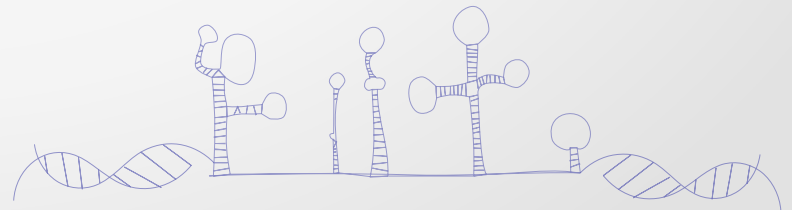


Transcriptome landscape of human cells infected with Reston and Zaire ebolavirus: Focus in ncRNAs

Nelly F. Mostajo^{1,2}, Nadine Biedenkopf¹,
Dirk Becker¹, Martin Hoelzer², Stephan
Becker¹, Manja Marz²

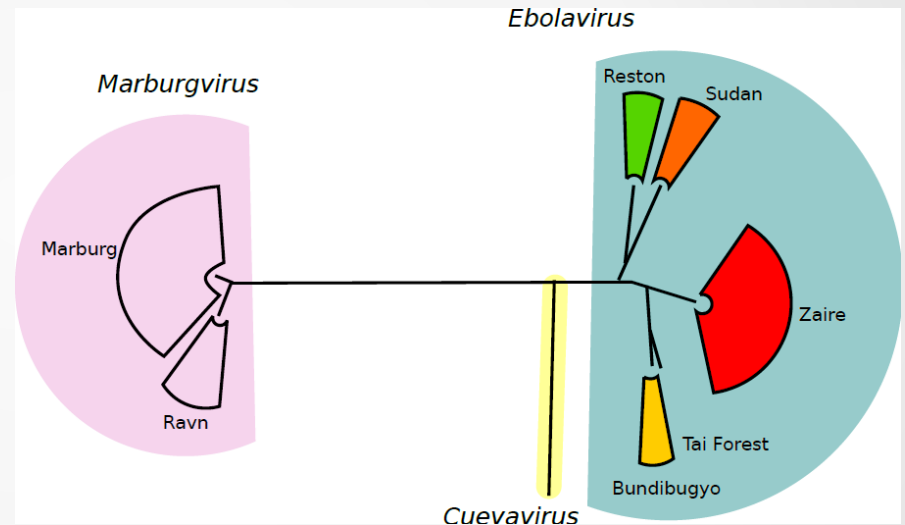
1. Institute of Virology, Philipps University Marburg

2. RNA Bioinformatics and High Throughput Analysis, Faculty of
Mathematics and Computer Science, Friedrich Schiller
University Jena,

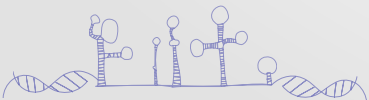


Ebola virus

- Order: *Mononegavirales* (-ssRNA)
- Family: *Filoviridae*
- Genus: *Ebolavirus*
- Species:
 - *Bundibugyo ebolavirus*
 - *Sudan ebolavirus*
 - *Zaire ebolavirus*
 - *Tai Forest ebolavirus*
 - *Reston ebolavirus*



Adapted from Se-Ran Jun et al. FEMS Microbiol Rev 2015





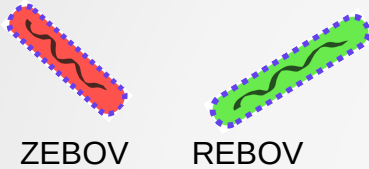
Hypothesis

There are differences in the host response that lead to the different pathogenesis during infection with REBOV and ZEBOV respectively



