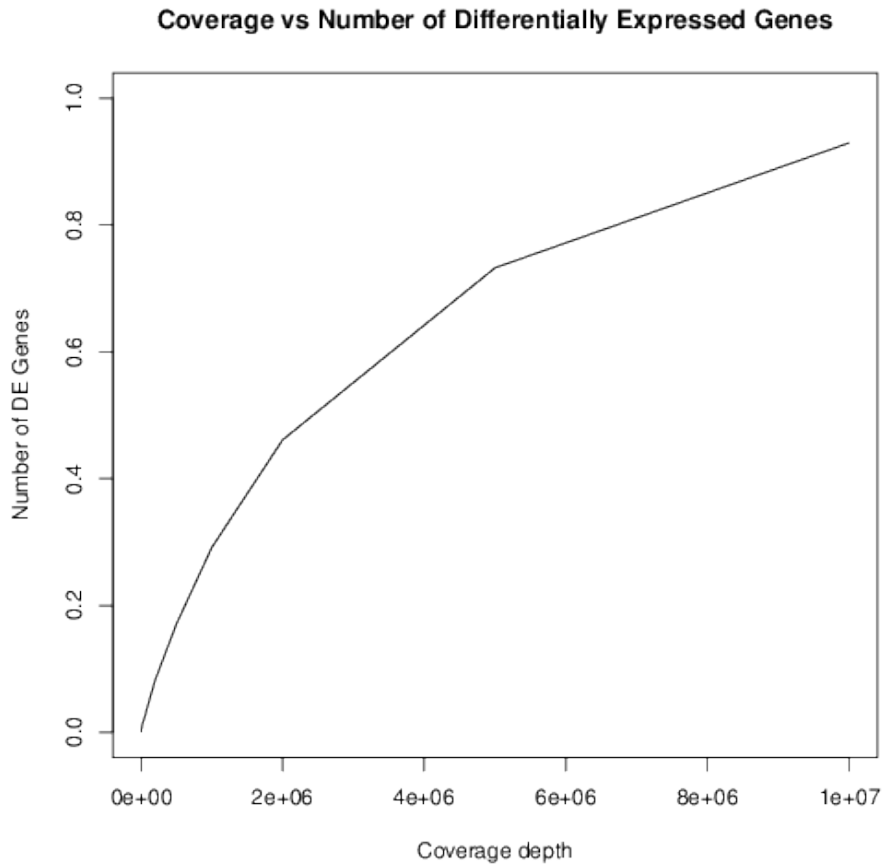
`featureCounts`

`TMM`

`exactTest, plotMDS, plotBCV`

Effect of Sequencing Depth

- Trade off between multiplexing and statistical power
- Conclusion: Recommend 48x multiplexing (5M reads)



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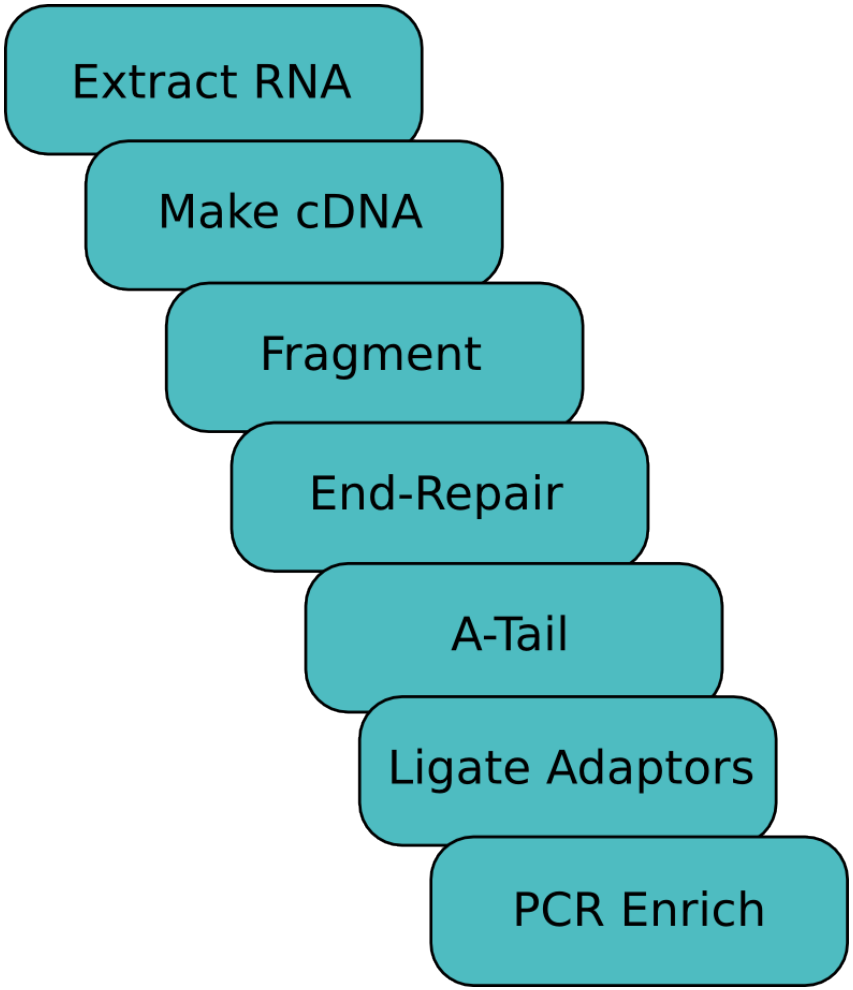

