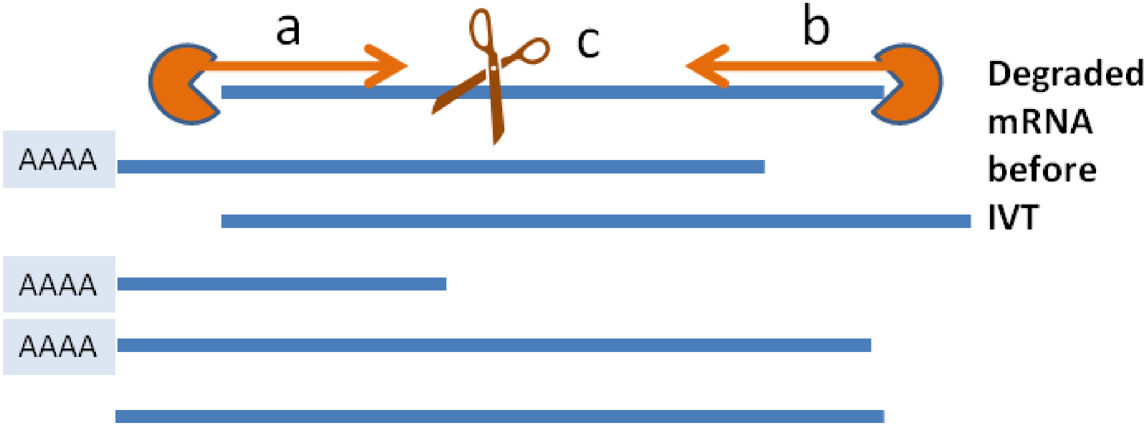
Learning about RNA Degradation (from Microarray Data)

EFRE: Europäischer Fonds für regionale Entwicklung
im Rahmen der Sächsischen Landesexzellenzinitiative

Synopsis

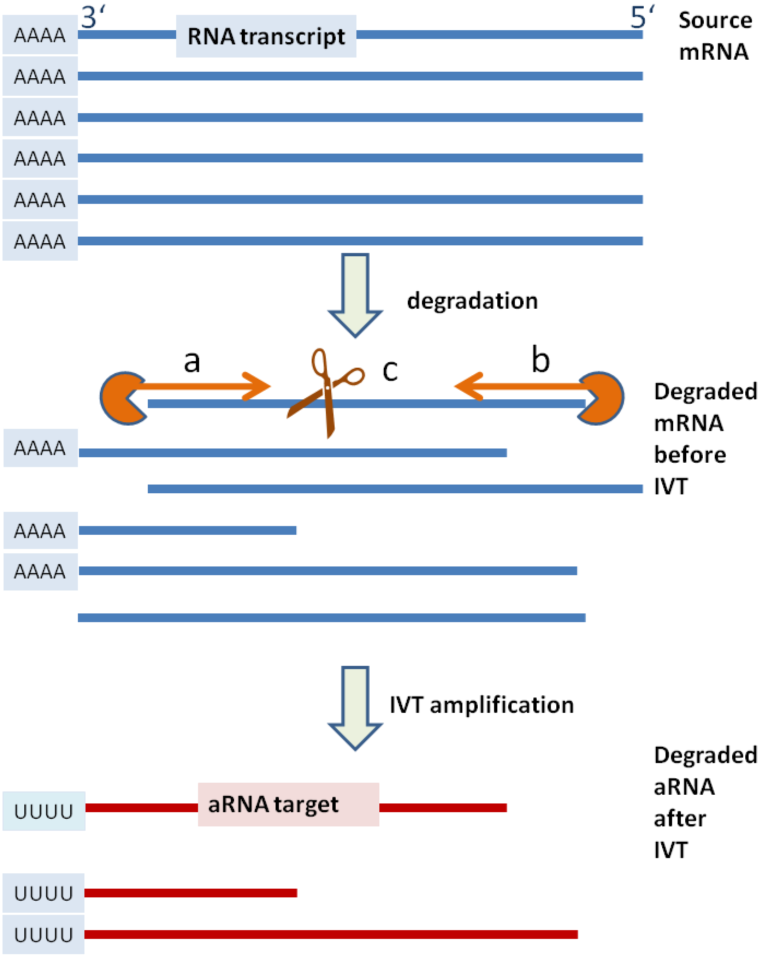
- RNA Degradation Basics (one slide)
- A quantitative analysis of RNA degradation based on microarray data
- Qualitative analysis of RNA degradation
- Time runs out

RNA degradation - on a single slide



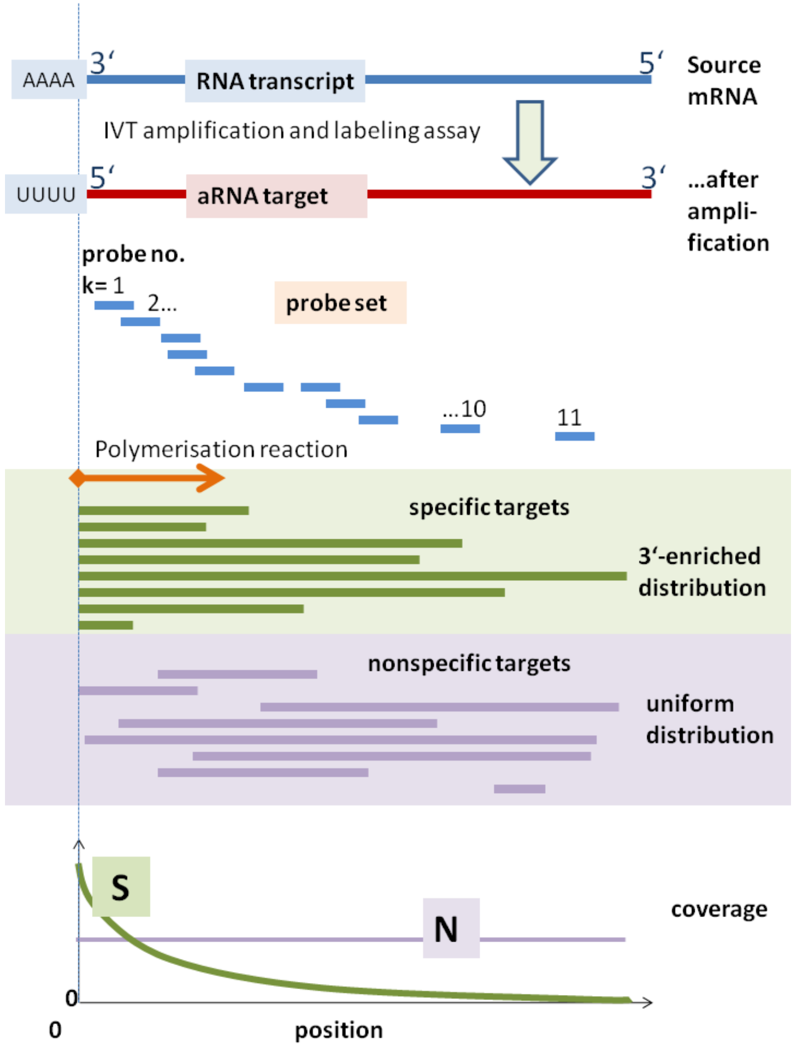
- Complex; controlled by many pathways
- Must be avoided for all transcriptomics experiments – assessment mandatory

