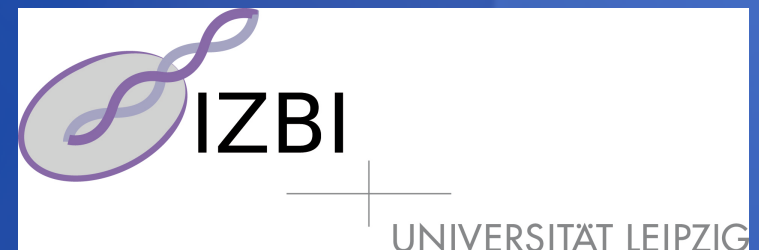


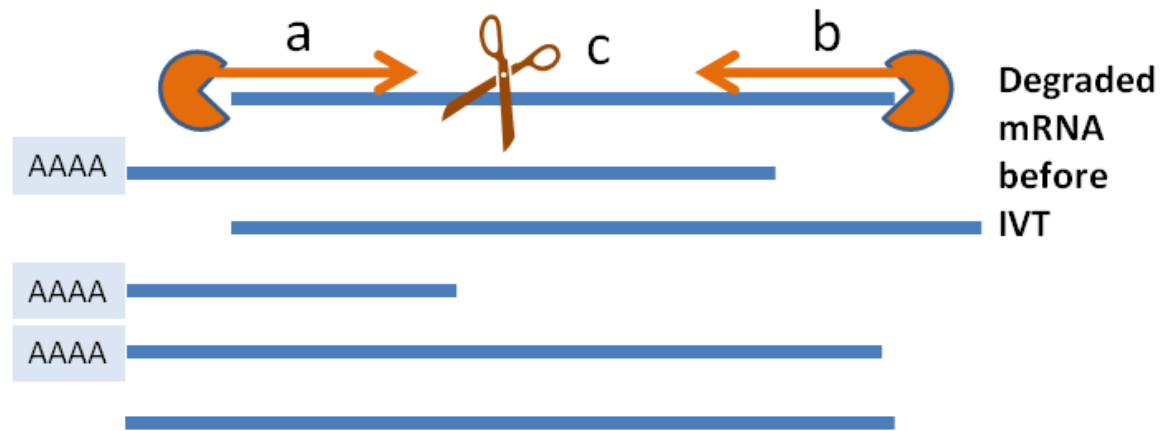
# Learning about RNA Degradation (from Microarray Data)



# Synopsis

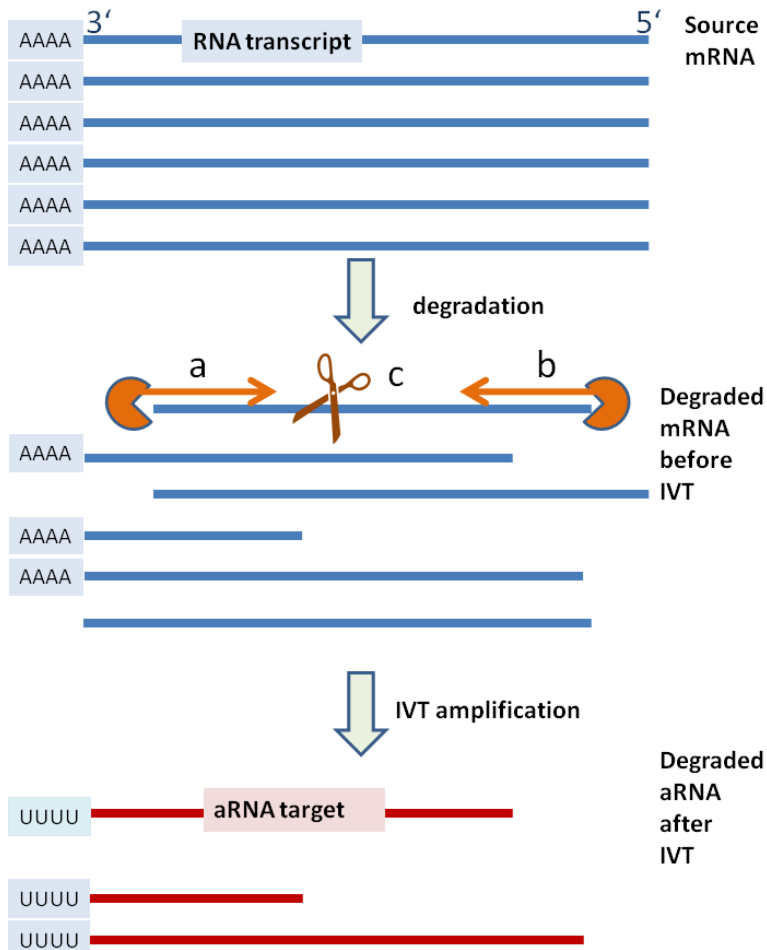
- RNA Degradation Basics (one slide)
- A quantitative analysis of RNA degradation based on microarray data
- Qualitative analysis of RNA degradation
- Time runs out

# RNA degradation - on a single slide



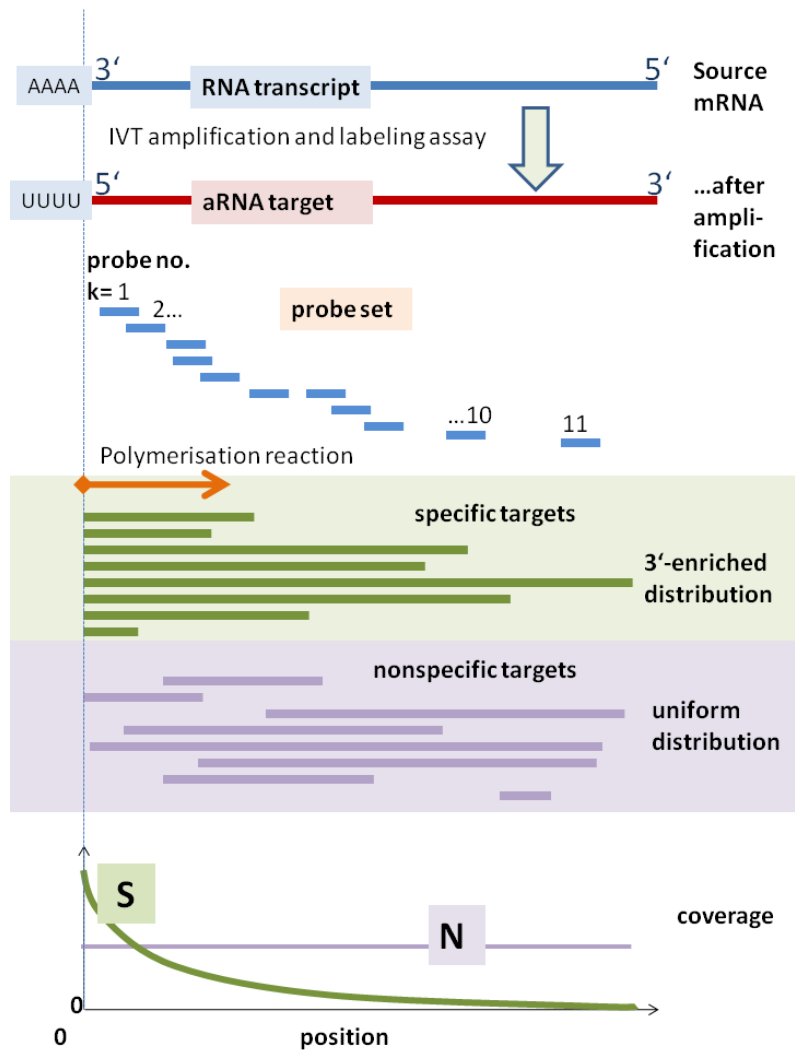
- Complex; controlled by many pathways
- Must be avoided for all transcriptomics experiments – assessment mandatory