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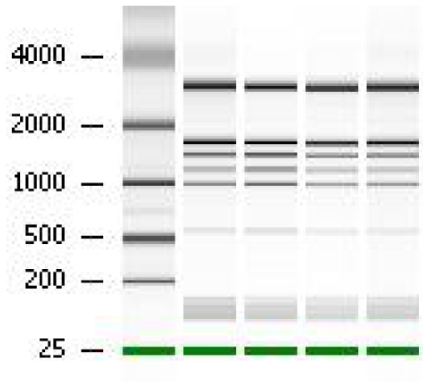
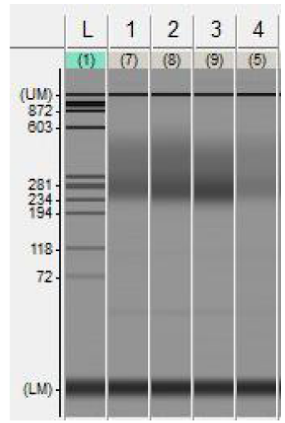
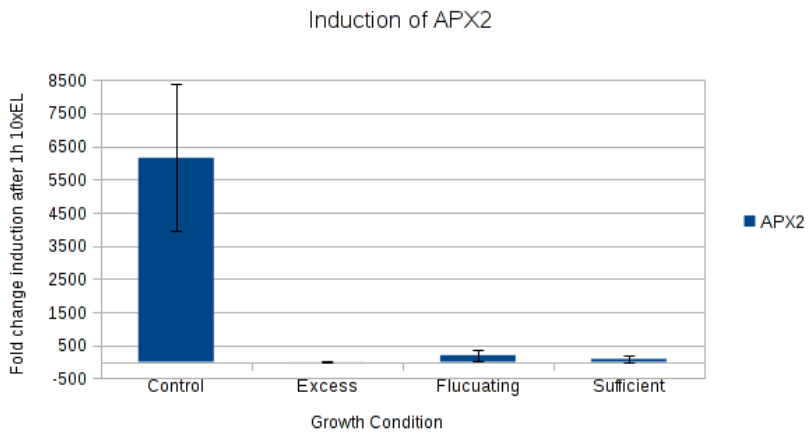