Affymetrix 3' IVT protocol is practical for degradation analysis



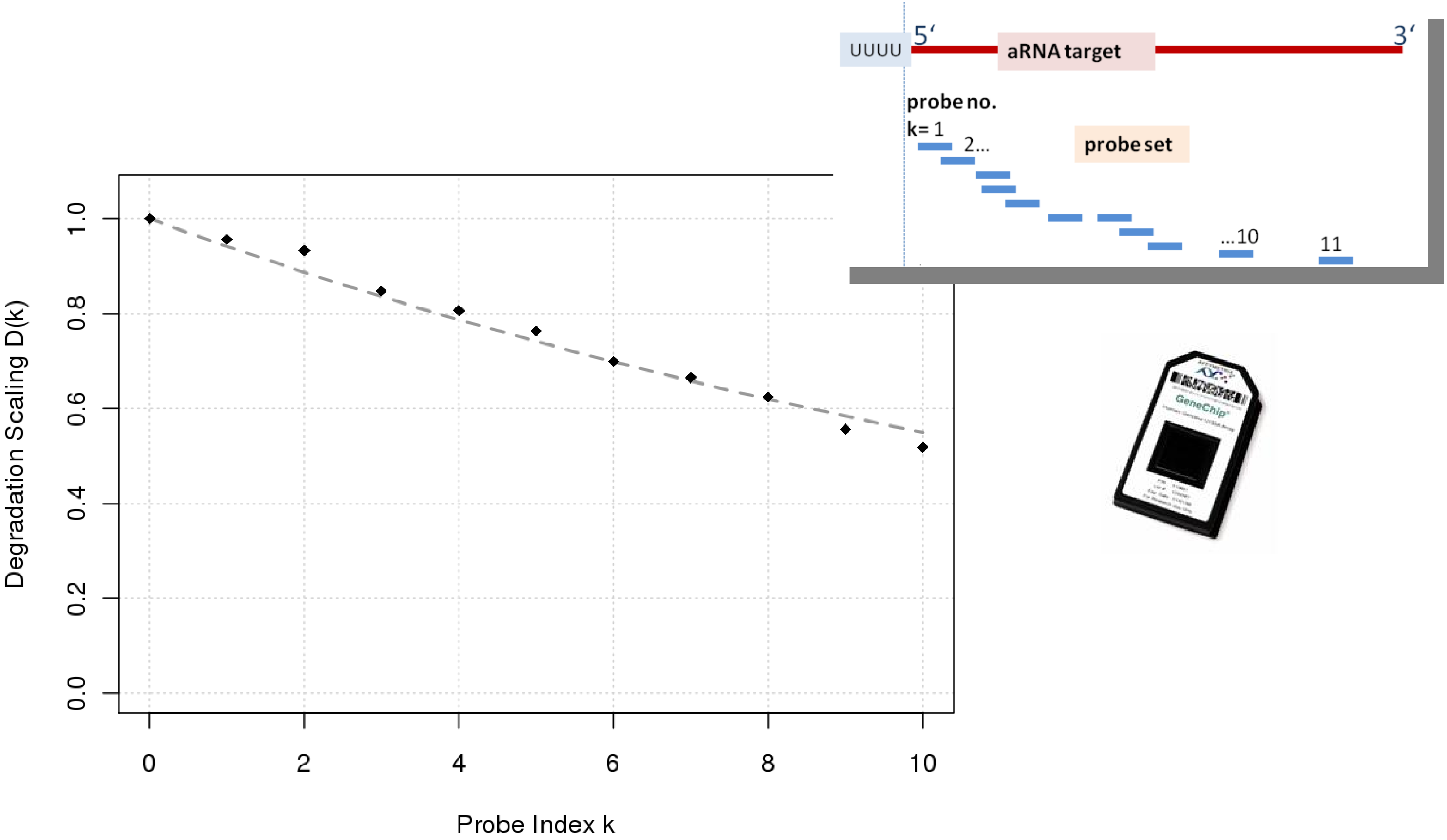
- In-vitro transcription protocol leads to uniform aRNA amplification directed from 3' end of mRNA

