Microarray analysis of viral infections

Dipl. Biol. Stefanie Müller

Department of Virology

Bernhard Nocht Institute for Tropical Medicine

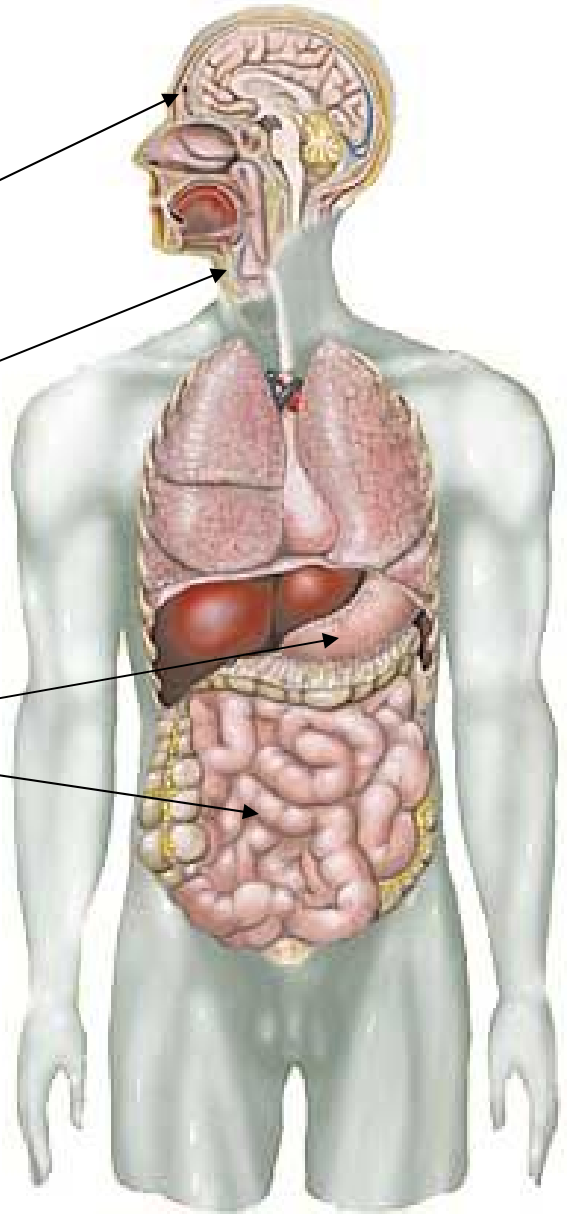
- Possible investigations of viral infections

- Description of symptoms → patient
- Analysis of affected organs→ Pathology, blood
- Investigation of affected cells → Microarray analysis, Protein analysis

Lassa infection

Symptoms:

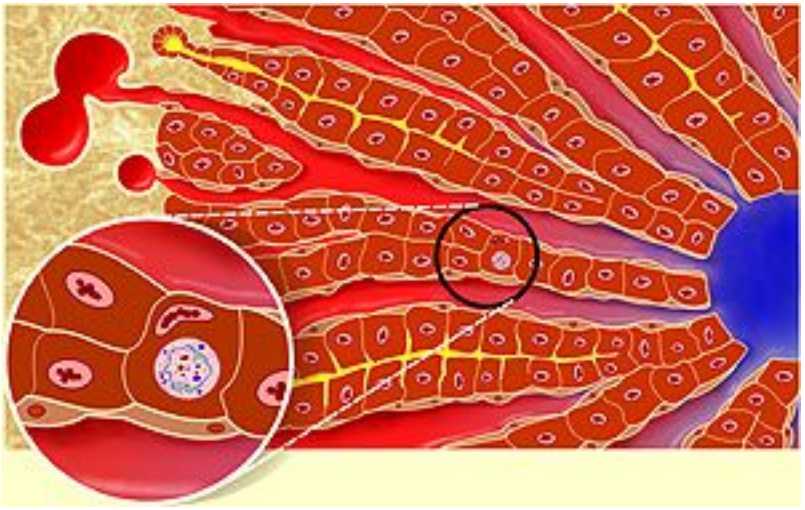
- fever
- severe headache, sore throat
- abdominal pain
- Hepatitis
- hemorraghe



Lassa infection

- Affected organs

- example liver
- pathological diagnosis



Lassa infection

- Investigation of affected cells
- Electron microscopy:

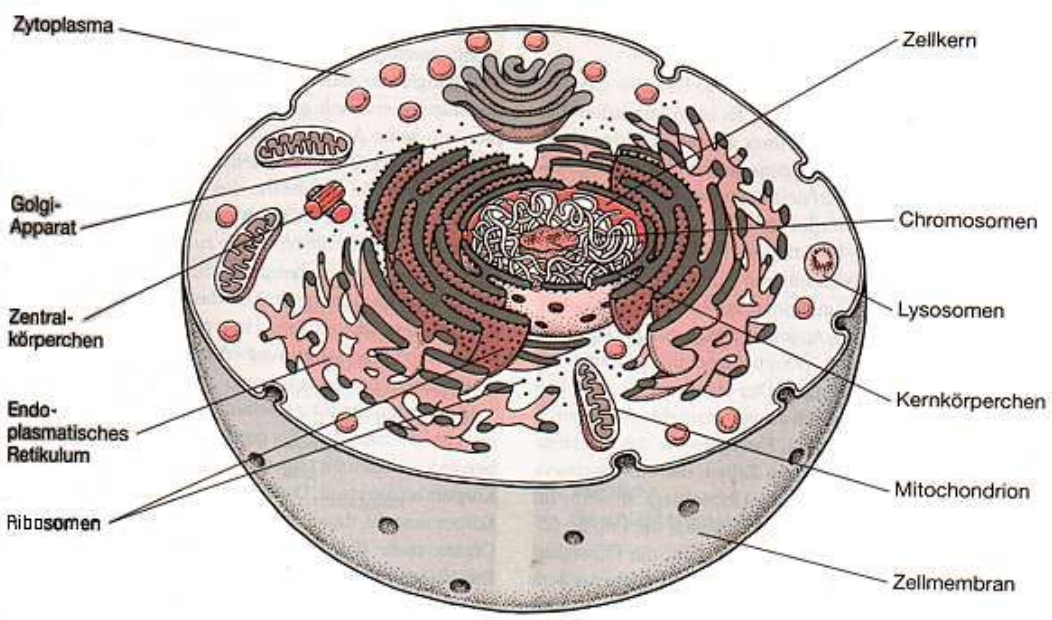
  cell shape

  CPE

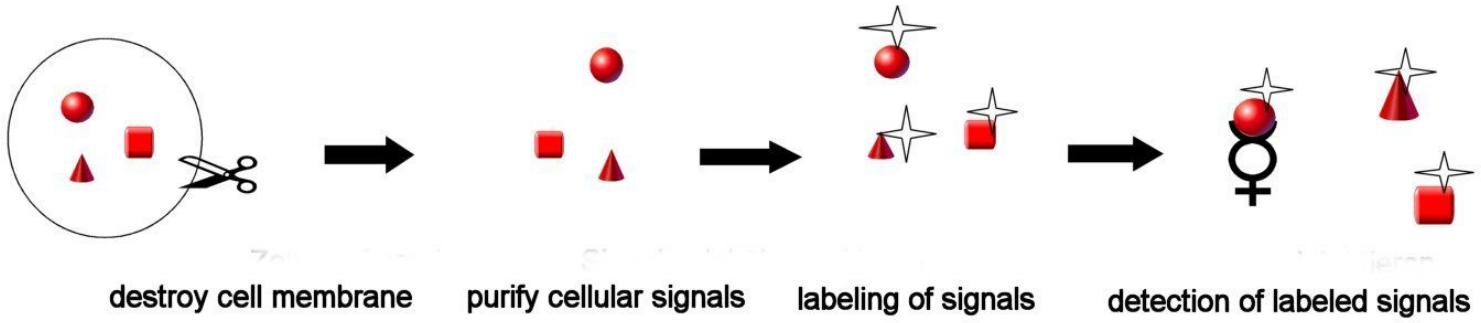
  viral particles

Lassa infection

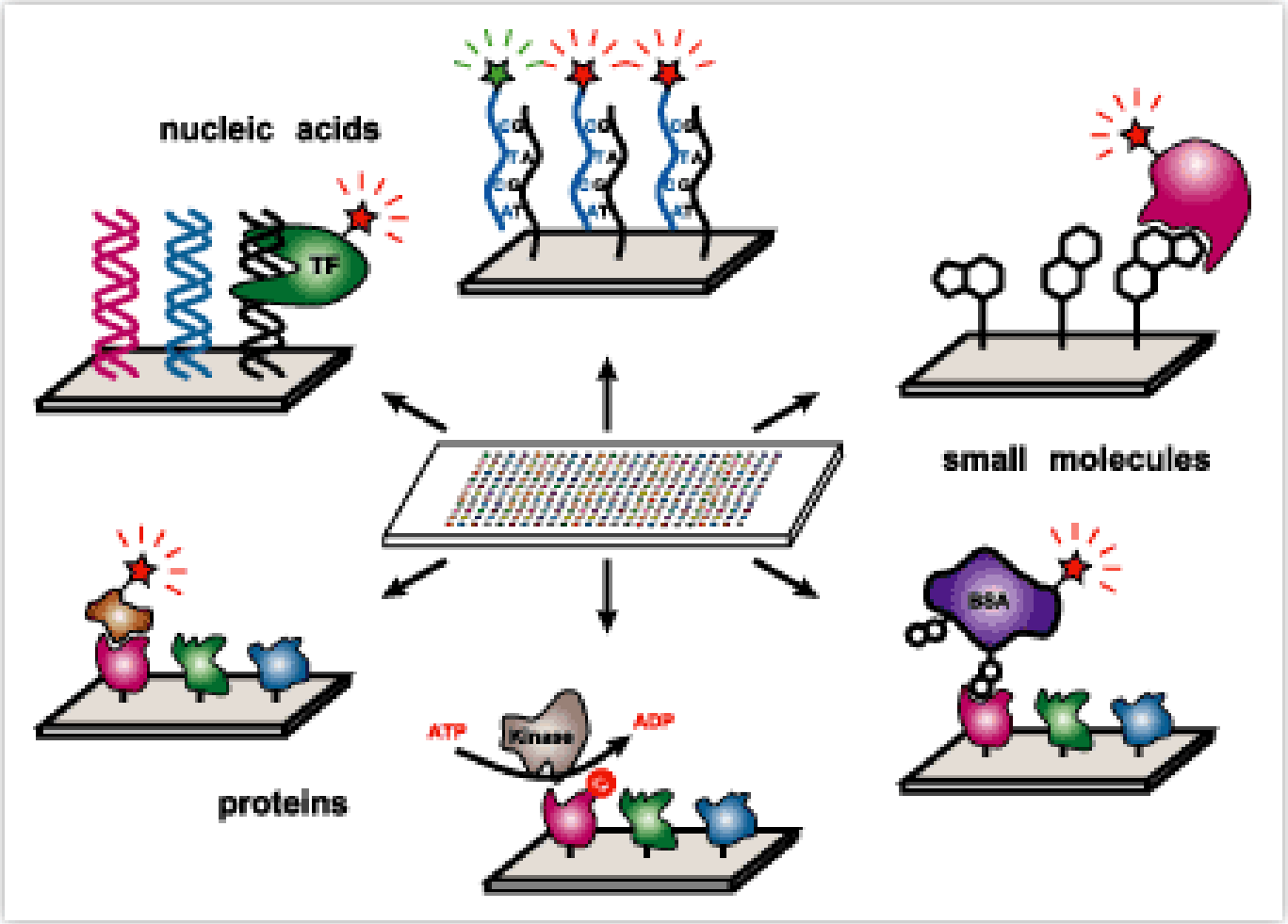
- Events inside the infected cells
- Visualisation problem
- Possibility to solve the problem: analyse the cellular signals with DNA microarrays



Scheme of experimental procedure



- What are microarrays?

