

A microarray analysis of dormancy states in *Arabidopsis thaliana*

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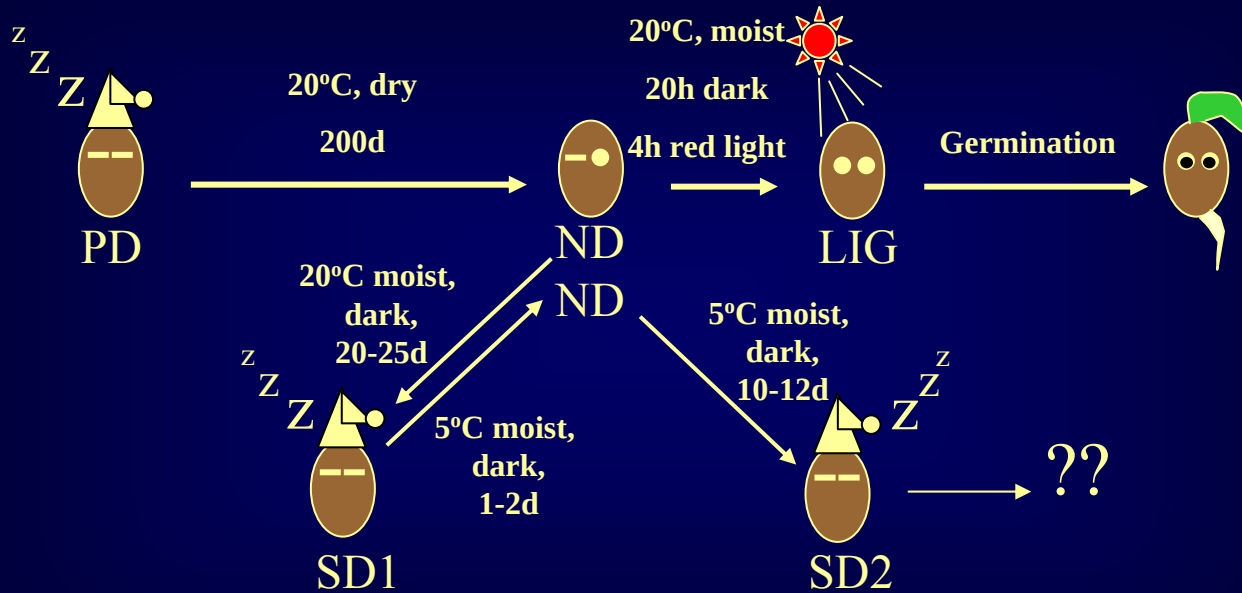
H. Hilhorst, Wageningen University, The Netherlands

- Induction and release of dormancy can be triggered by diverse environmental and physiological cues