# Affymetrix 3' IVT protocol is practical for degradation analysis



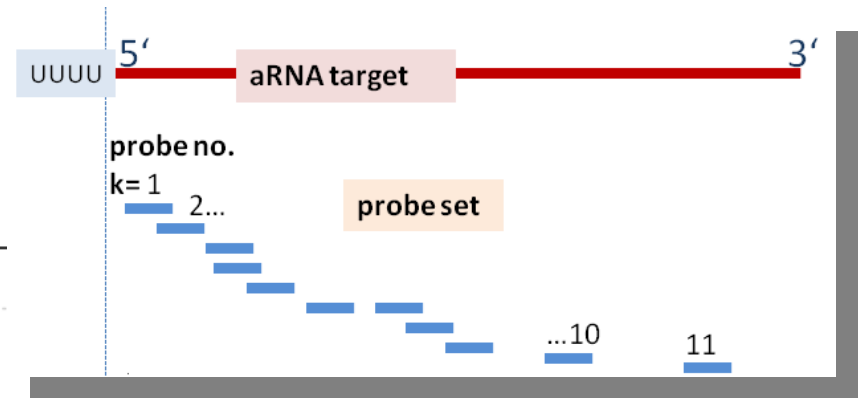
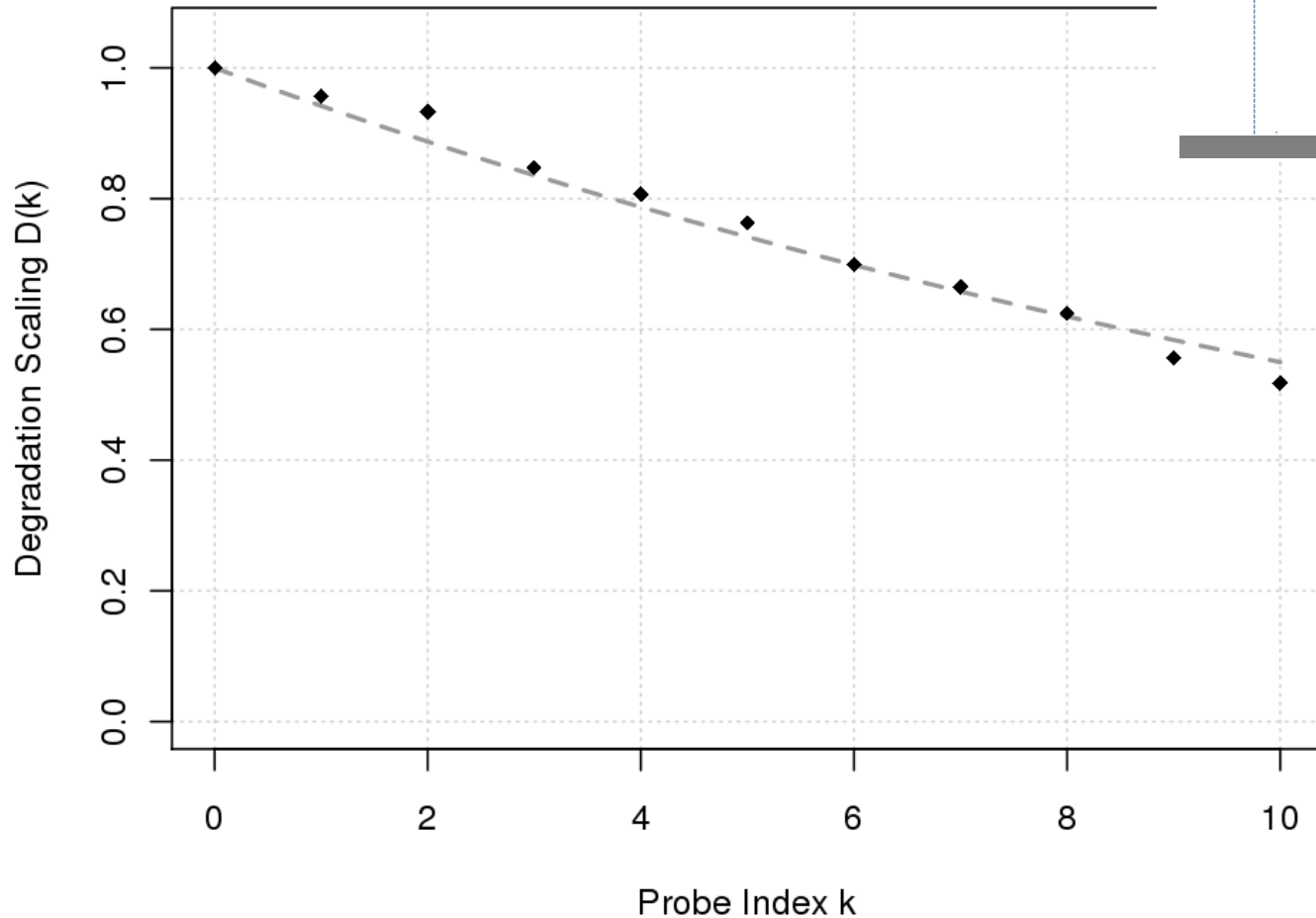
- In-vitro transcription protocol leads to uniform aRNA amplification directed from 3' end of mRNA