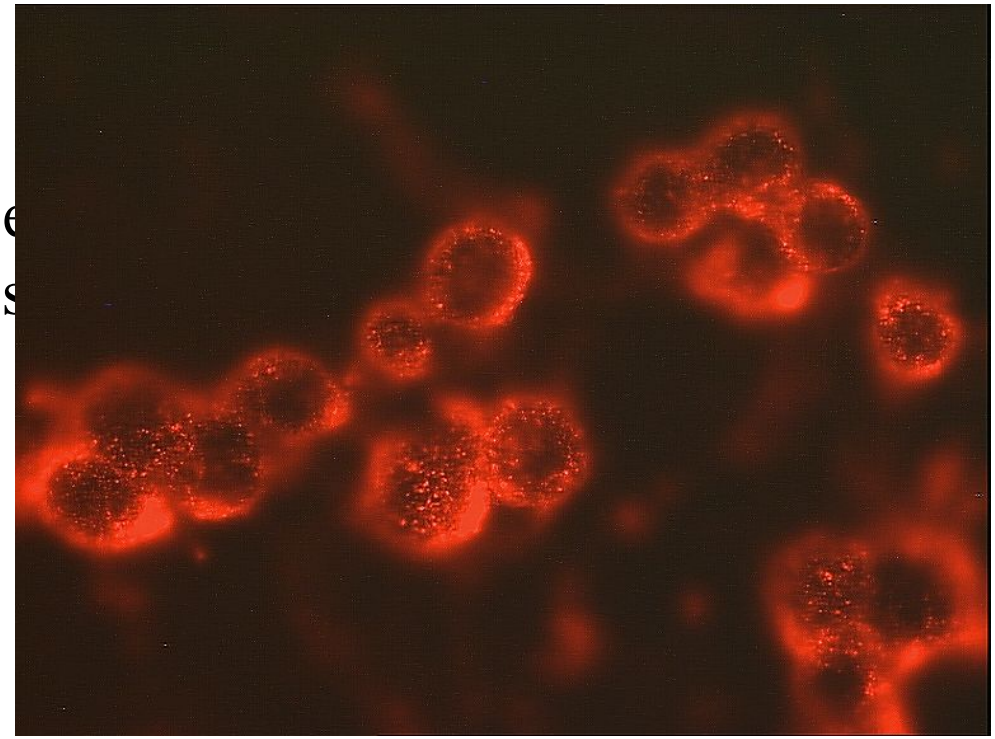


- **Microarray Experiments**

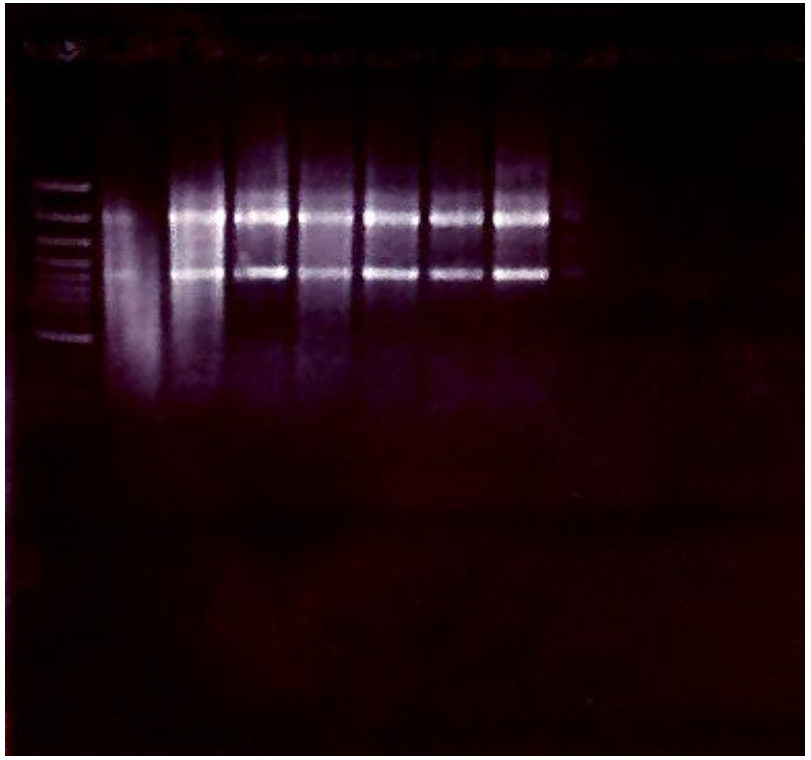
- Infection of model cells with Lassa
- Isolation of mRNA from the infected cells
- Labelling of mRNA
- Hybridisation of mRNA to the array
- Detection of the signals
- Computer analysis of the signals

- **Cell culture and virus infection**
- Growth of cells on media
- Application of virus
- Growth of virus