Null Hypothesis

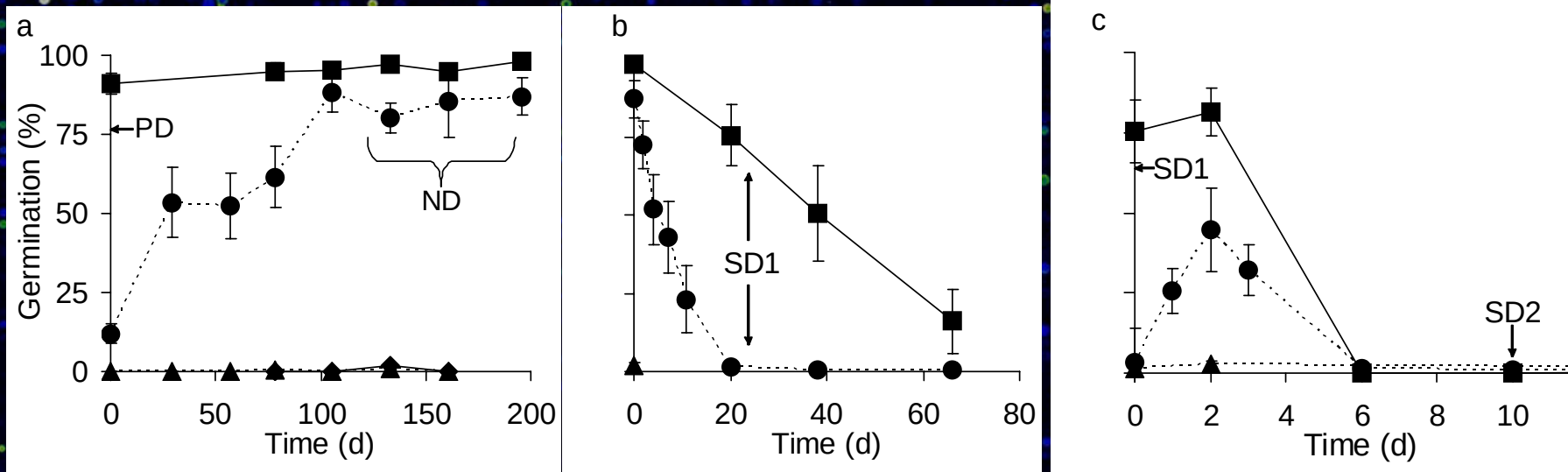
- Dormant states induced by different environmental conditions are identical at the molecular level

Dormancy Cycling in *Arabidopsis thaliana* Cvi



Germination of *A. thaliana* ecotype Cvi.

Nitrate (solid lines) - without nitrate (dotted lines) - white light at 20 °C (■●) - dark (▲◆)