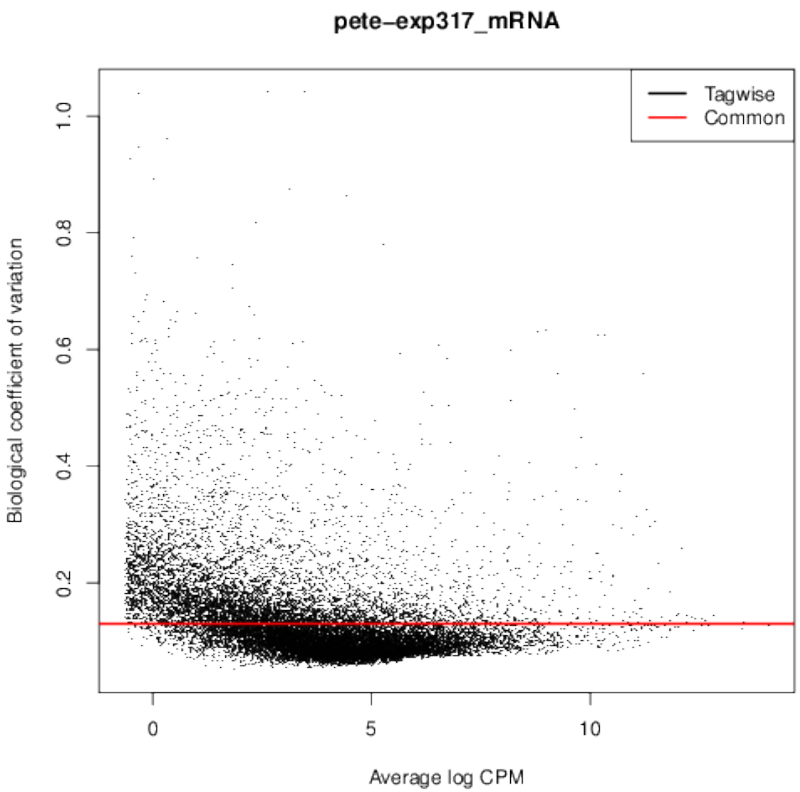
qPCR analysis tells a small story

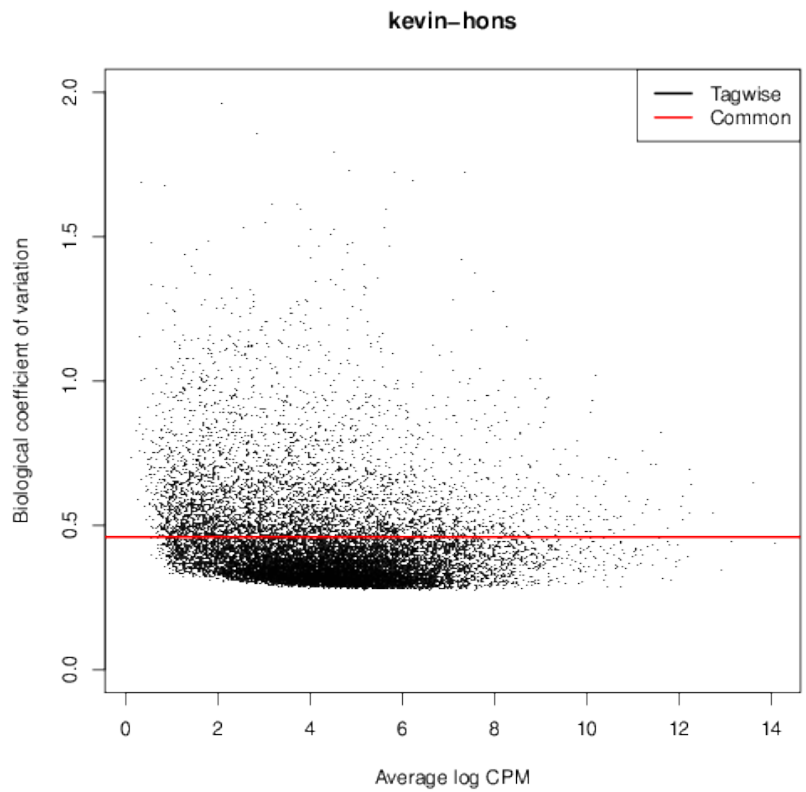
- Examine expression of known excess light responsive genes
- Show reduced induction and increased steady state expression
- Hypotheses appear mostly correct