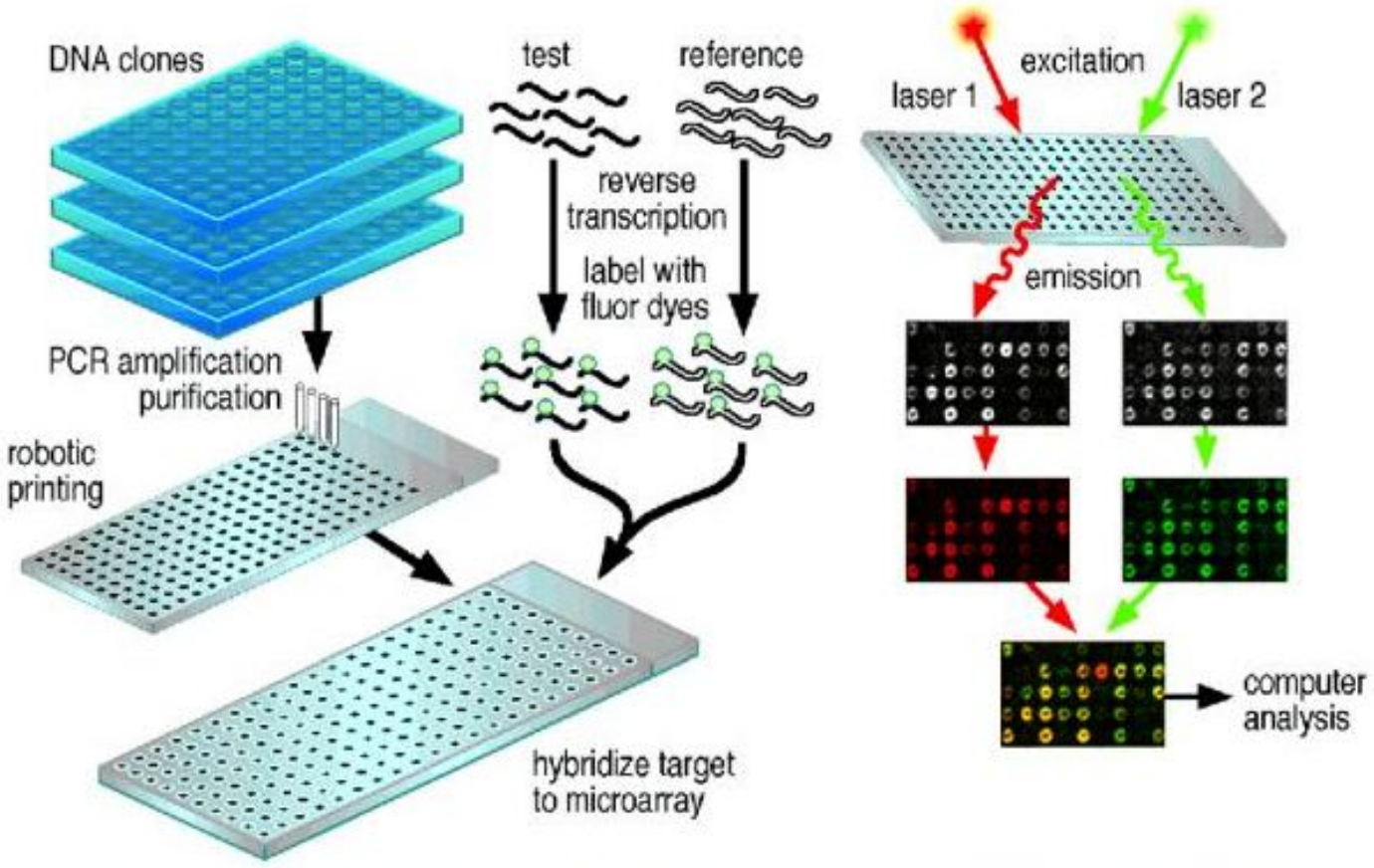
- **Control of virus infection**
- Detection of virus inside the cells with antibodies



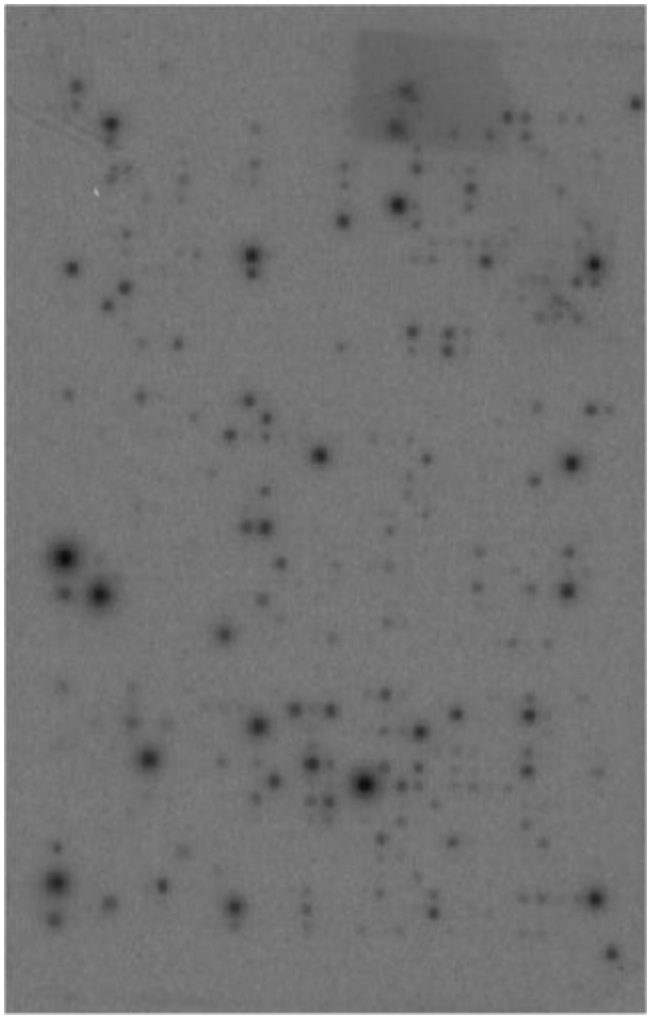
- **Isolation of mRNA**
- Destruction of cell membrane
- Isolation of mRNA from the cytoplasm
- Detection of mRNA-control of isolation