# Affymetrix probe design is practical for degradation analysis

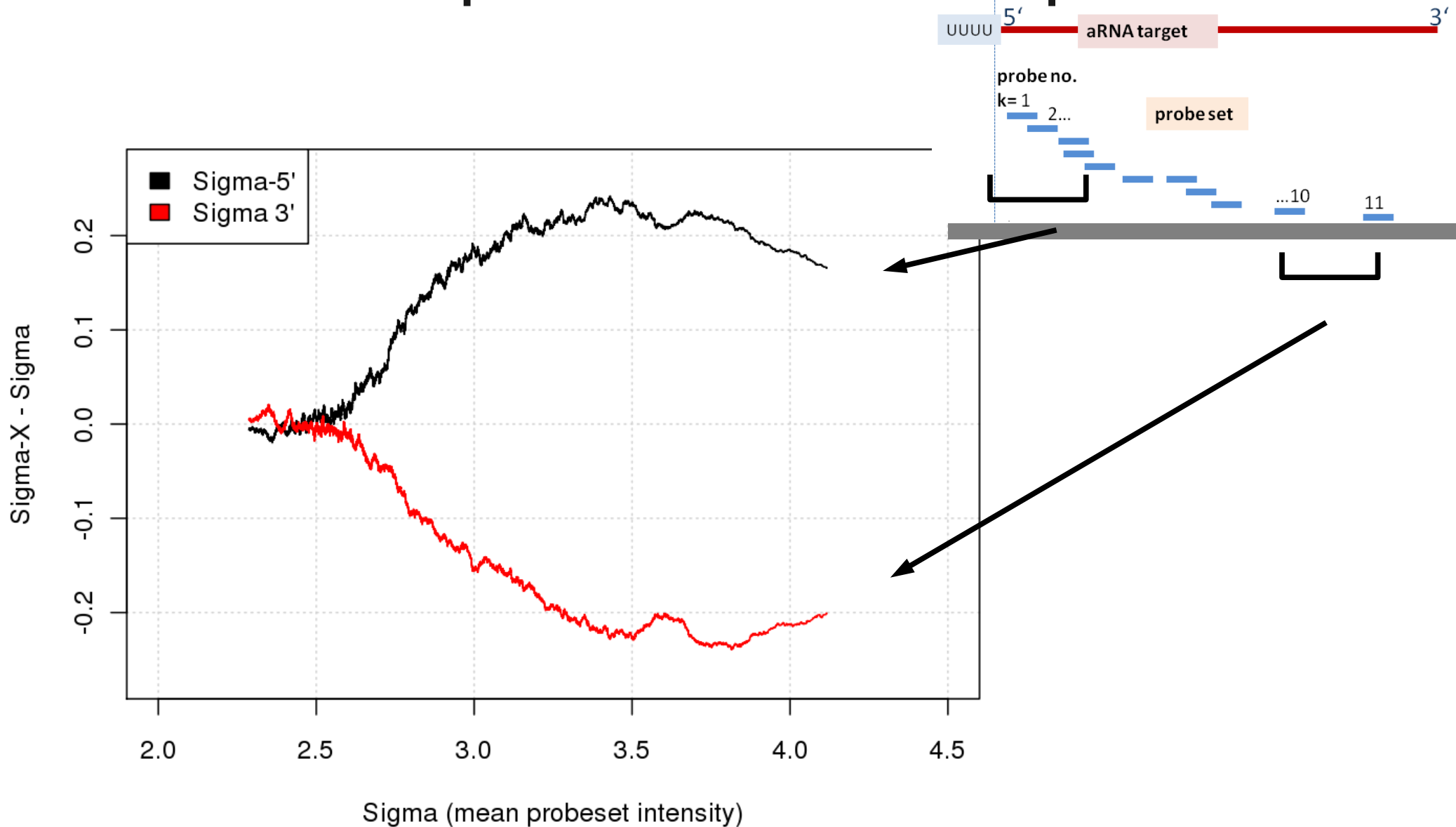


- 11 or more probes target one transcript at varying positions

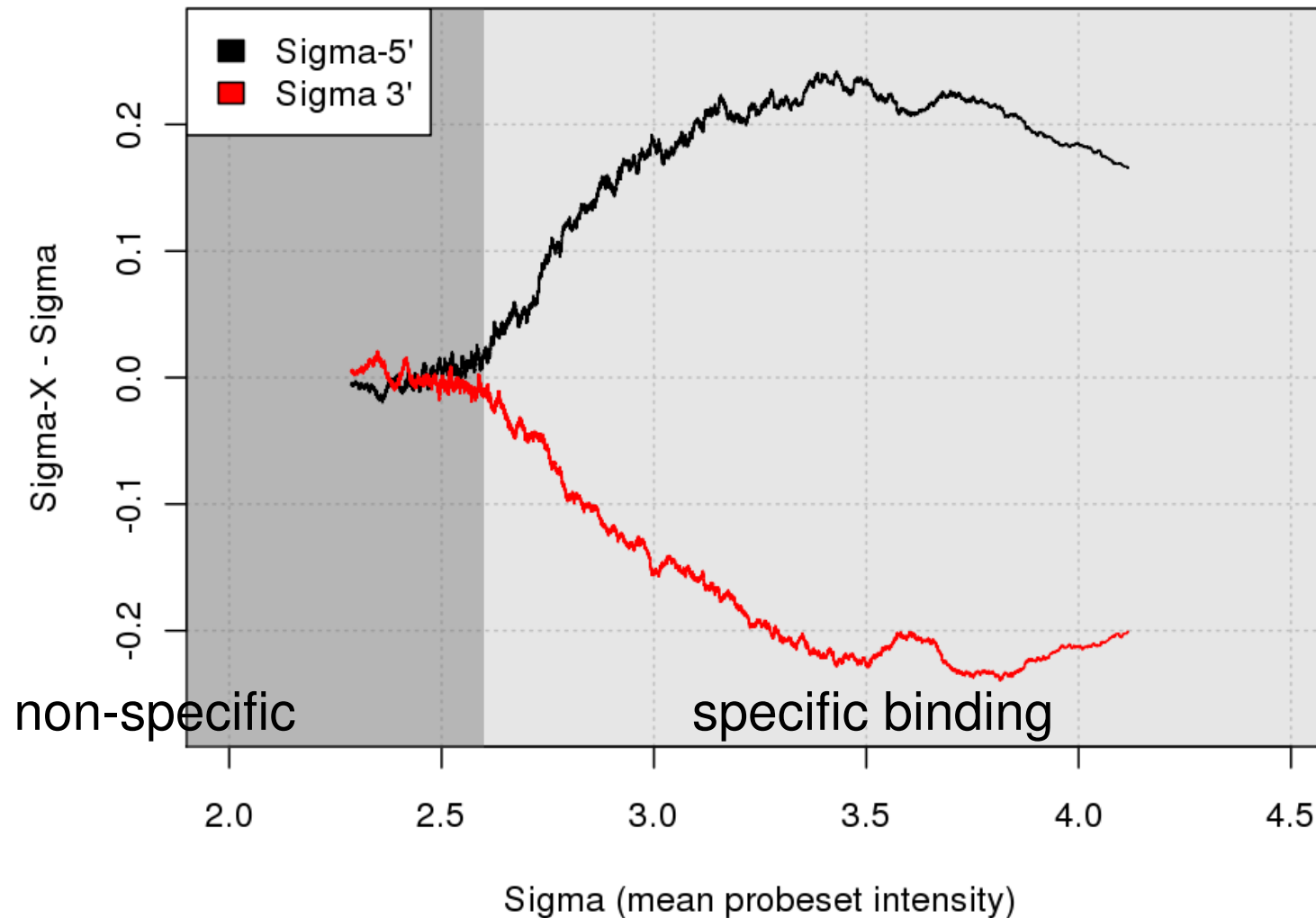
# Basic measure: average of all probe intensities over probe index



