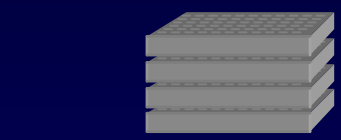
DNA Microarrays of Fission Yeast

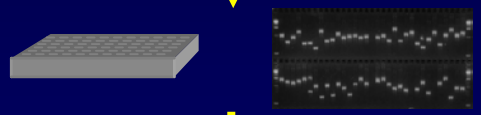
http://www.sanger.ac.uk/PostGenomics/S_pombe

The Wellcome Trust Sanger Institute / Cancer Research UK

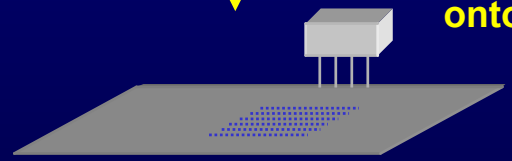
Steps for DNA microarray experiments



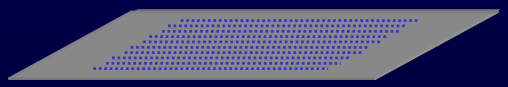
gene-specific primer pairs



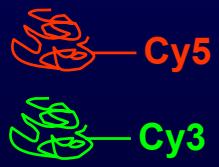
PCR products



DNA probes spotted onto glass slides



DNA fixed to slides, array processing



+

fluorescent ss cDNA targets

pooled targets, differential hybridization

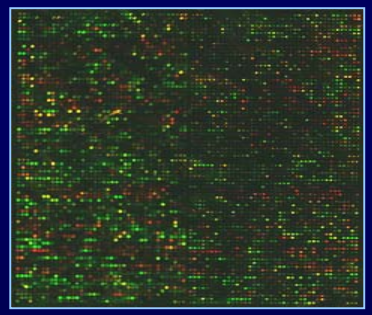
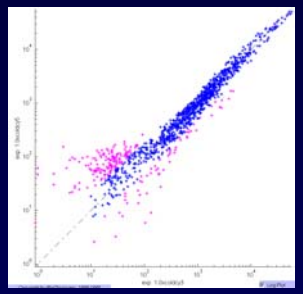
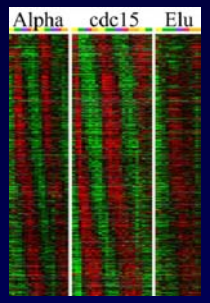


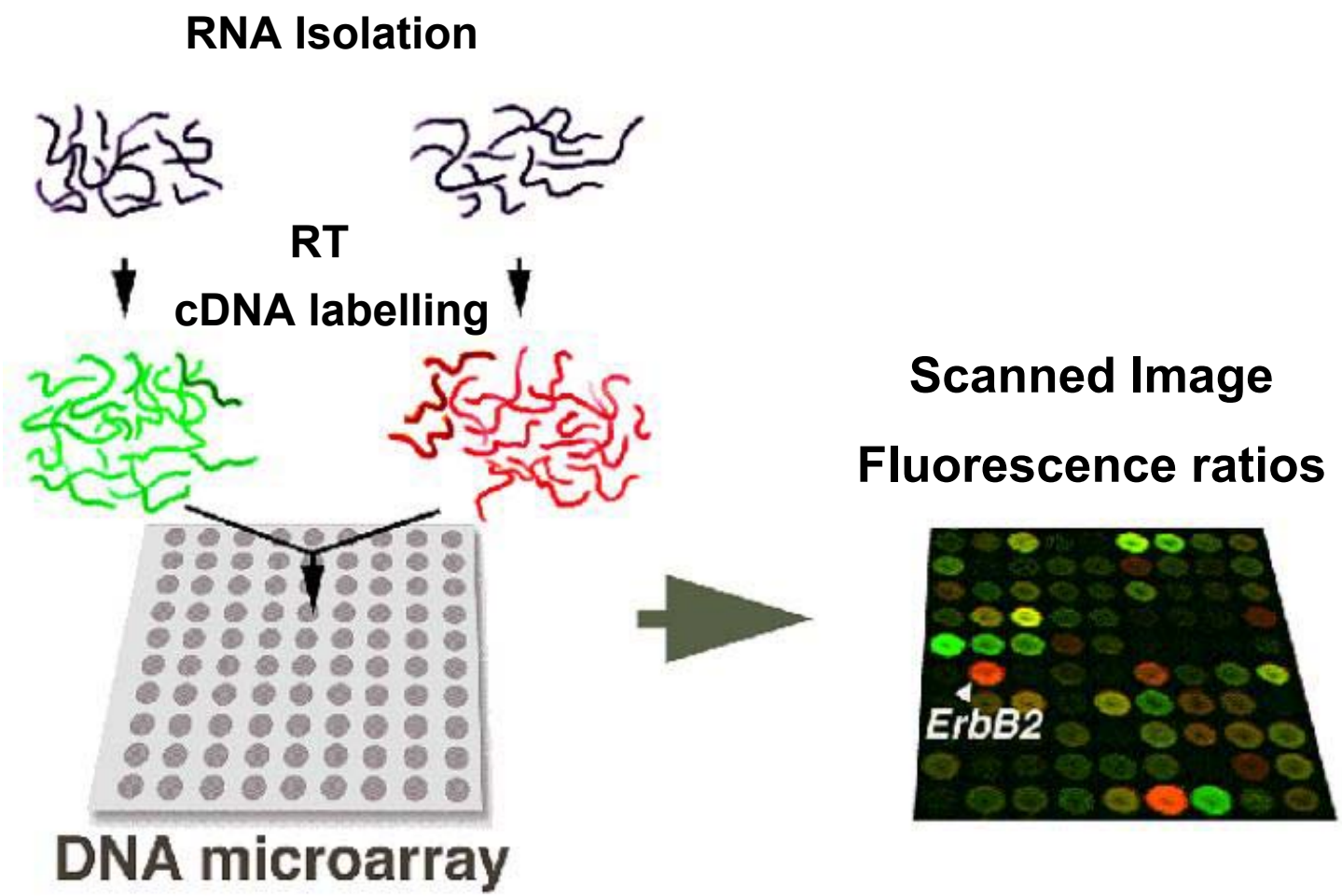
image acquisition, scanner



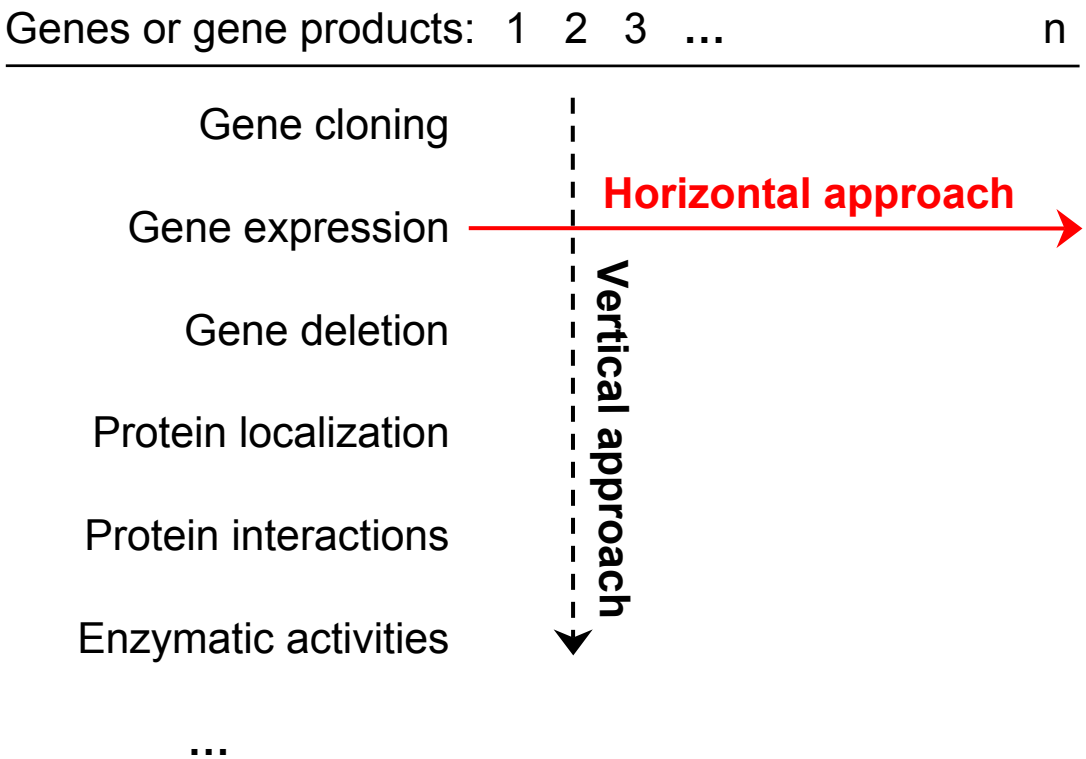


ratio calculation & data evaluation

Principle of Differential Hybridization



Post-genomic vs traditional experiments:

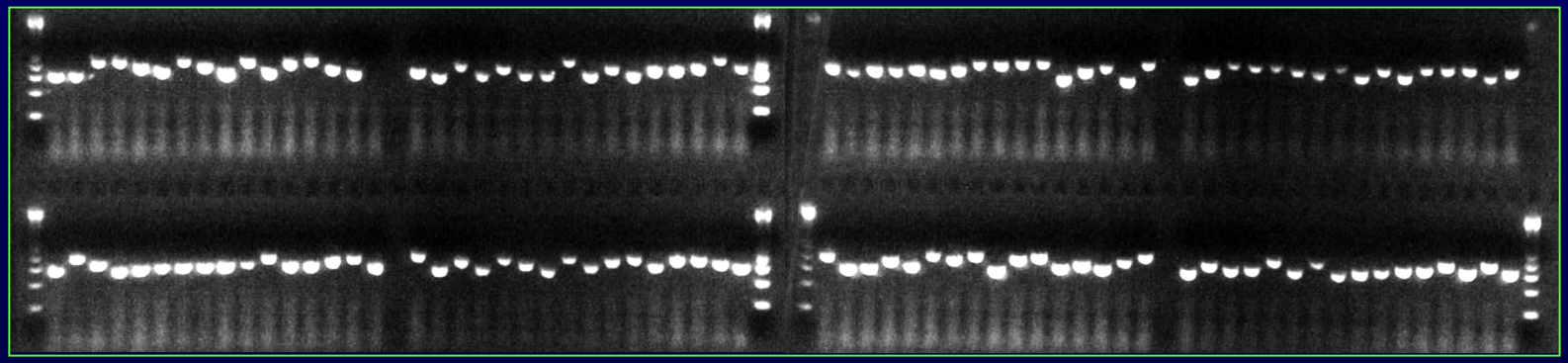


Primer Design

- Primer length: 18-22 bp
  (+ 8 bp universal sequence for forward primers)
- Annealing temperature: 58-62°C; GC content: 40–60%
- Product length: 200–500 bp; <2500 bp from gene end
- Products are 100% exon sequence
- Products have Blast score <400
  with other fission yeast sequences
- All ORFs (nuclear and mitochondrial),
  'pseudogenes', introns, bacterial control genes

Probe Preparation

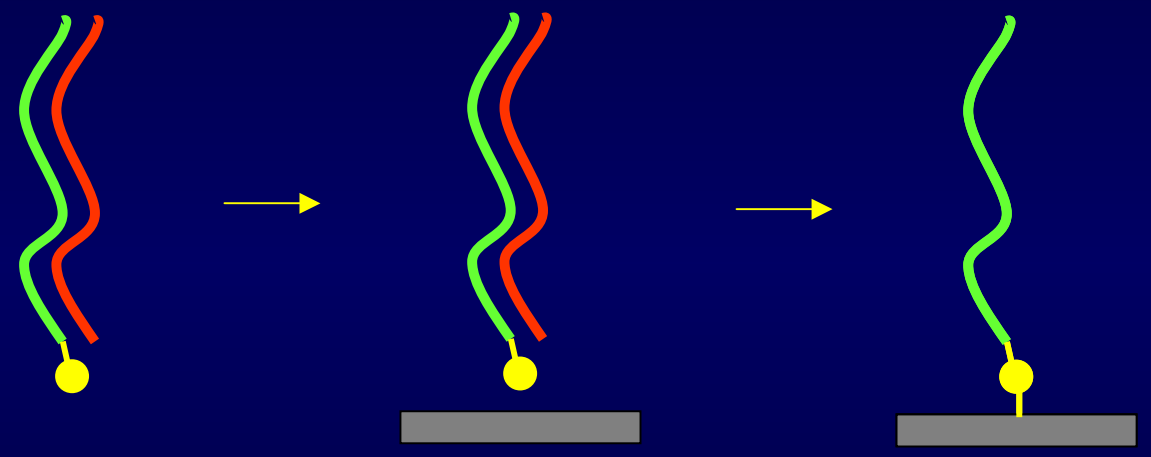
- 1st round PCR: gene-specific primer pairs, forward primer with universal sequence
- 2nd round PCR: 5'-aminolink universal forward primer, gene-specific reverse primer
- All PCR products checked on agarose gels: ~3% failures



- Add spotting buffer (currently ~6,000)

5’-aminolink surface chemistry

- covalent attachment of DNA via 5’-aminolink modification
- single-stranded probes can be made after attachment



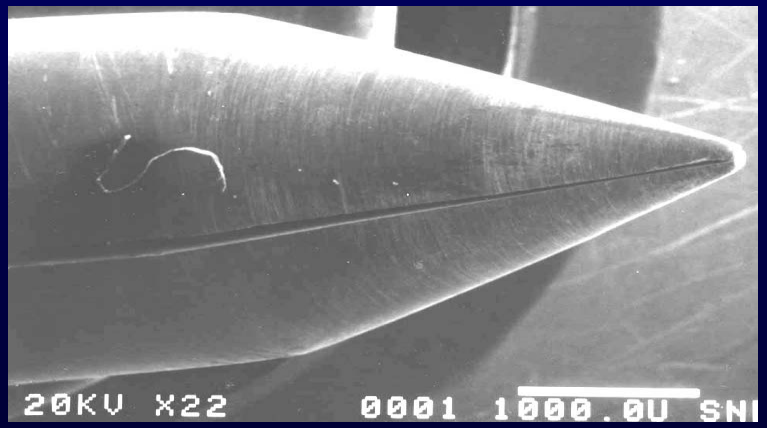
- entire length of probe accessible for hybridization
- high binding efficiency of DNA to the slide (80-90%)
- high sensitivity

Arrayer

Biorobotics MicroGrid II

- 48 pins
- 200 μm spots, 250 μm spacing
- >20,000 spots/slide
- 100 slide capacity

Split pin used for printing



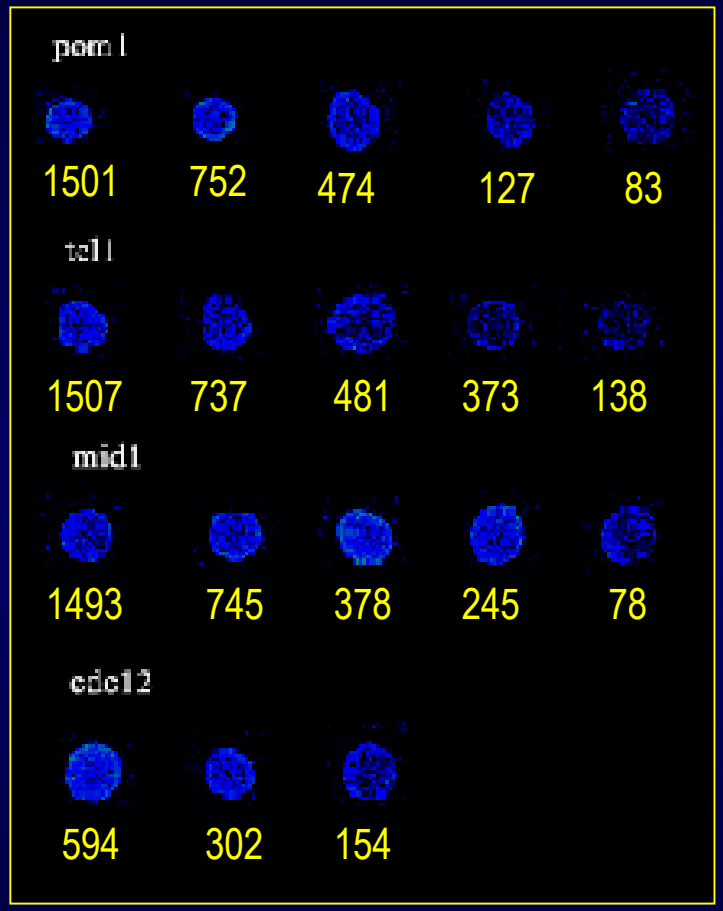
Scanner

- 5µm resolution
- simultaneous dual laser scanning
- GenePix analysis software

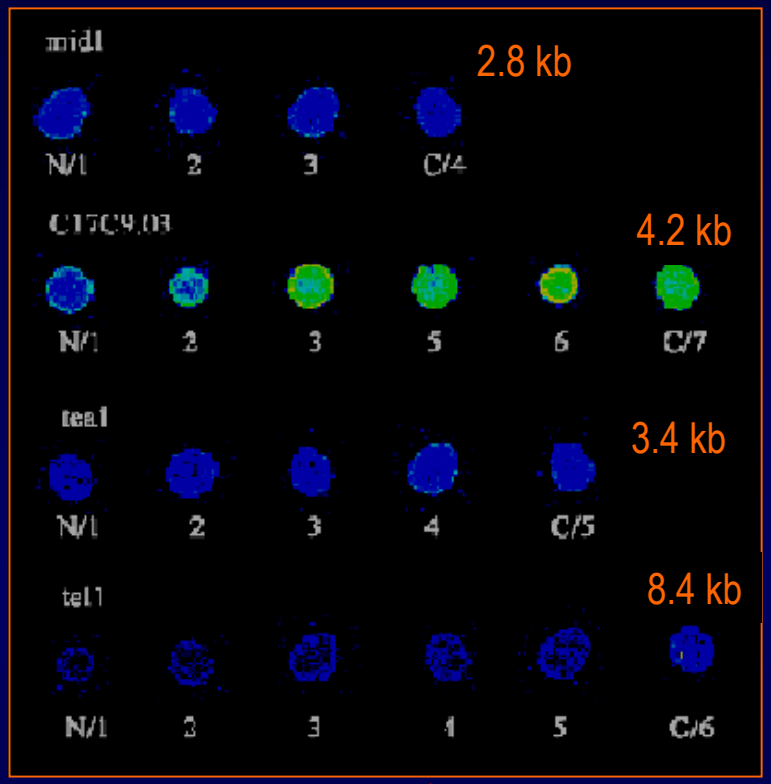
Genepix 4000B (Axon)

Various tests and controls

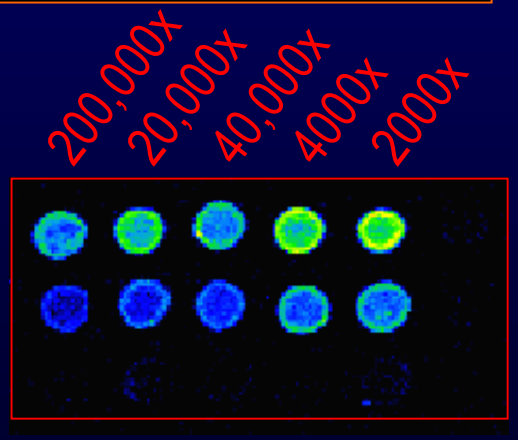
Probe size and signal strength:



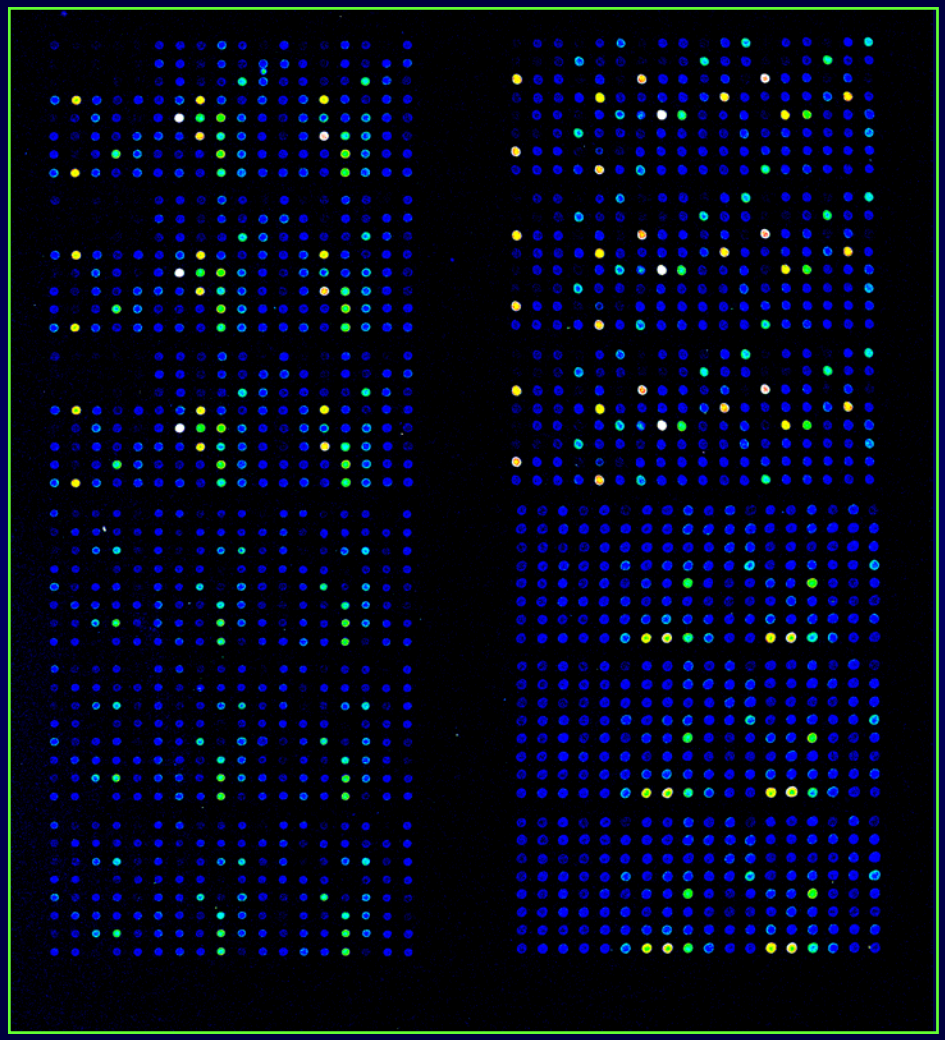
Probe position and signal strength:



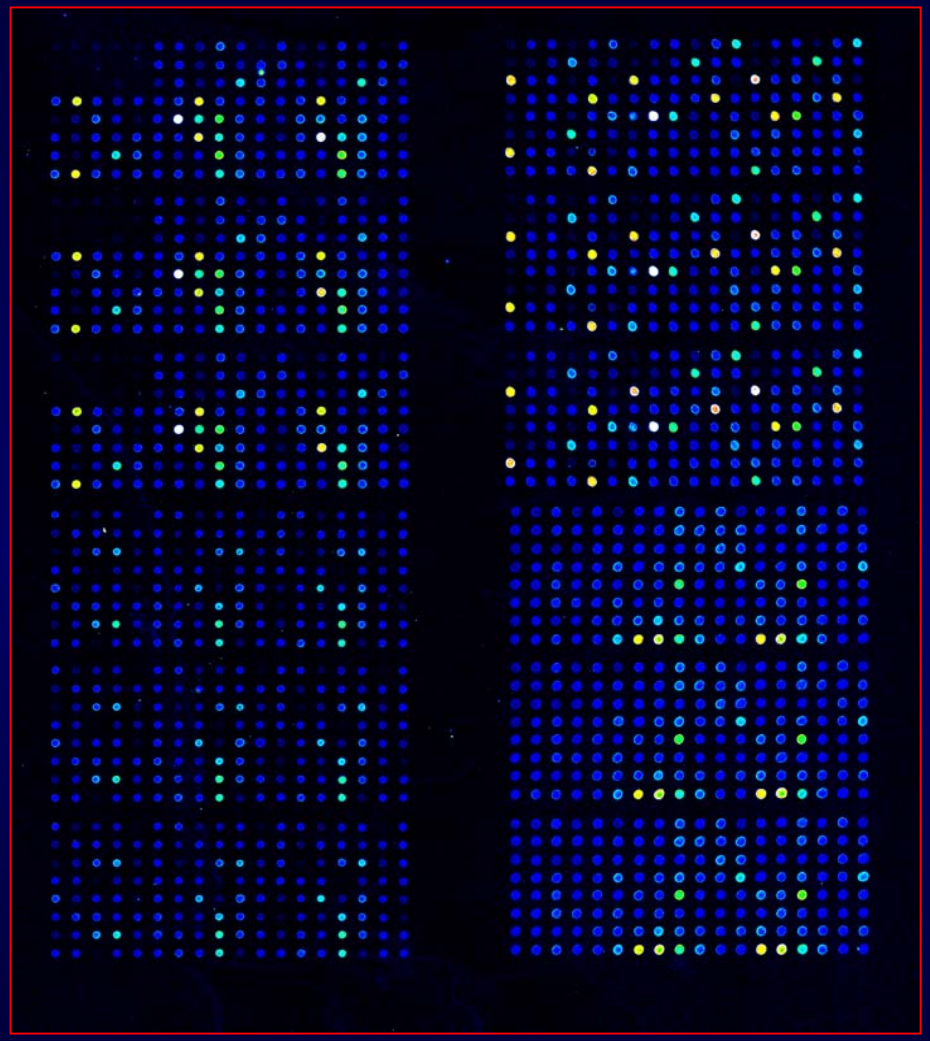
Positive and negative controls:



Self vs Self: same target



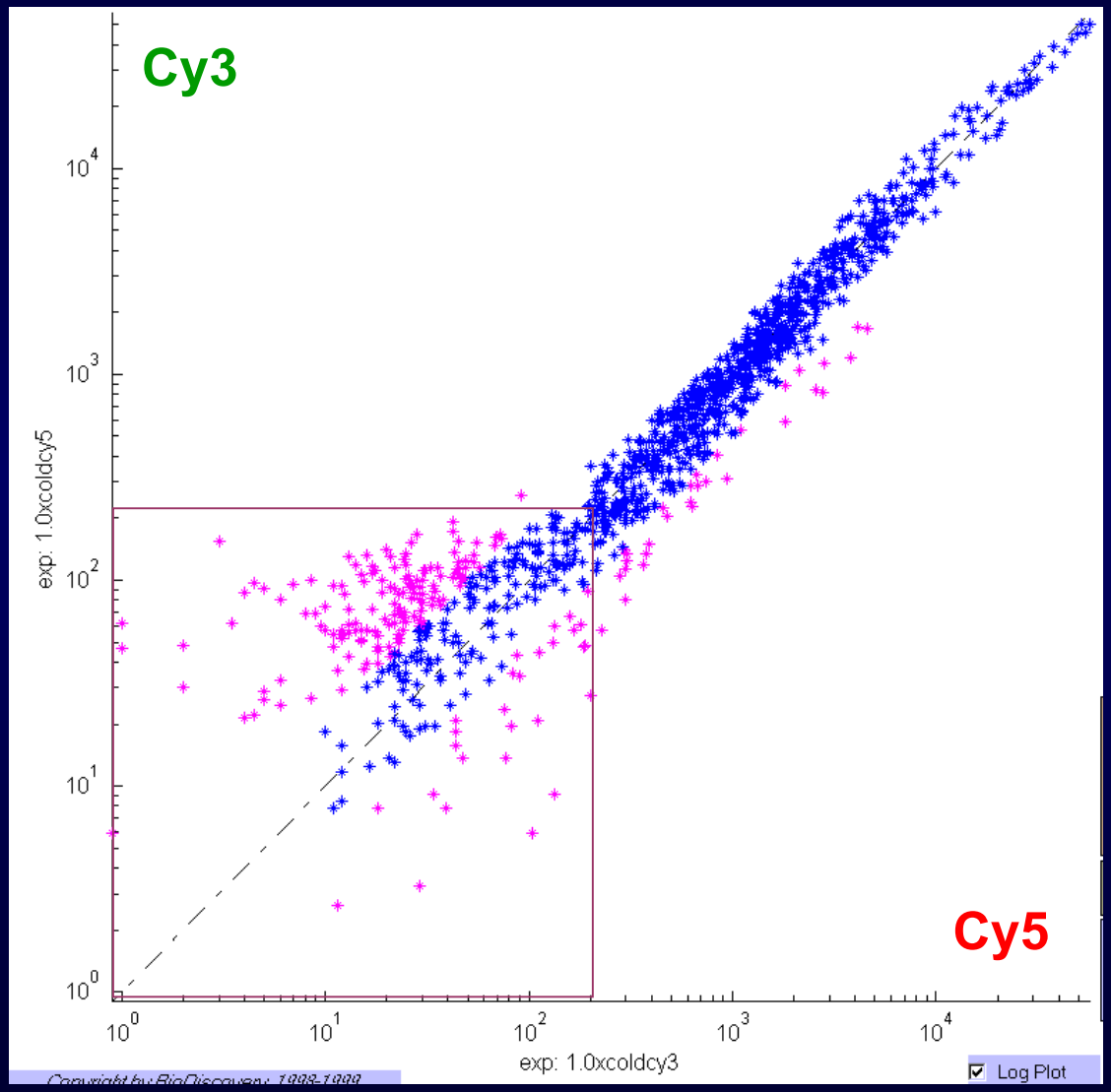
Cy3



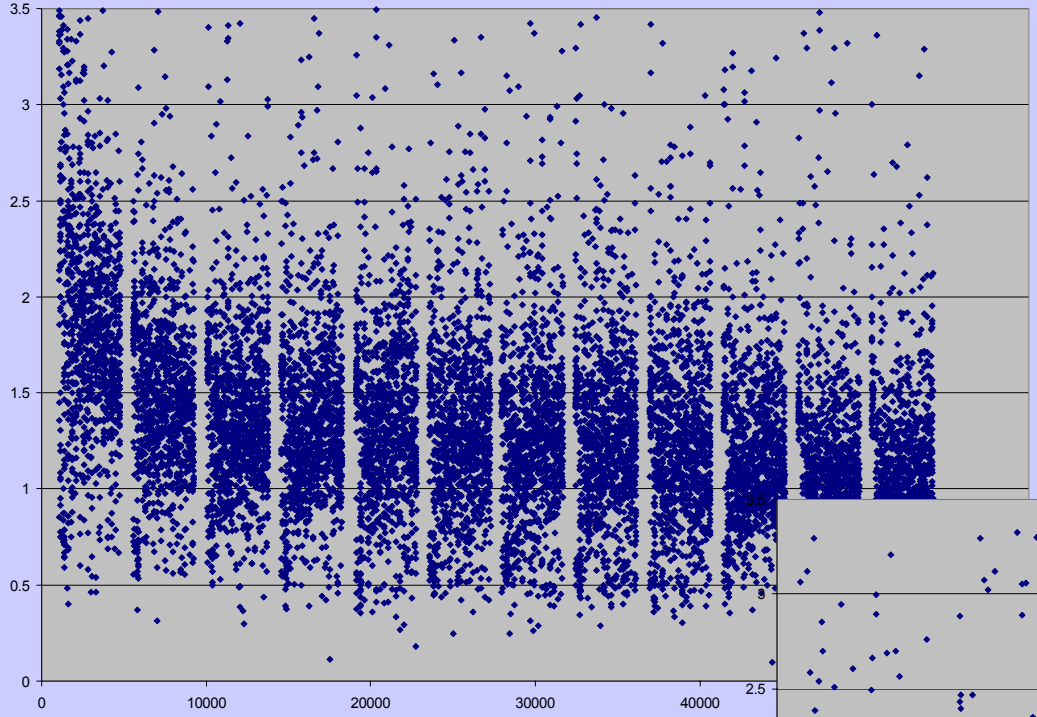
Cy5

Self vs Self: Quantitation

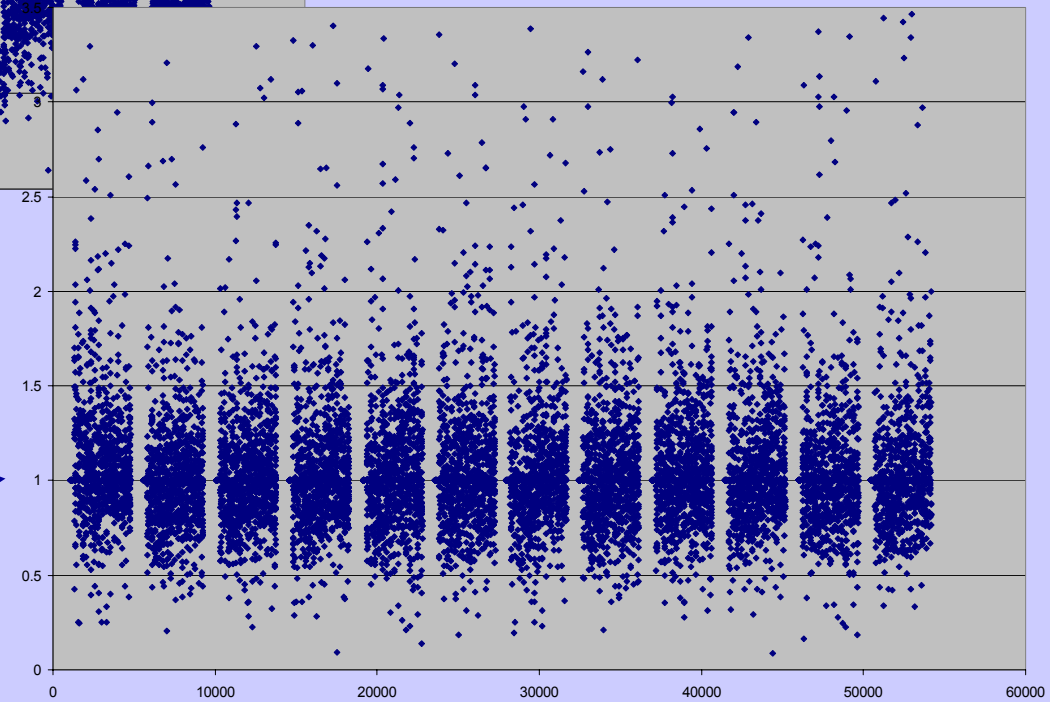
- 90% of genes show less than 2-fold variation
- 20% of genes show no or very low expression – 50% of these genes show greater than 2-fold variation
- 80 % of genes show mod. low to high expression – 99% of these genes show less than 2-fold variation
- ↓ expression level of a gene, ↓ confidence that expression differences are significant



Localized Normalization:

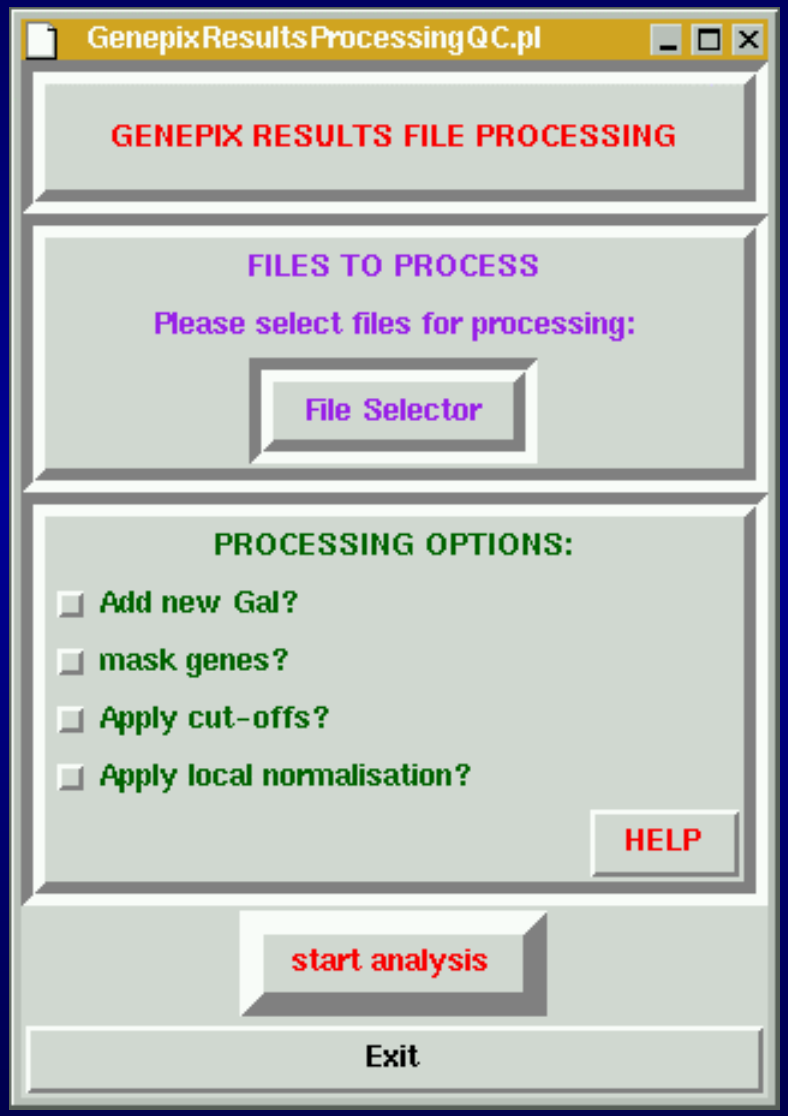


ratios depend
on slide position

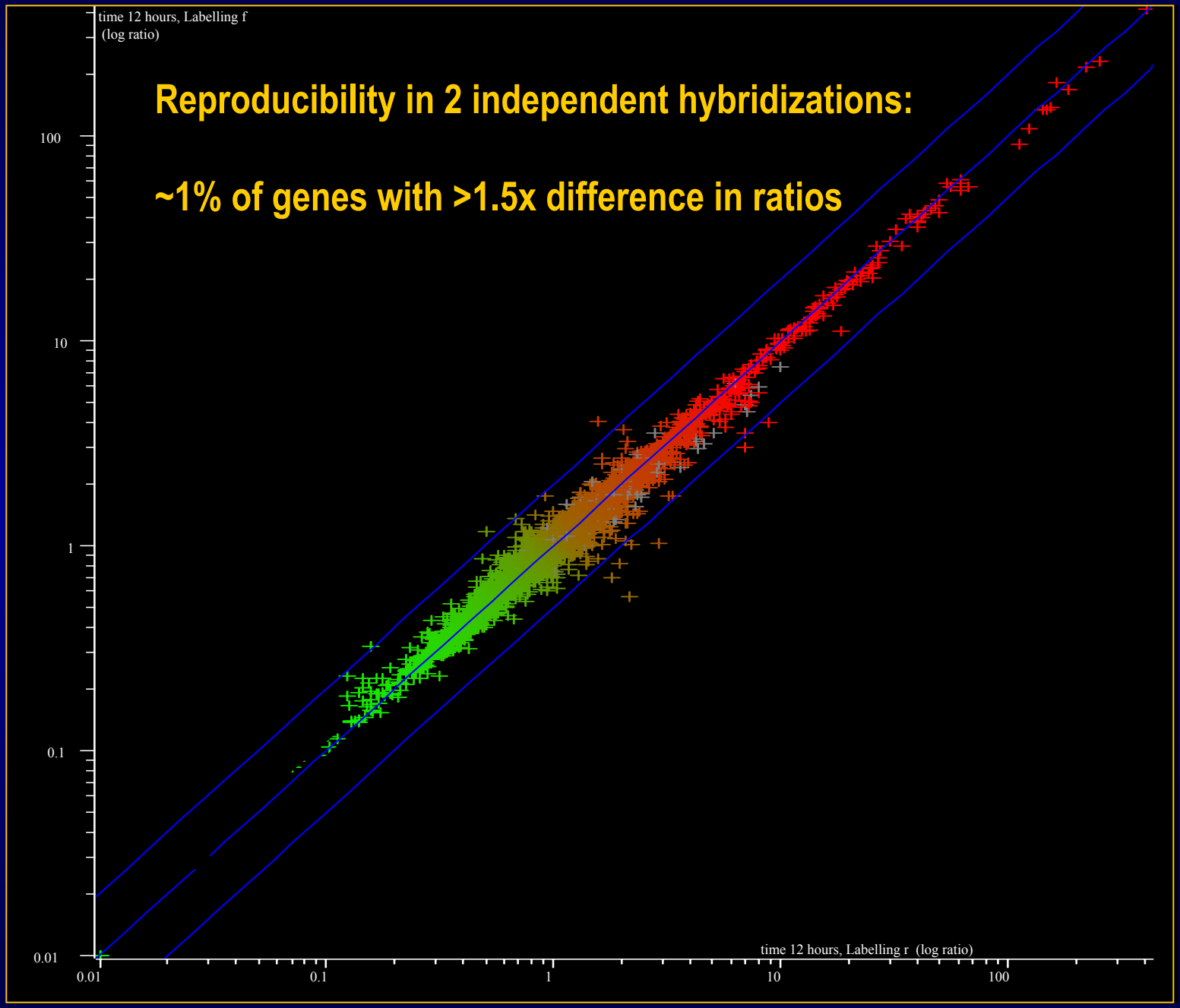


after normalization
(running window
of ~1000 spots)

Initial Data Processing



- InHouse program to perform initial data processing
- Failed primers are flagged as “absent”
- Spots with < 50% of pixels >2 SD above background are flagged as “absent”.
- Local normalisation to correct for asymmetries between cy3 and cy5 dyes.
- A quality control report is produced which reports the number of poor quality spots on the array and duplicated spots showing poor reproducibility



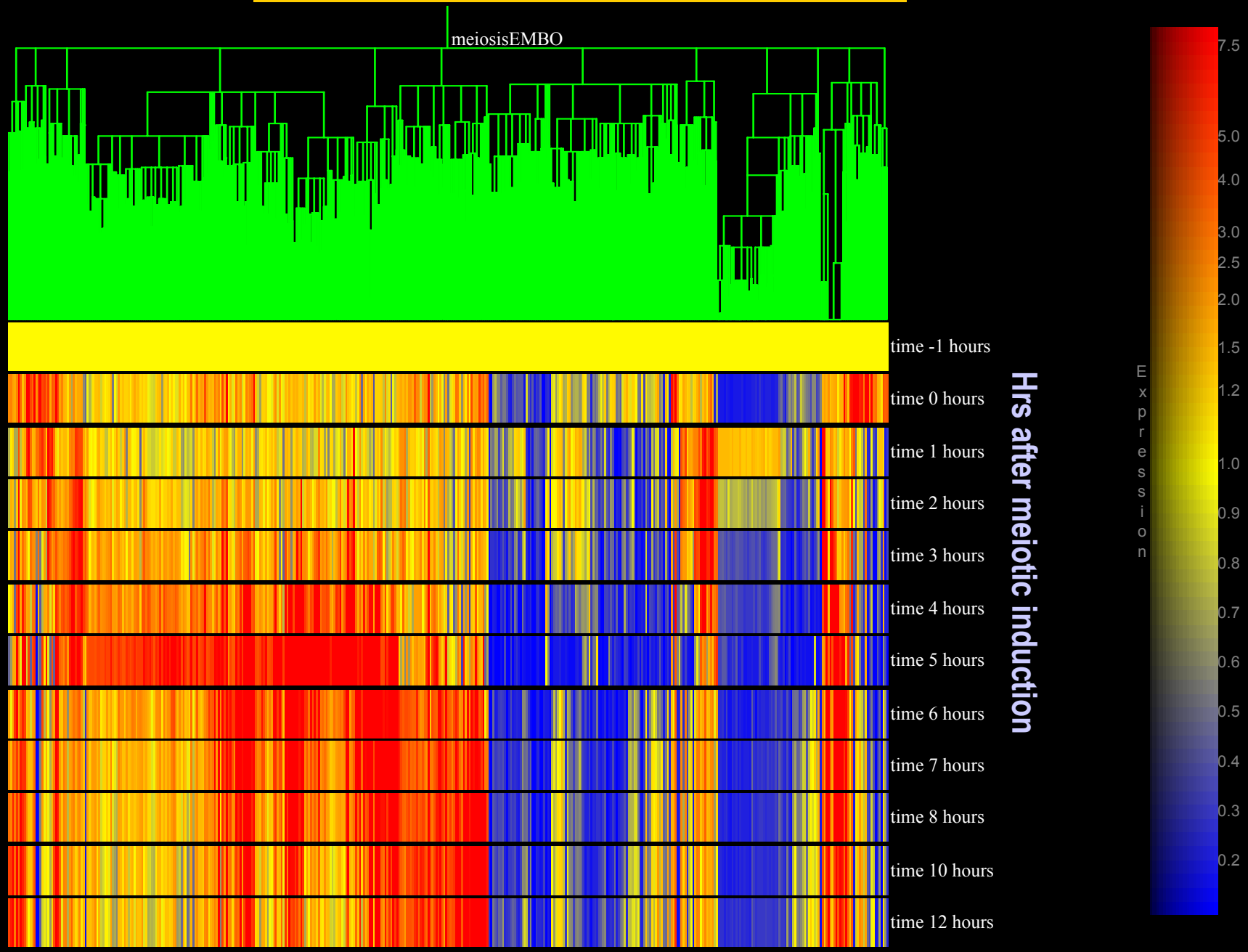
Reproducibility in 2 independent hybridizations:

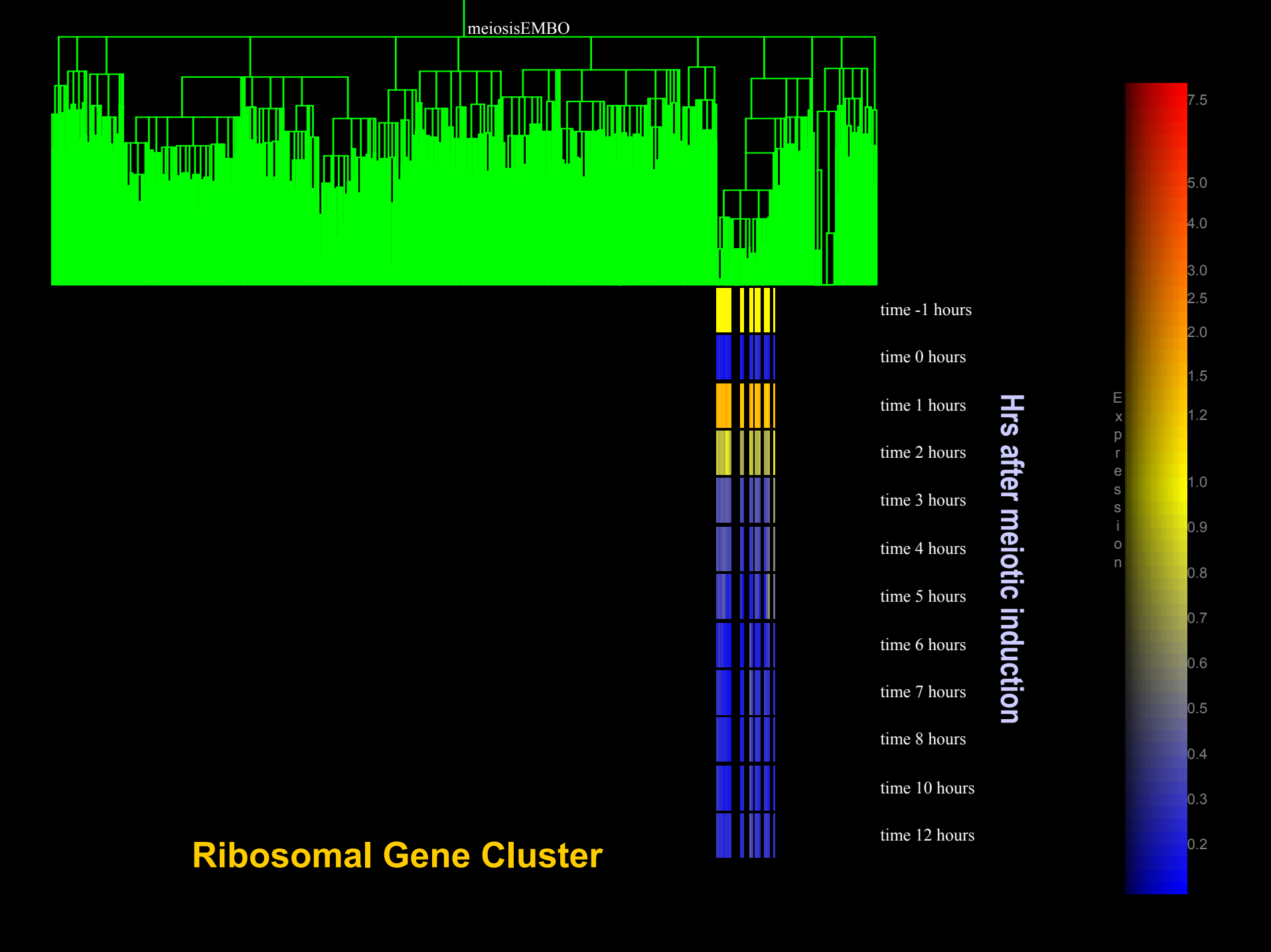
~1% of genes with >1.5x difference in ratios