PD: Requires light plus nitrate

ND: Requires light

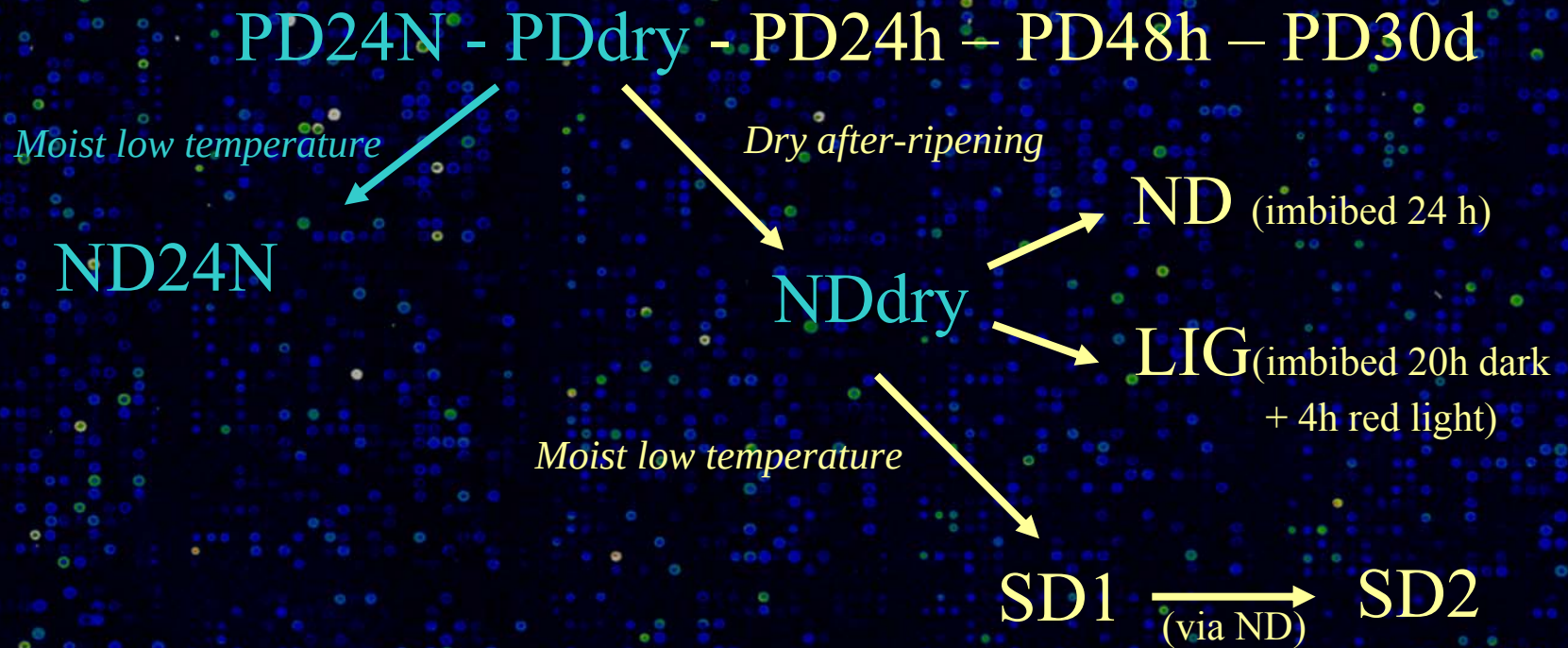
SD1: Requires light plus nitrate

SD2: Requires light plus nitrate plus ?

Dormancy is not broken by low temperature, high temperature or alternating temperatures

Dormancy can be broken by Fluridone + GA (100 % viable after 200 days)

Samples for Microarrays



Sisymbrium officinale; Brassica oleracea

Methods of study

- Affymetrix and CATMA microarrays used to study changes in gene expression patterns in different *Arabidopsis thaliana* Cvi seed dormancy states
- Quantitative PCR used to confirm gene expression patterns of individual genes identified from microarray experiments

Affymetrix Microarrays

- RNA of 4 biological replicates submitted to NASC Affymetrix Service *
- ATH1 Genome Array: >22,000 probe sets represent ~24,000 gene sequences