# Degradation can only be measured for expressed transcripts

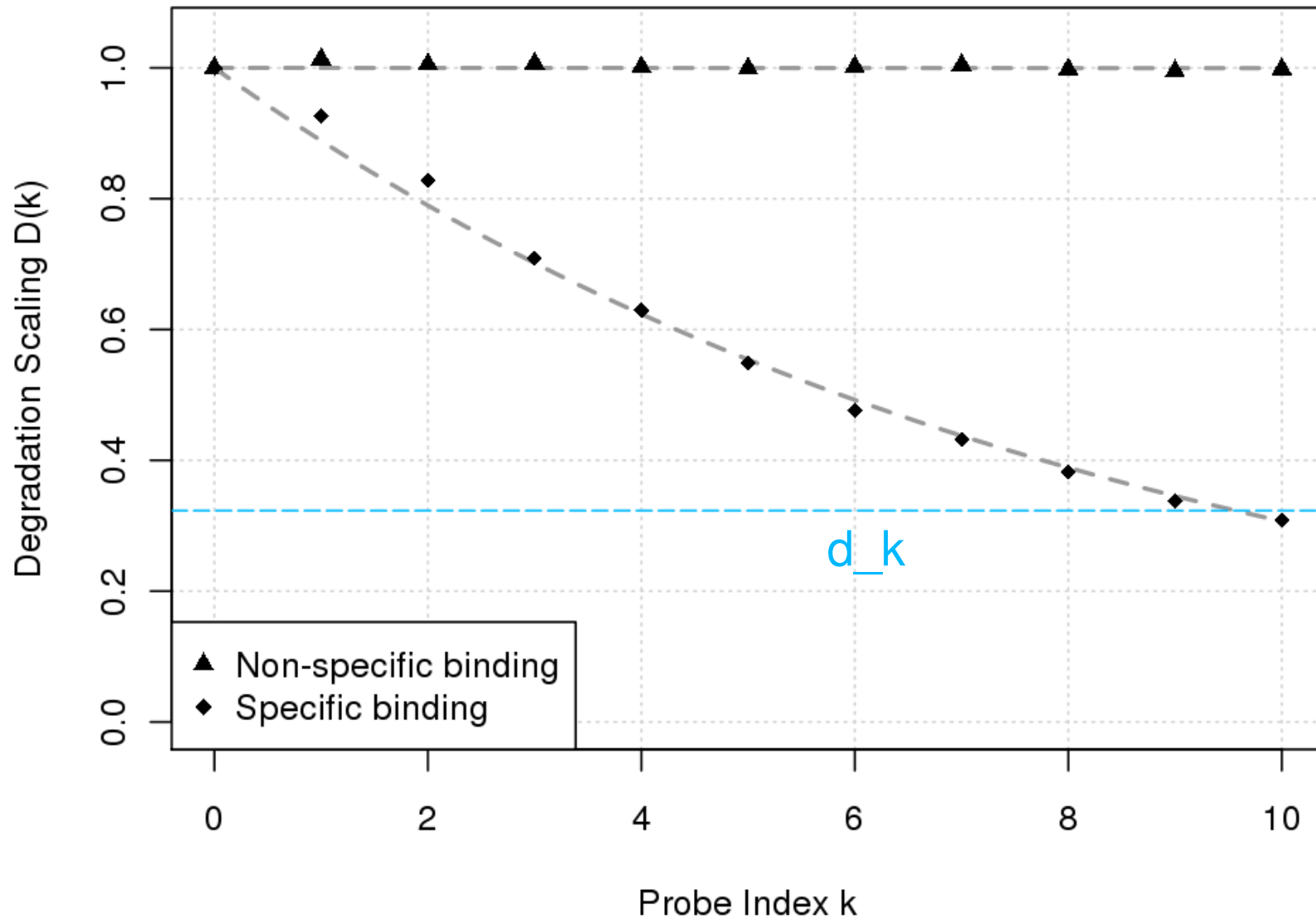


# Degradation can only be measured for expressed transcripts

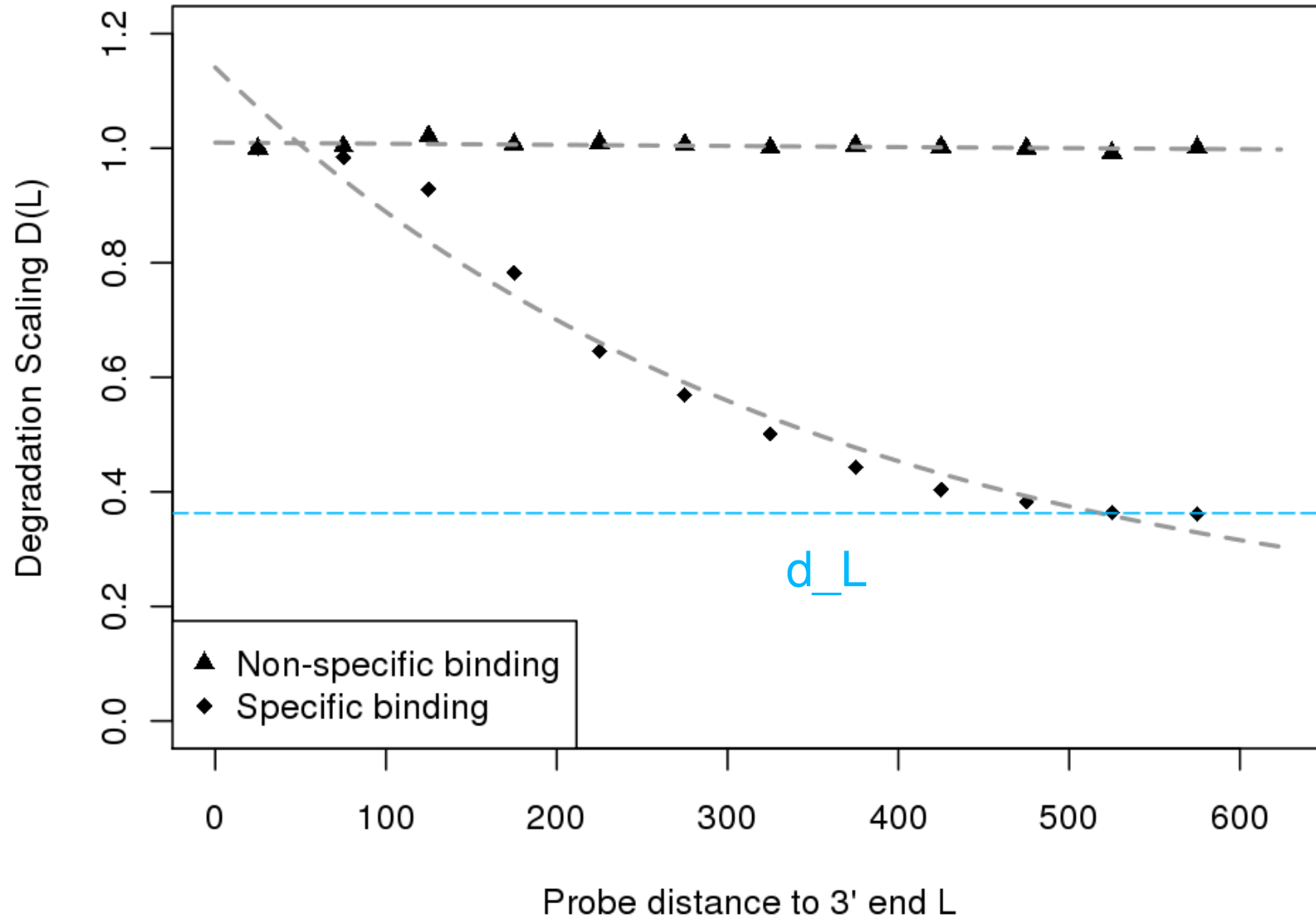




# Degradation = intensity decrease for specific transcripts

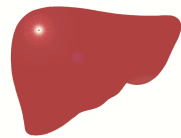


# Using the actual alignment of the probes to the target mRNA





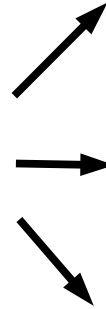
# The „Rat Experiment”



Liver



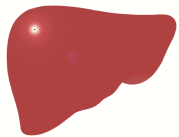
Incubation  
0-300min 37°C



RNA later



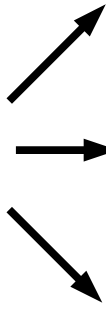
RNA Isolation



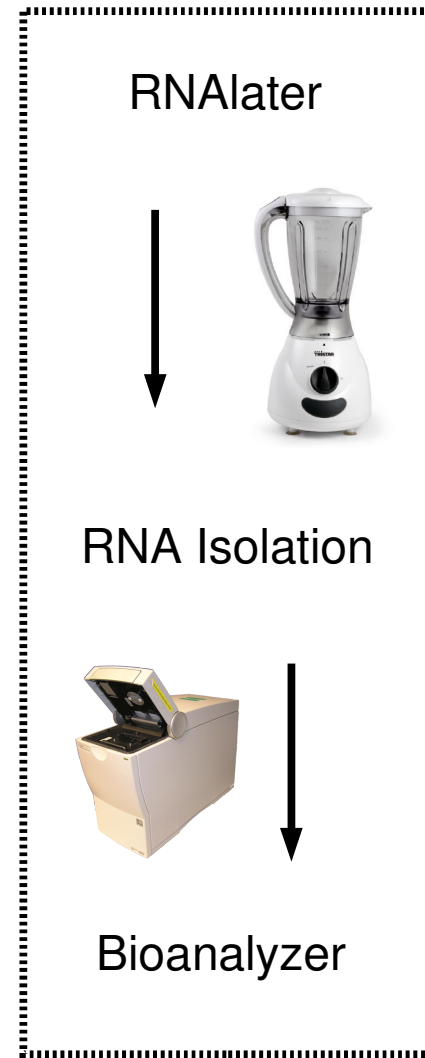
Snap frozen -70°C.  
Thawing.