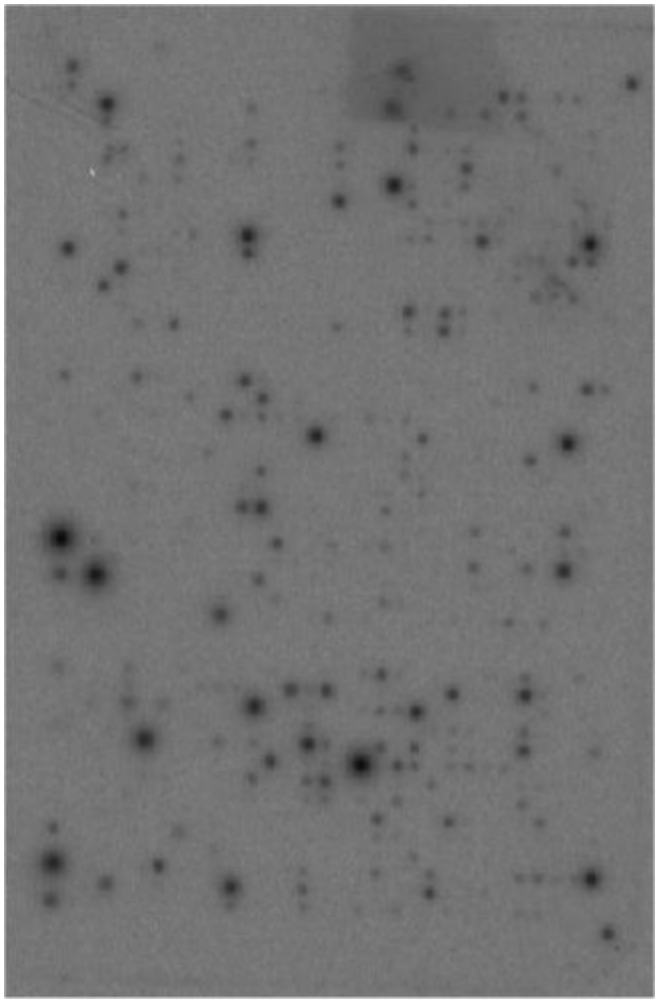
- **Microarray Analysis-scheme**



- Array

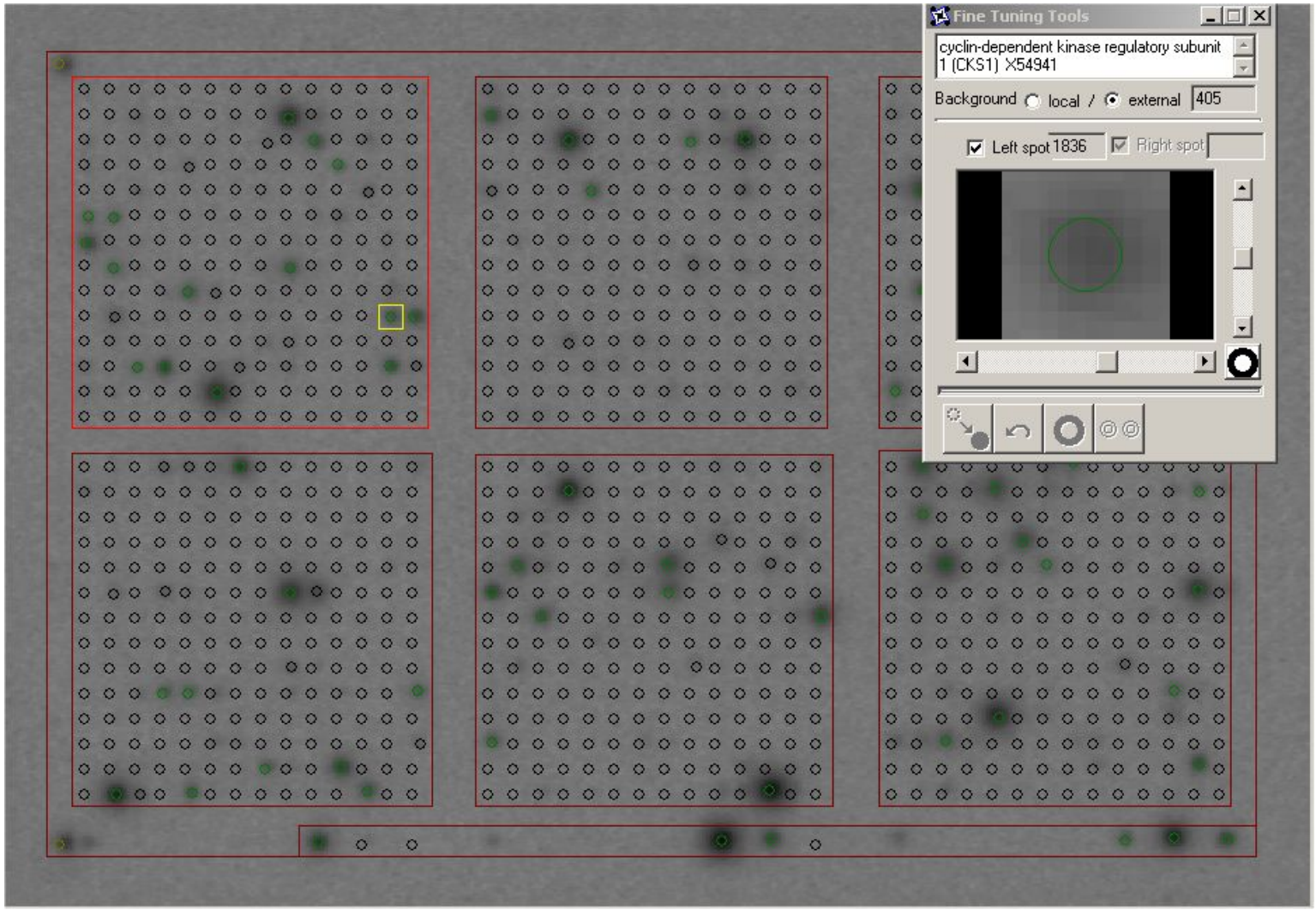


Lassa array I

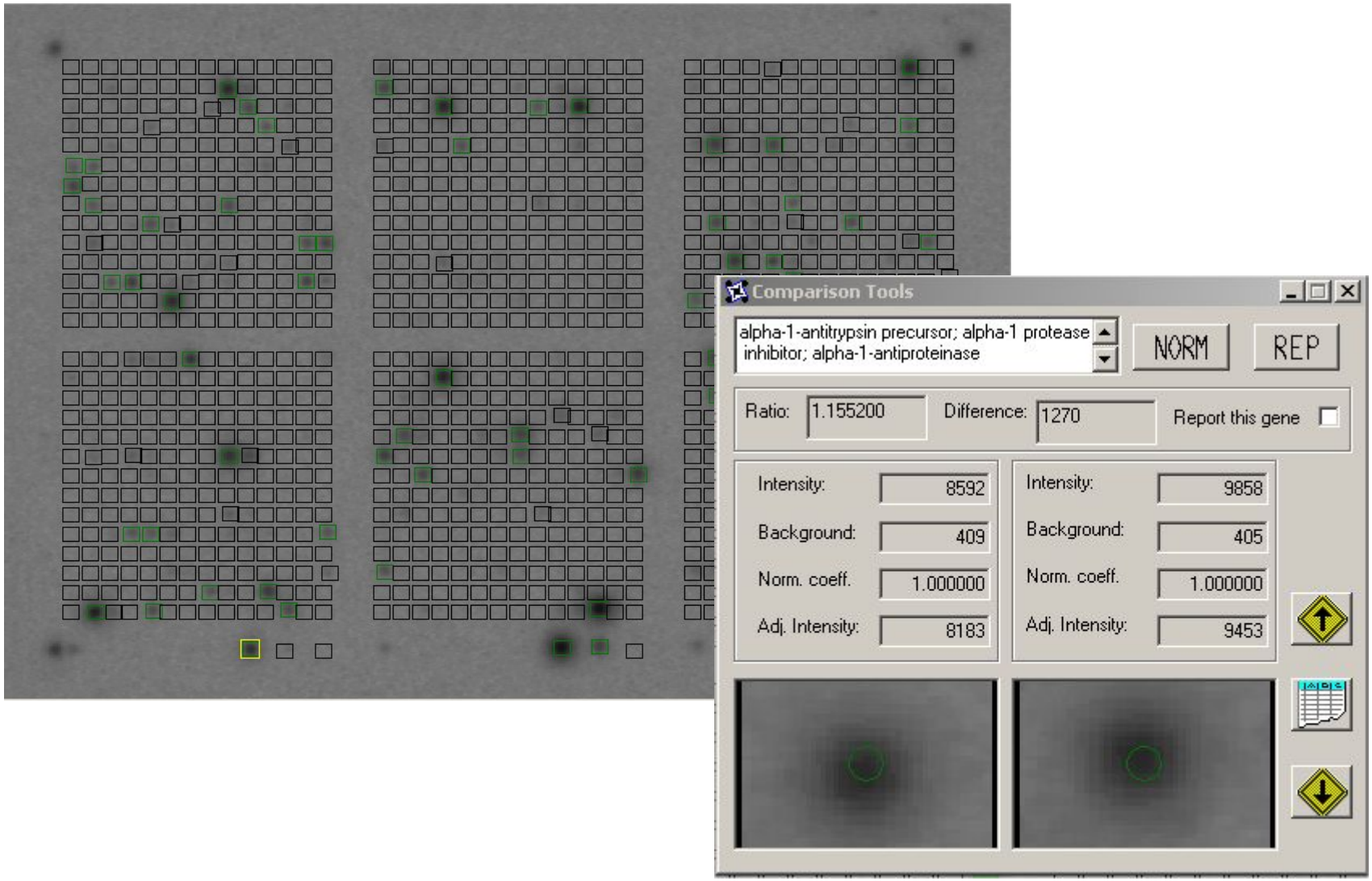