Data Normalisation

- Variance Stabilisation and Normalisation (VSN)**

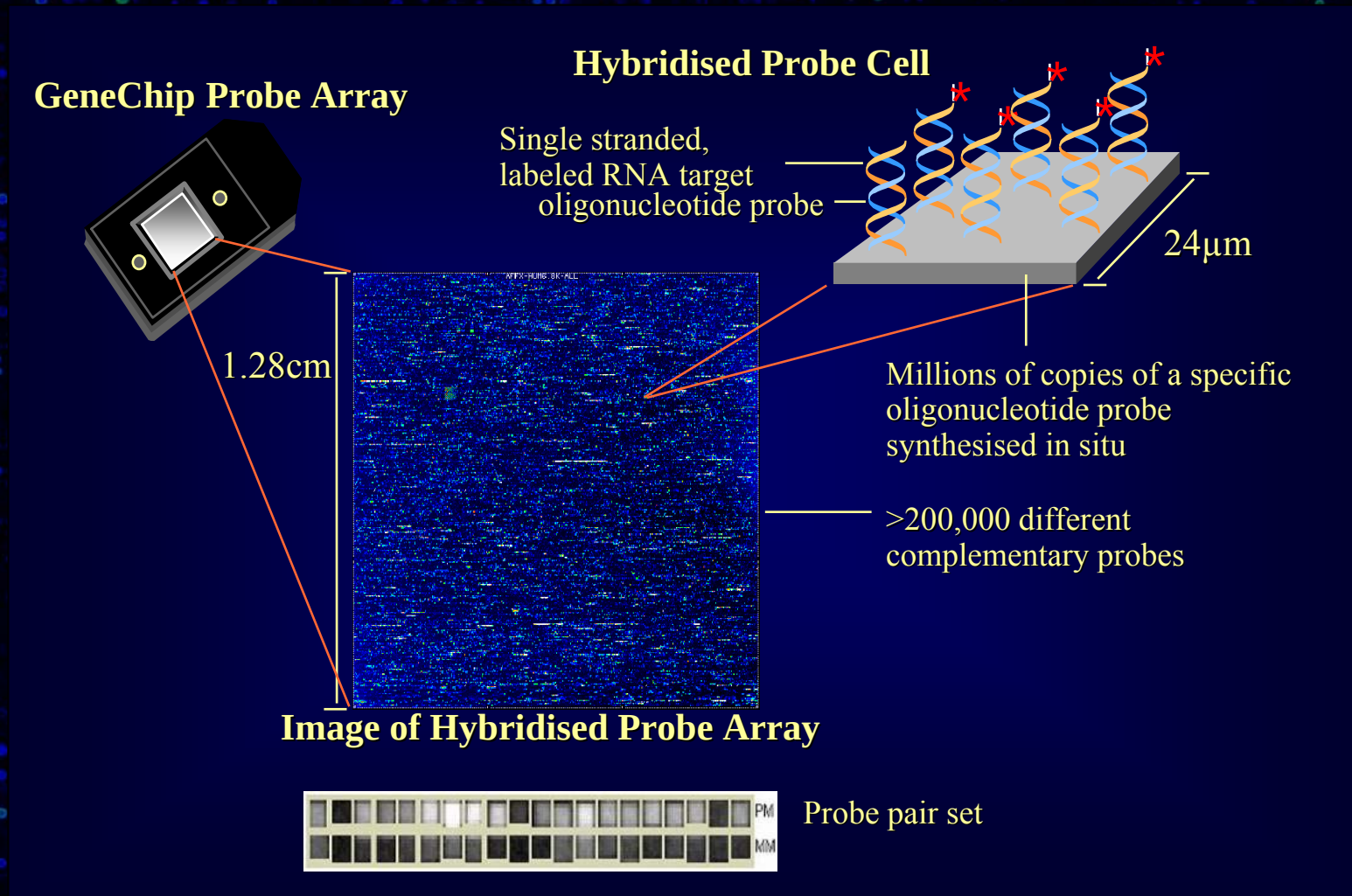
Quantitative RT-PCR

- Quantitative RT-PCR confirms patterns of expression shown by the microarrays

*Craigon DJ., James N., Okyere J., Higgins J., Jotham J., May S. NASCArrays: A repository for Microarray Data generated by NASC's Transcriptomics Service. Nucleic Acids Research 32: D575-D577, 2004.