RNAseq shows the whole picture

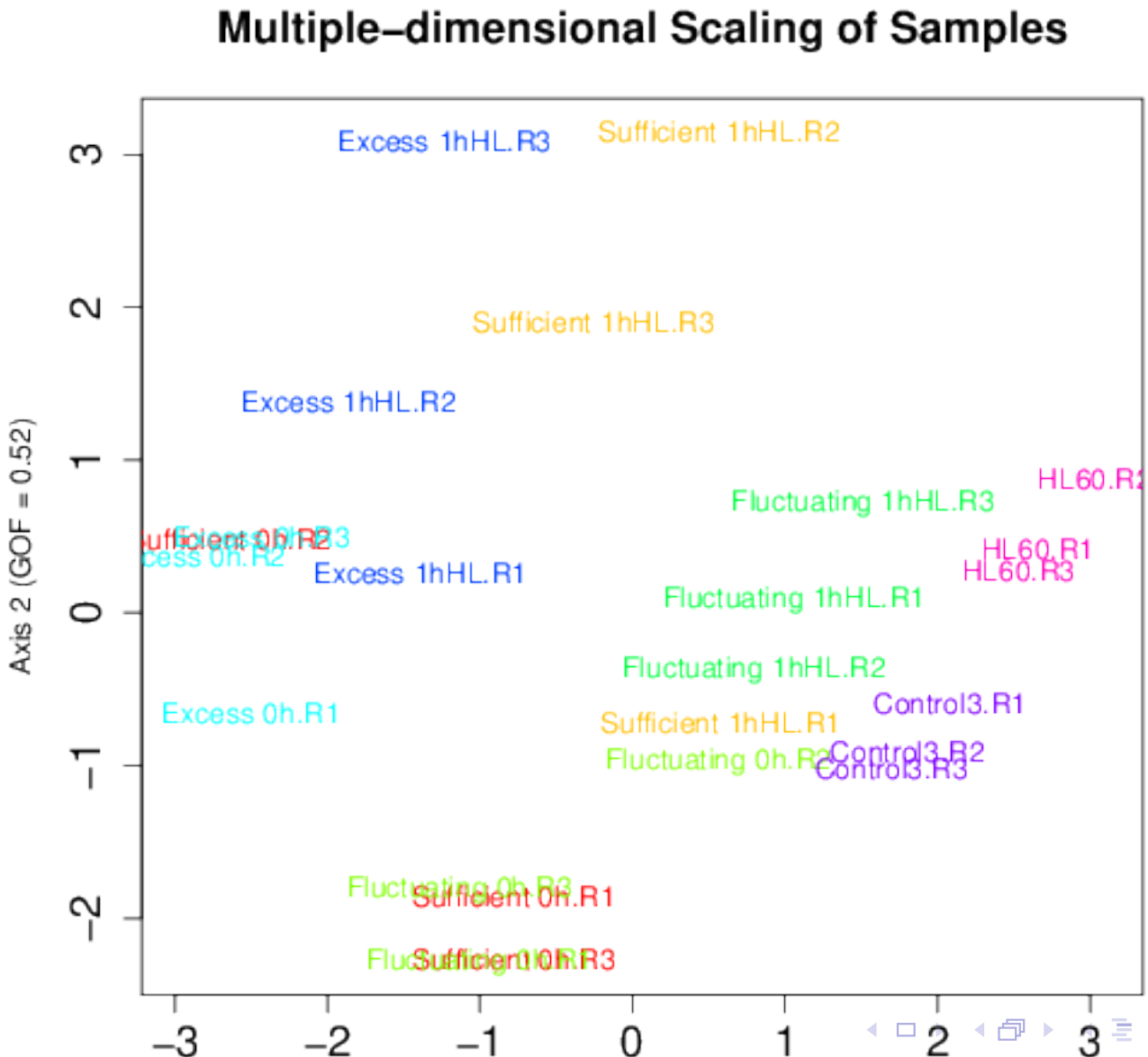
- Overall, high variation amongst samples