Control array I

- Alignment Lassa Array



- Comparison Lassa-control



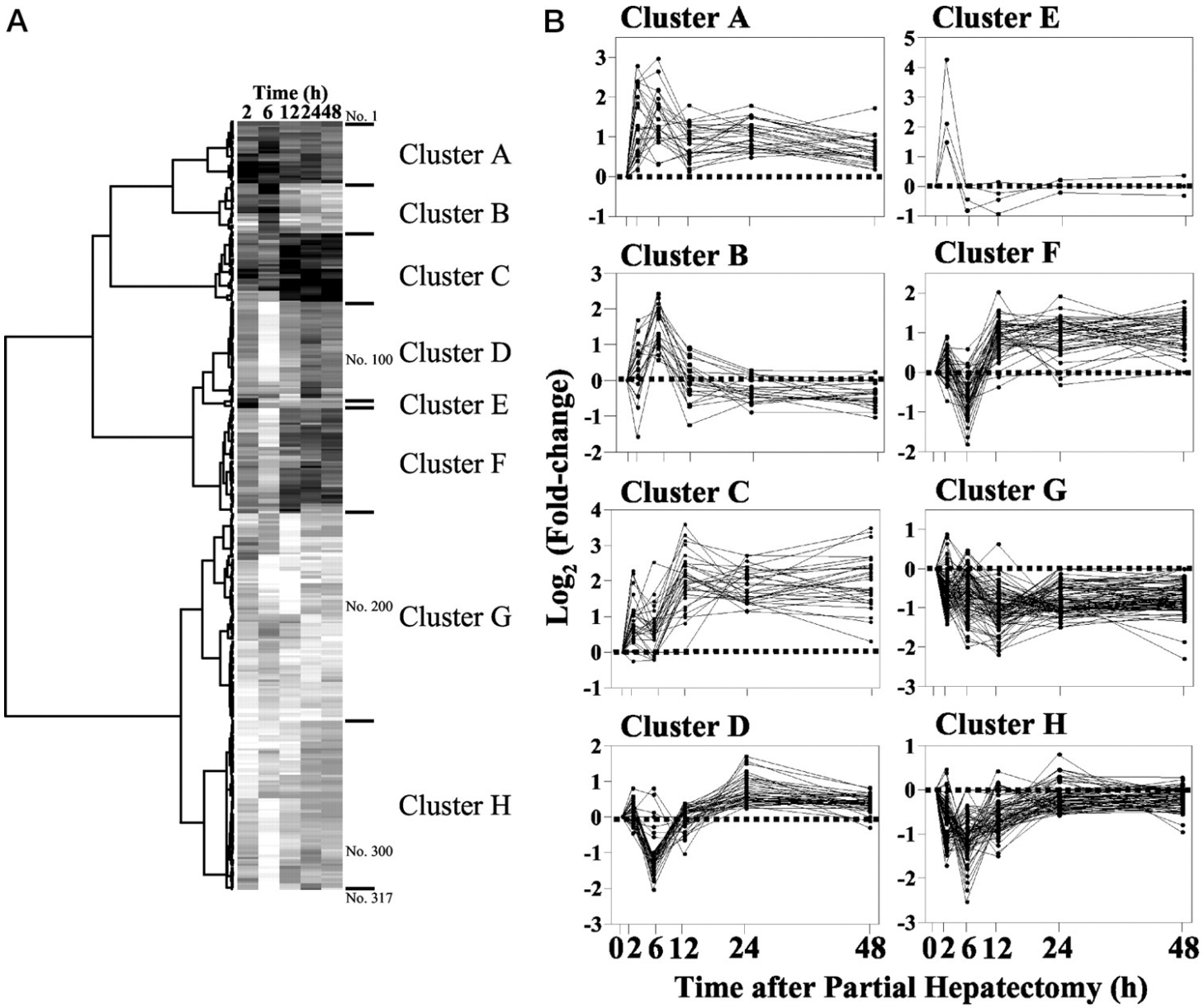
- Data analysis

- Comparison of infected cells and control cells