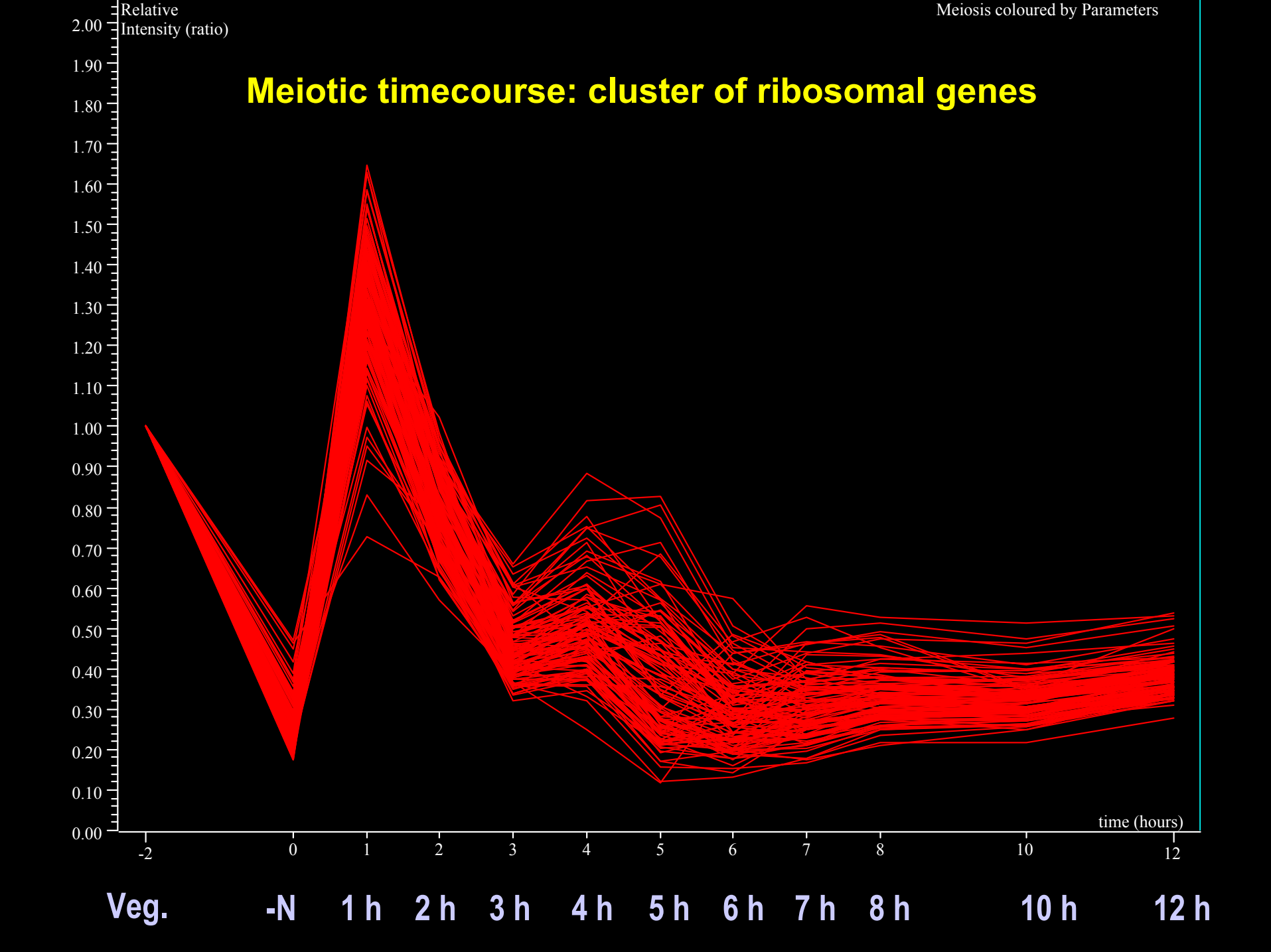
Data Processing Pipeline

- GenePix Image Analysis Software
- InHouse program for further data processing
- Data analysis using Genespring (Silicon Genetics) and some other software (Cluster, SAM, ArrayMiner…)
- Oracle Database – MIDAS:
  Plate tracking and storage of raw data

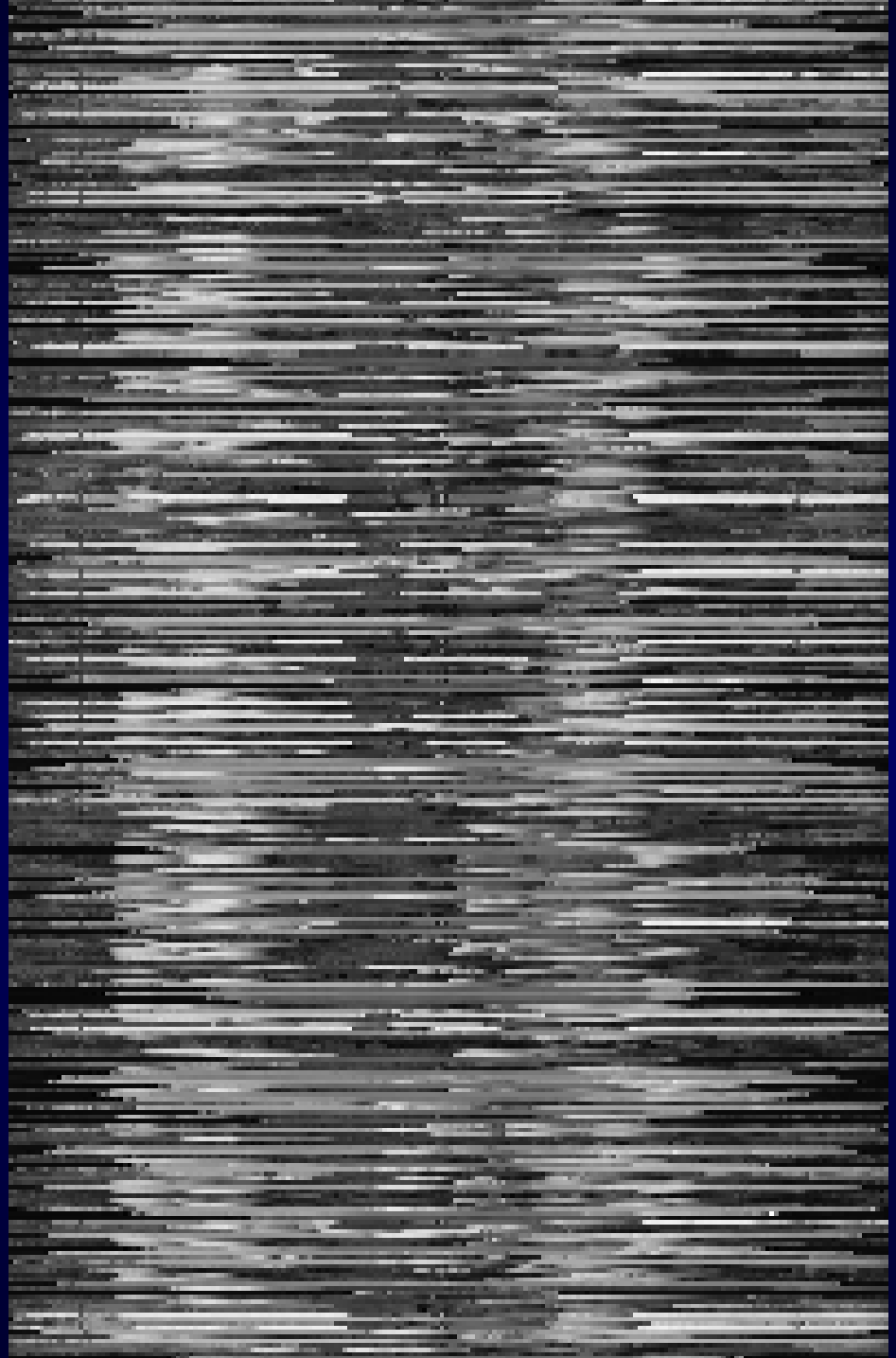
Hierarchical Gene Clustering:

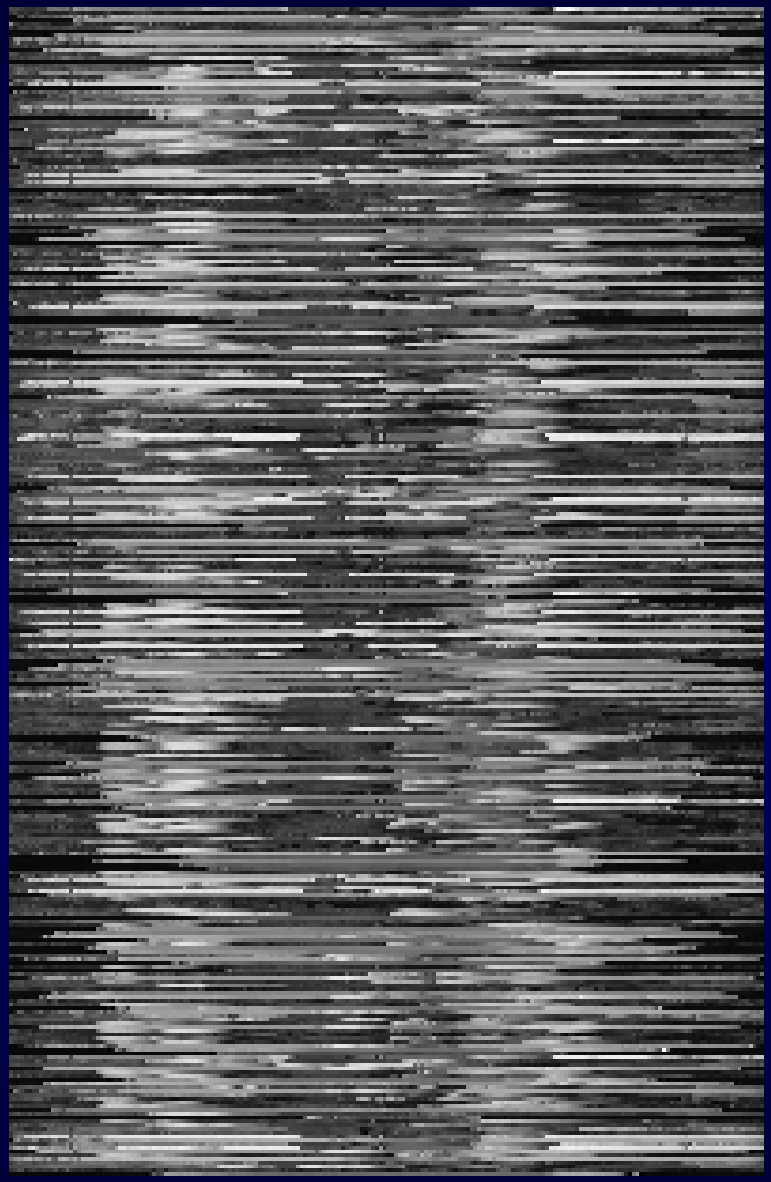


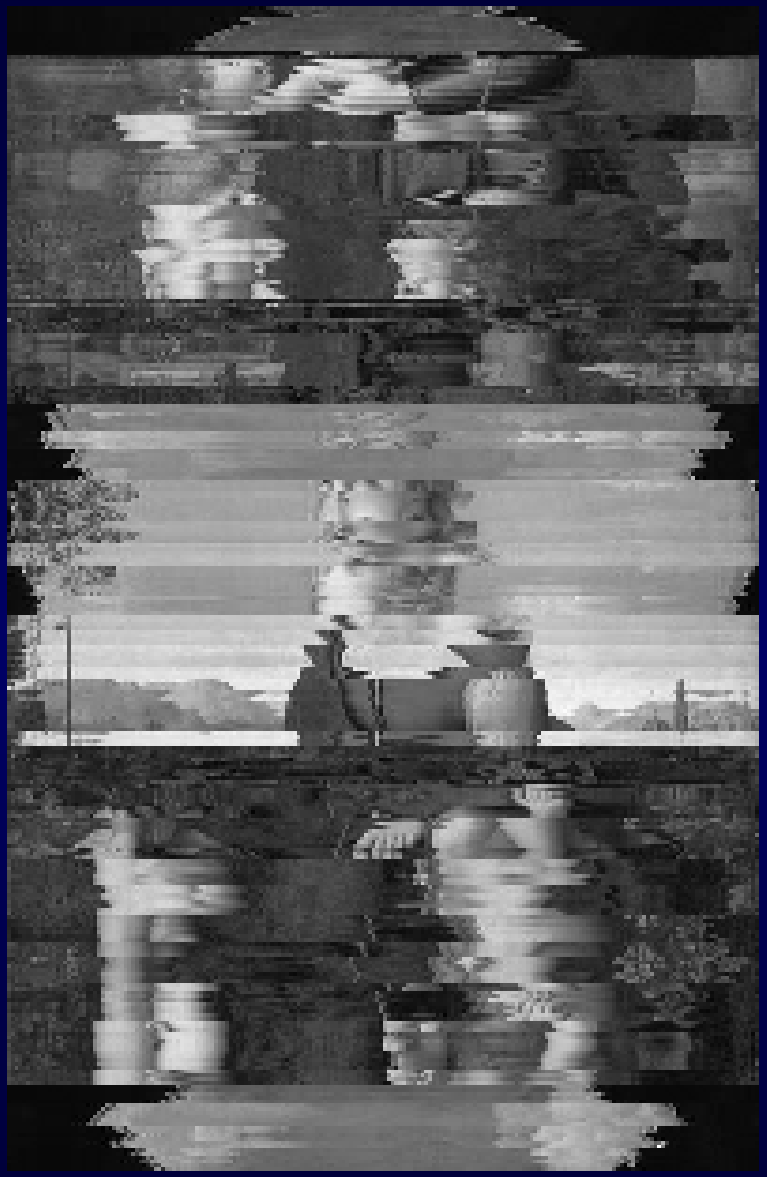

meiosisEMBO
time -1 hours
time 0 hours
time 1 hours
time 2 hours
time 3 hours
time 4 hours
time 5 hours
time 6 hours
time 7 hours
time 8 hours
time 10 hours
time 12 hours
Hrs after meiotic induction
Expression
7.5
5.0
4.0
3.0
2.5
2.0
1.5
1.2
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
Ribosomal Gene Cluster

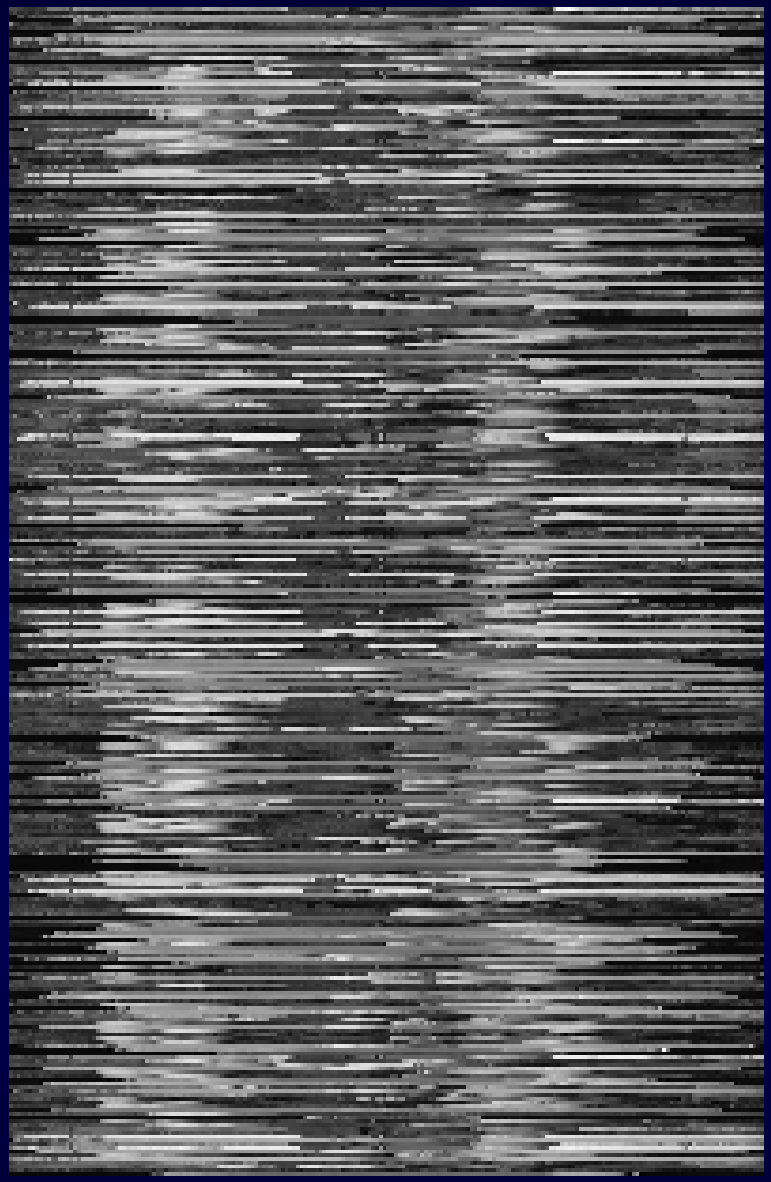

Meiotic timecourse: cluster of ribosomal genes
Relative
Intensity (ratio)
Meiosis coloured by Parameters
2.00
1.90
1.80
1.70
1.60
1.50
1.40
1.30
1.20
1.10
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
time (hours)
-2
0
1
2
3
4
5
6
7
8
10
12
Veg.
-N
1 h
2 h
3 h
4 h
5 h
6 h
7 h
8 h
10 h
12 h

Mike Eisen's demonstration of clustering process:

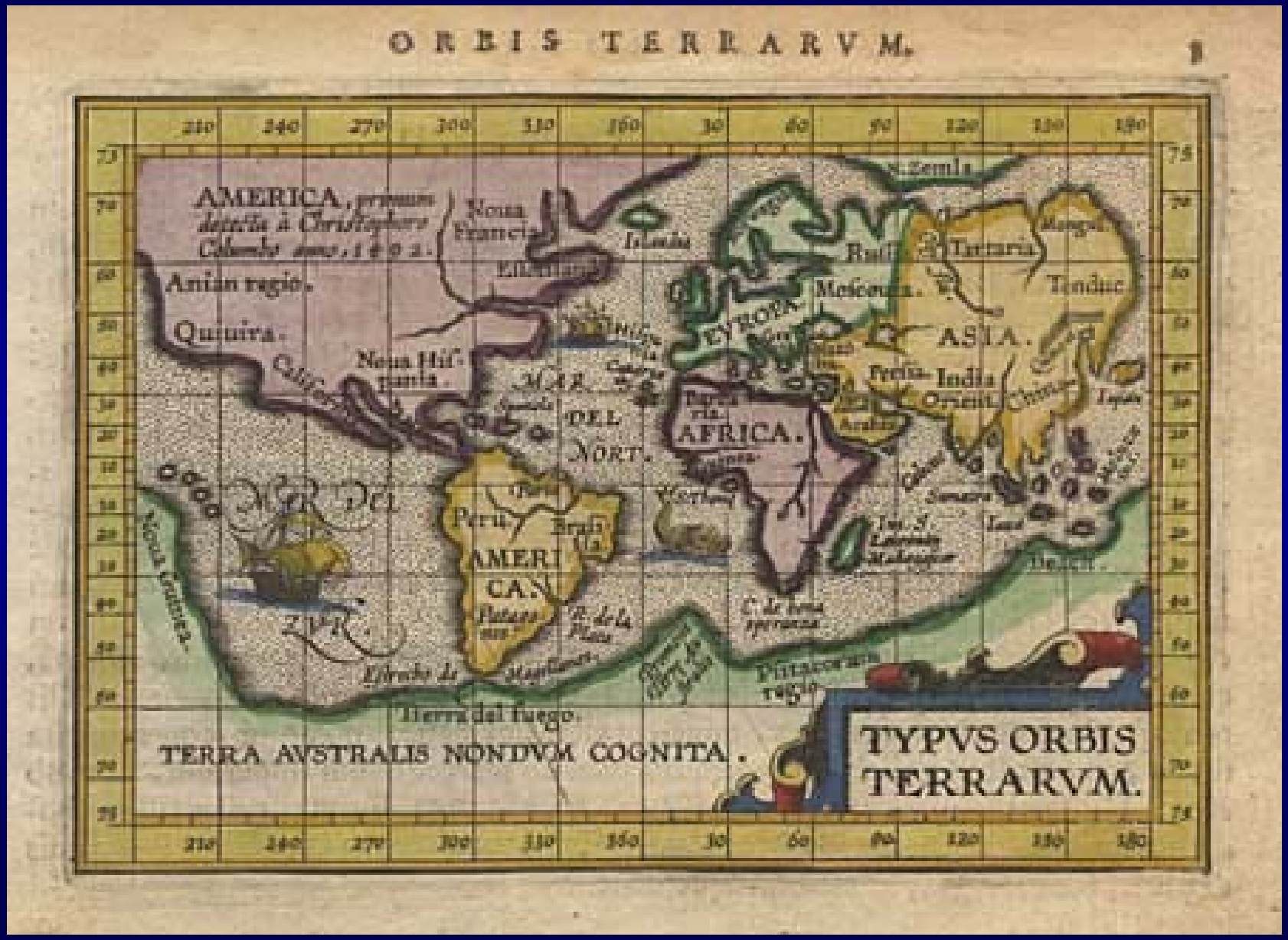


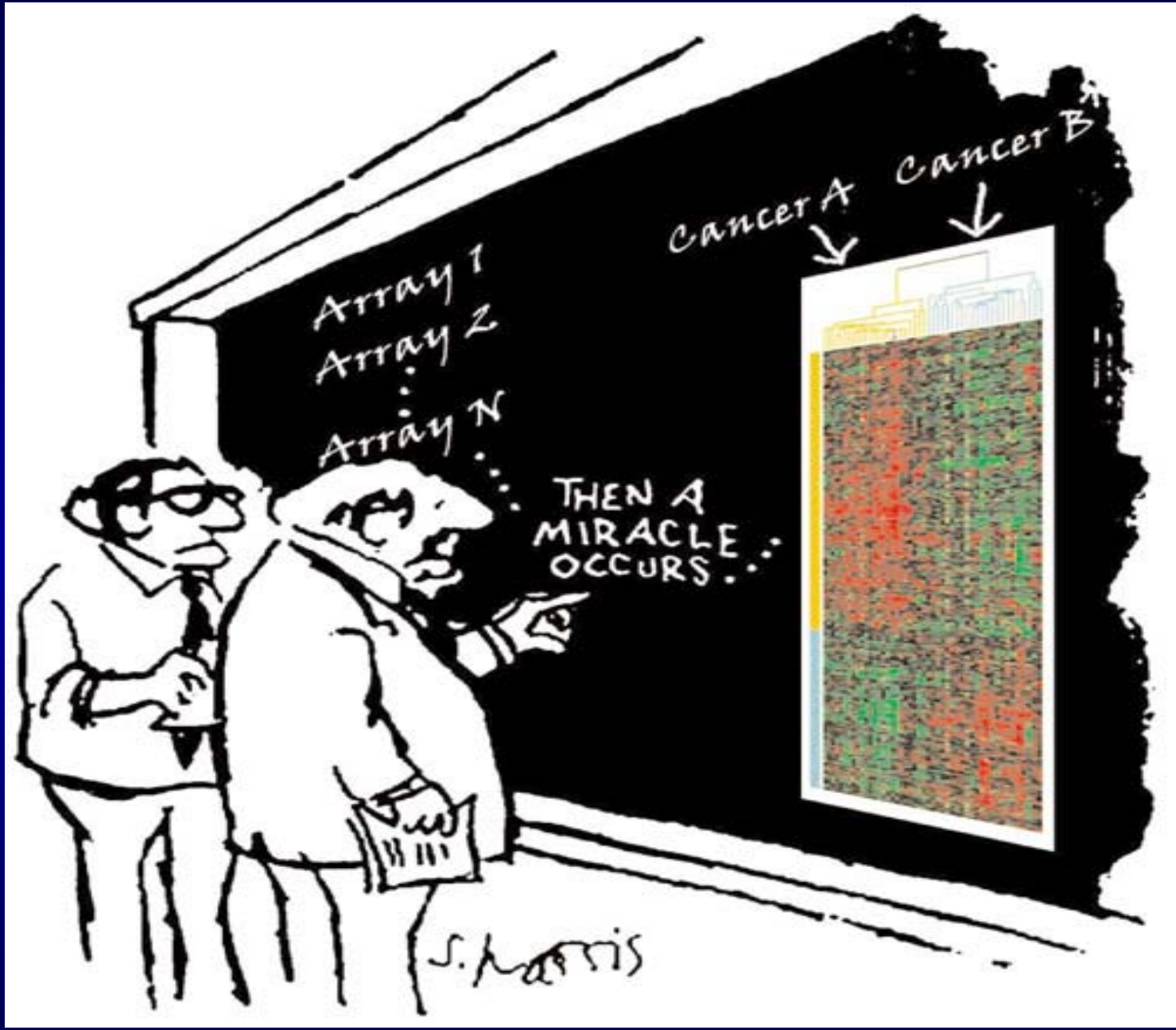






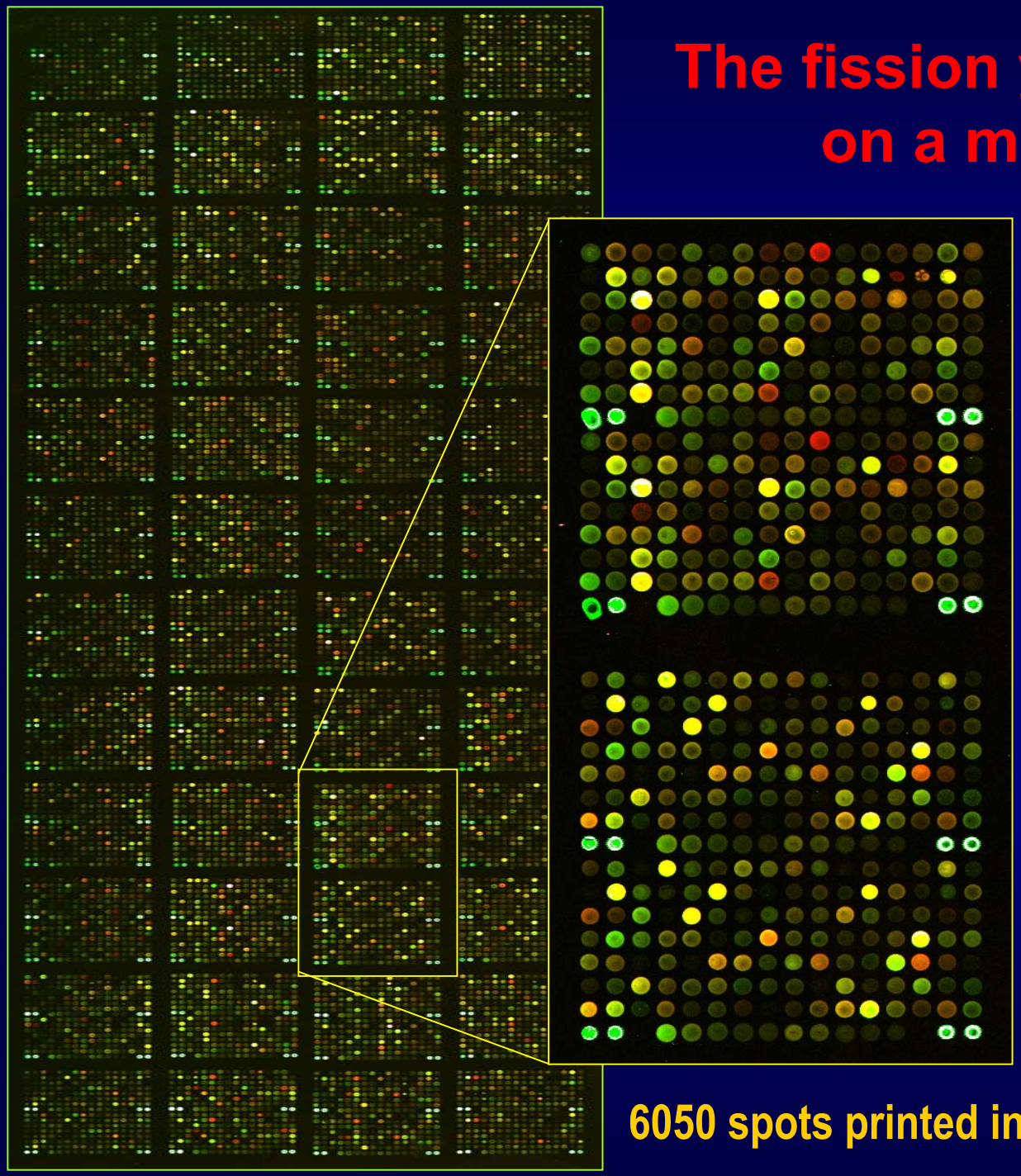
Global map as an approximation of reality:





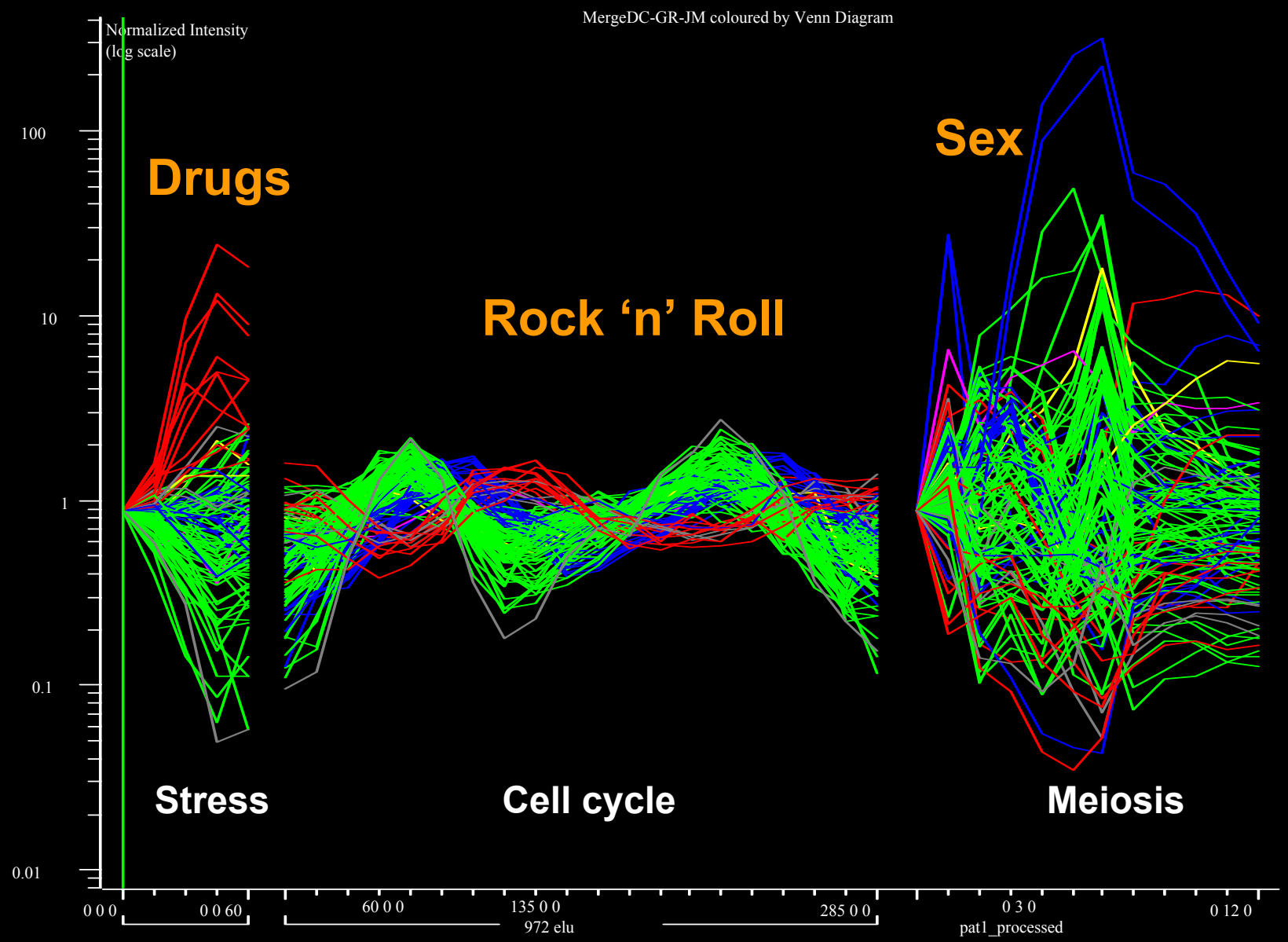
"I think you should be more explicit here in step 2."

The fission yeast genome on a microarray



6050 spots printed in duplicate: 12,100 spots

Three main projects:



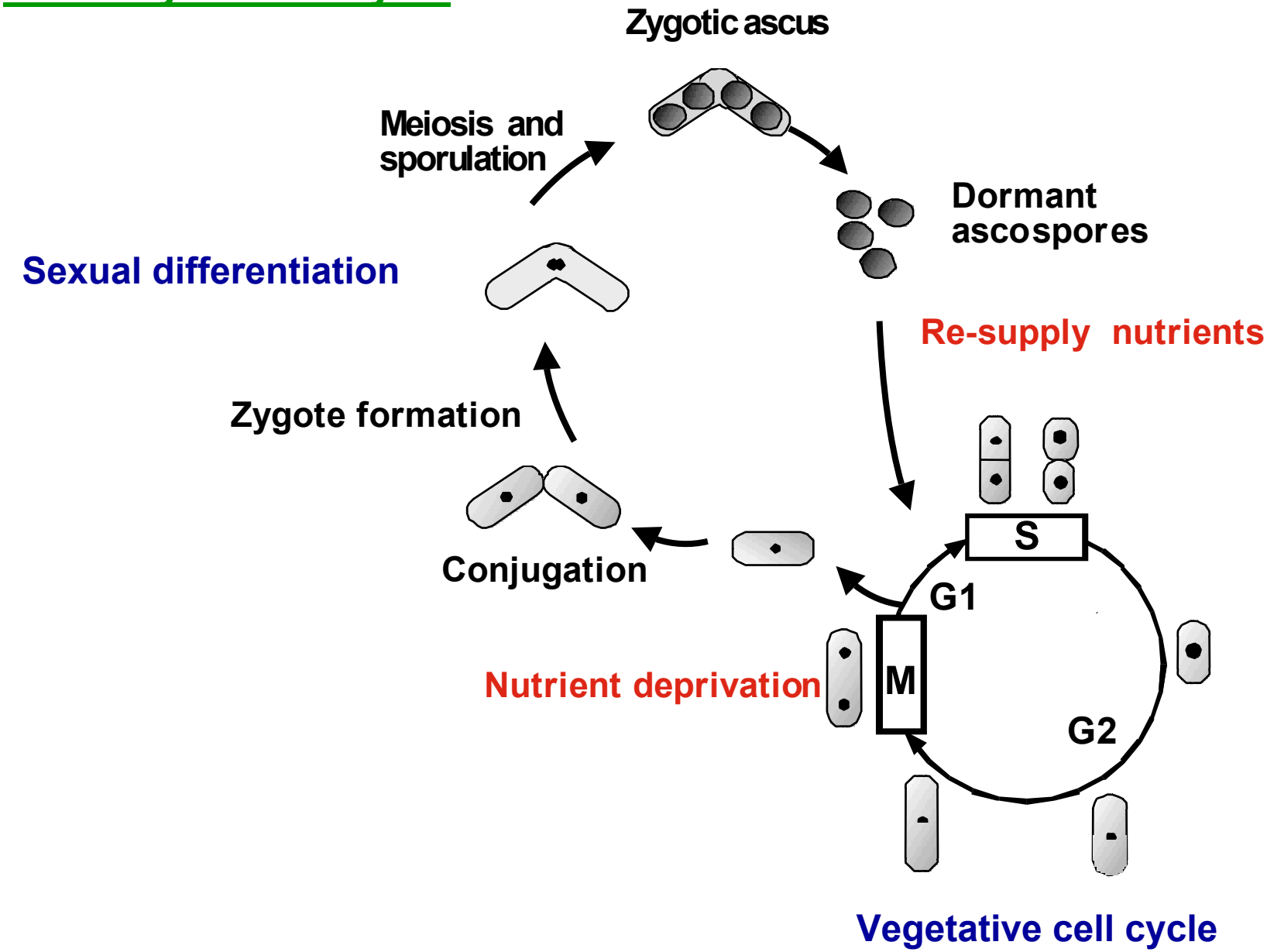
Expression Profiling During Sexual Differentiation:

Juan Mata

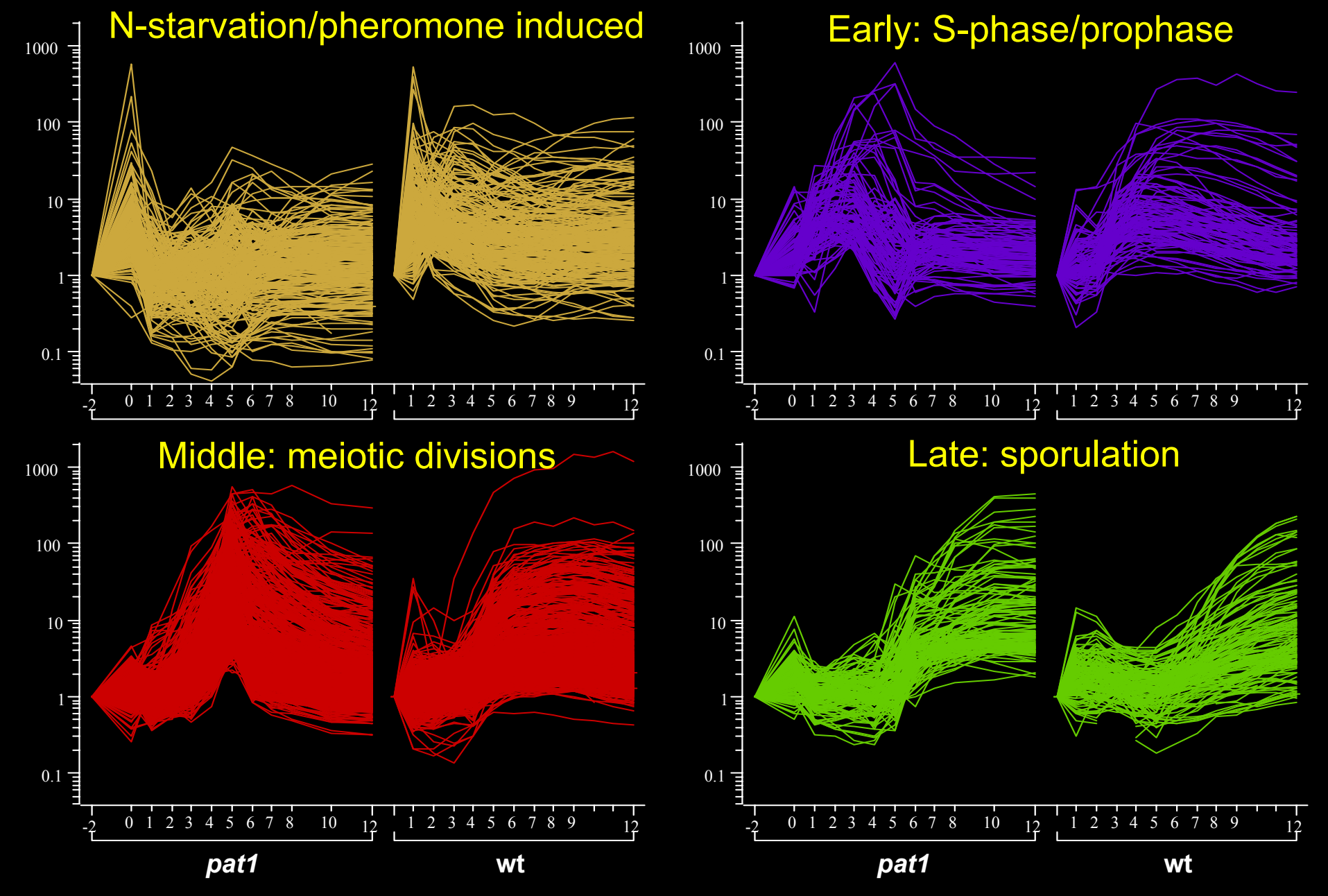
- synchronized meiotic cell cultures
- transcription factor mutants

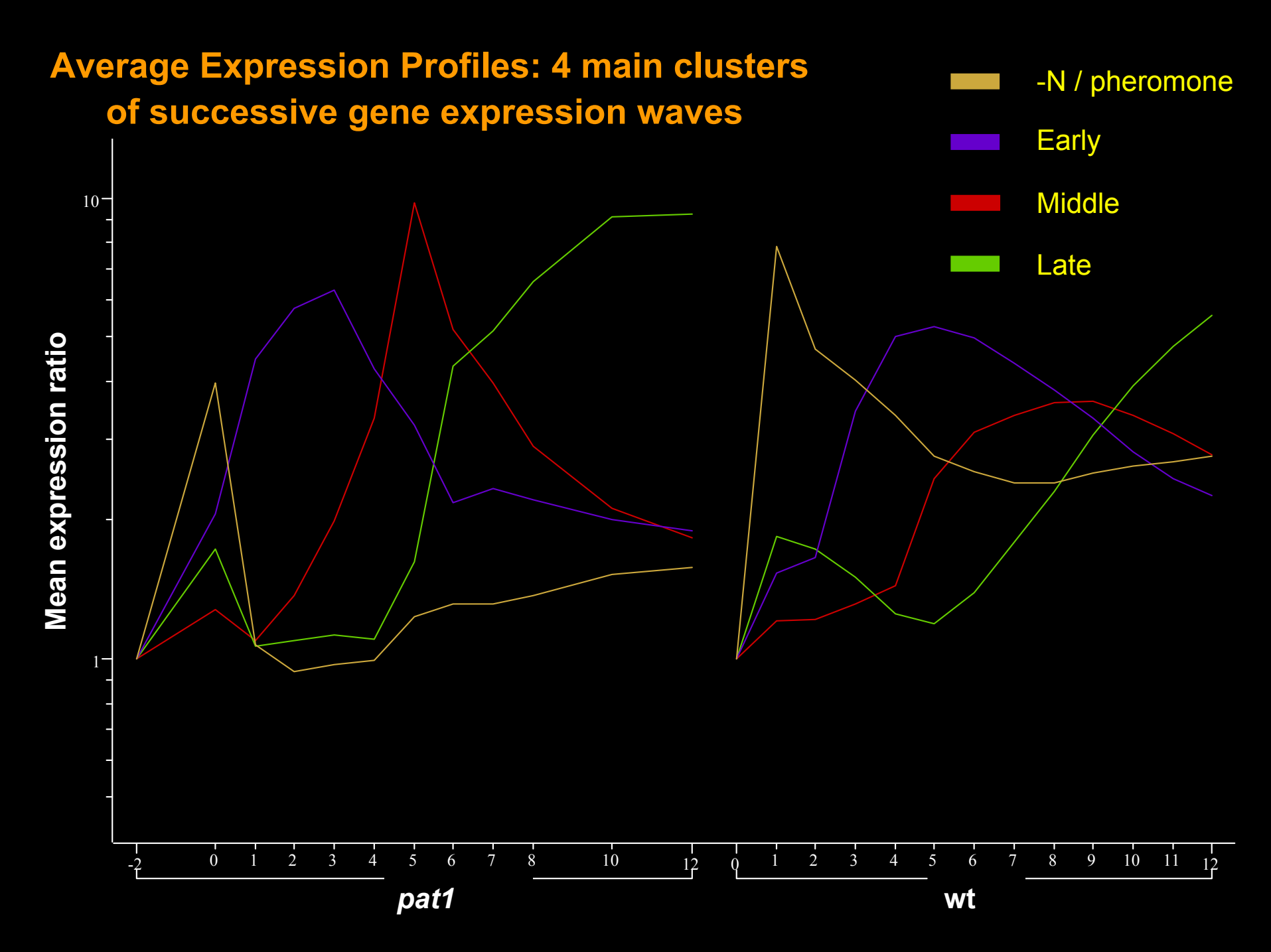
- genome-wide transcriptional program
- stage-specific gene expression
- regulatory sequence motifs and circuits

Fission yeast life cycle:

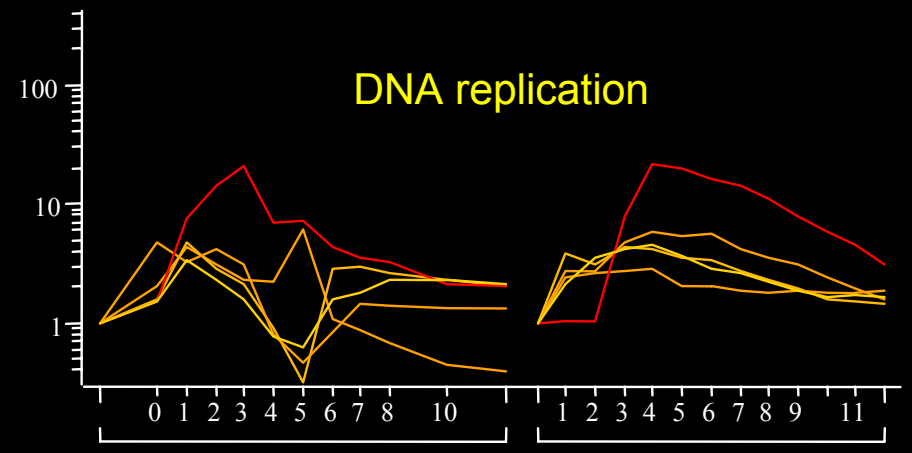


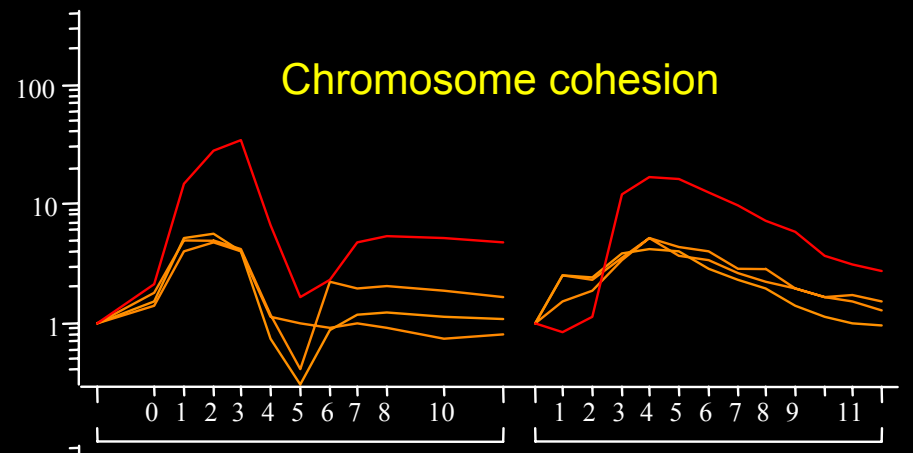
Meiotic timecourses: major clusters of gene expression

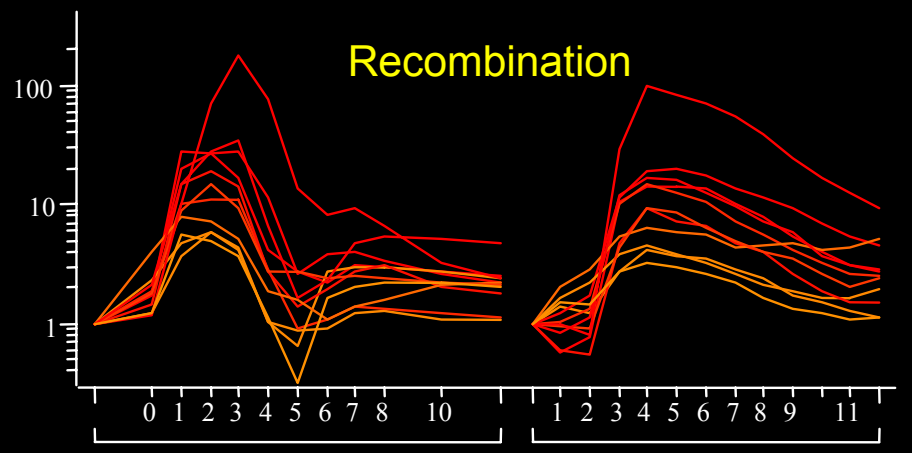


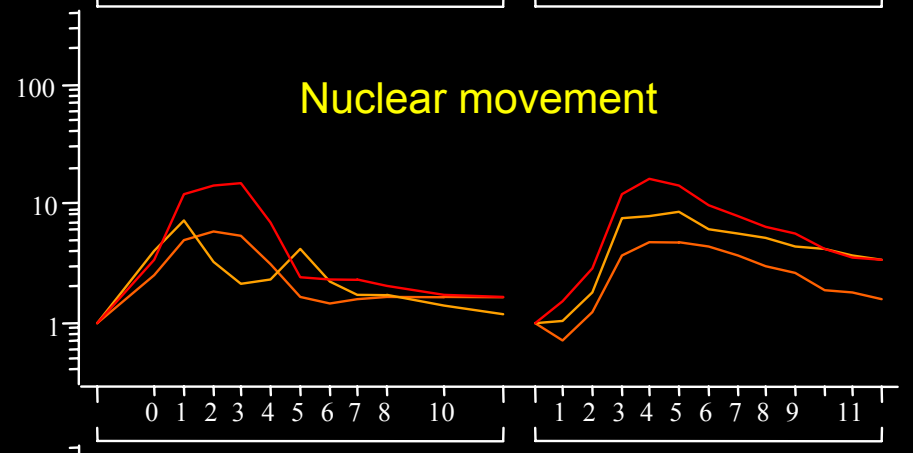

Average Expression Profiles: 4 main clusters
of successive gene expression waves
-N / pheromone
Early
Middle
Late
Mean expression ratio
10
1
-2
0
1
2
3
4
5
6
7
8
10
12
0
1
2
3
4
5
6
7
8
9
10
11
12
pat1
wt