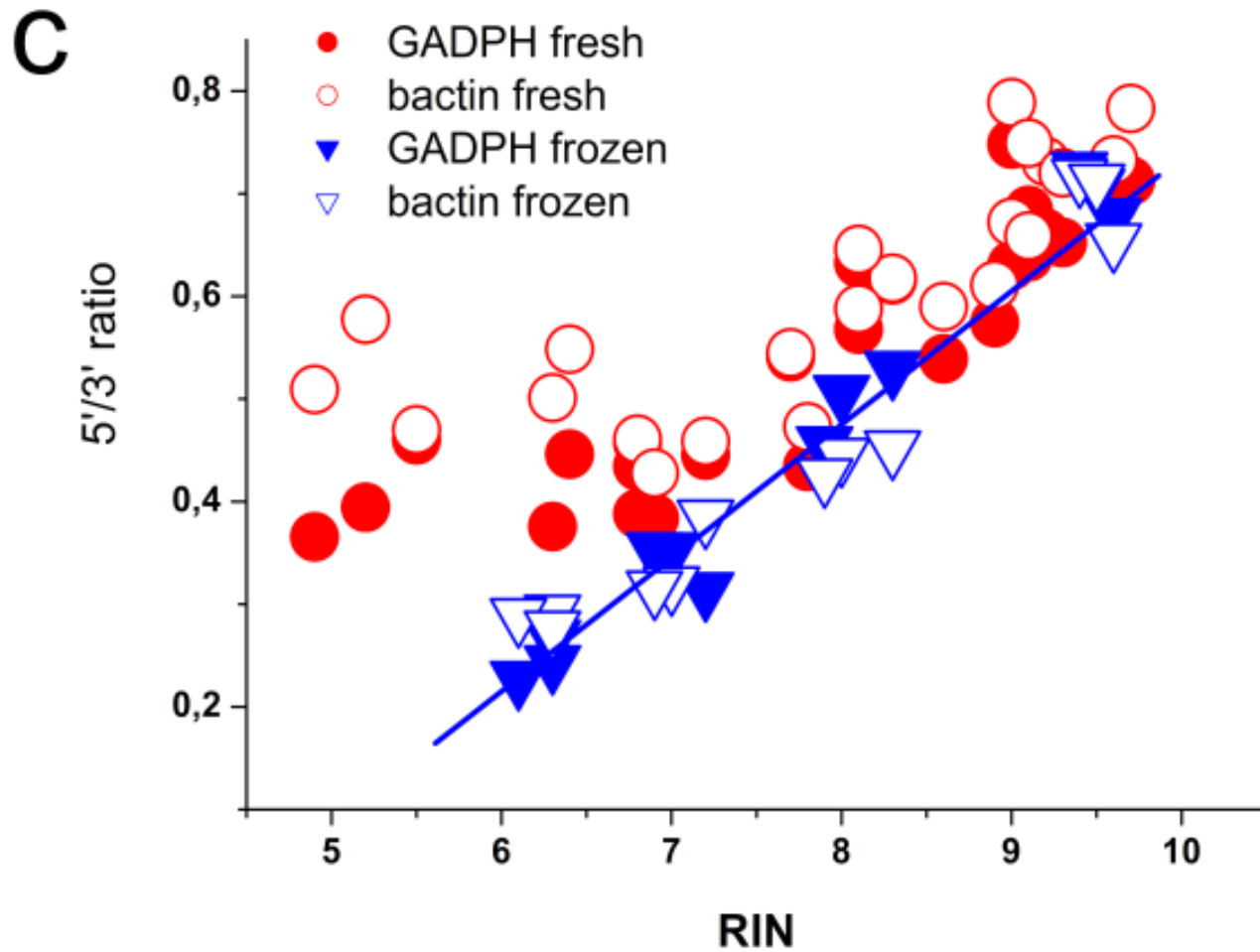
Incubation  
0-60min



Bioanalyzer

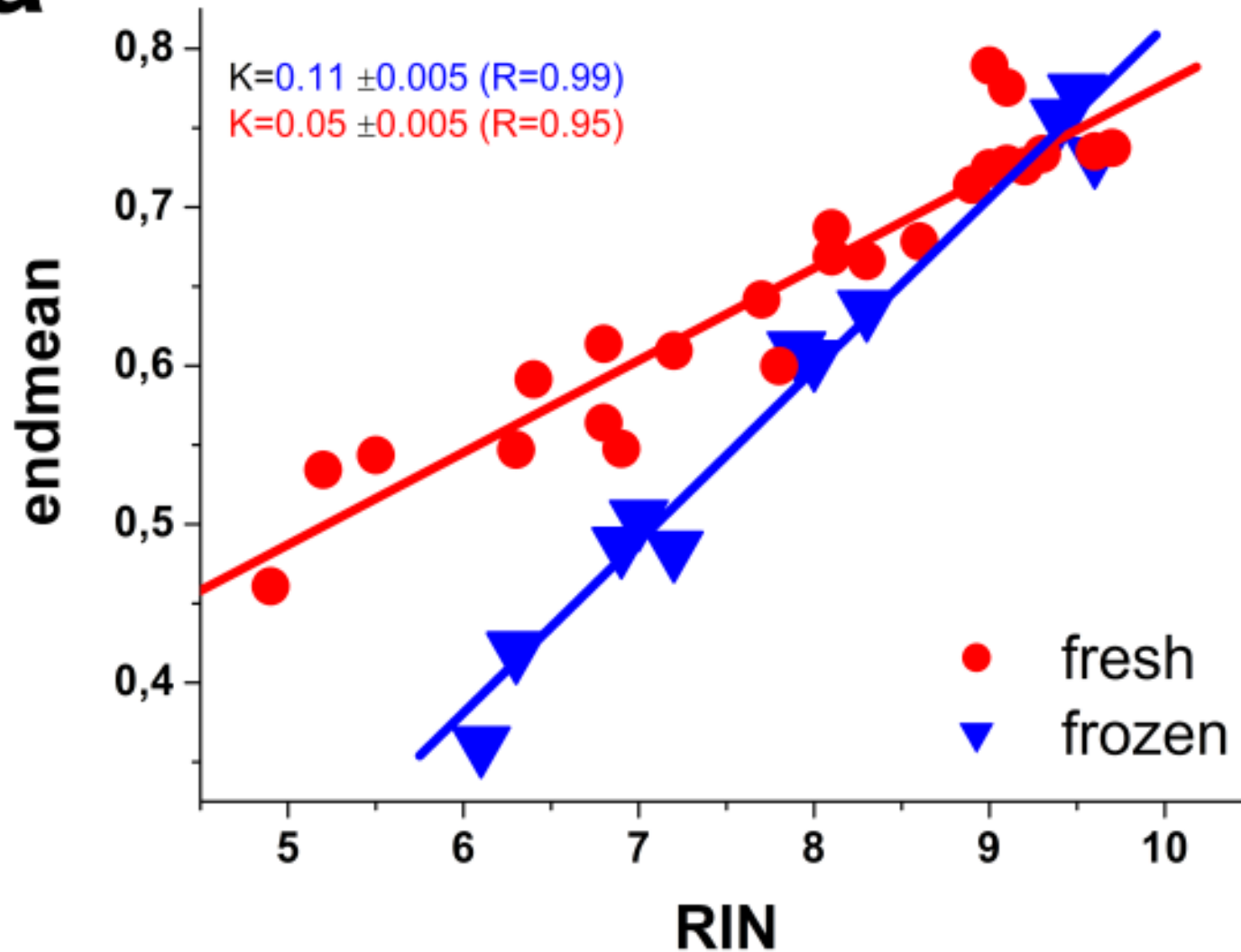


# Affymetrix Marker vs. Bioanalyzer

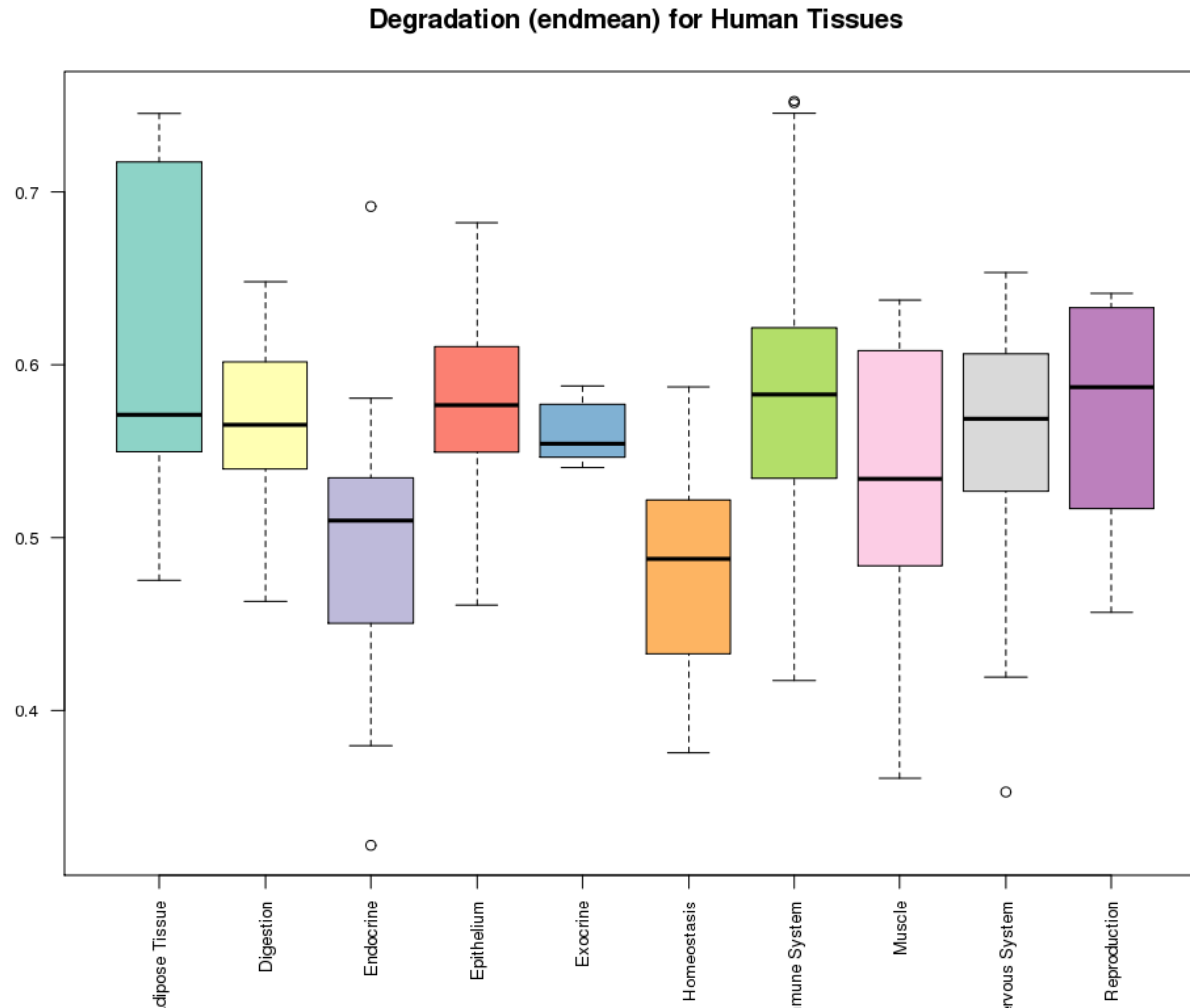


# D\_k vs. Bioanalyzer

**a**



# Example result: degradation is tissue-specific



# Qualitative assessment of degradation

- Idea: certain biological processes might 'correlate' with degradation in a time dependent manner
- Use  $d_k$  as an indicator
- Can we build gene sets that are a marker for degradation?