- Comparison of several experiments:
- Question:

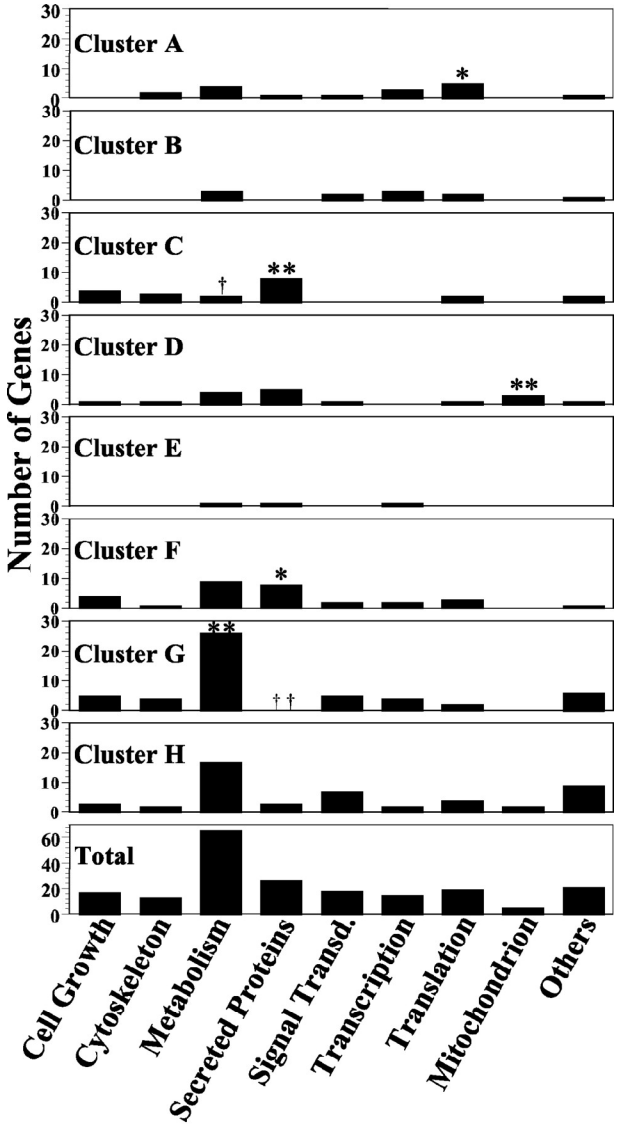
  Which genes are affected by the same external parameters?