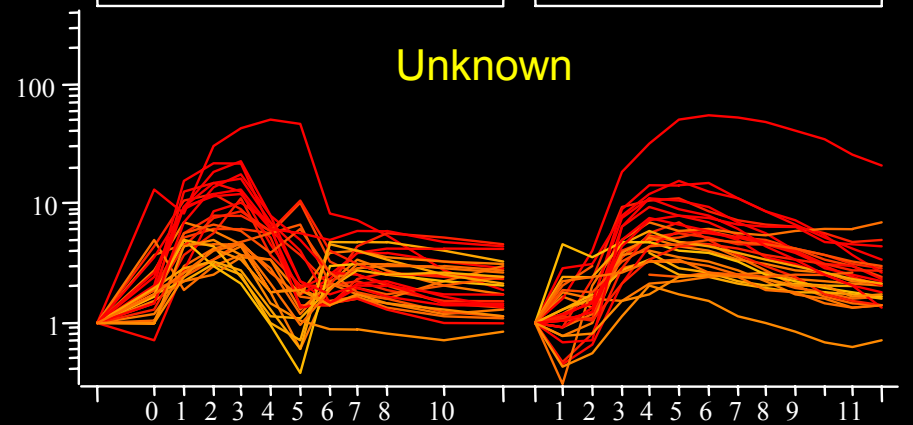
Functions of early genes:



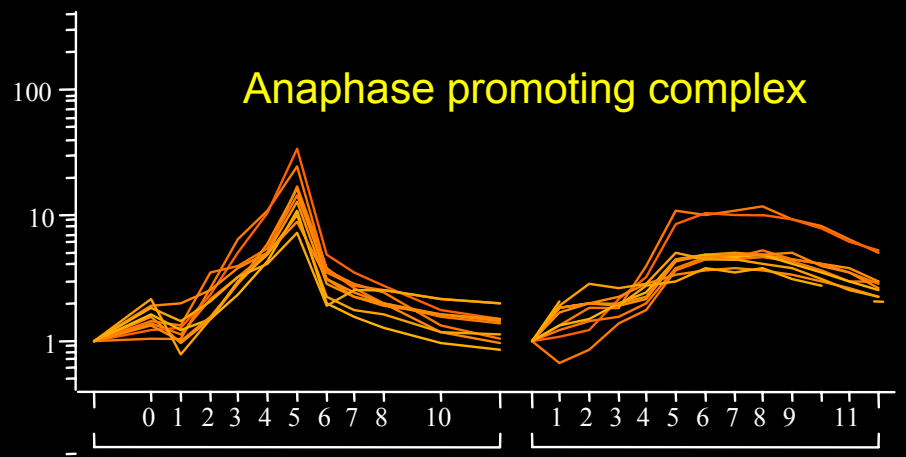


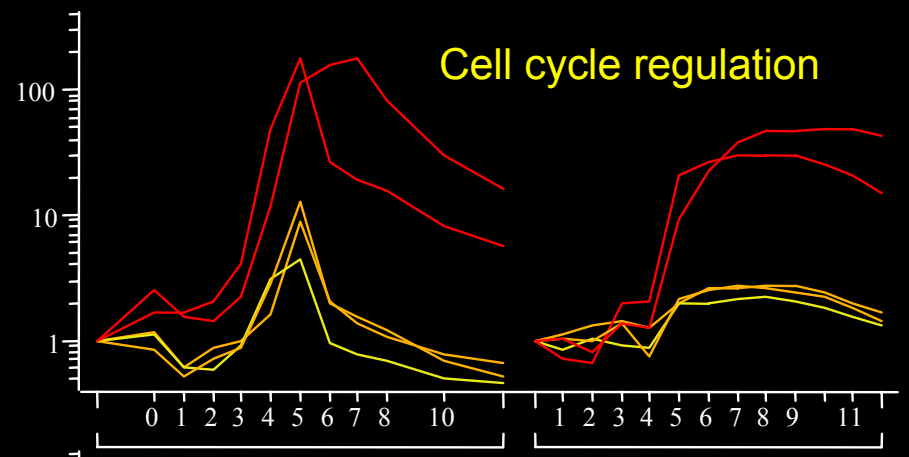


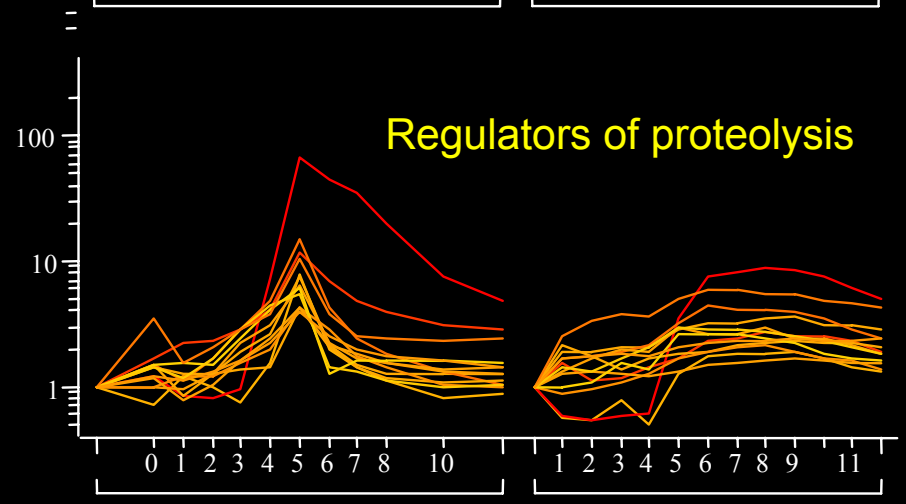


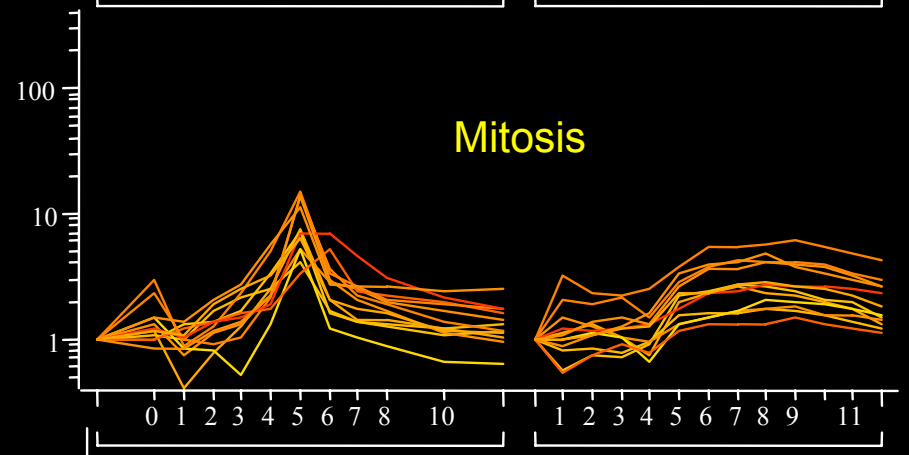


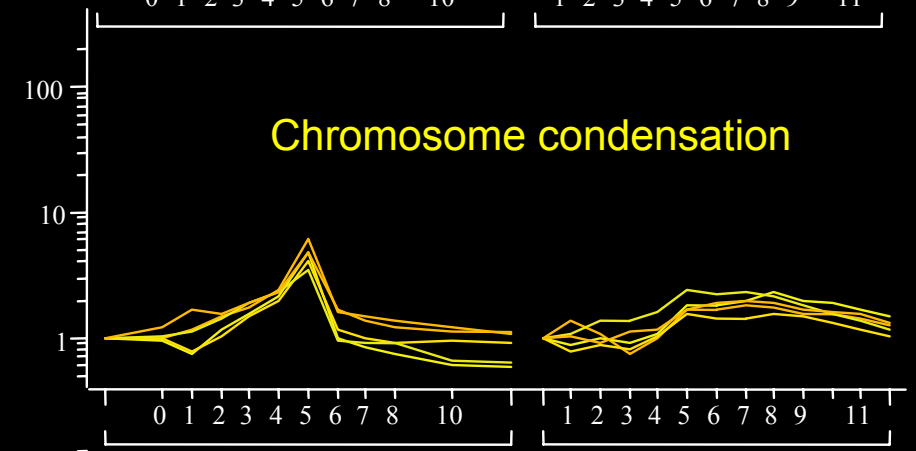
Functions of middle genes:



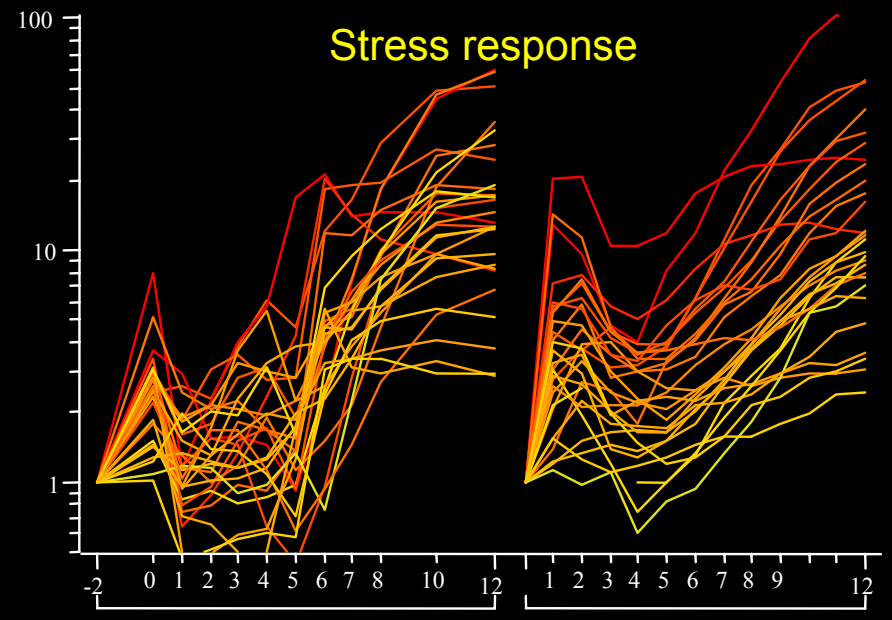


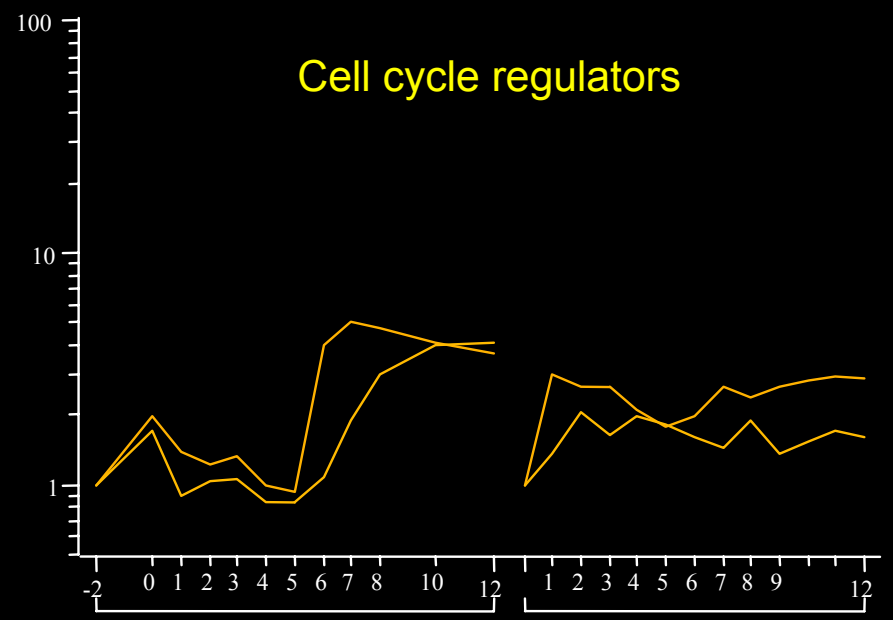


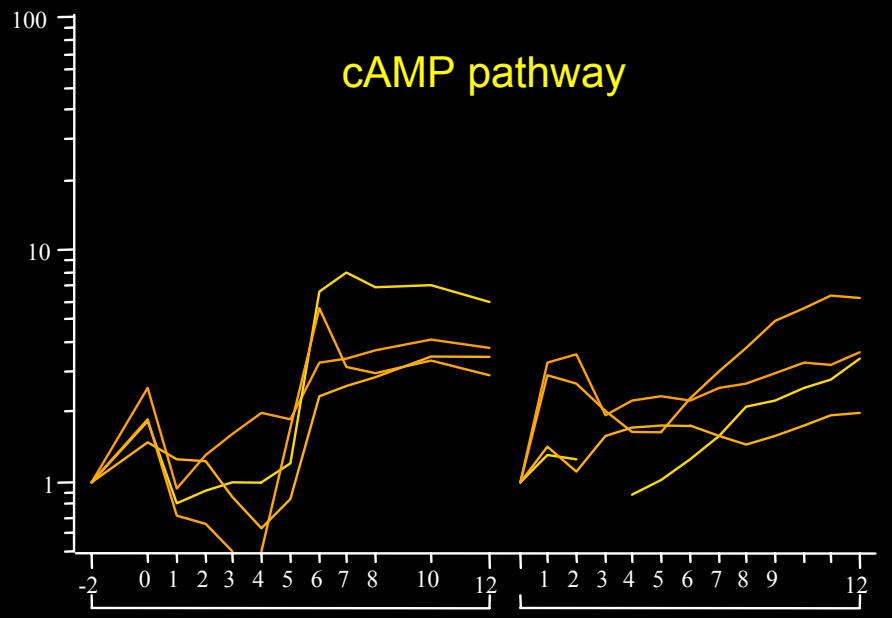


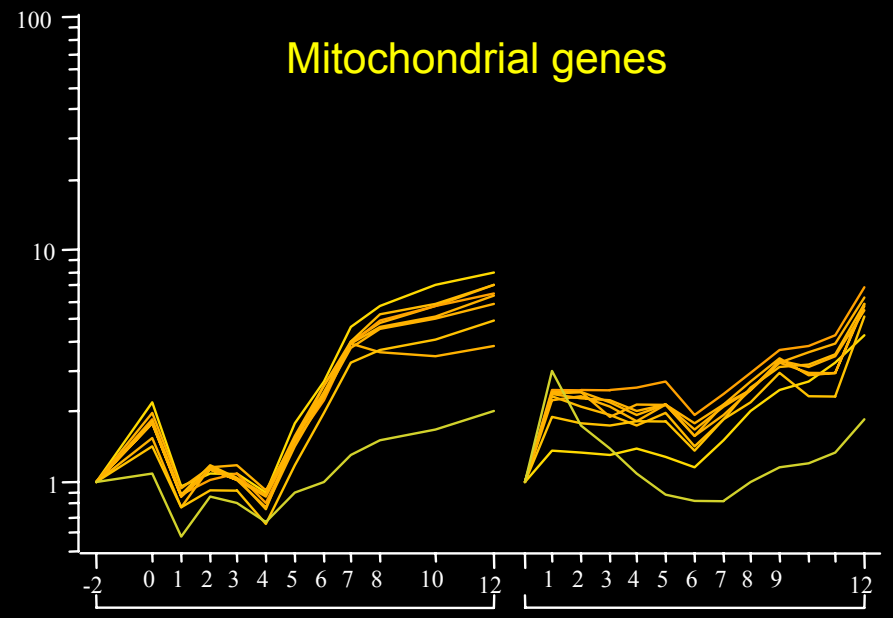


Functions of late genes:

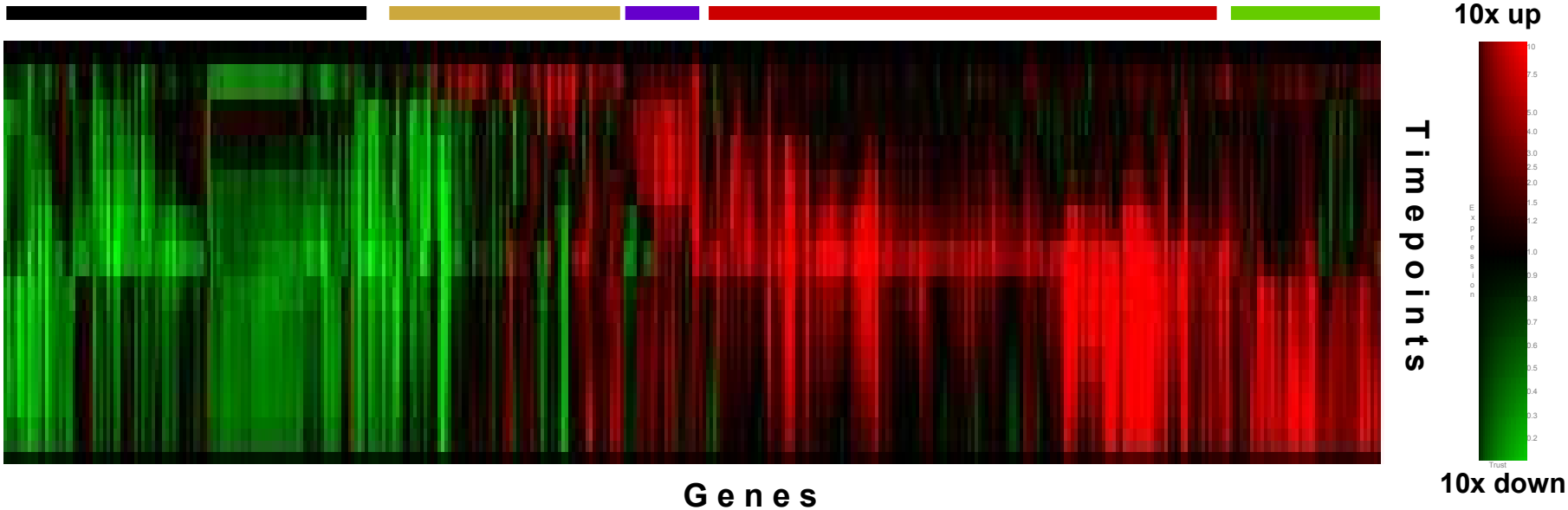


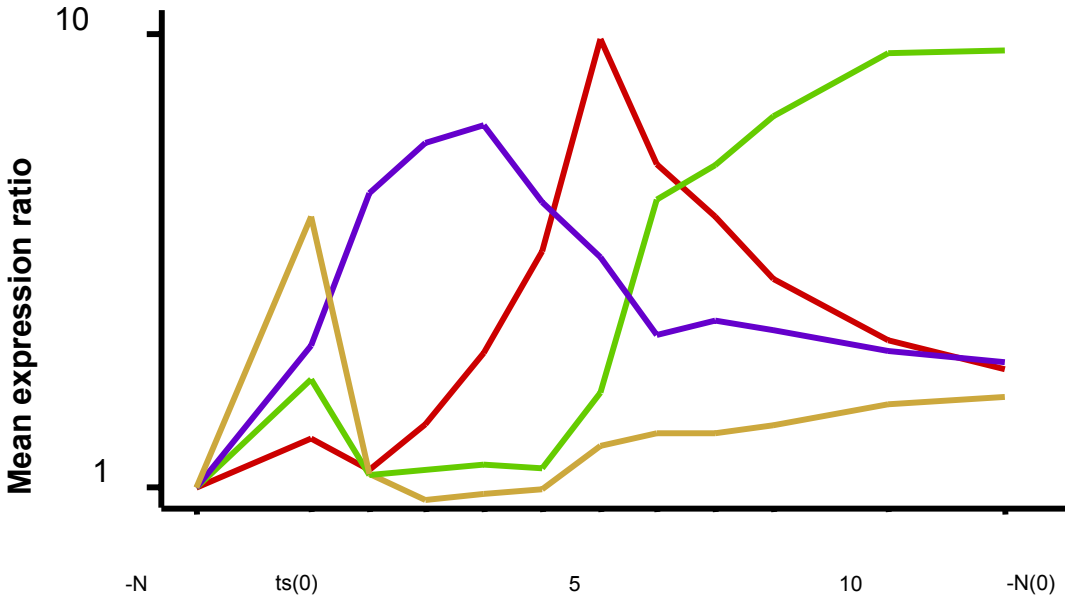




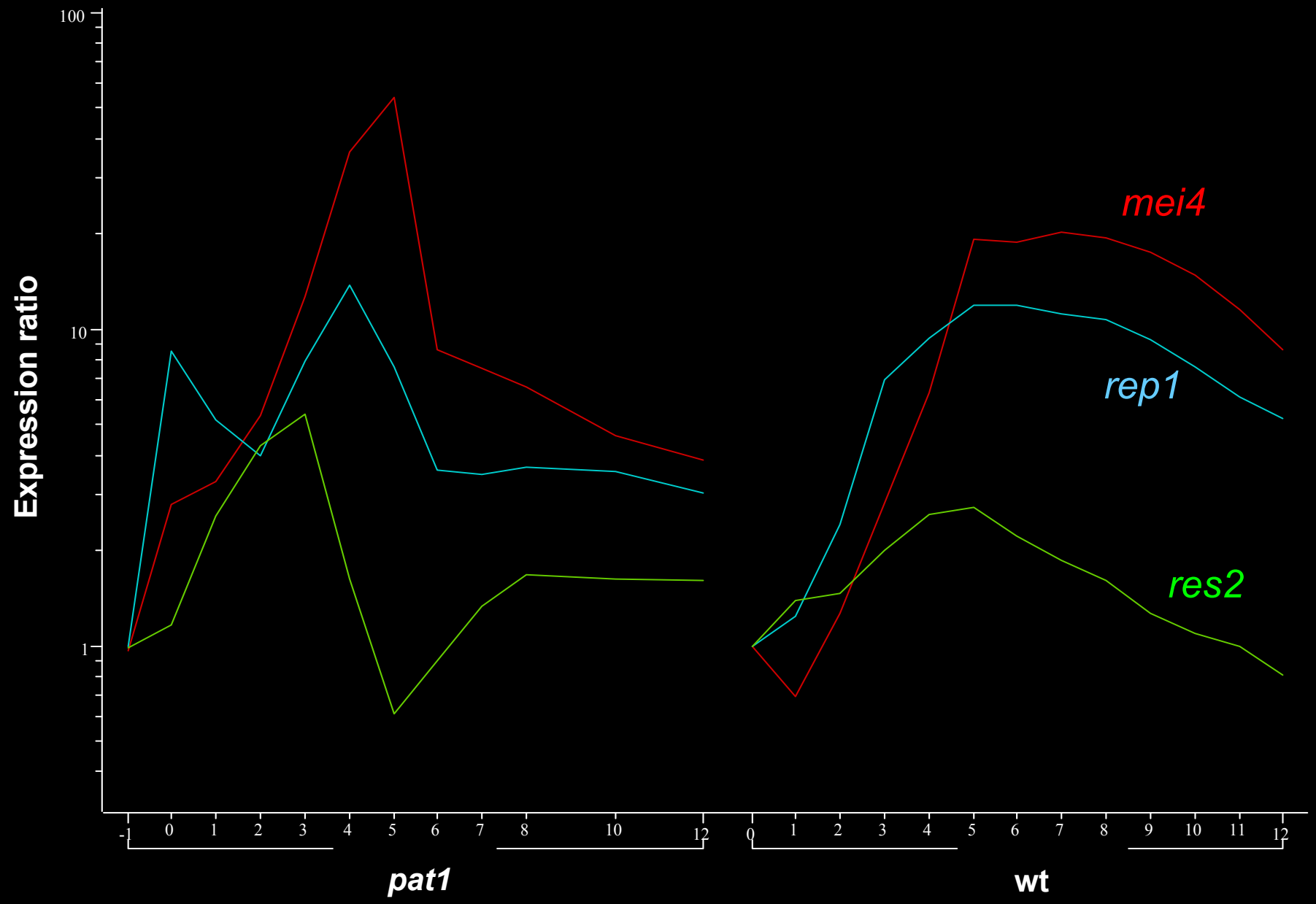


Meiotic Gene Expression Clusters:

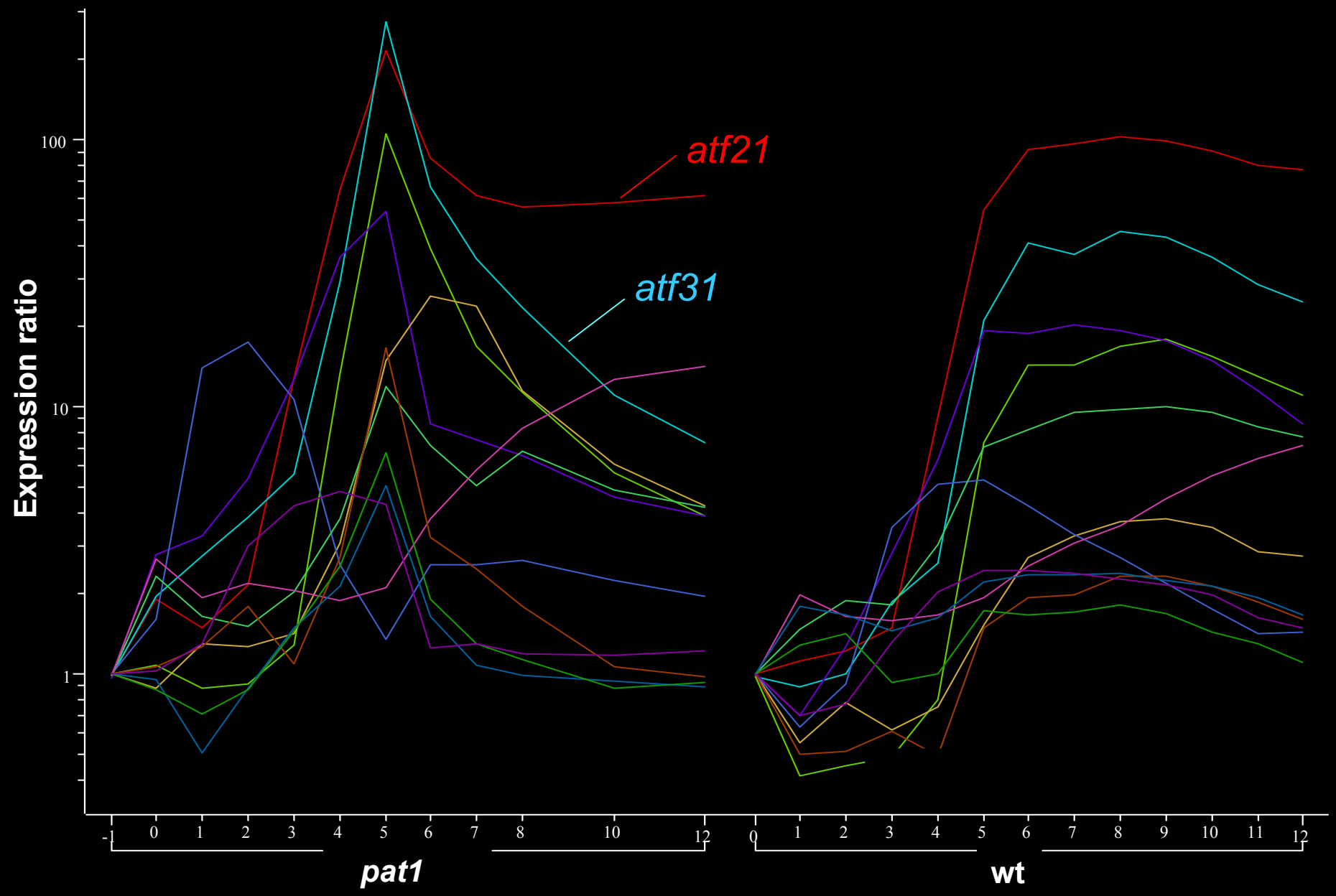




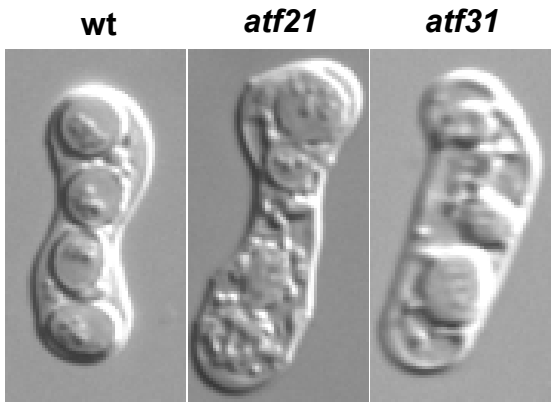
Known meiotic transcription factors are themselves regulated:

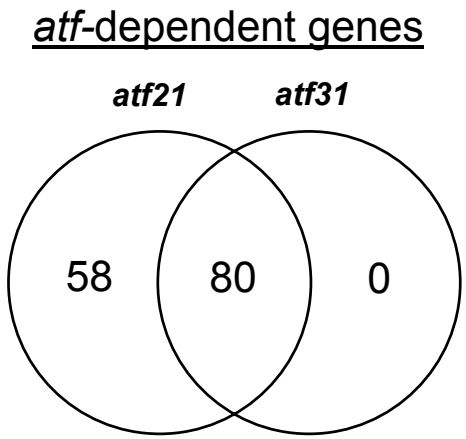


Other transcription factors up-regulated during meiosis:

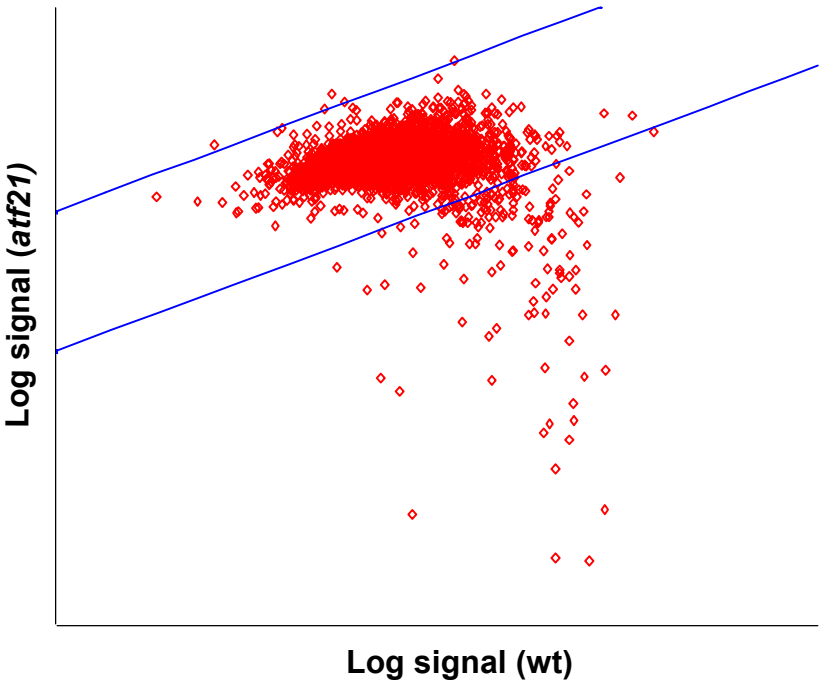


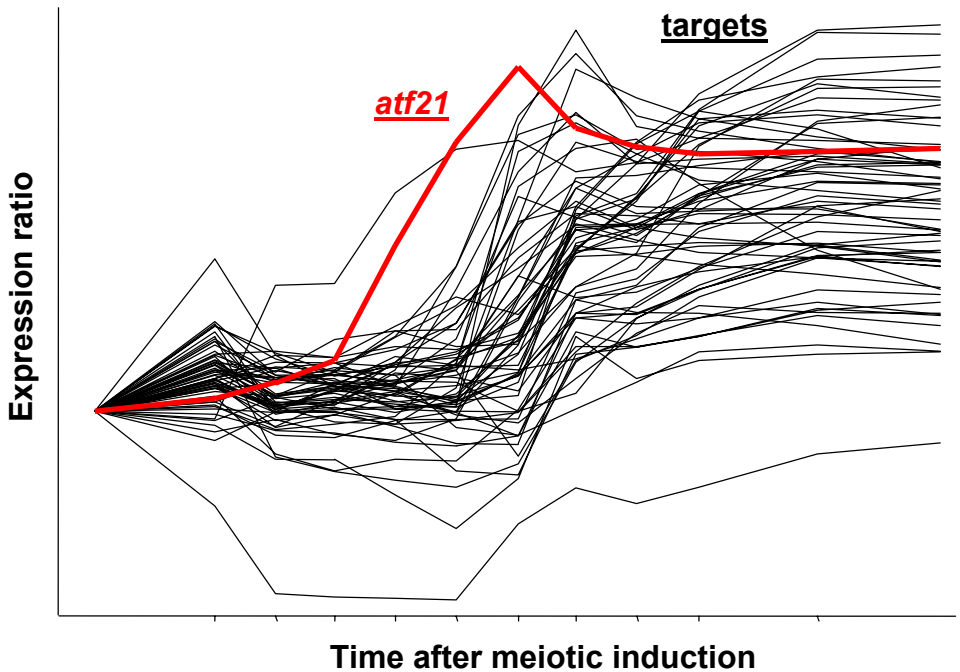
Characterization of *atf* function during meiosis:



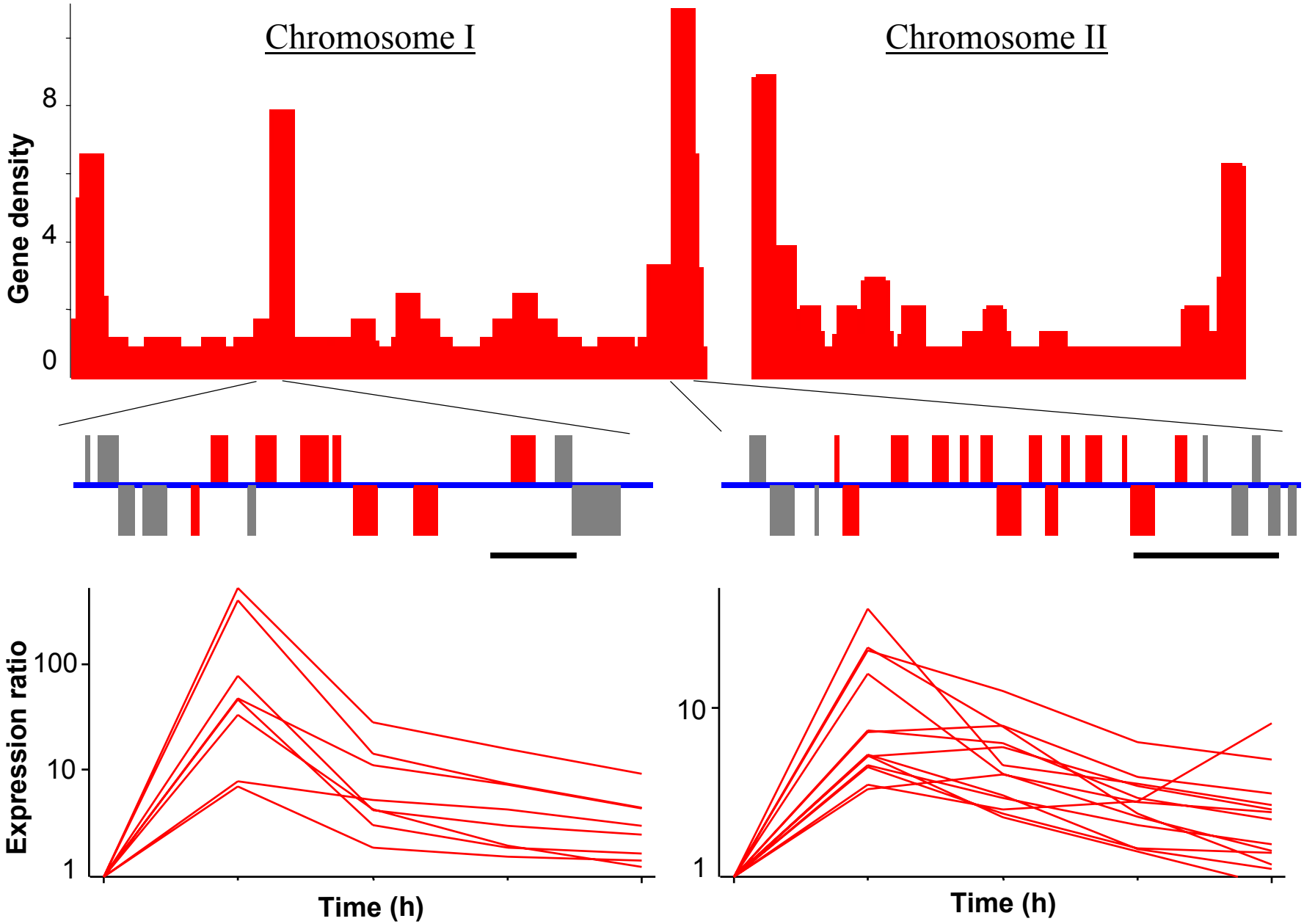


~55% of late genes are *atf21/31* dependent





Global regulation of genes up-regulated in N-starvation:



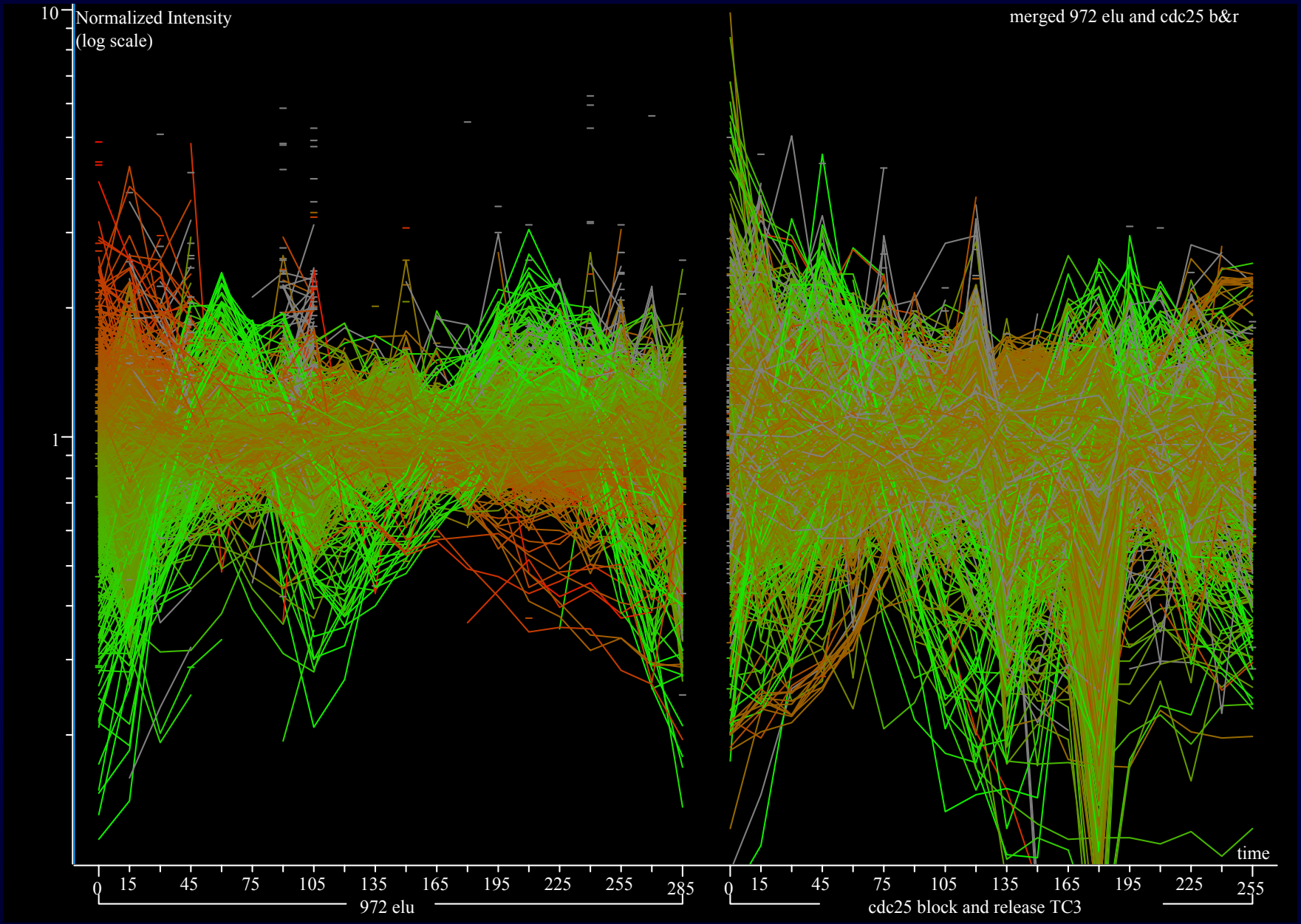
Expression Profiling During Cell Cycle:

Gabriella Rustici

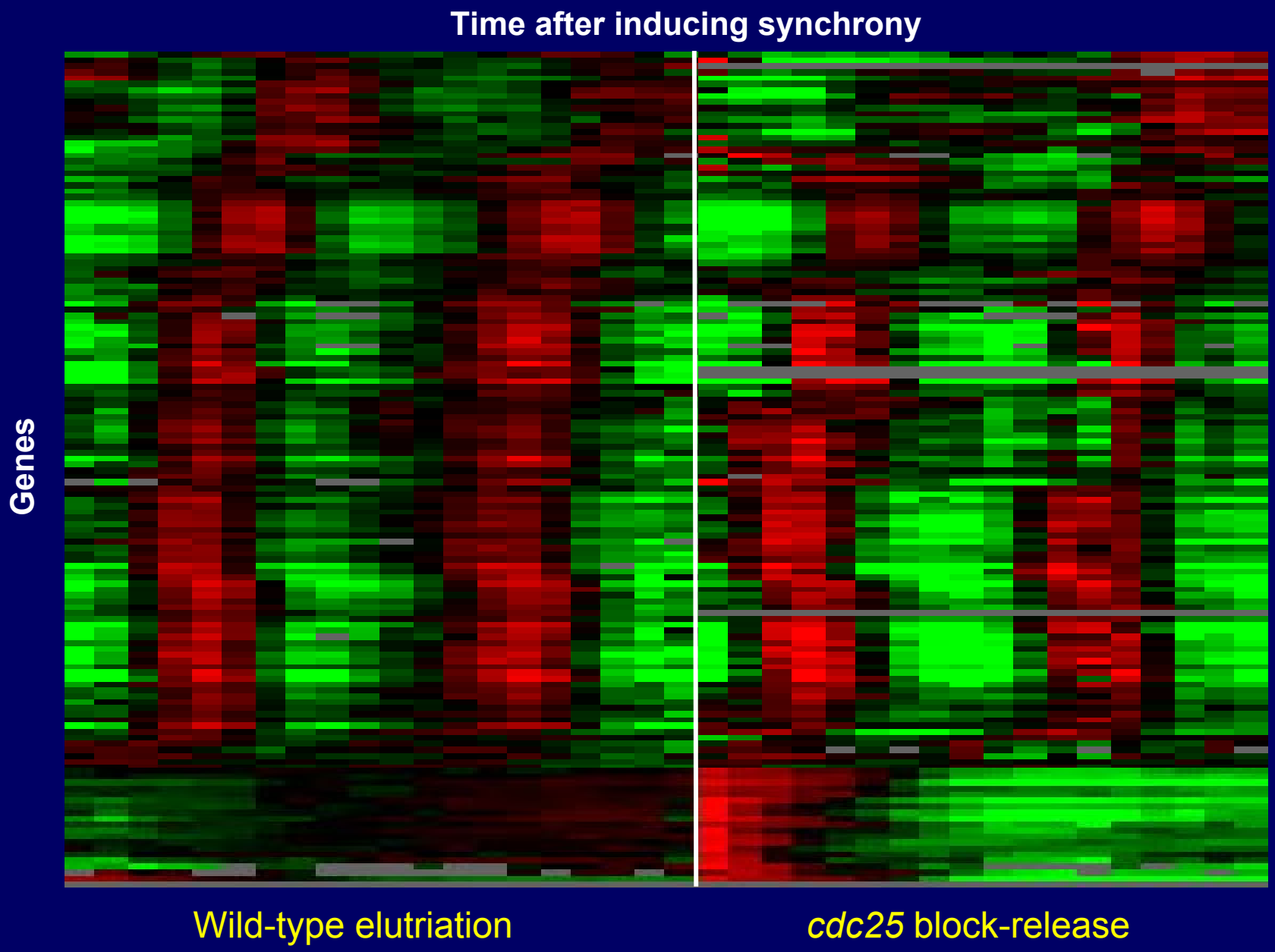
- synchronized cell cultures
- cell cycle and transcription factor mutants

- periodic (stage-specific) gene expression
- gene function ('guilt by association')
- regulatory sequence motifs and circuits

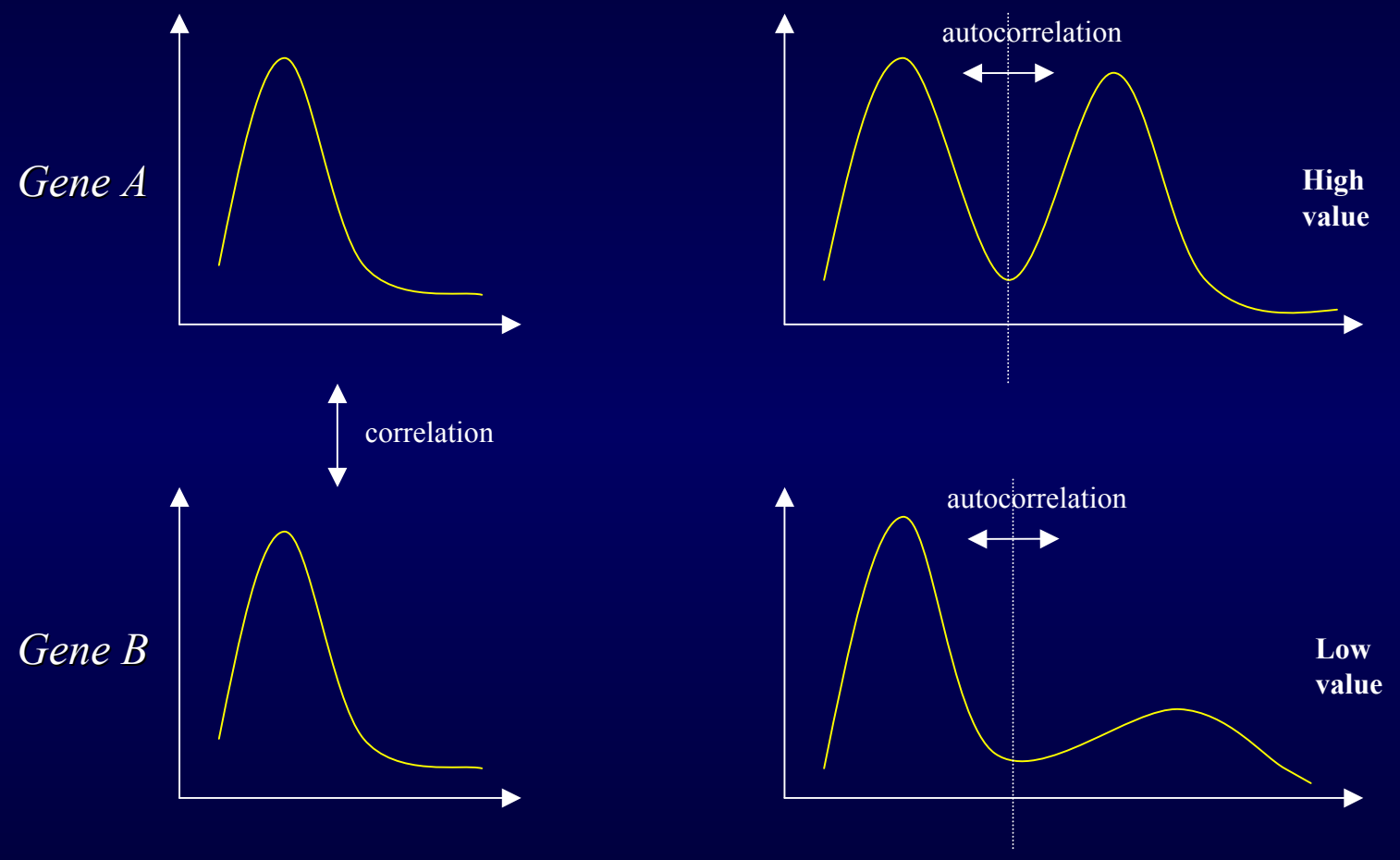
All genes: 2 experiments



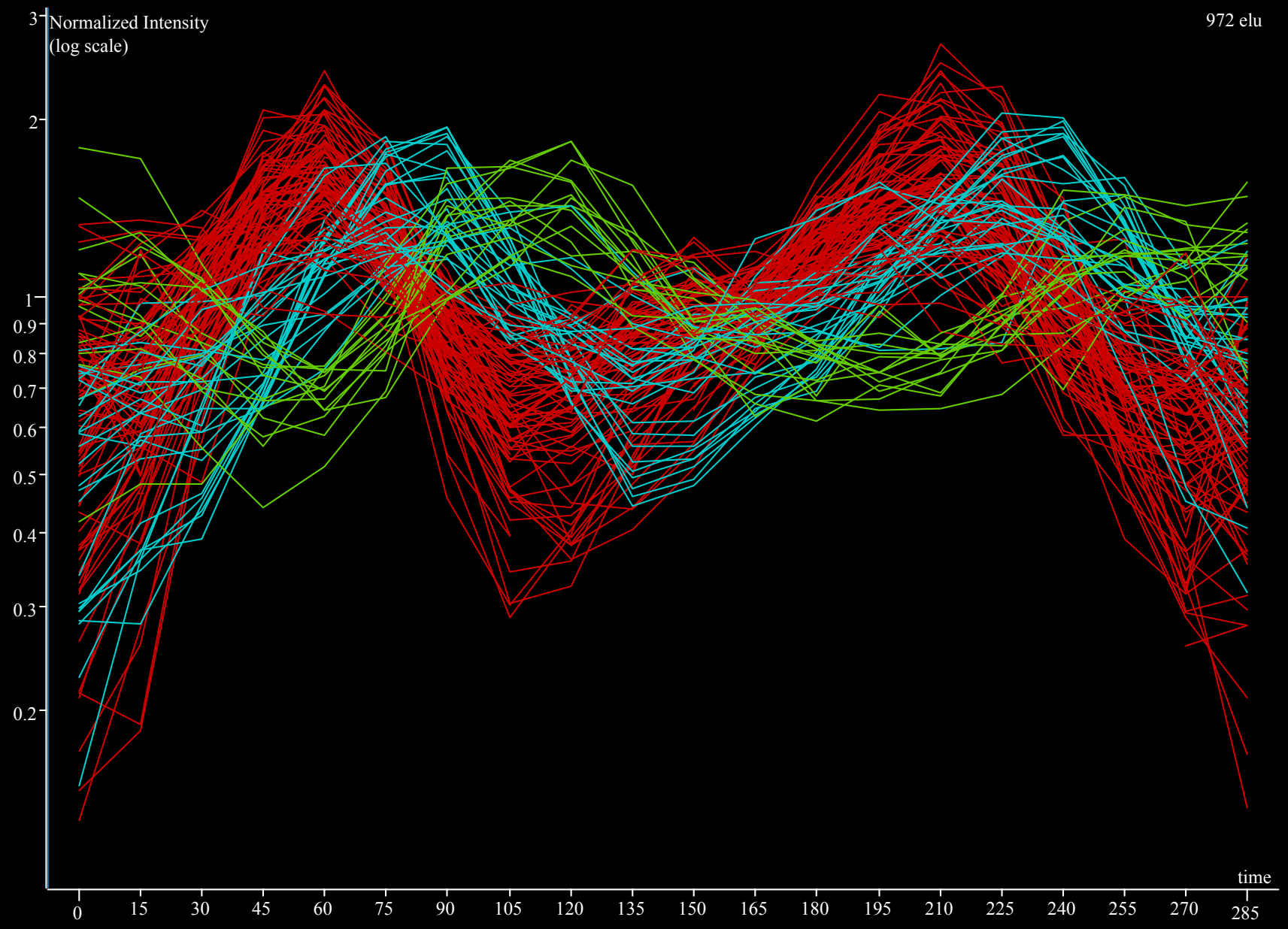
Hierarchical Clustering of Genes Regulated During Cell Cycle:



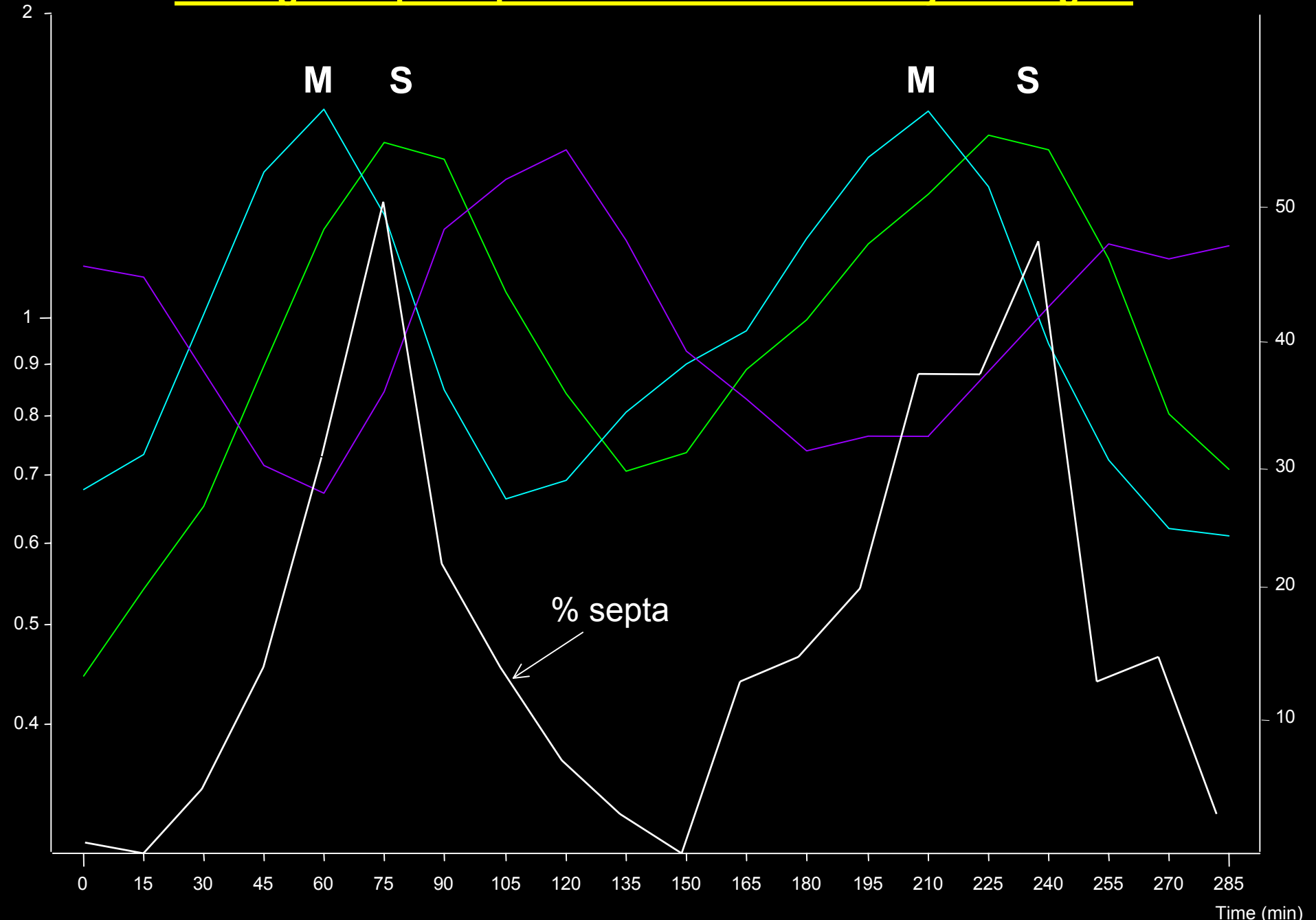
Autocorrelation script



Periodic gene expression during cell cycle:



Average temporal patterns relative to cell cycle stages:



Preliminary conclusions

- 3 distinct waves of transcription, with gap during much of G2 phase where no genes seem to be regulated

- First wave: highest number of genes and peaks shortly before septation. Contains known target genes of at least 2 transcription factors

- Second wave: peaks during S-phase; histones and some other genes

- Third wave: peaks during early G2 phase; mainly uncharacterized genes

Stress Response Mechanisms:

Dongrong Chen

Mark Toone, Nic Jones (Paterson, Manchester)

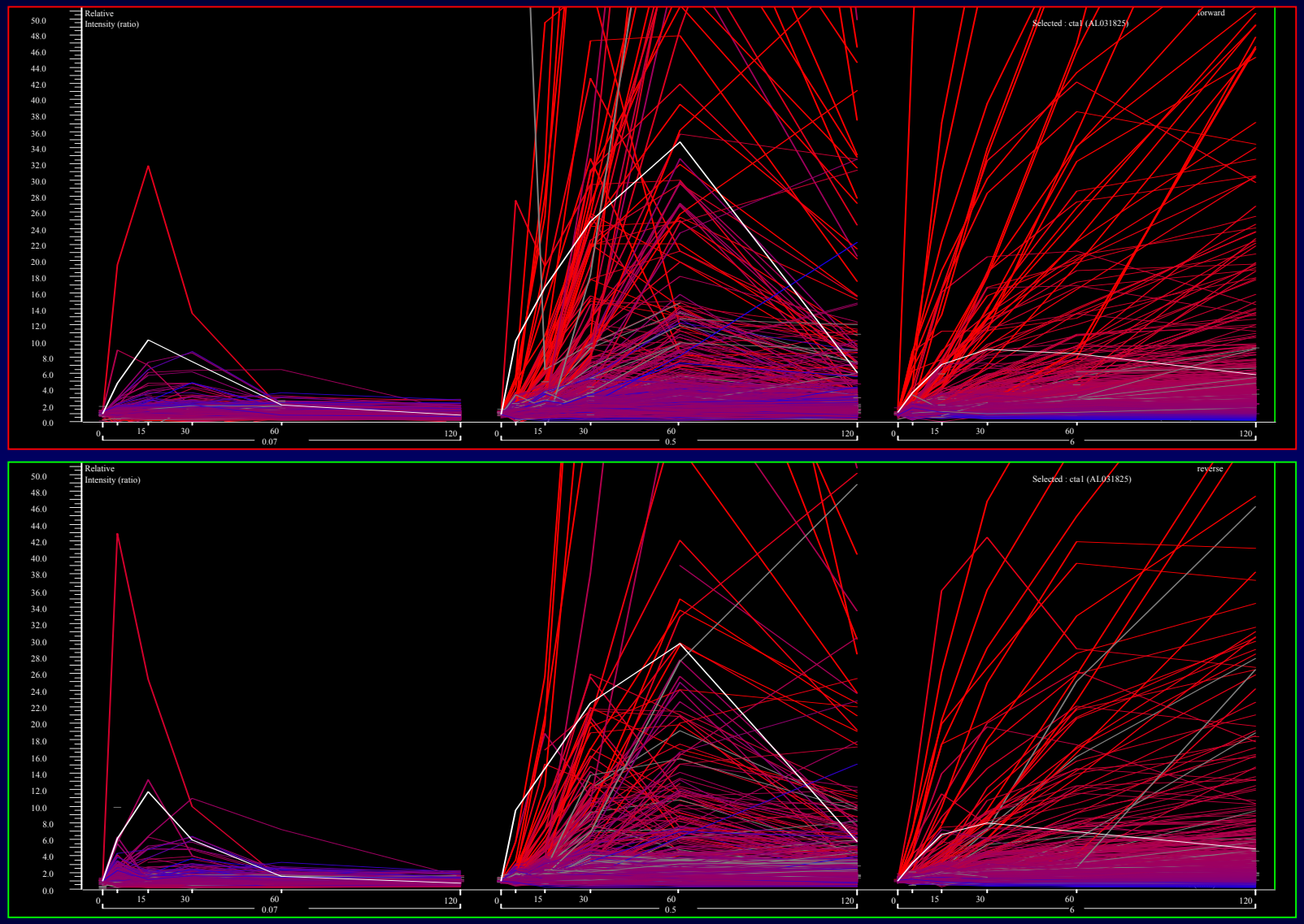
- oxidative stress: time, dose, different oxidants
- other abuses: heat, osmotic, toxic metals, EMR …
- constant stress, >100 generations (chemostat)

  - acute vs adaptive stress response
  - general vs specific stress responses
  - long-term adaptation / evolution
  - regulatory sequence motifs and circuits

Timecourses after addition of various doses of H_20_2:

For

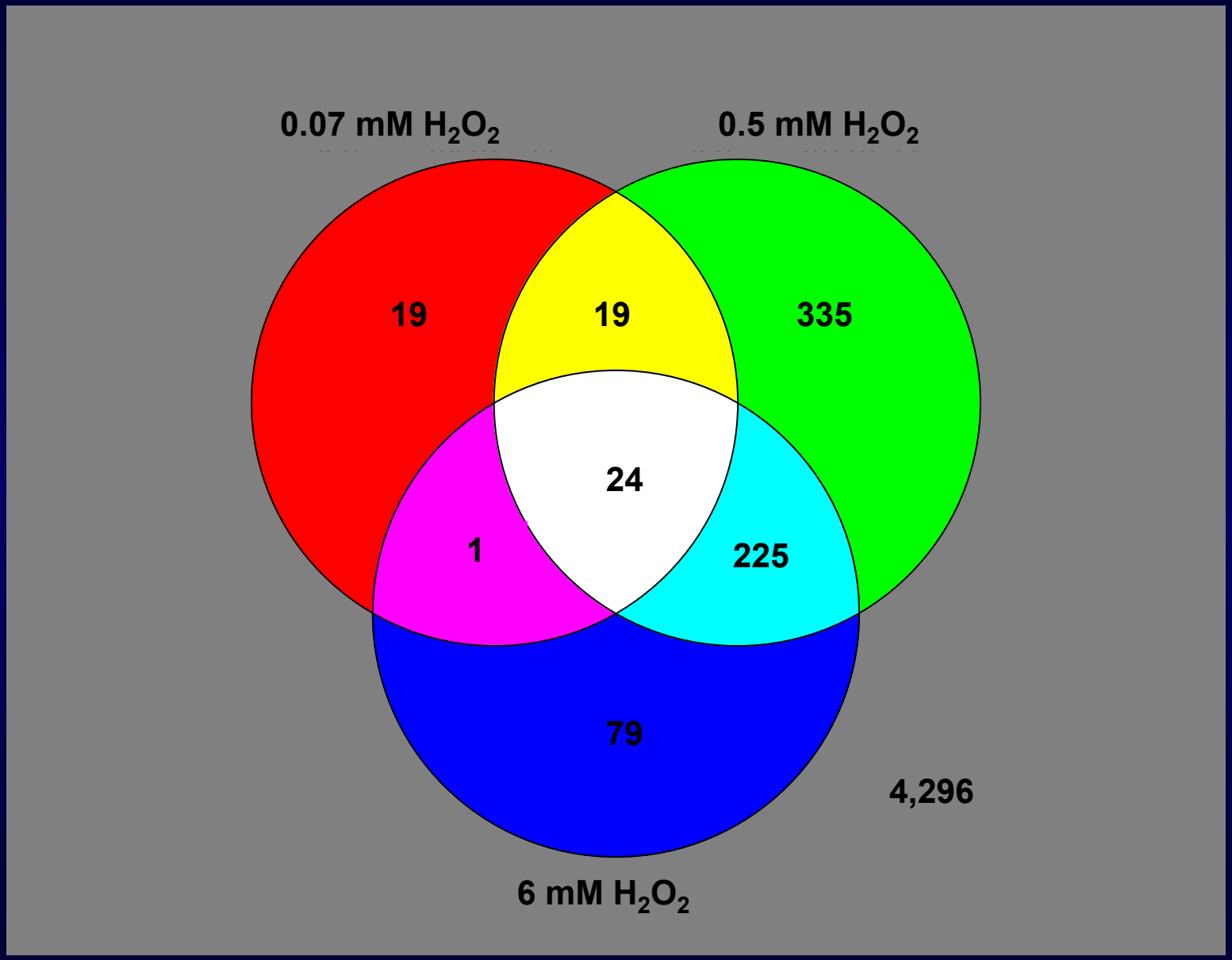
Rev



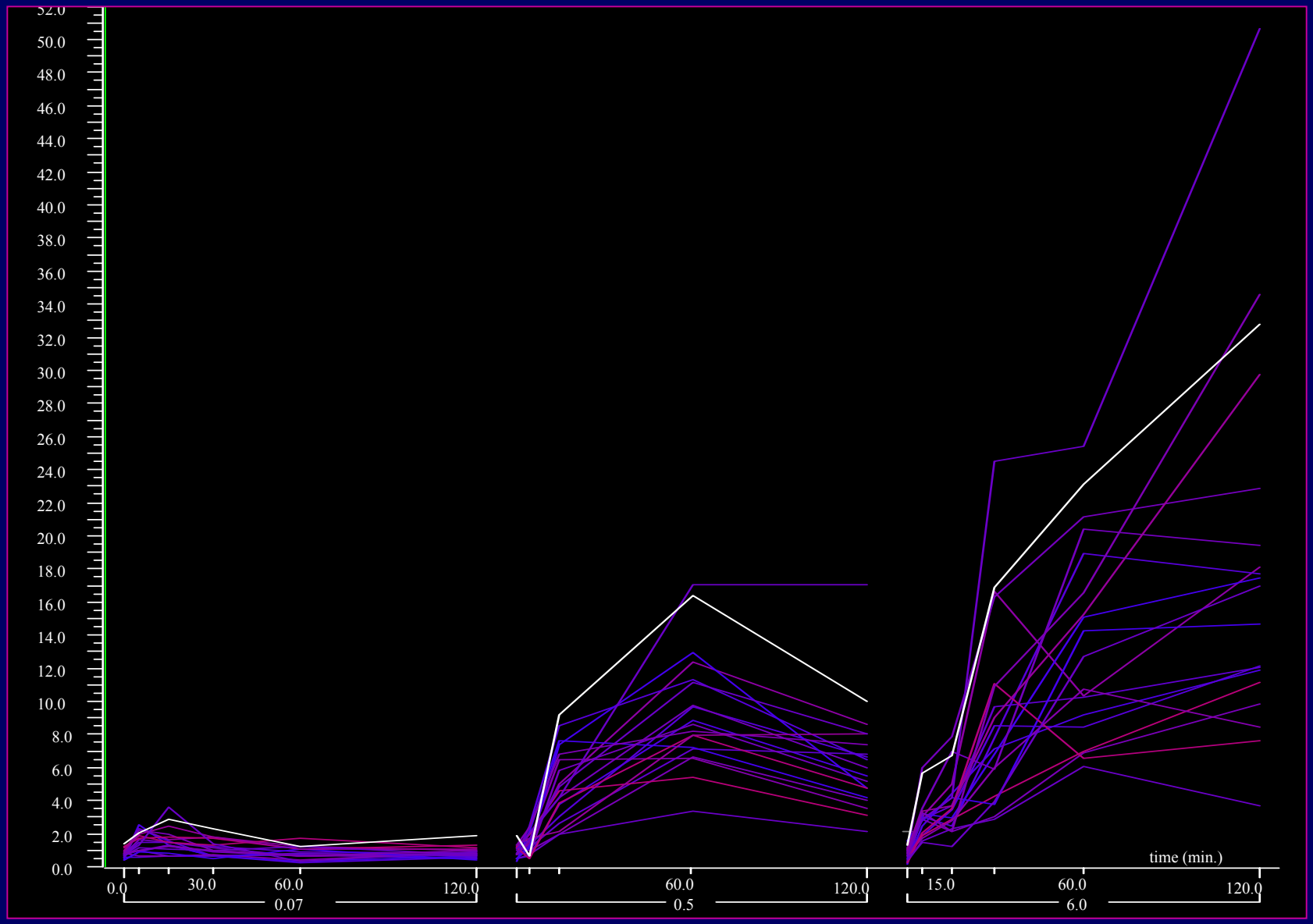
Min after stress: 0 5 15 30 60 120 0 5 15 30 60 120 0 5 15 30 60 120

Oxidant dosis: **low** **medium** **high**

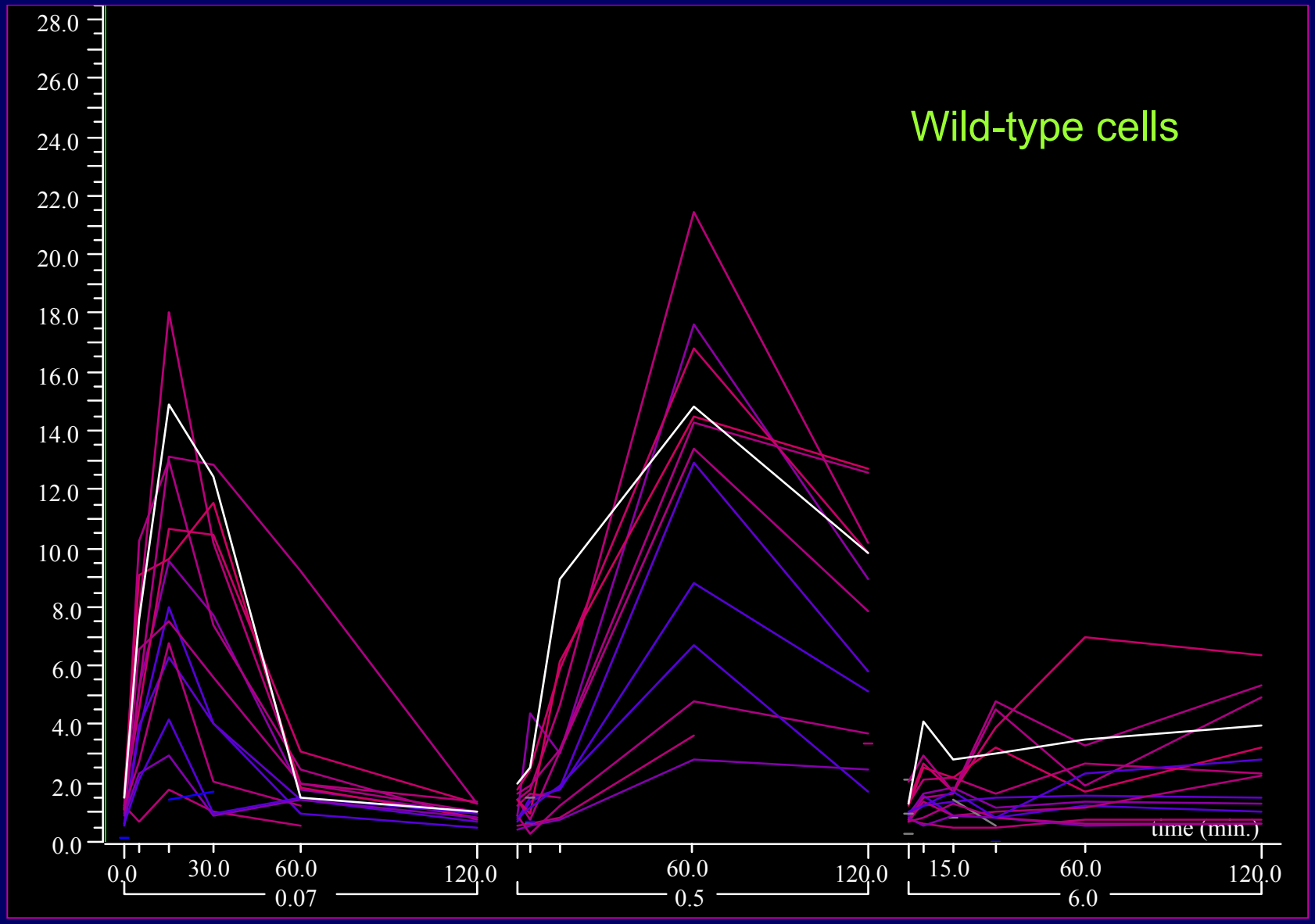
Genes up-regulated with various doses of oxidant