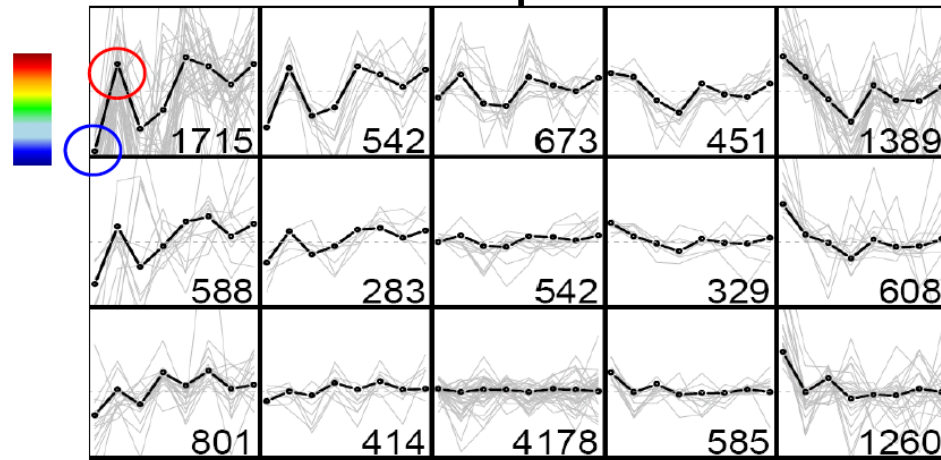
# Self Organizing Maps

Raw Data

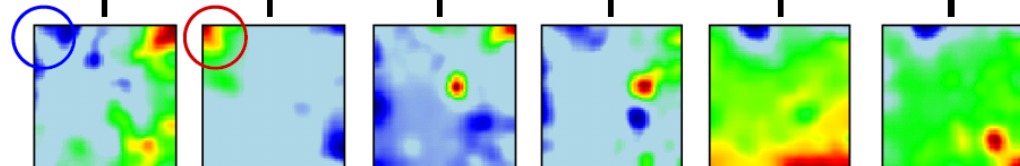


Preprocessing

Metagenes

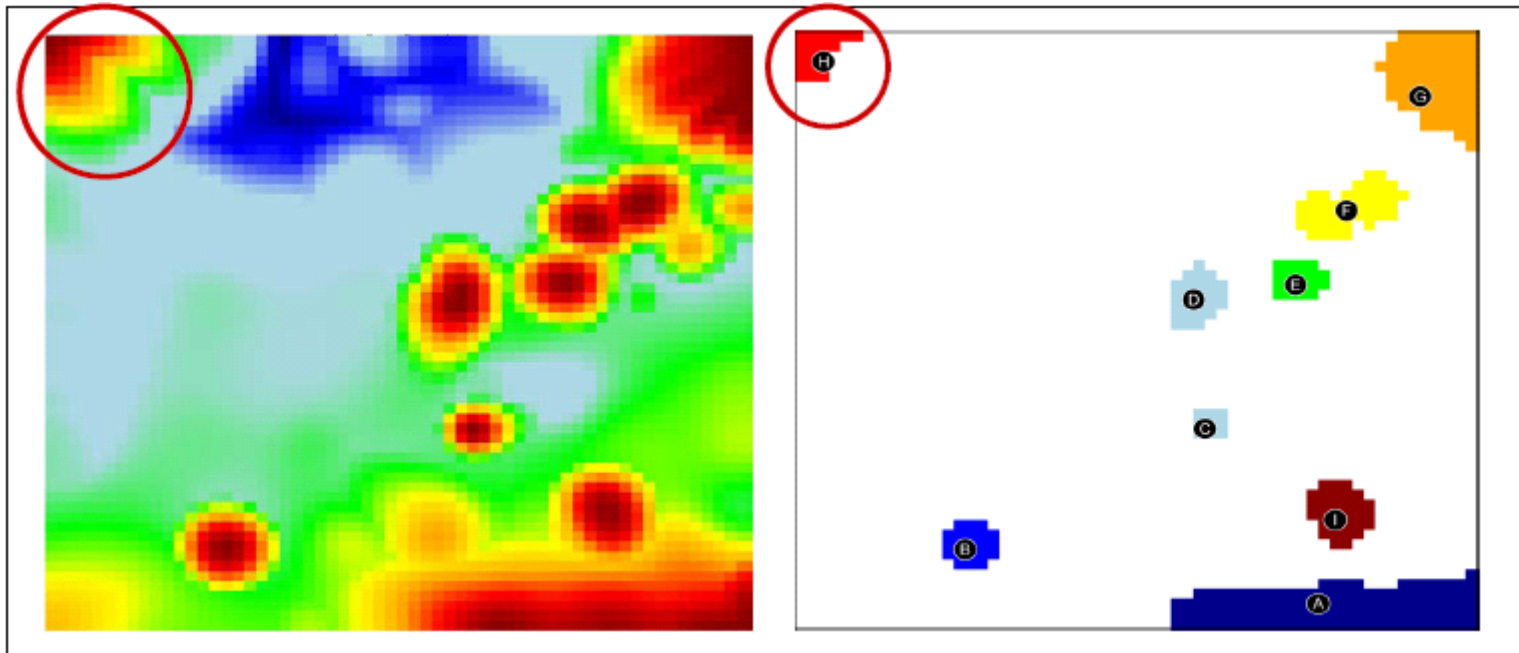


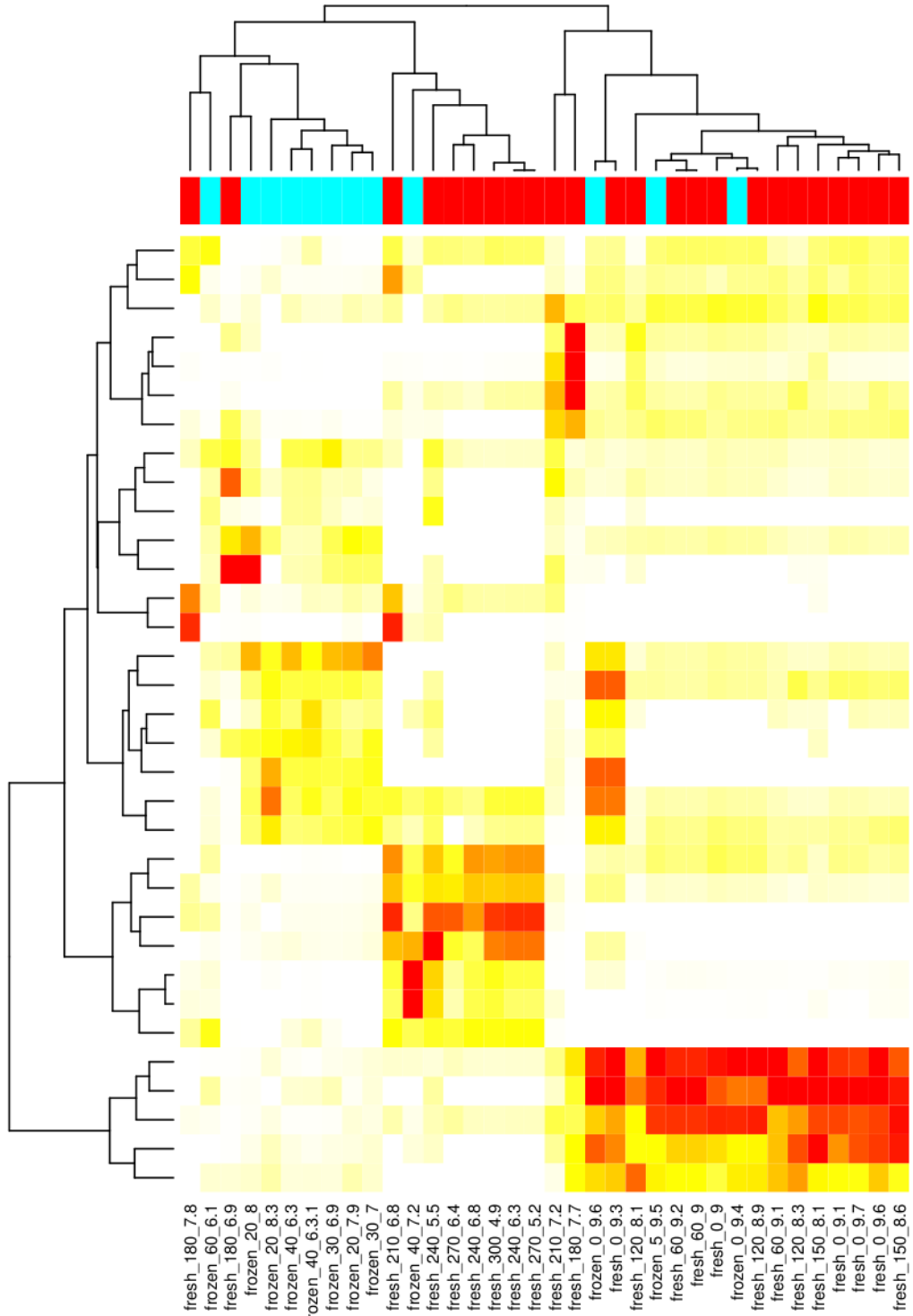
Expression Profiles





# Expression Profiles and Spots

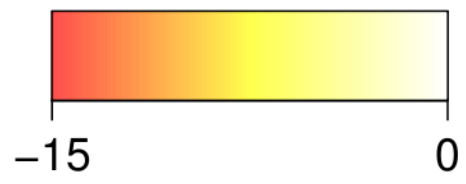




HG  
log( p-values )

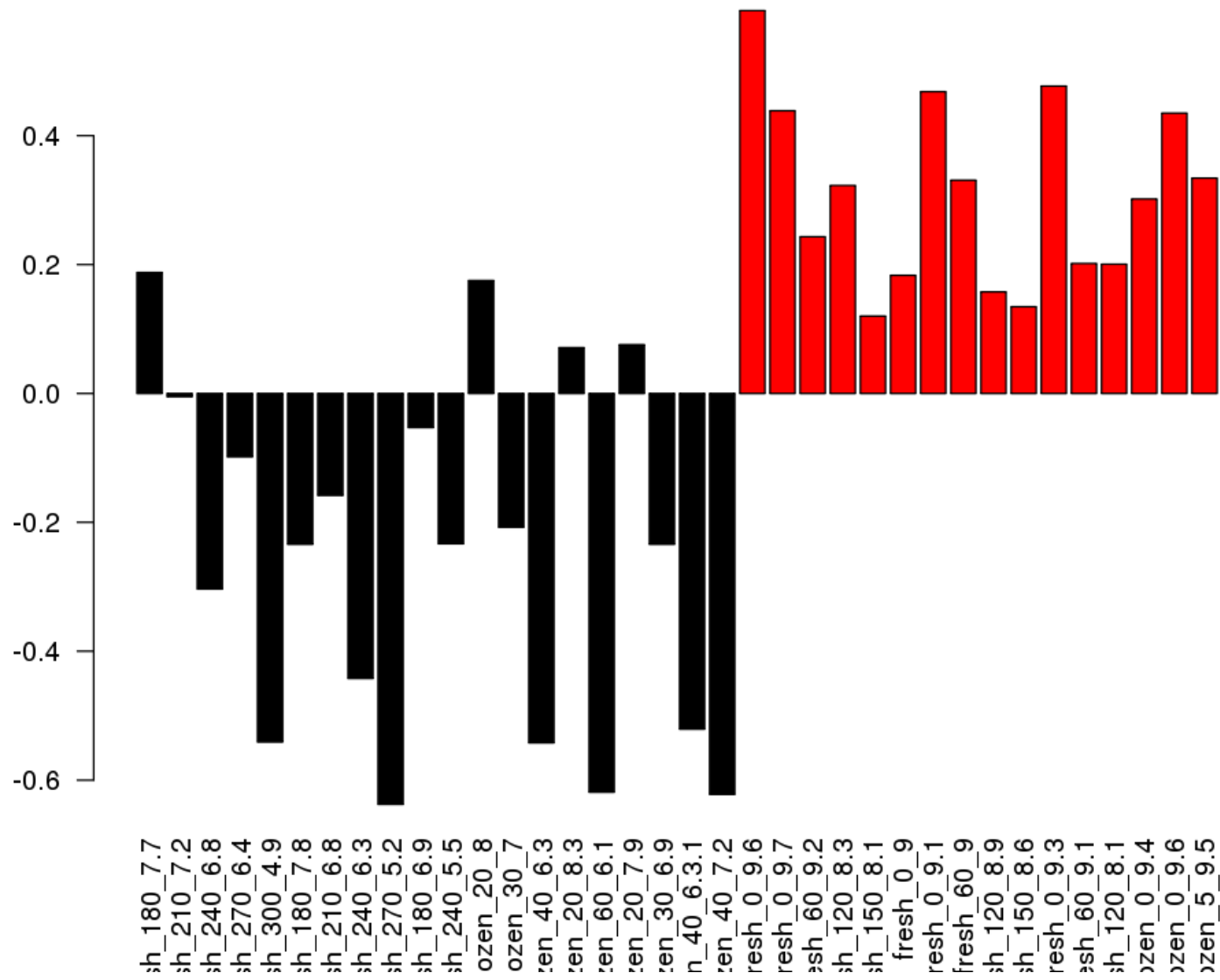
- regulation of transcription, DNA-dependent
- response to activity
- embryonic placenta development
- protein targeting to mitochondrion
- metabolic process
- cell redox homeostasis
- ubiquitin-dependent protein catabolic process
- chromatin modification
- mRNA transport
- androgen receptor signaling pathway
- microtubule-based process
- RNA processing
- anterior/posterior pattern formation
- sensory perception of smell
- bone mineralization
- T cell differentiation
- leukocyte cell-cell adhesion
- transcription, DNA-dependent
- actin filament bundle assembly
- response to vitamin D
- positive regulation of protein amino acid phosphorylation
- synaptic transmission
- muscle organ development
- adult walking behavior
- regulation of cell growth
- potassium ion transport
- ion transport
- feeding behavior
- cellular response to insulin stimulus
- cholesterol homeostasis
- positive regulation of transcription
- positive regulation of smooth muscle cell proliferation
- regulation of protein catabolic process