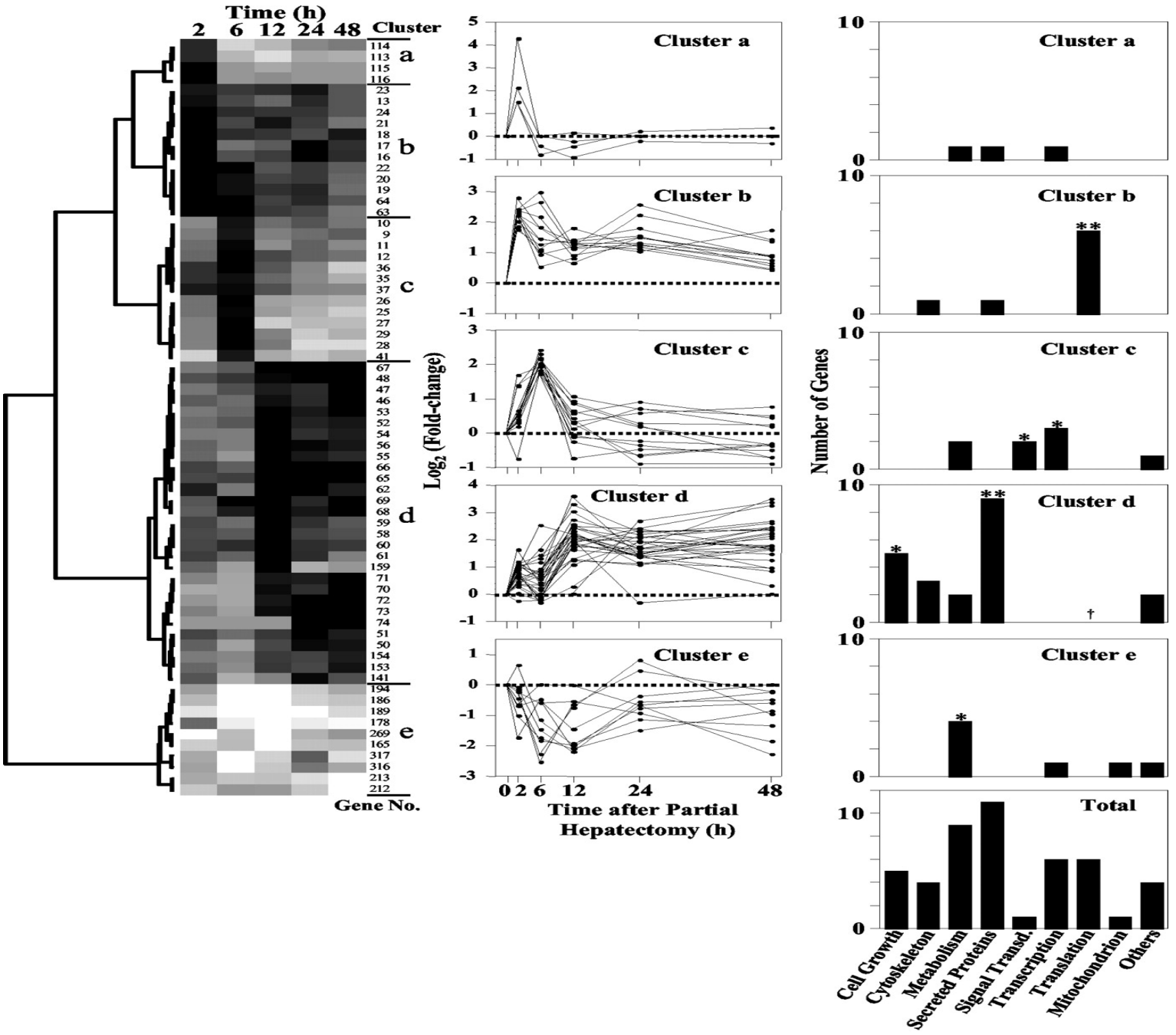
  Are there gene clusters regulated together?

- Technical solution: cluster analysis


A
Time (h)
2 6 12 24 48
No. 1
Cluster A
Cluster B
Cluster C
No. 100
Cluster D
Cluster E
Cluster F
No. 200
Cluster G
Cluster H
No. 300
No. 317
B
Cluster A
Cluster E
Cluster B
Cluster F
Cluster C
Cluster G
Cluster D
Cluster H
Log2 (Fold-change)
0 2 6 12 24 48
Time after Partial Hepatectomy (h)

- Do the genes within the cluster belong to special biological groups
- Clustering because of biological function




Time (h)
2 6 12 24 48
Cluster
a
b
c
d
e
Gene No.
Log2 (Fold-change)
Cluster a
Cluster b
Cluster c
Cluster d
Cluster e
0 2 6 12 24 48
Time after Partial Hepatectomy (h)
Number of Genes
Total
Cell Growth
Cytoskeleton
Metabolism
Secreted Proteins
Signal Transd.
Transcription
Translation
Mitochondrion
Others

- **Add-ons**
- working with Lassa virus
- because of high mortality rate of 30% and the lack of an accurate therapy Lassa virus is classified as BSL4 organism
- This implicates working under special conditions

The biosafty levels and classification of organisms

- 1: not harmful for humans and environment
  E. coli, Streptomyces spc
- 2: little danger for humans and environment
  S. pneumonie
- 3: high danger for humans and environment
  HIV
- 4: extremely dangerous for humans and environment
  Lassa, Ebola, Marburg

- Working in a BSL4 laboratory
- special laboratories, 2x in Germany
- special air systems, restricted area, decontamination of personal and material
- special equipment for people working in the lab

The Laboratory

preparing

Working in the Lab: moving

Working in the lab

microscopy

Communication and co-operation