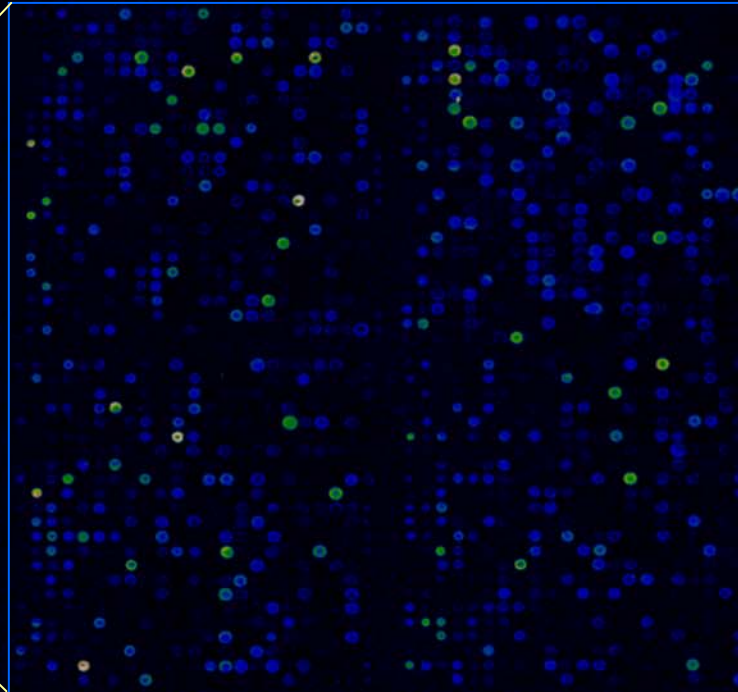
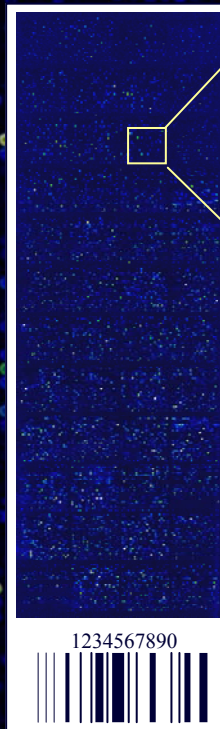
**Huber, W., von Heydebreck, A., Sültmann, H., Poustka, A. and Vingron, M. Variance stabilization applied to microarray data calibration and to the quantification of differential expression. Bioinformatics 18: S96-S104, 2002