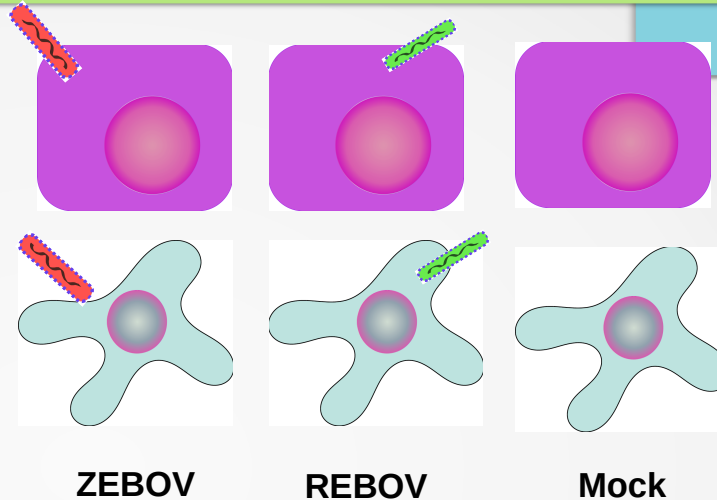
Establishment of the experimental setup

High Quality of viral stock



MOI:
5

100% of cells infected



3 x (3 h p.i. and 24h p.i.)

Cell
Harvesting
+ RNA
extraction

smallRNAseq

Microarray

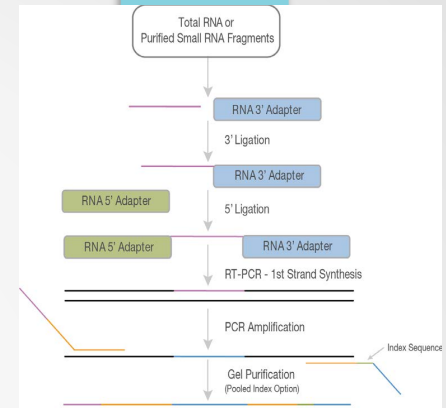


smallRNAseq



Processing

- Library preparation:
 - TruSeq Small RNA
 - 3' OH adapter + 5'P
- Filtering:
 - Adaptor removal
 - Quality depuration
 - Size 15-51nt
 - Mapping: No gaps, 1 mismatch allowed
 - Genomes: Human+Zaire+Reston



Results

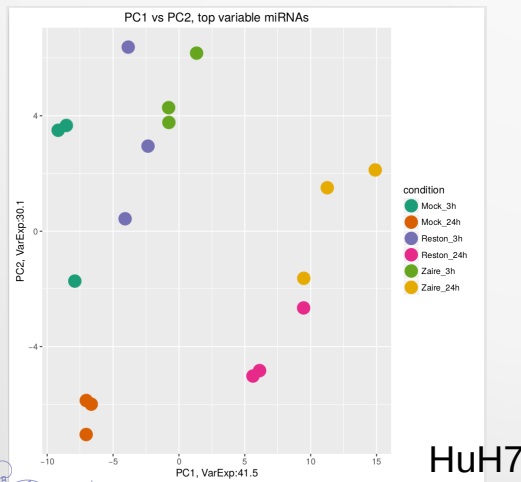
- THP1:
 - Total reads: >7,8millions
 - Mapping: >96%
 - rRNA: 6% > (one sample with 13%)
 - mitRNA: 2,5% >
 - Viral RNA: 1% >
- HuH7:
 - Total reads: >9,6millions
 - Mapping: >95%
 - rRNA: 6,3% >
 - mitRNA: 19% >
 - Viral RNA: 1% >

*Tip: mitRNA % is tissue specific

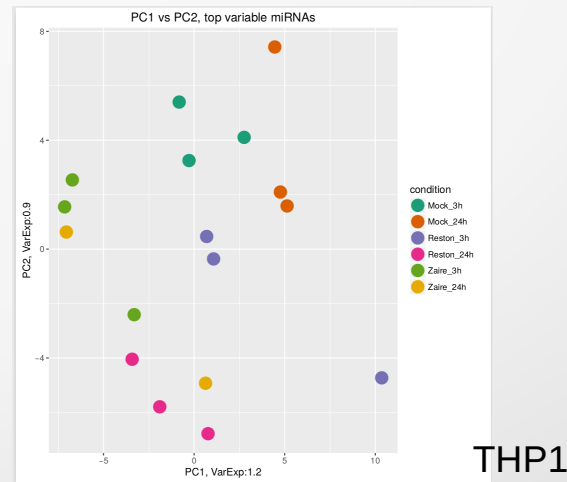


Methods

- *De novo* prediction of genes was performed with BlockClust tool
- Genes included current human annotation and potential new genes predicted with BlockClust
- Counting tool: FeatureCounts → ~47% to ~56%
 - Results showed DEG being covered by coding regions. There were no miRNAs in this group (e.g. PCA)