Affymetrix probe design is practical for degradation analysis

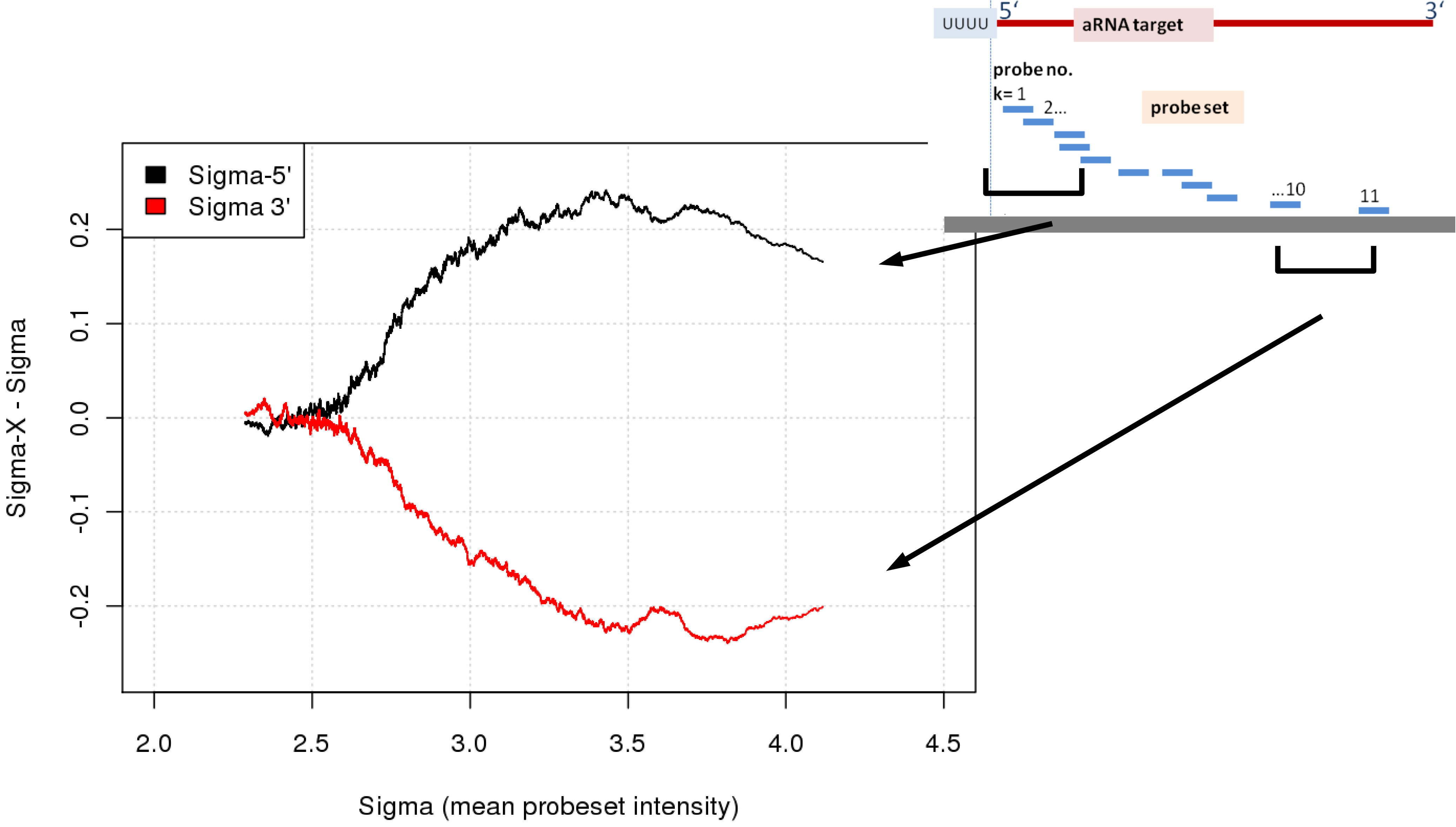


- 11 or more probes target one transcript at varying positions

Basic measure: average of all probe intensities over probe index