1. Generate biotin-labelled cDNA from messenger RNA
2. Fragment and hybridise to GeneChip expression array
3. Wash, stain, scan and quantitate



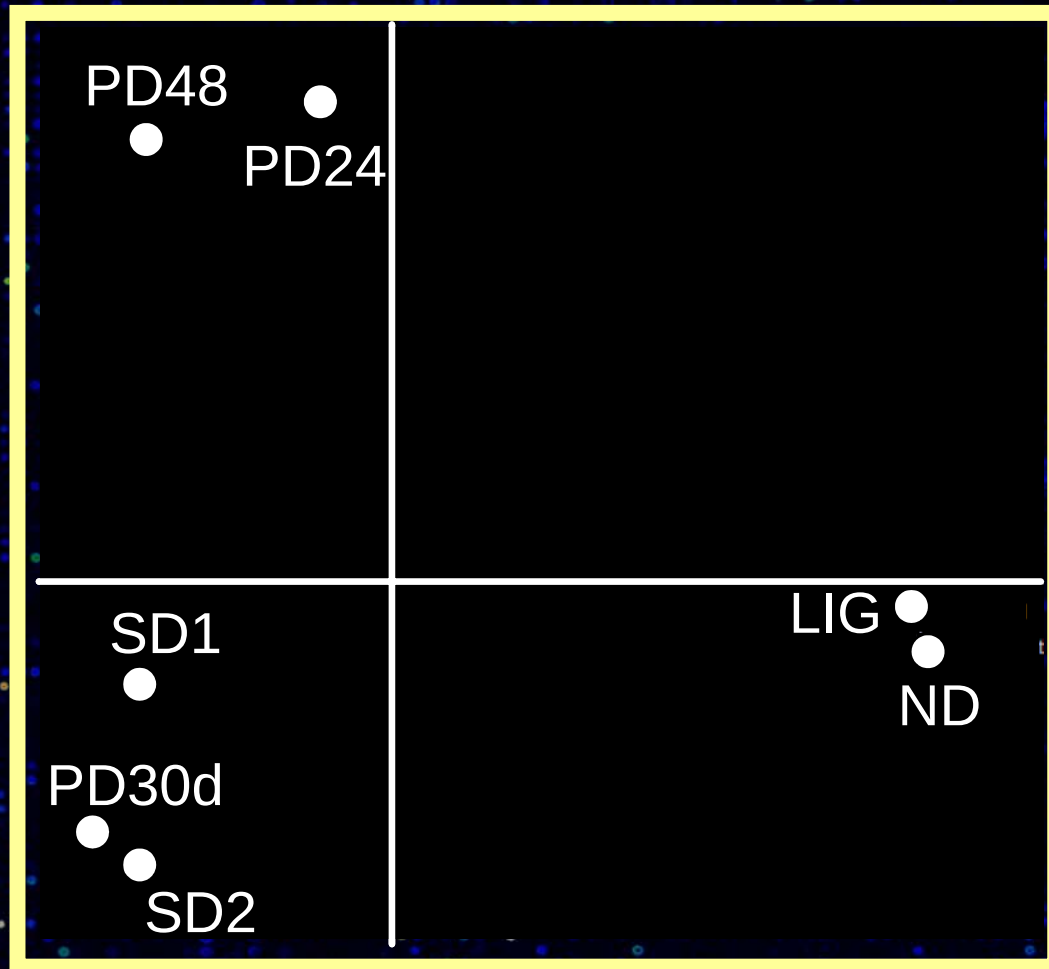
CATMA

- A Complete Arabidopsis Transcriptome MicroArray
- Spotted GSTs (150-500n) represent ~25,000 genes