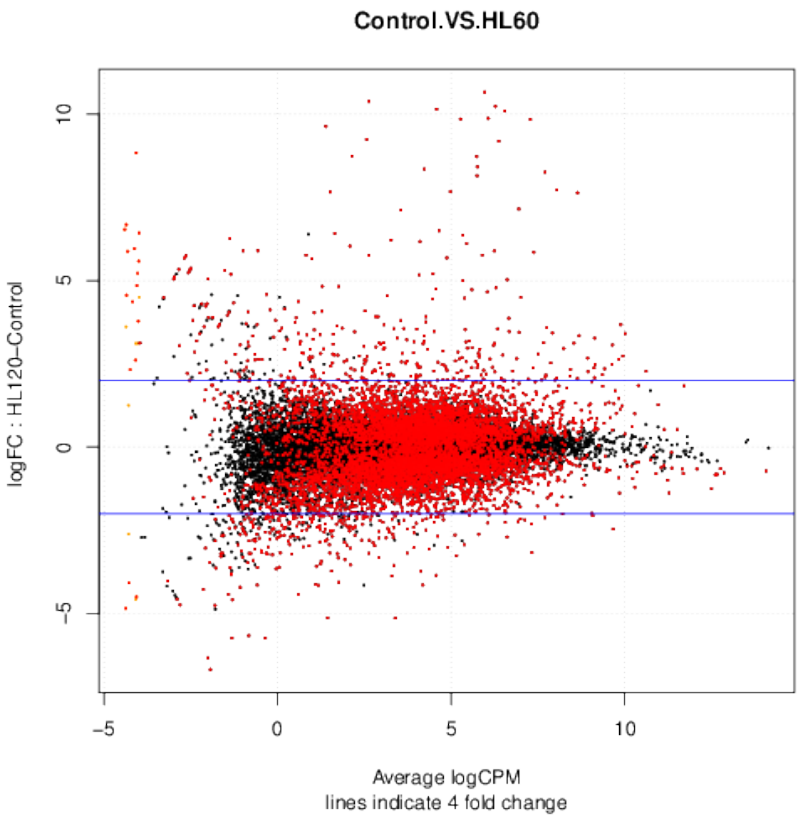
RNAseq shows the whole picture

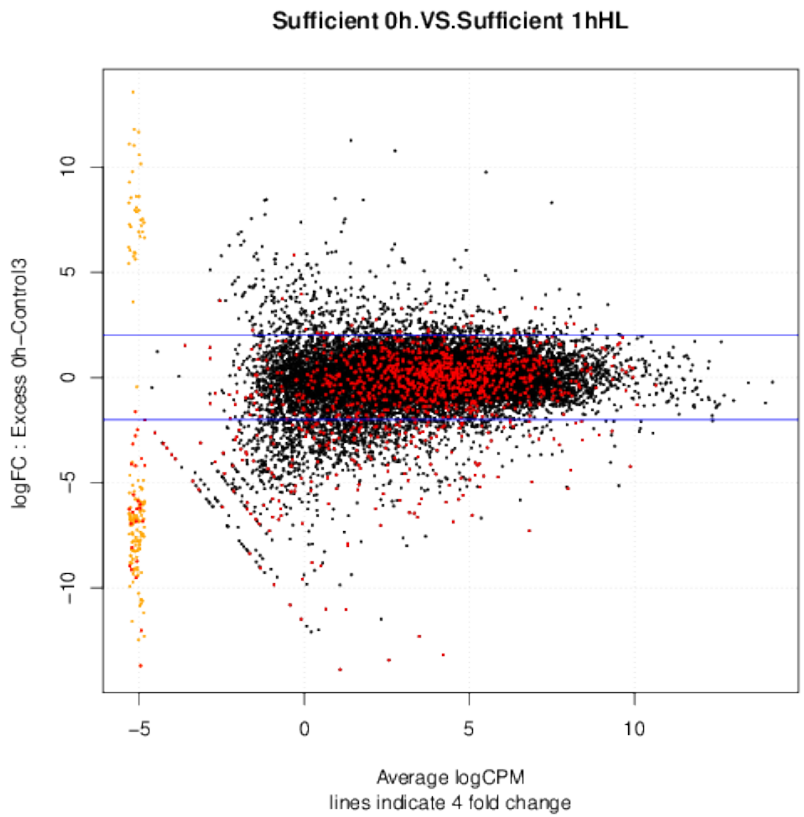
- Amongst a noisy response, a pattern emerges



RNAseq shows the whole picture

- Less differential expression than expected





DE table

- This is how many genes are DE
- This is what they have in common
- Analysis/interpretation is ongoing - (GSEA)

Conclusions

- Dynamic growth conditions give interesting biology
- Patterns of differential expression seen, more replicates needed
- RNAseq is here, and easier than you might think