fresh\_180\_7.8  
frozen\_60\_6.1  
fresh\_180\_6.9  
frozen\_20\_8  
frozen\_20\_8.3  
frozen\_40\_6.3  
frozen\_40\_6.3.1  
frozen\_30\_6.9  
frozen\_20\_7.9  
frozen\_30\_7  
fresh\_210\_6.8  
frozen\_40\_7.2  
fresh\_240\_5.5  
fresh\_270\_6.4  
fresh\_240\_6.8  
fresh\_300\_4.9  
fresh\_240\_6.3  
fresh\_270\_5.2  
fresh\_210\_7.2  
fresh\_180\_7.7  
frozen\_0\_9.6  
fresh\_0\_9.3  
fresh\_120\_8.1  
frozen\_5\_9.5  
fresh\_60\_9.2  
fresh\_60\_9  
fresh\_0\_9  
frozen\_0\_9.4  
fresh\_120\_8.9  
fresh\_60\_9.1  
fresh\_120\_8.3  
fresh\_150\_8.1  
fresh\_0\_9.1  
fresh\_0\_9.7  
fresh\_0\_9.6  
fresh\_150\_8.6

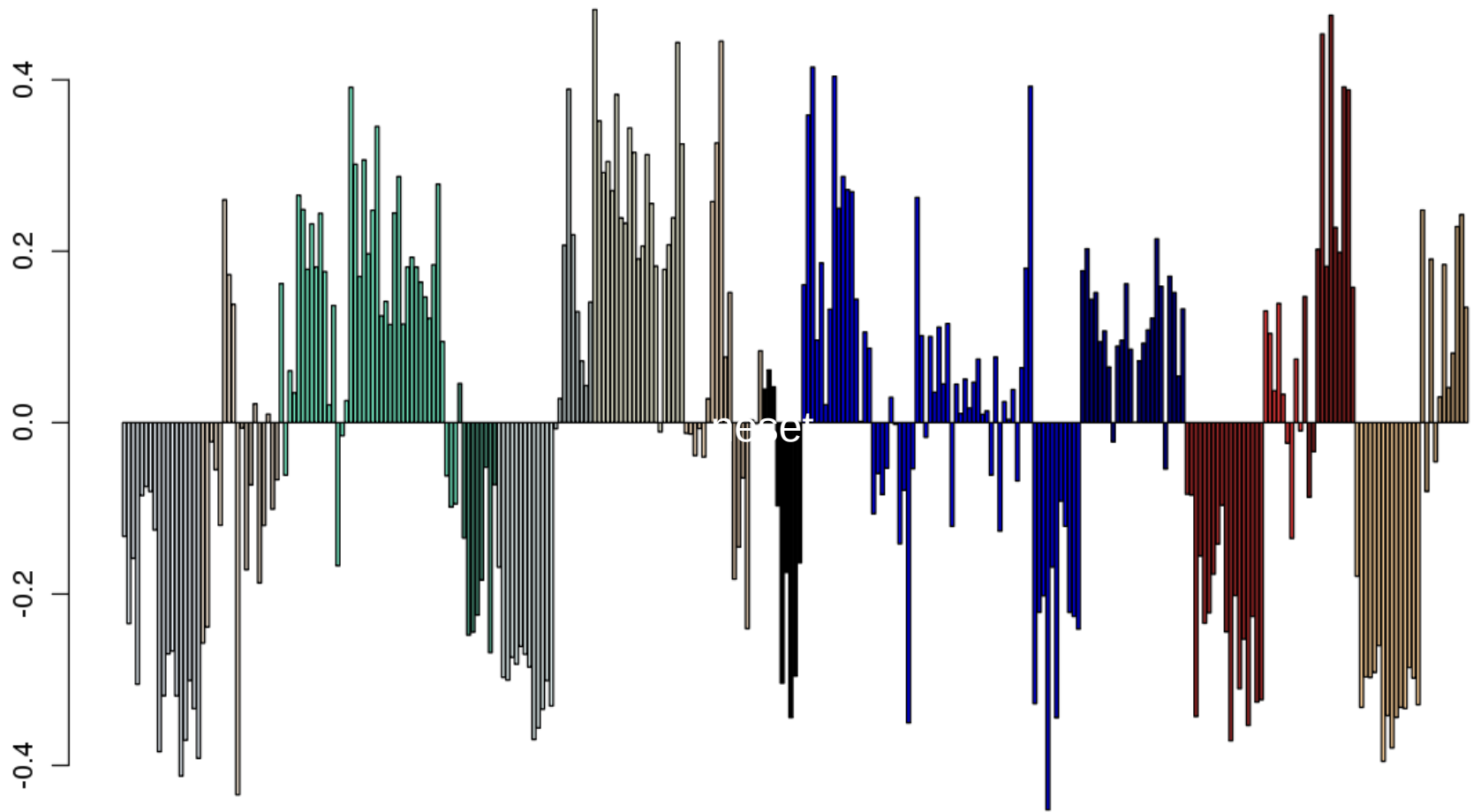


SOM  
GO-Enrichment  
Heatmap from  
Rat Experiment

# Fold-changes of marker geneset 1 in the „Rat Experiment”



# Fold-changes of marker geneset 1 (mapped) in human tissues dataset



# Take Home

- Degradation must be taken care of in any transcriptomics experiment
- It can well be assessed in Affymetrix 3' GeneChip microarrays
  - Use our degradation measure, available via an R package (see <http://www.izbi.de>)
- Maybe other platform-independent markers for degradation can be found

# Acknowledgments

- Hans Binder



- Henry Wirth



- Thanks for their ideas and support: Jan Bruecker, Stephan Preibisch, Markus Riester, Peter Menzel, Gunner Boldhaus, Jörg Hackermüller, Peter Stadler...