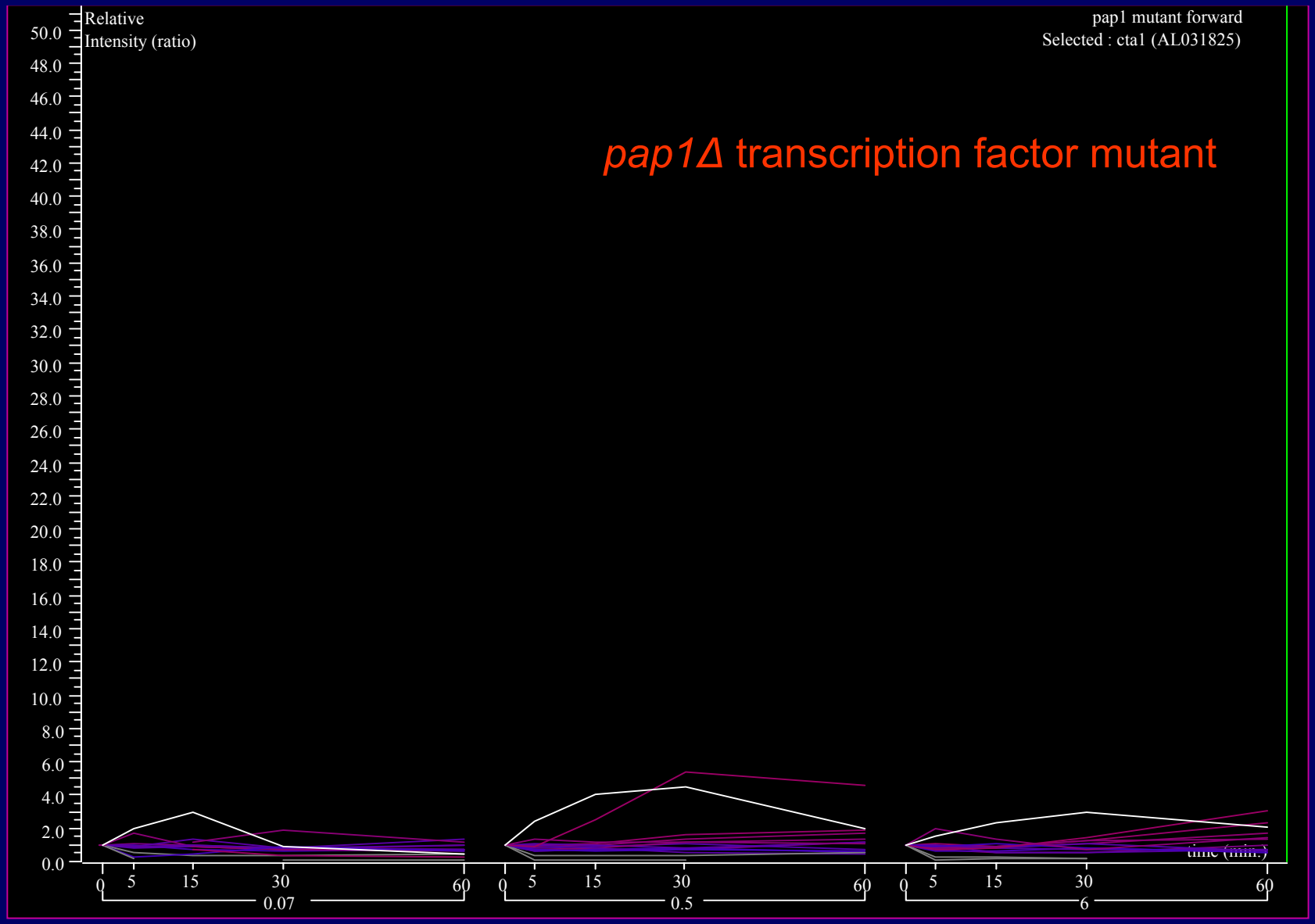
Genes up-regulated at high doses:



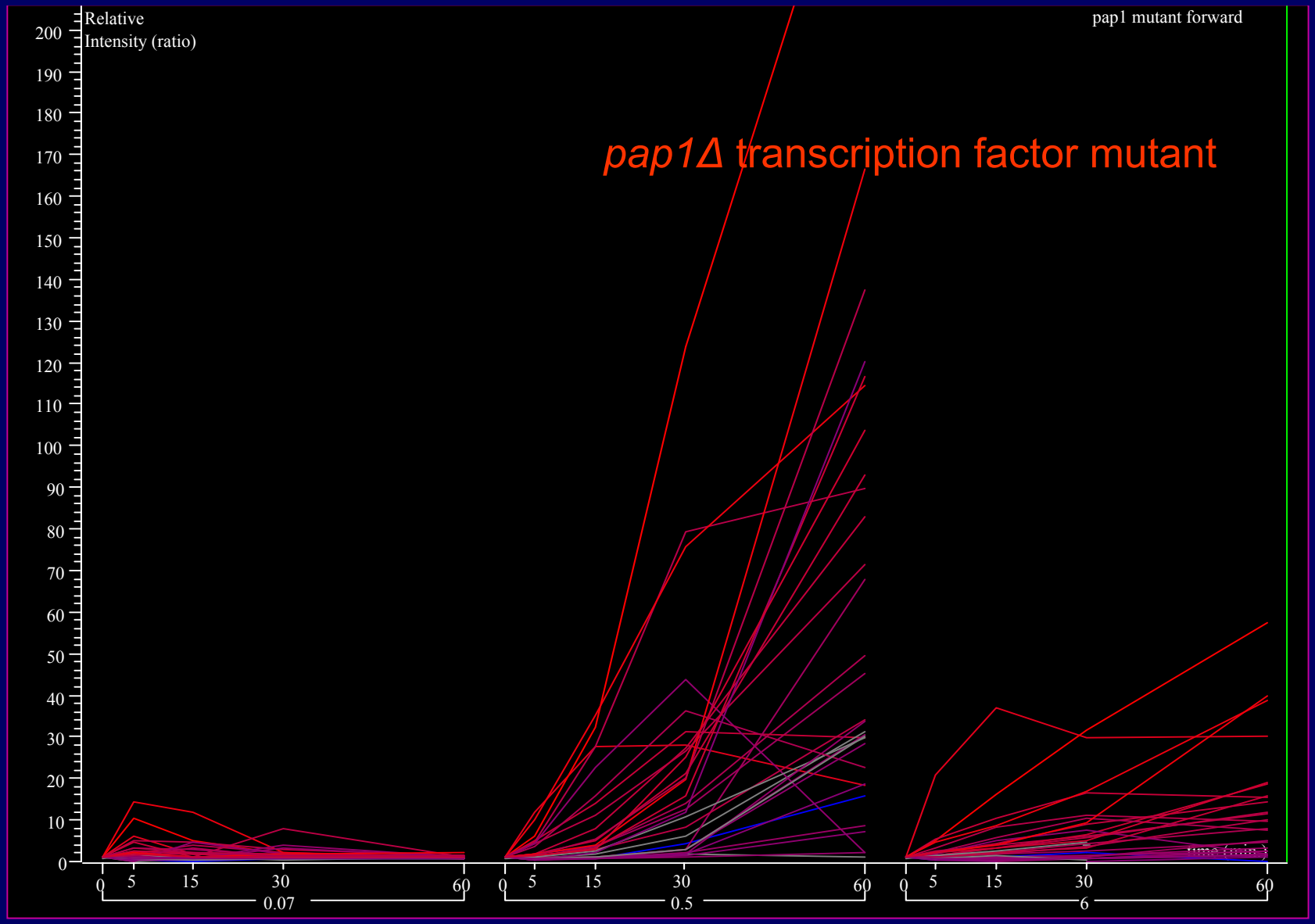
Genes up-regulated at low doses:



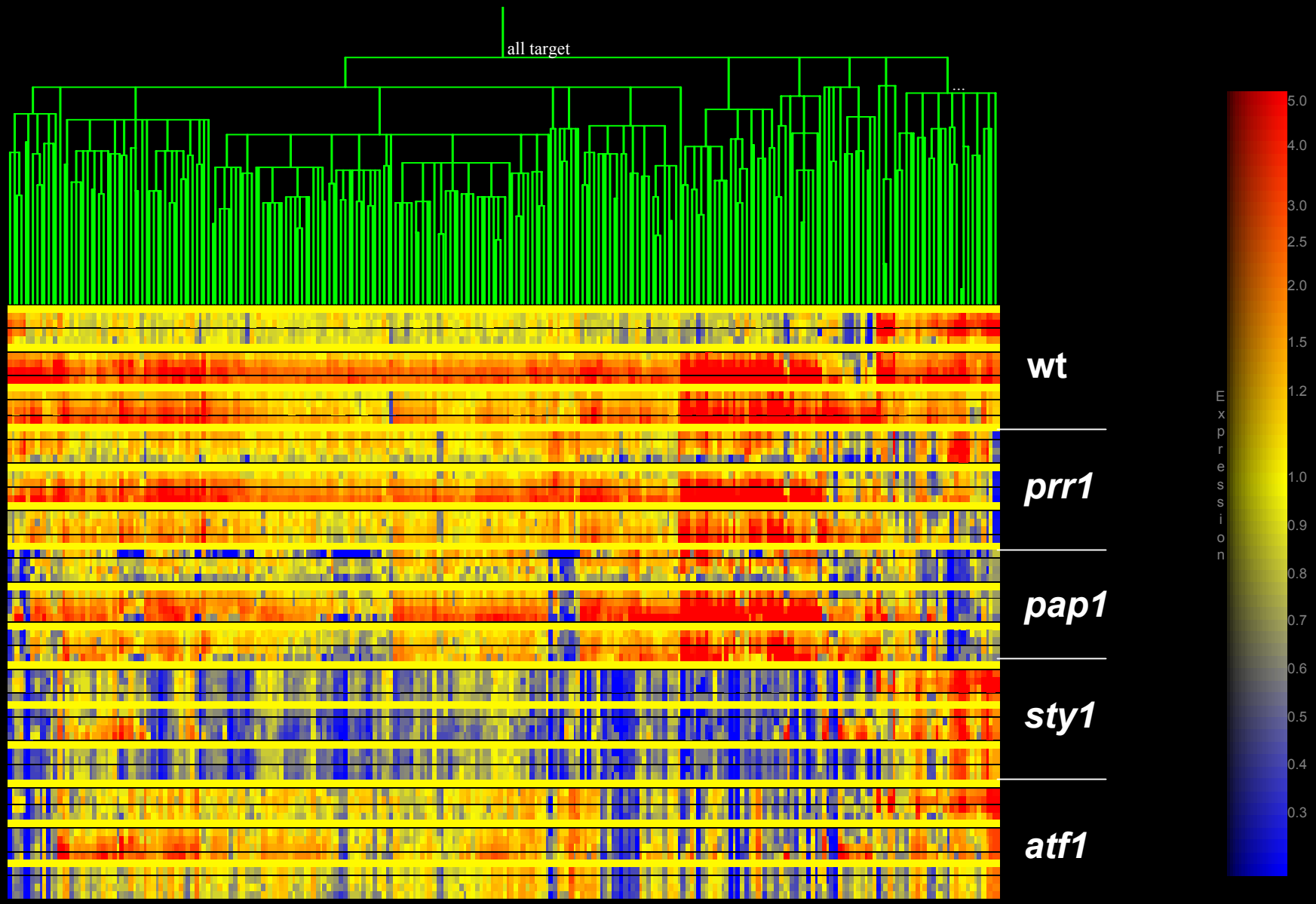
Genes up-regulated at low doses:



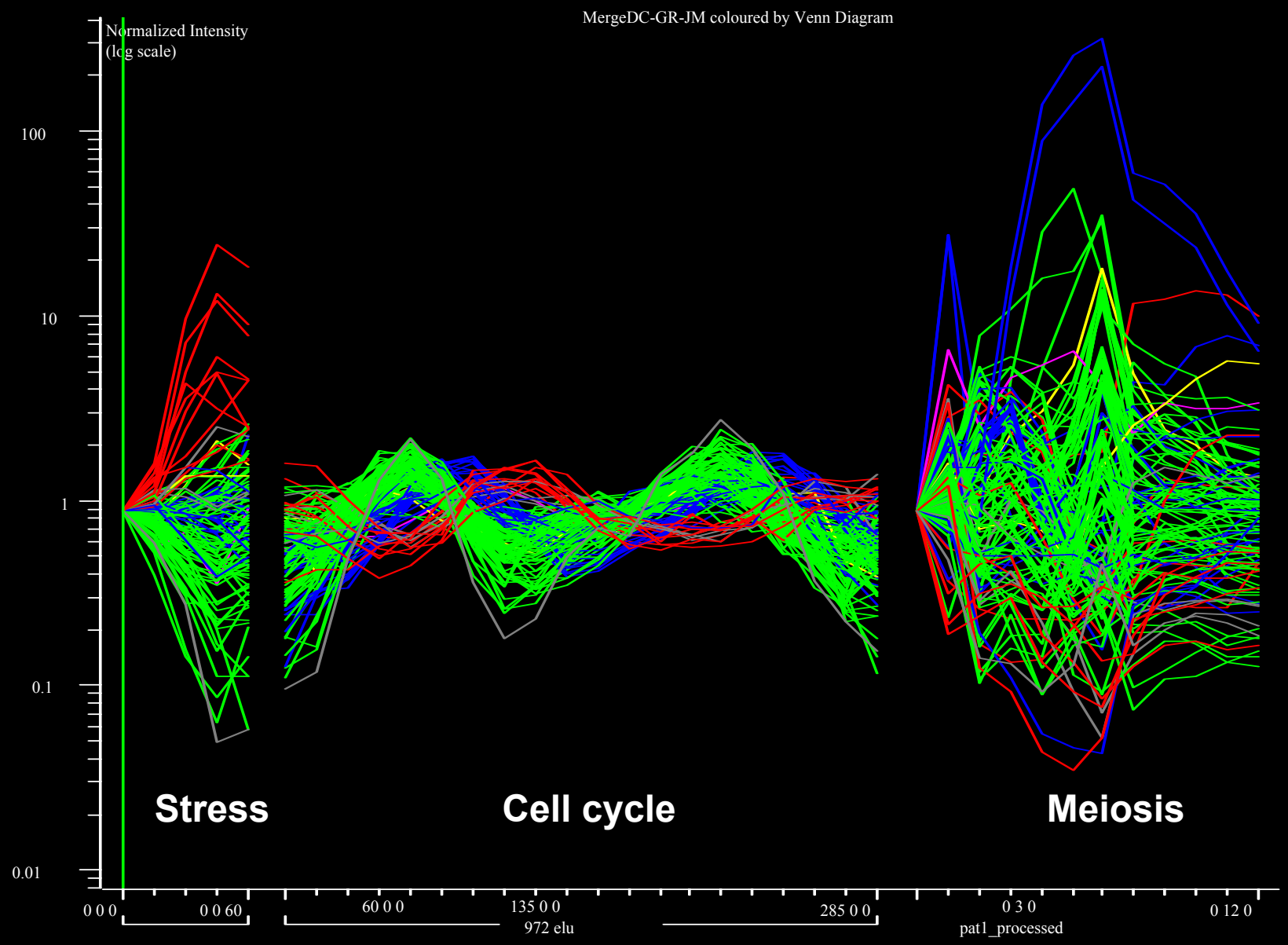
Genes up-regulated at high doses:



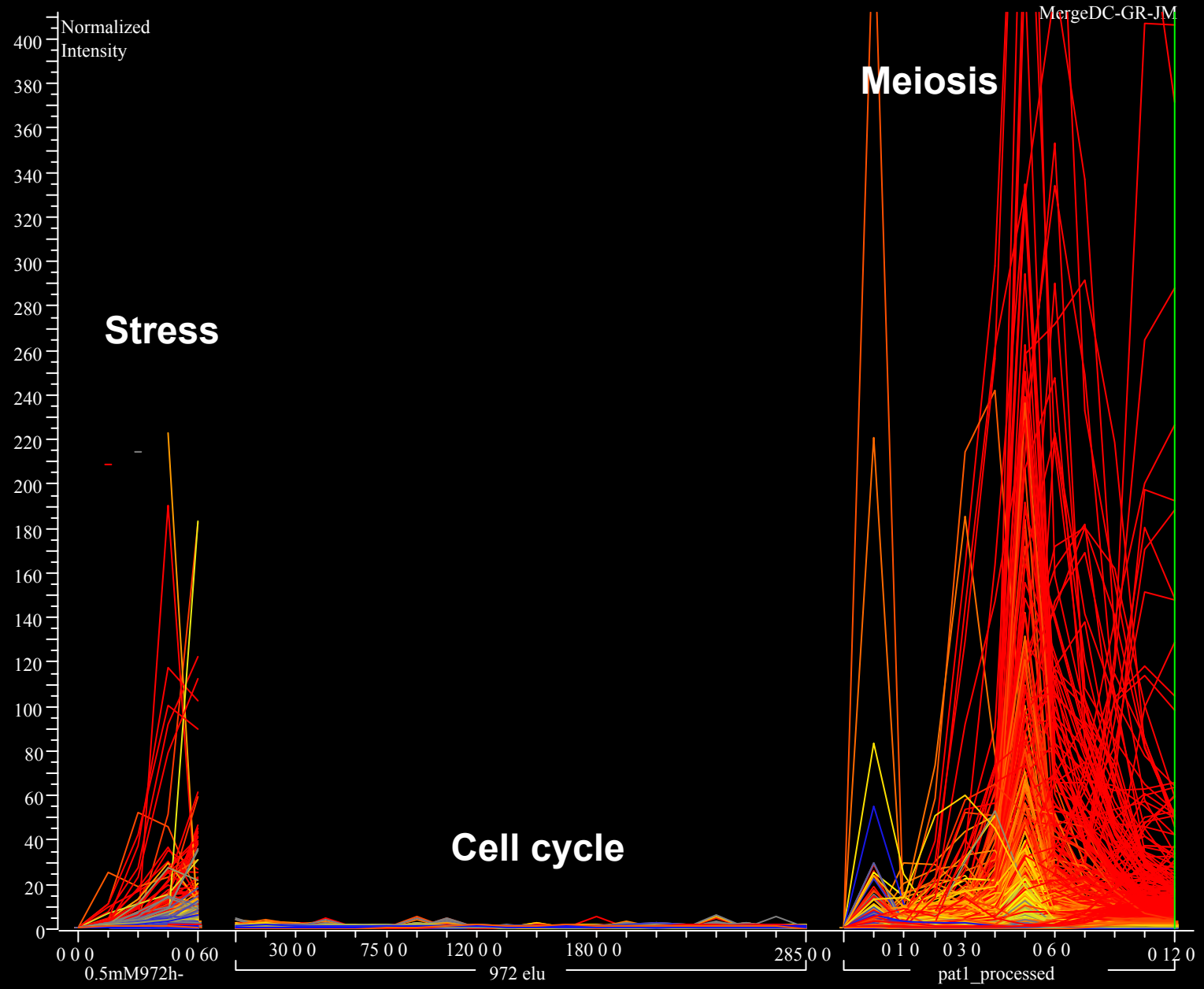
Clustering of genes differentially expressed during oxidative stress:



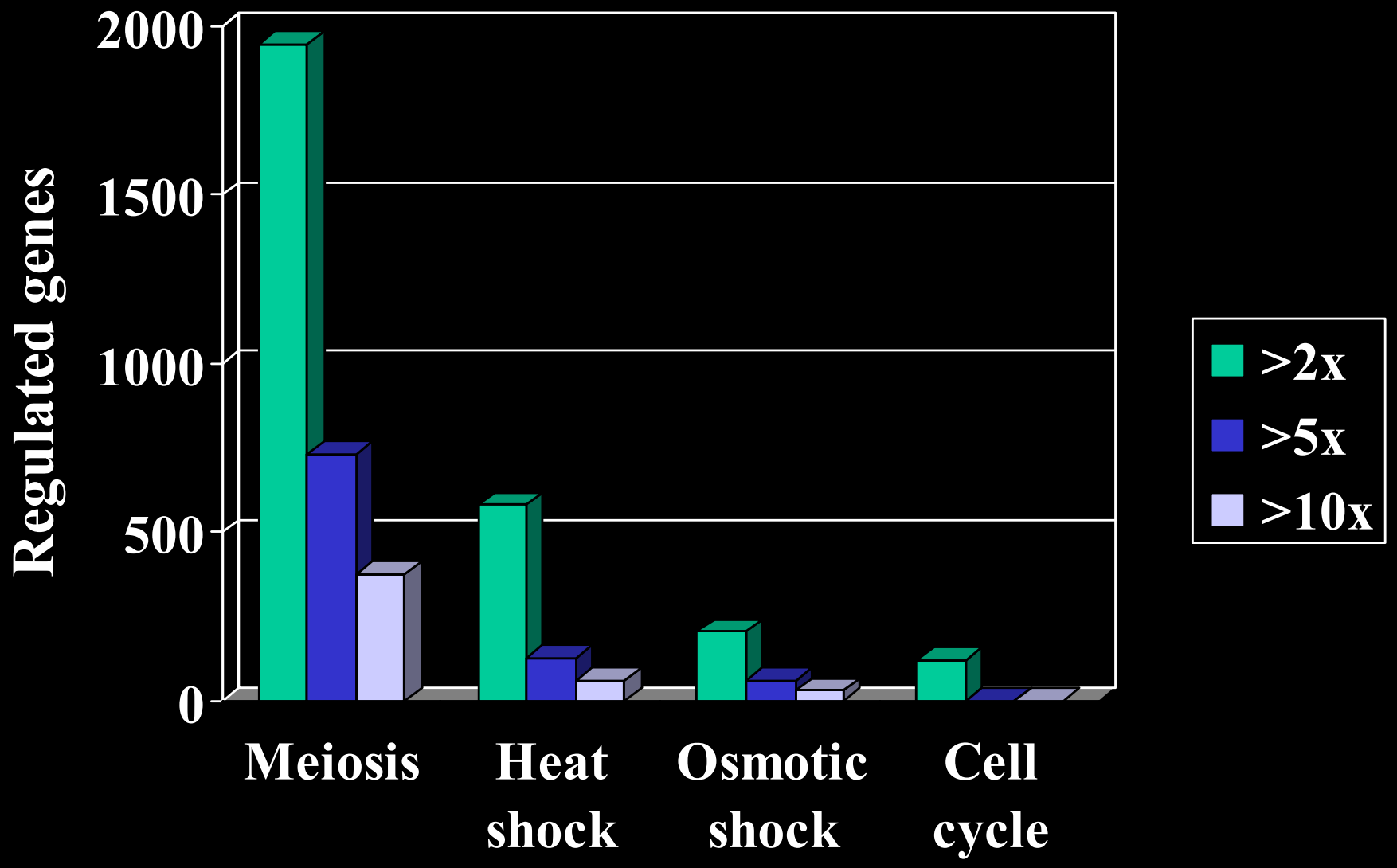
Comparison of data from different microarray experiments:



Comparison of gene expression in different experiments:



Gene expression under various conditions:



Chromosome 'Tiling' Microarray:

- Goal: all DNA without gaps (~1 kb pieces)
- Focus first on inter-genic regions
- ChIP: immunoprecipitate DNA bd. protein, use bound DNA as target for hybridization
  - map protein binding sites globally along chromosomes
  - identify ORFs
  - map transcript lengths
  - ...

General and simple recommendations:

- Repeat biological experiments to get statistically sound data
- Plan and design experiments carefully / controlled and standardized conditions
- Compare data from different experiments The more data the better!
- Explore data with various tools

Microarrays and I?

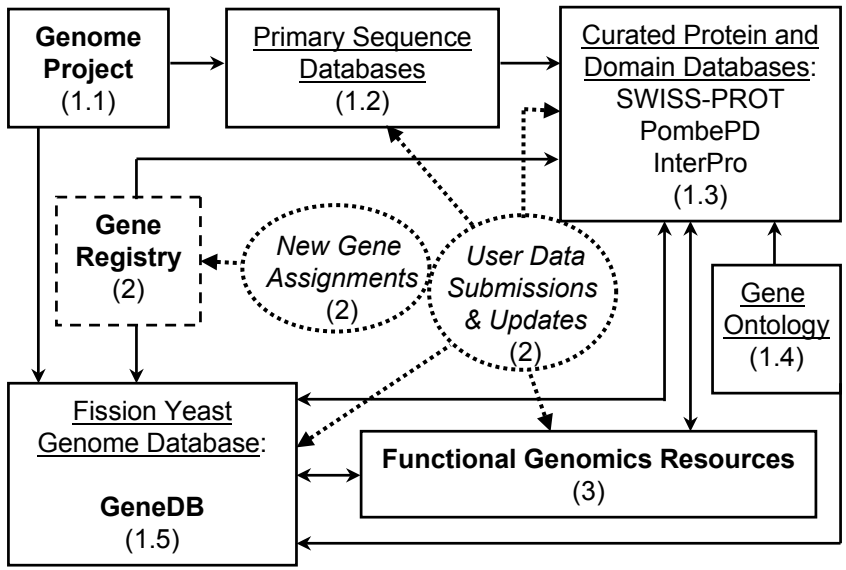
- Even if you are not equipped to do microarrays yourself, you can make good use of available data

- Examples:

  Genes expressed at stage of interest:
  Toth et al. (2000) Cell 103:1155

  Genes co-expressed with genes of interest:
  Colman-Lerner et al. (2001) Cell 107:739

Everything you always wanted to know about fission yeast data resources, but were afraid to ask:



http://www.sanger.ac.uk/PostGenomics/S_pombe/

-> Preprint: Wood & Bähler

Thank you!

Gavin Burns
Rachel Lyne
Gabriella Rustici
Dongrong Chen
Juan Mata
Jürg Bähler

Dave Vetrie (Sanger)
Valerie Wood (Sanger)
Mike Lyne (Sanger)
Alvis Brazma (EBI)
Jaak Vilo (EBI)
Aengus Stewart (CRUK)
John Sgouros (CRUK)

M. Toone / N. Jones (Paterson)
J. Hayles / P. Nurse (CRUK)

Cancer Research UK

DNA Microarrays of Fission Yeast

http://www.sanger.ac.uk/PostGenomics/S_pombe

The Wellcome Trust Sanger Institute / Cancer Research UK