Future Work

- Optimise 96-well RNAseq protocol
- Analyse QTL mapping set:
  - RNAseq to map QTLs for expression of stress responsive genes
  - Find phenomic traits e.g. anthocyanin accumulation
- Repeat entire experiment with improved sampling techniques - increase statistical power

Acknowledgements

- Pogson Lab
- Borevitz lab

Old/Dead slides

Project Overview

- What I've spent my year doing:

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- Design & implement data analysis pipeline for RNAseq

Light Quantity

- Plants need a "happy medium" of light
- Too little = sub-optimal growth
- Too much = photooxidative damage
- Interesting model system for examining dynamic growth conditions

Light Response & Transcriptomics

- Excess light invokes known transcriptional response
- Many genes induced in excess light constitutively expressed in field
- Transcriptomics sensitive to subtle or fast changes

History of Light Transcriptomics

- Pogson, Nagashi, Karpinski, Jannsen

Aims

1. Develop a pipeline of software to analyse RNAseq datasets

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2. Determine the response of *Arabidopsis thaliana* to altered light intensity under dynamic growth conditions

Making a pipeline?

+

Making a pipeline?

+

Aim 1

Develop a pipeline of software to analyse RNAseq datasets

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Develop a pipeline of software to analyse RNAseq datasets

- Define & overcome shortcomings in existing analysis pipelines
- Experimentally define limitations of RNAseq experimental designs

Steps in an RNAseq Analysis

- Raw sequence data needs to be:
  - Filtered for quality
  - Aligned to the genome

Steps in an RNAseq Analysis

- Raw sequence data needs to be:
  - Filtered for quality
  - Aligned to the genome
- Once aligned, one needs to:
  - Quantify gene expression
  - Normalise counts
  - Perform statistical tests

Existing "Best Practice" Pipeline

- Settings optimised for non plants
- Not optimised for large/dramatic changes
- Result in hypothesised weird artefacts
- Can result in false positives or false negatives

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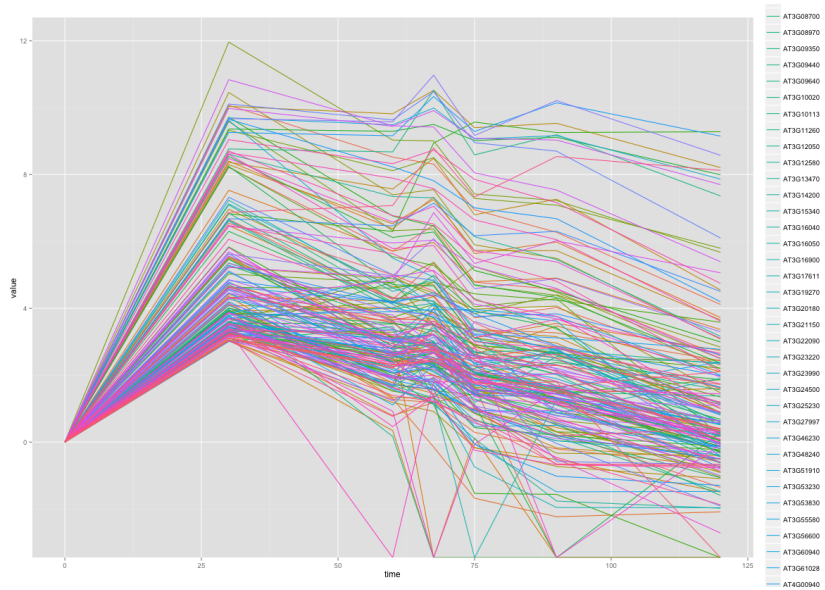

"Improved" plot

- Using edgeR, state of the art statistics
- Hypothesis was artefacts would be removed