Degradation can only be measured for expressed transcripts



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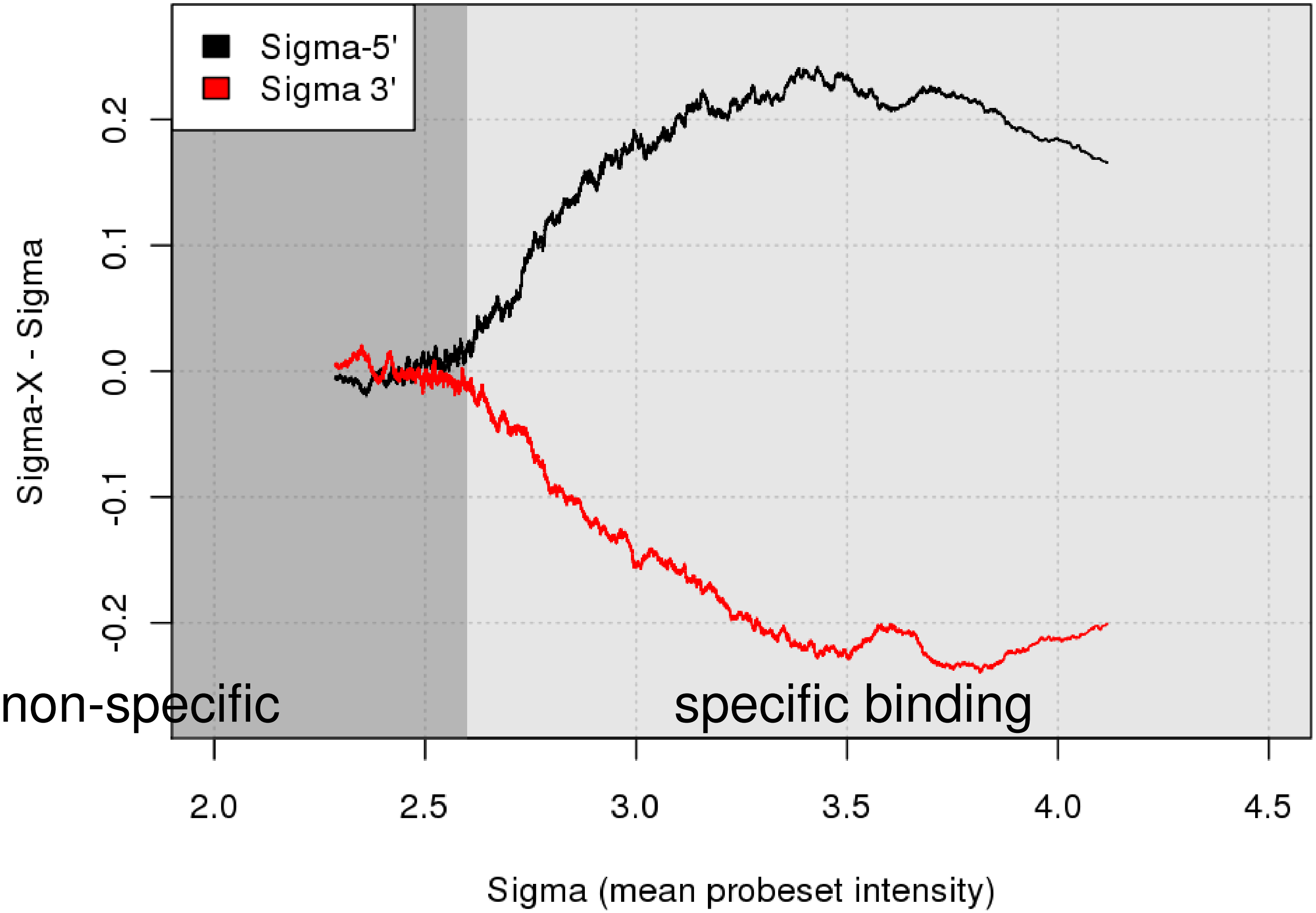
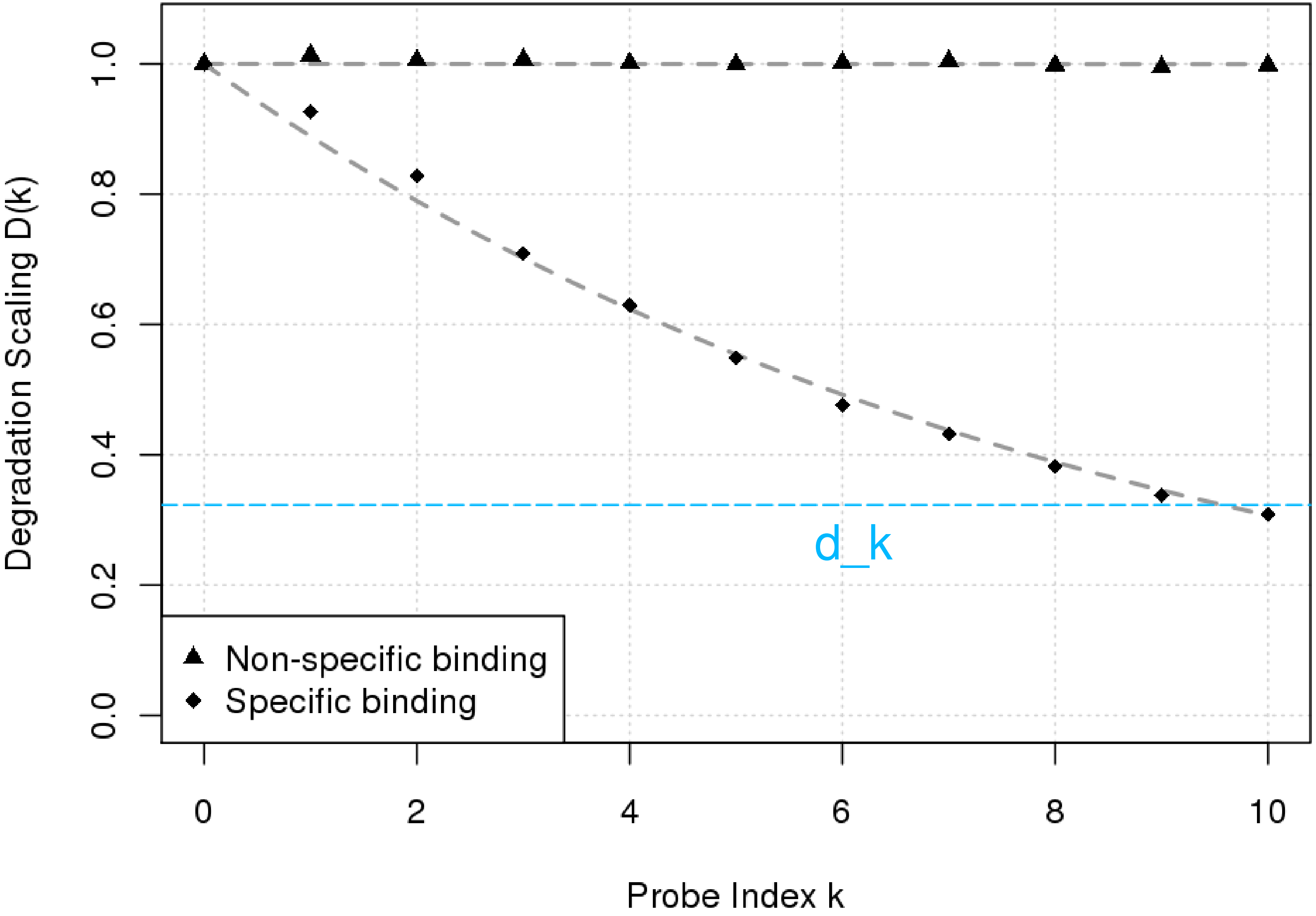
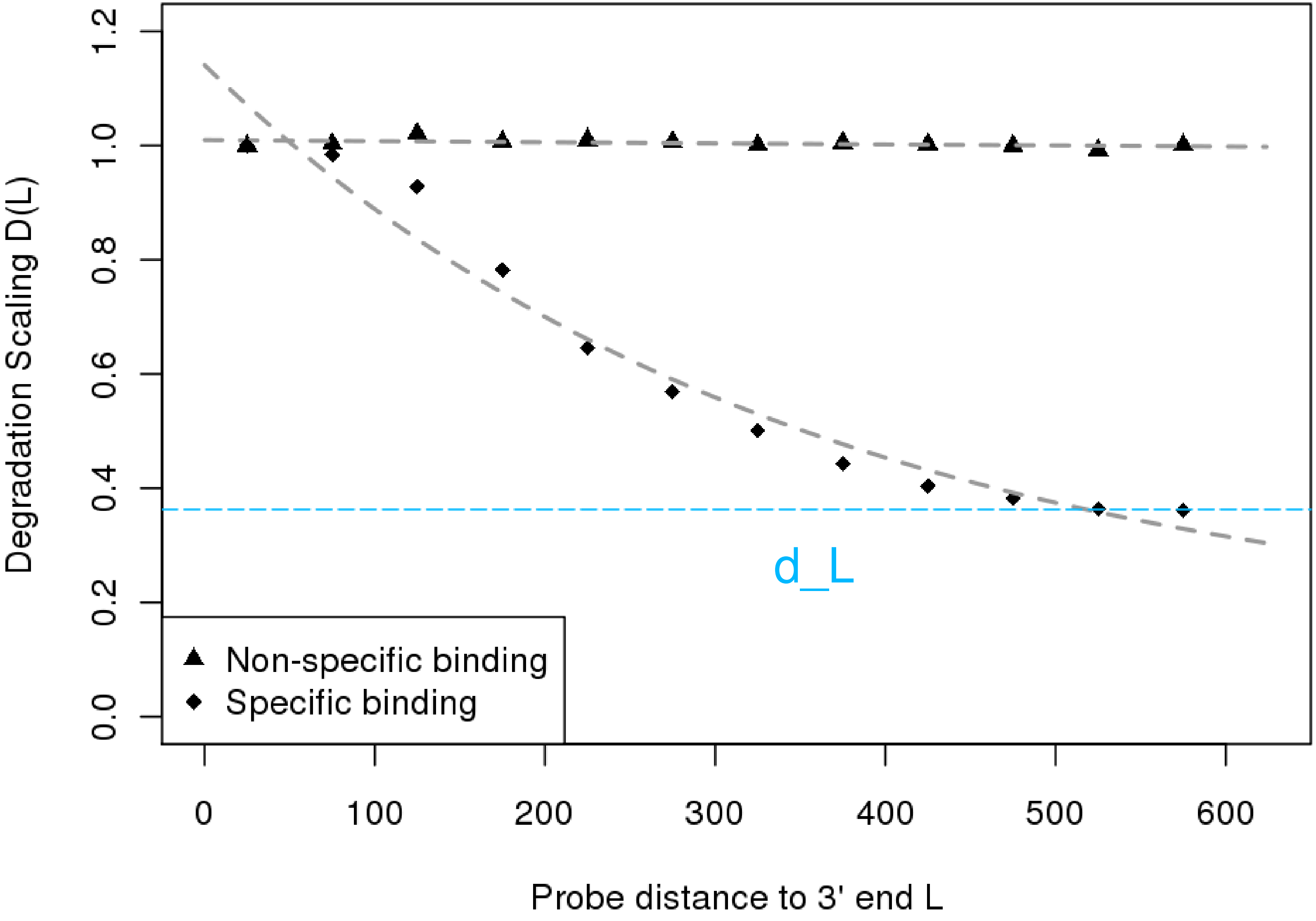