- Messenger RNA amplified, converted to cDNA, labelled with Cy3 or Cy5 and hybridised to CATMA chips

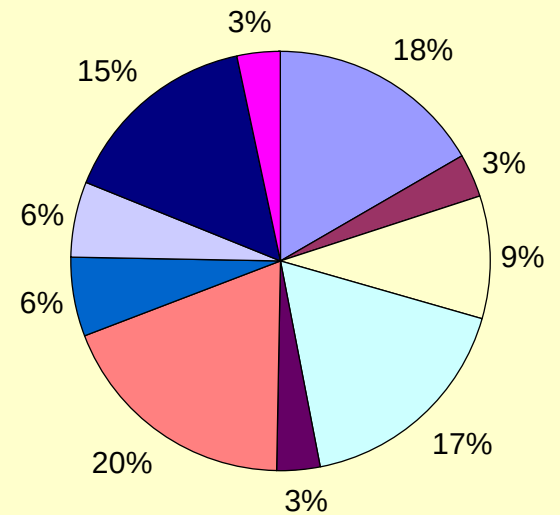
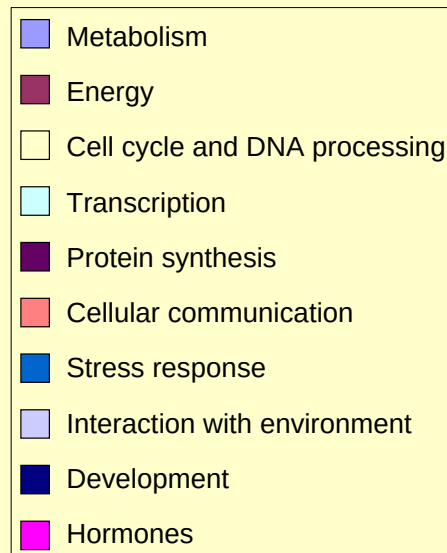
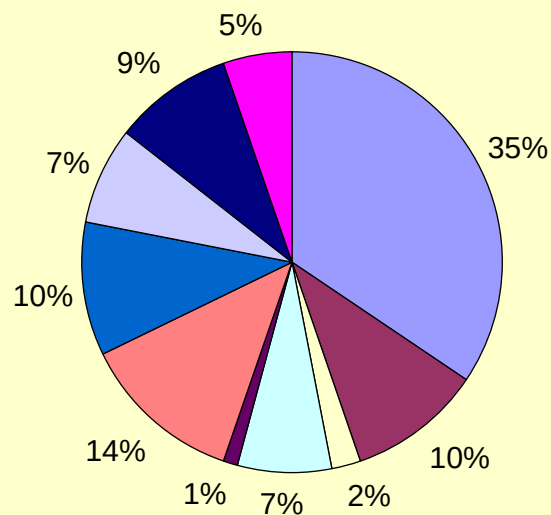
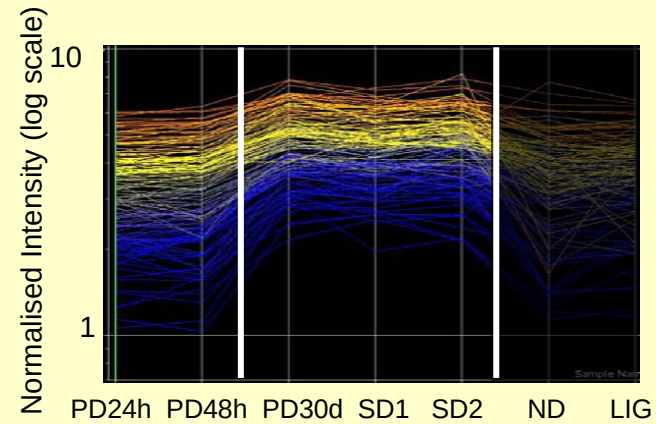
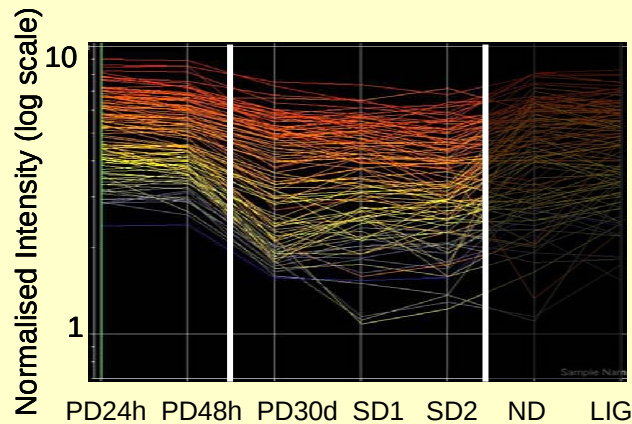
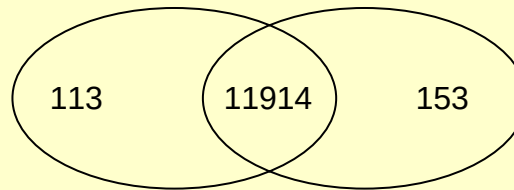