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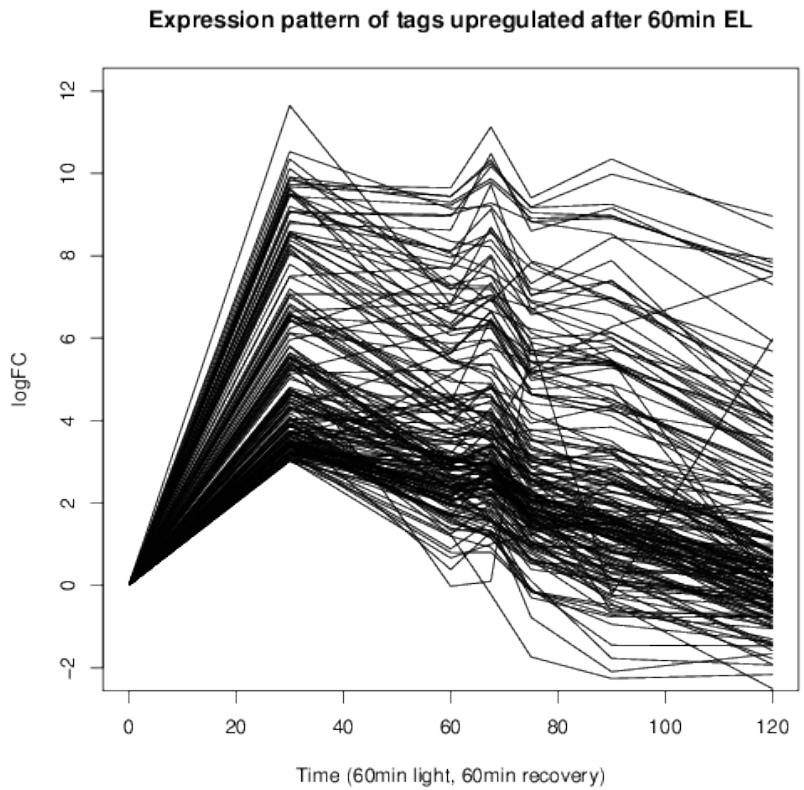
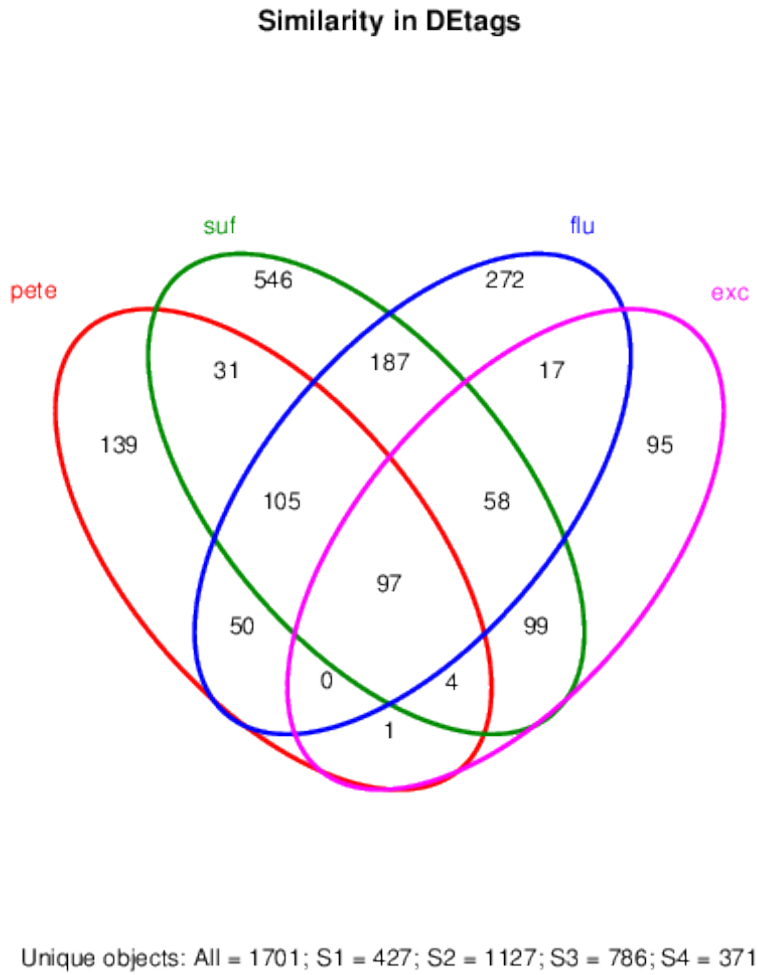


Venn Diagrams

- Hard to see, but there is most overlap between Sufficient and Control, and Sufficient and Fluctuating



QTL mapping aims

- Look for loci which control transcriptional response to abiotic stress