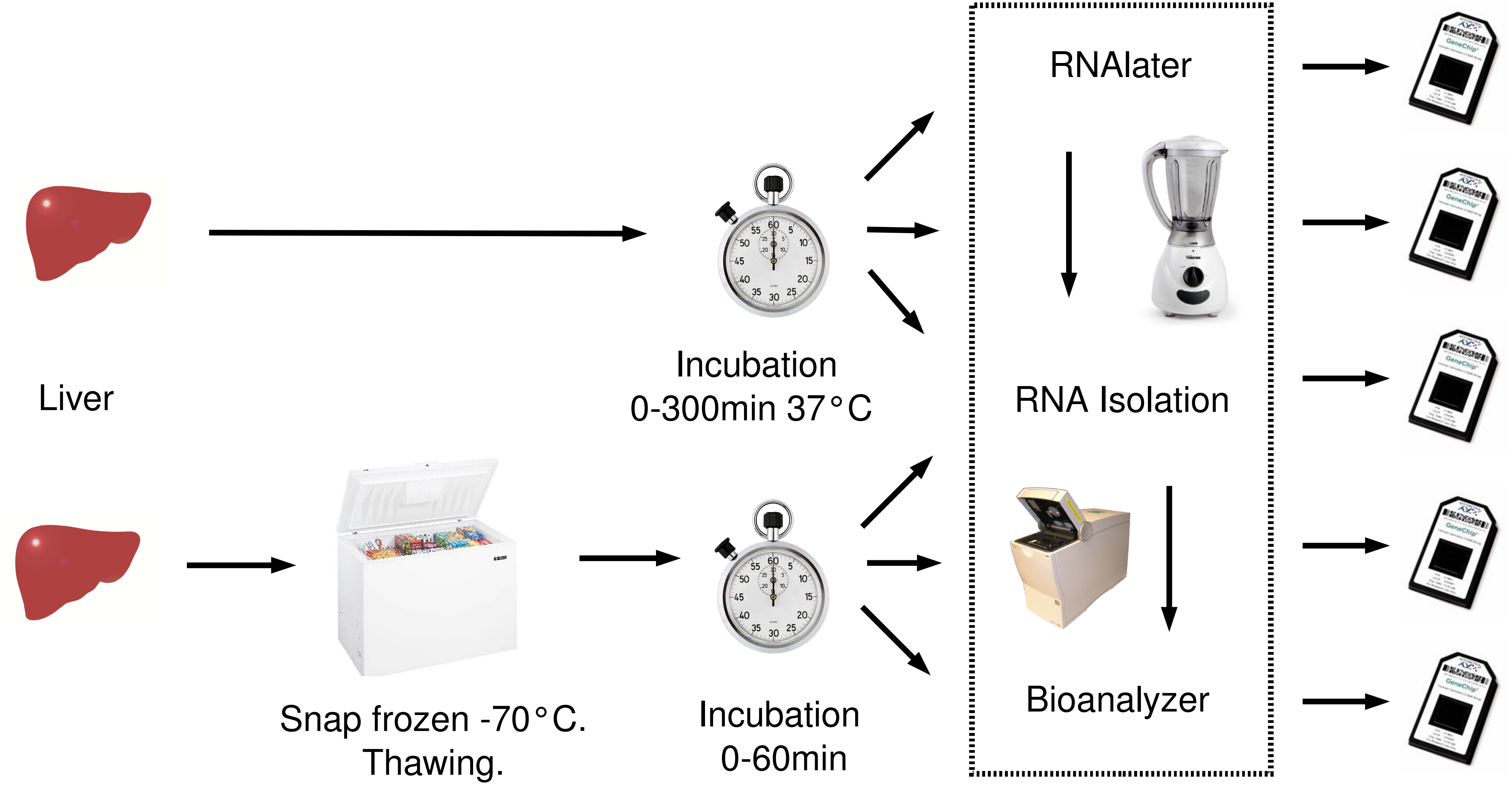
Degradation = intensity decrease for specific transcripts



Using the actual alignment of the probes to the target mRNA



The „Rat Experiment”



Liver

Incubation
0-300min 37 °C

Snap frozen -70 °C.
Thawing.