Crowe M.L., Serizet C., Thareau V., Aubourg S., Rouzé P., Beynon J.L., Hilson P., Weisbeek, P., Van Hummelen P., Reymond P., Paz-Ares J., Nietfeld W. and M. Trick (2003) CATMA - A complete *Arabidopsis* GST database. *Nucleic Acids Res* 31, 156-158.

Principal component analysis:

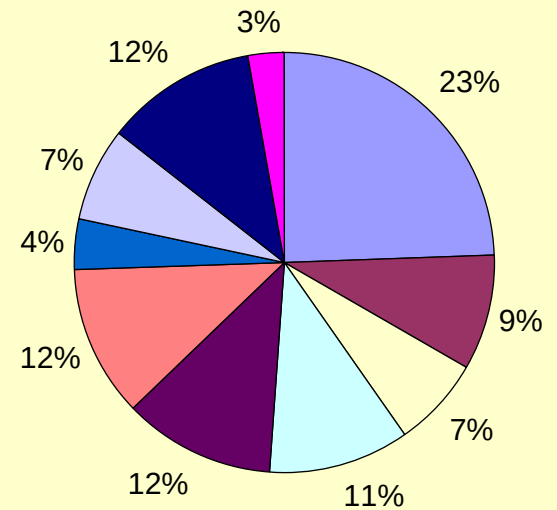
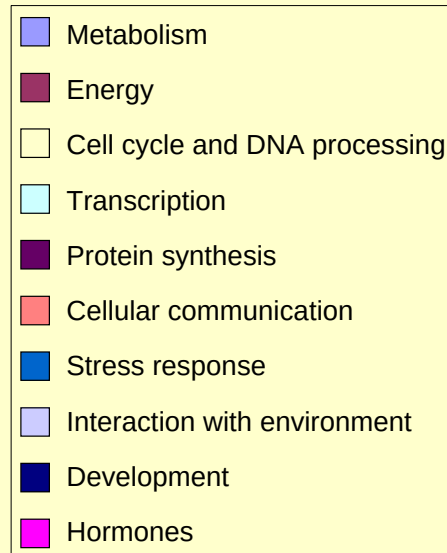
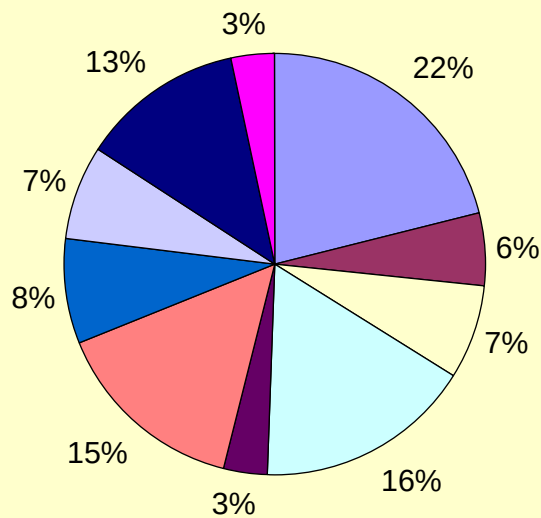
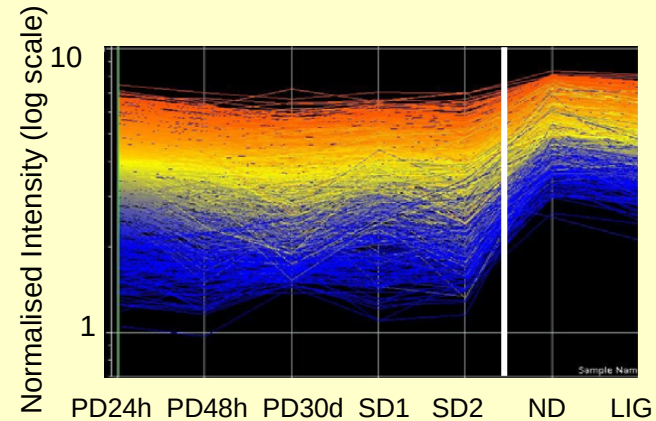
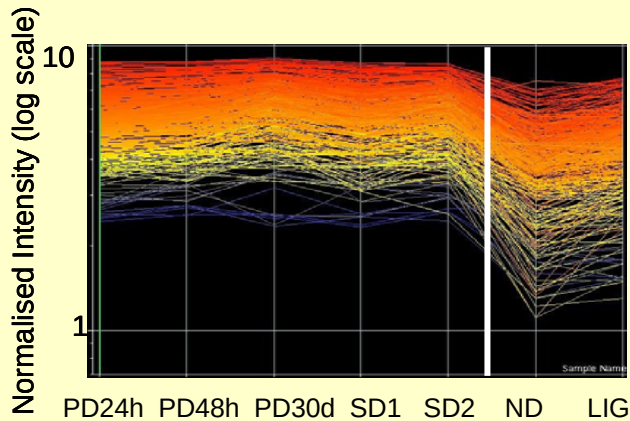
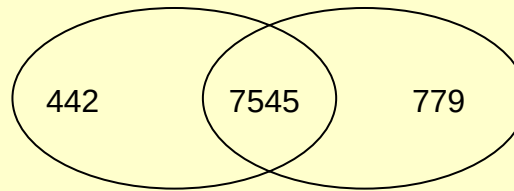


PD24h/PD48h PD30d/SD1/SD2



All 5 Dormant states

ND/LIG



Some tentative conclusions:

- PD imbibed for 24 and 48h is different from the maintained dormant state.
- Dormancy is not an inactive state in terms of transcription
- There appears to be a large switch in the gene expression programmes from dormant to ND states
- Genes associated with protein synthesis are up-regulated in ND compared to dormant states
- Data is consistent with synthesis of ABA to maintain dormancy
- Dormant up-regulated genes include those encoding heat-shock proteins and LEA proteins
- Non-dormant up-regulated genes include those encoding cell cycle proteins, ribosomal proteins, expansins and cell wall modifying proteins