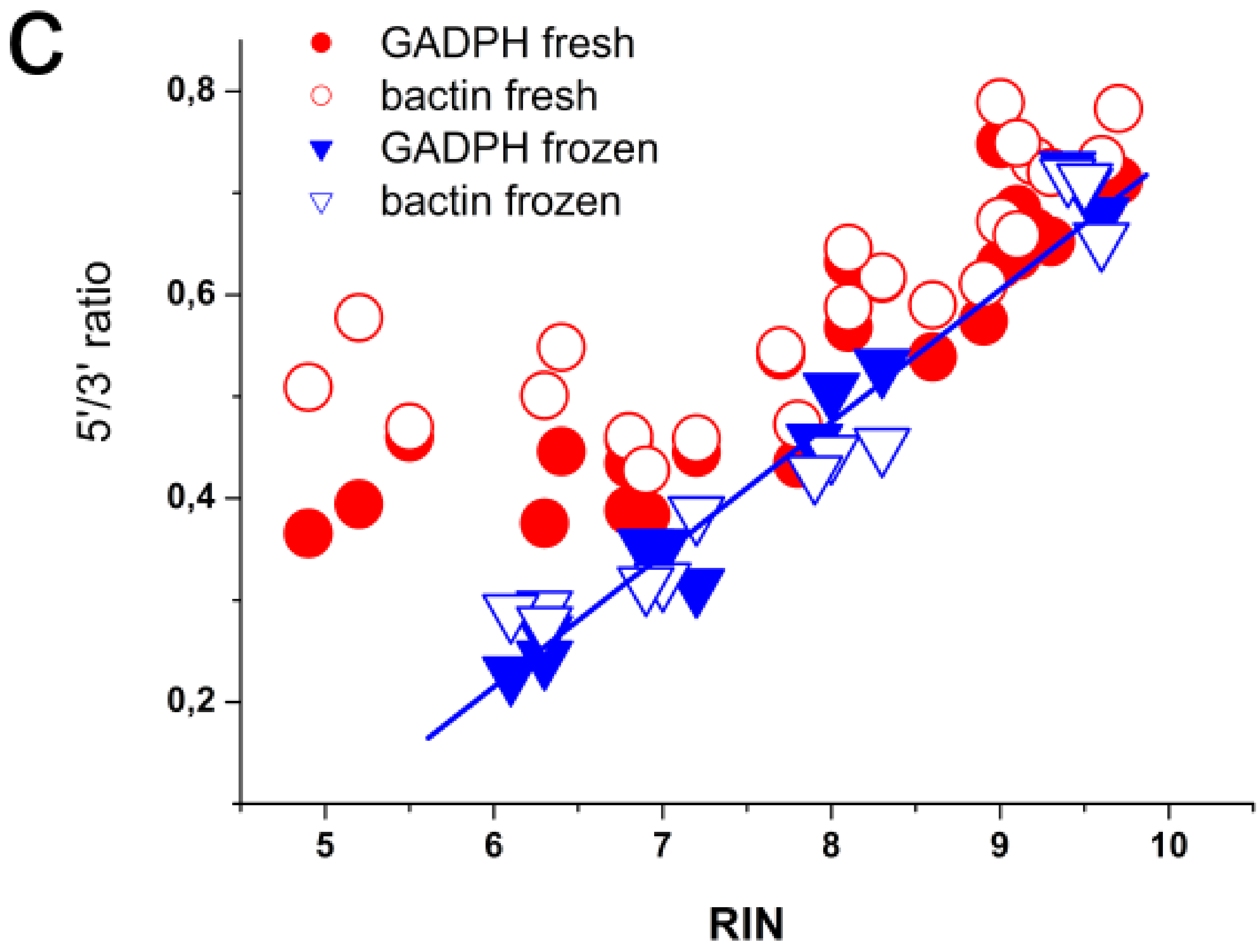
Incubation
0-60min

RNAlater

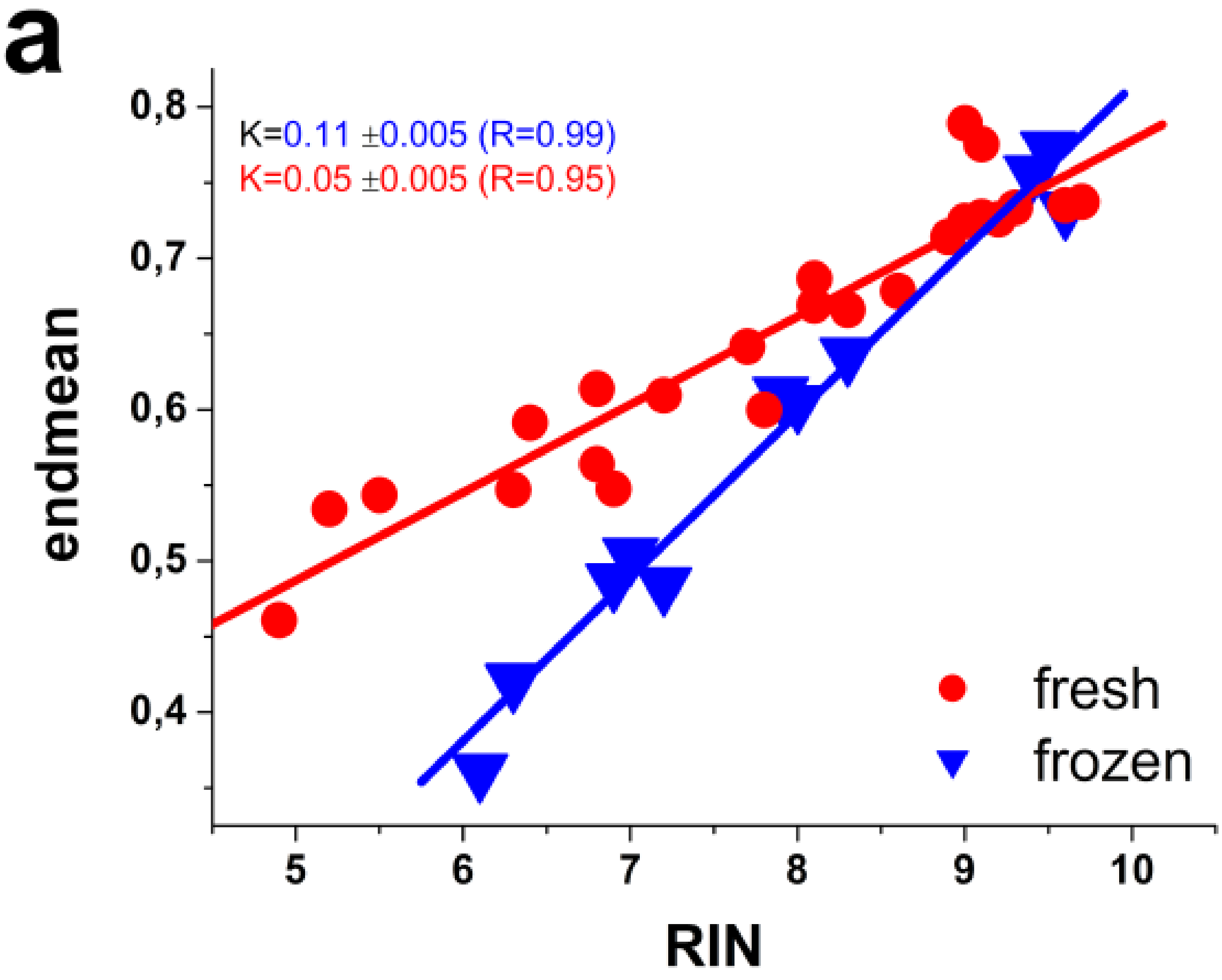
RNA Isolation

Bioanalyzer

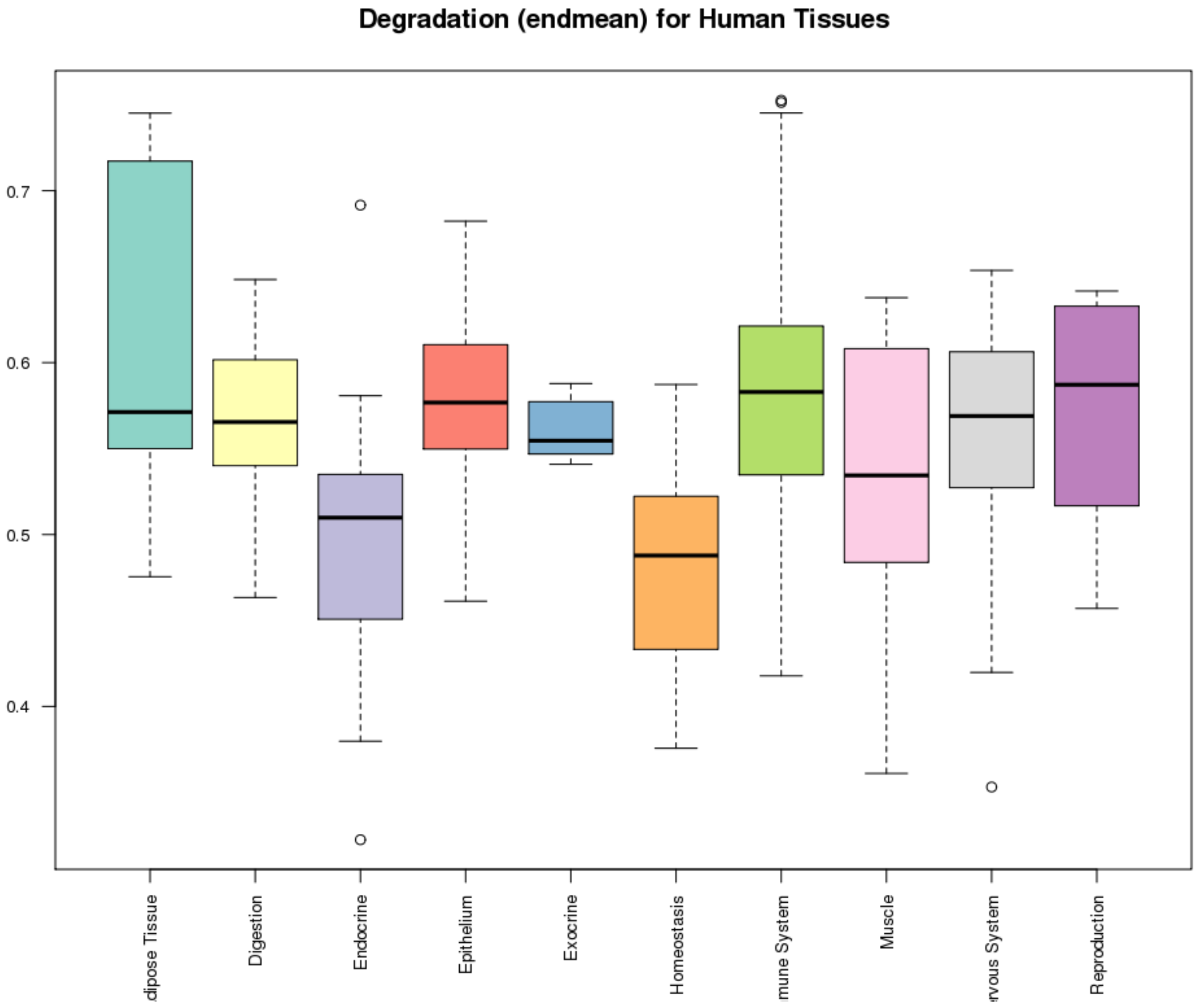
Affymetrix Marker vs. Bioanalyzer



D_k vs. Bioanalyzer



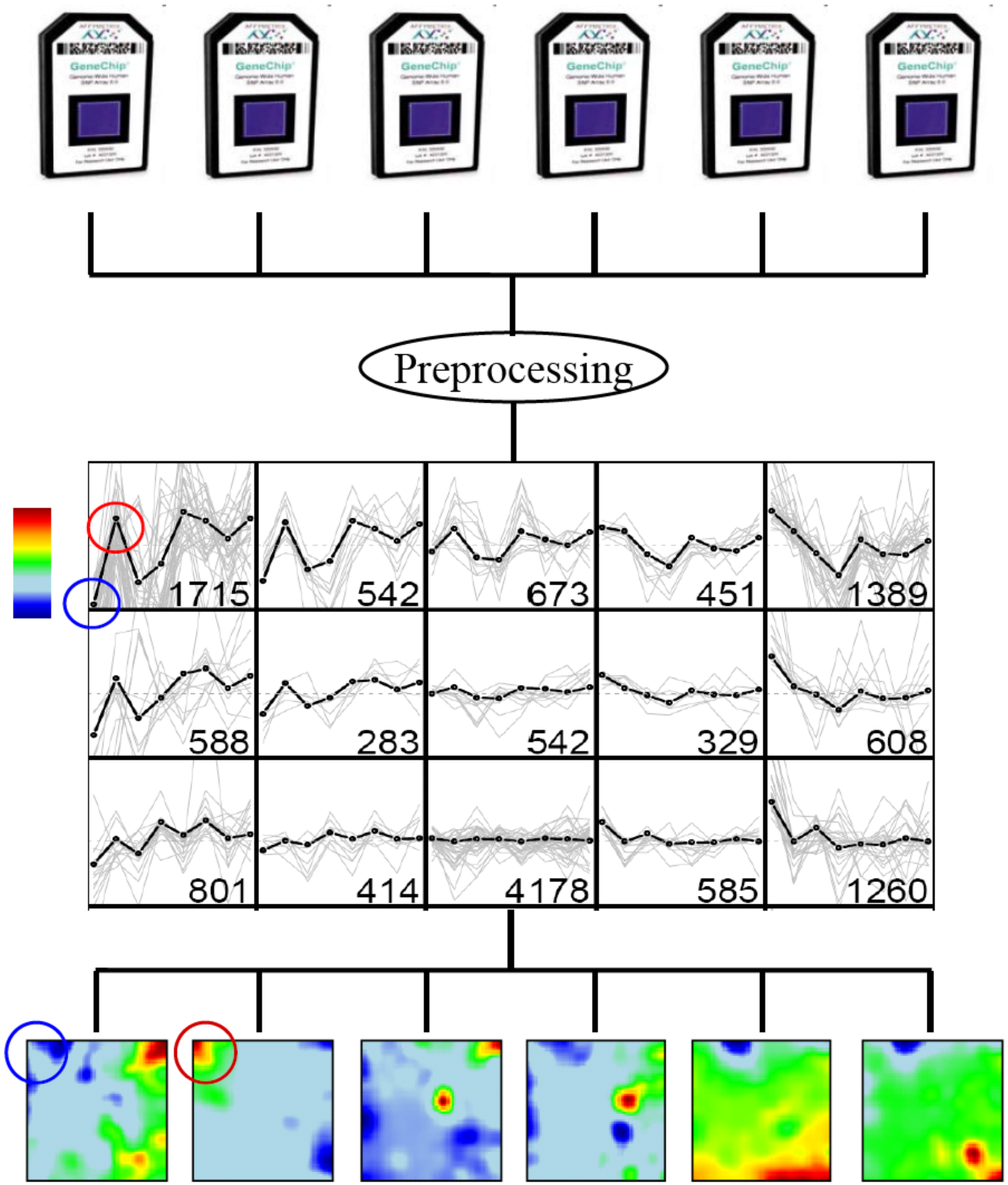
Example result: degradation is tissue-specific



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



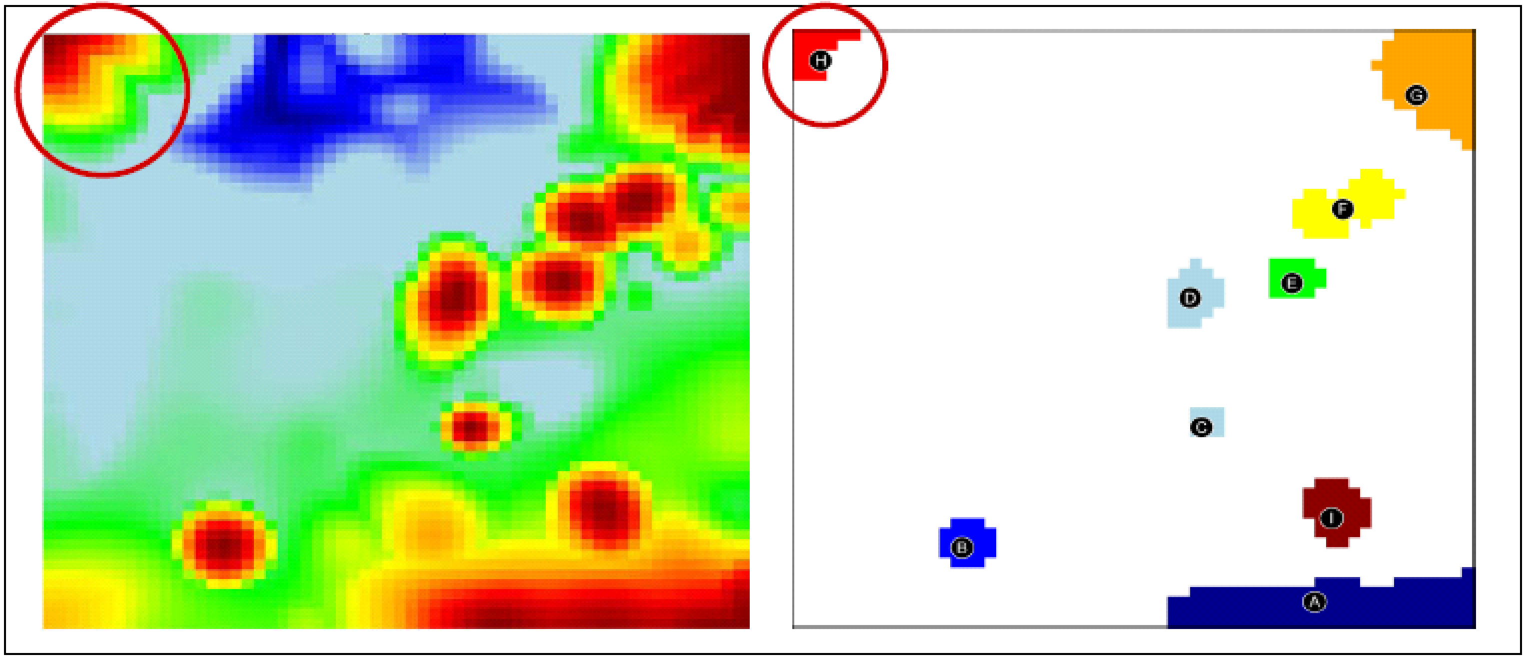
Raw Data

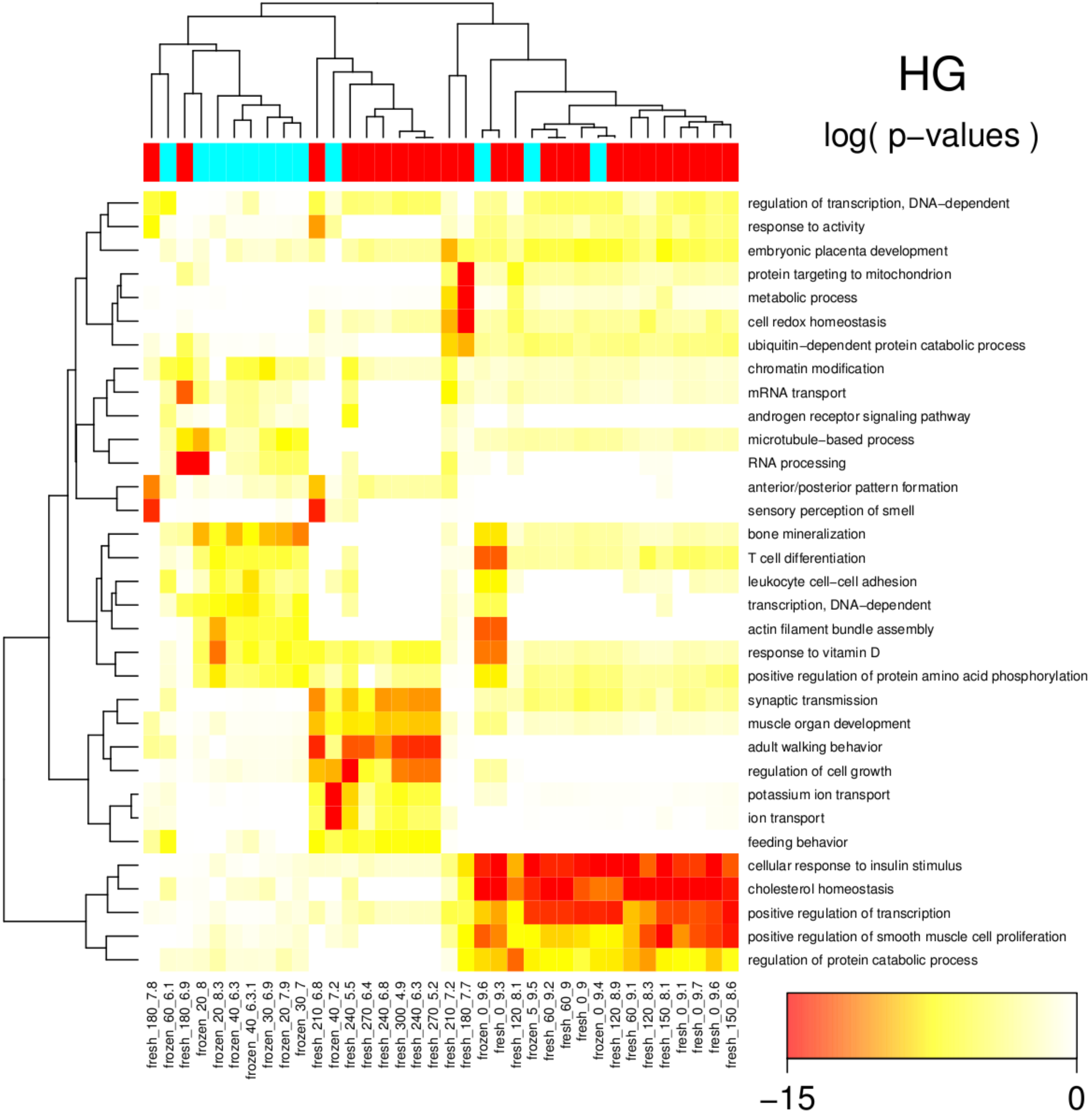
Preprocessing

Metagenes

Expression Profiles

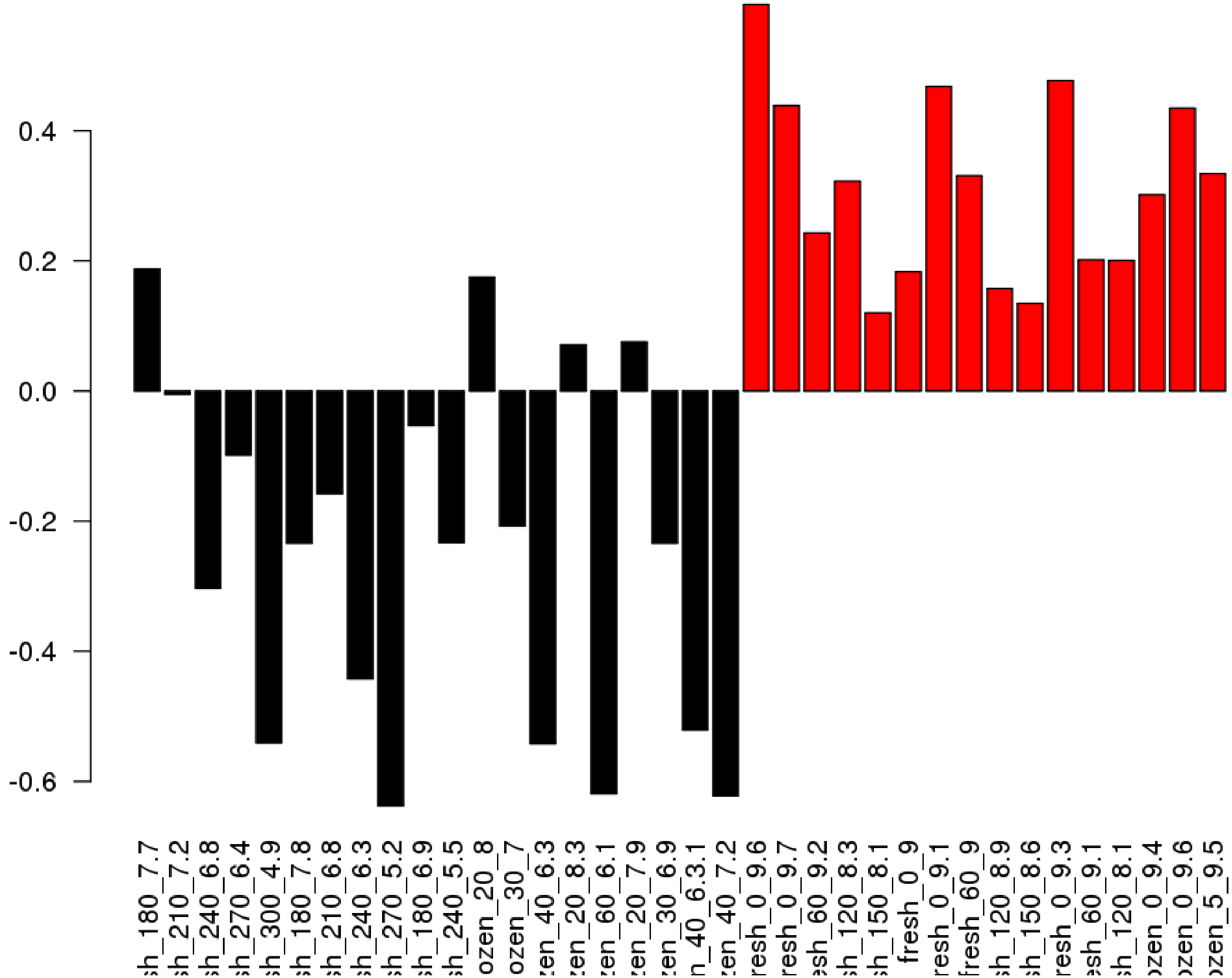
Expression Profiles and Spots



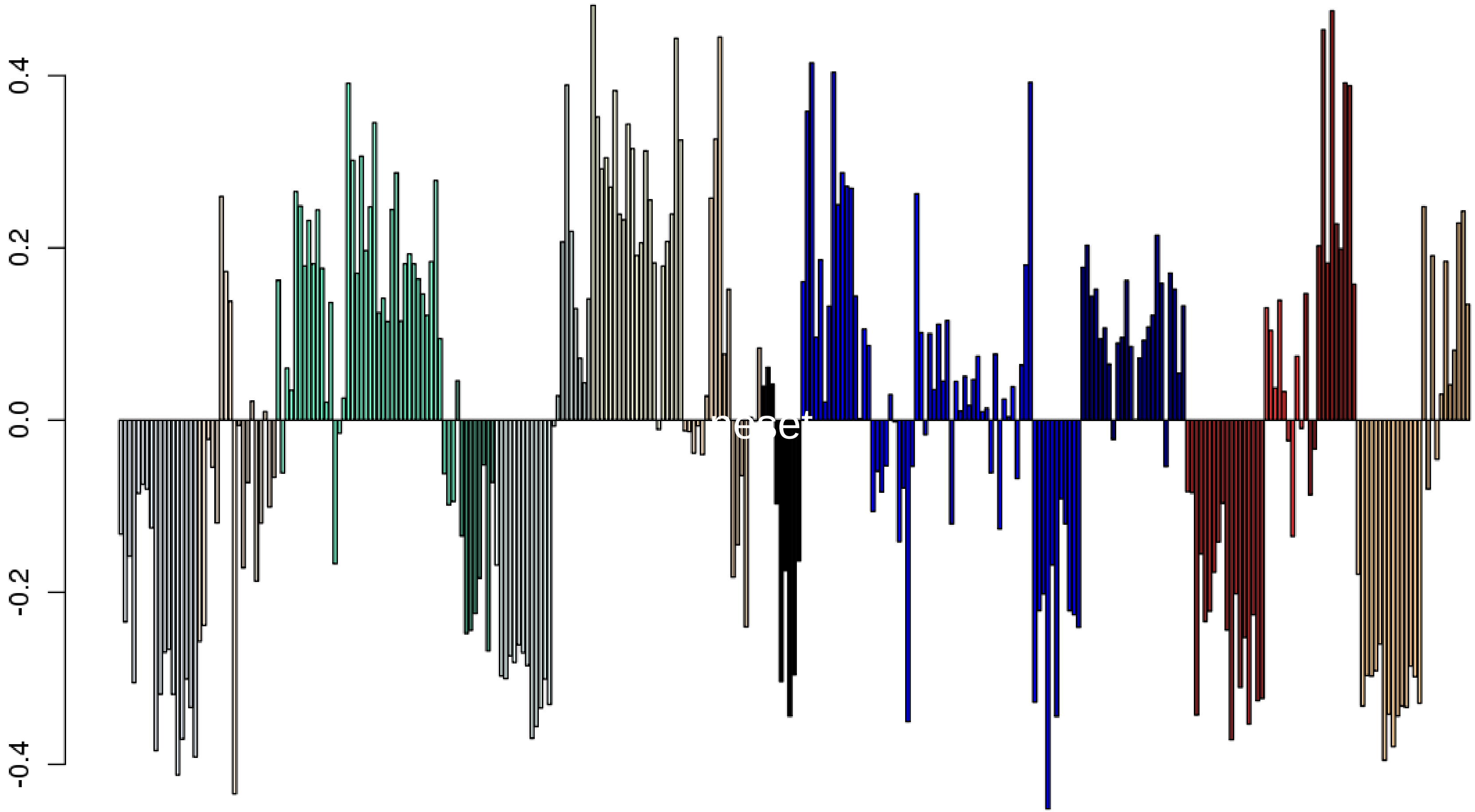


SOM GO-Enrichment Heatmap from Rat Experiment

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 experiemnt
- 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-indepenent markers for degradation can be found

Acknowledgments

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- Henry Wirth

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