HuH7



THP1

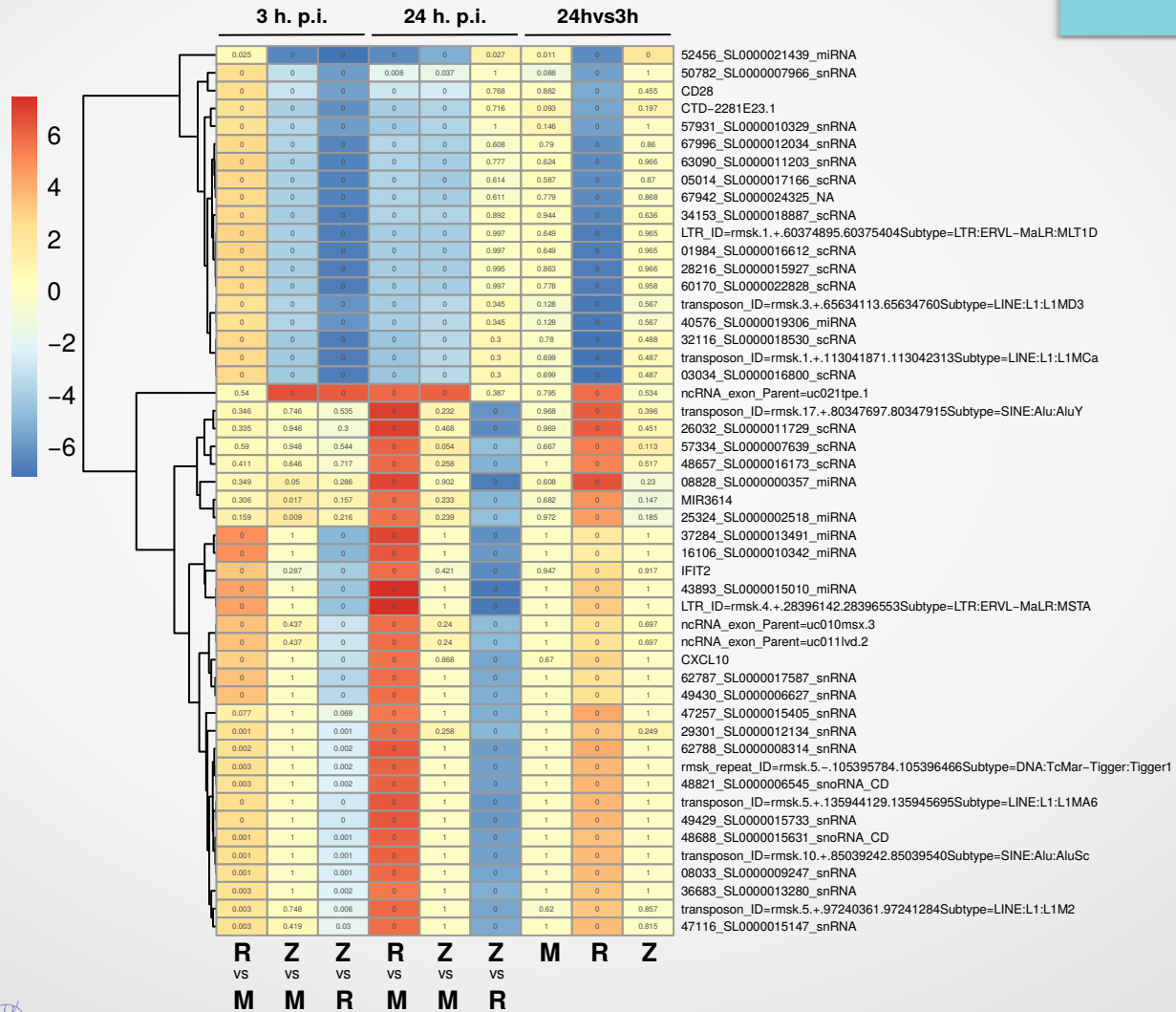


Updated methods

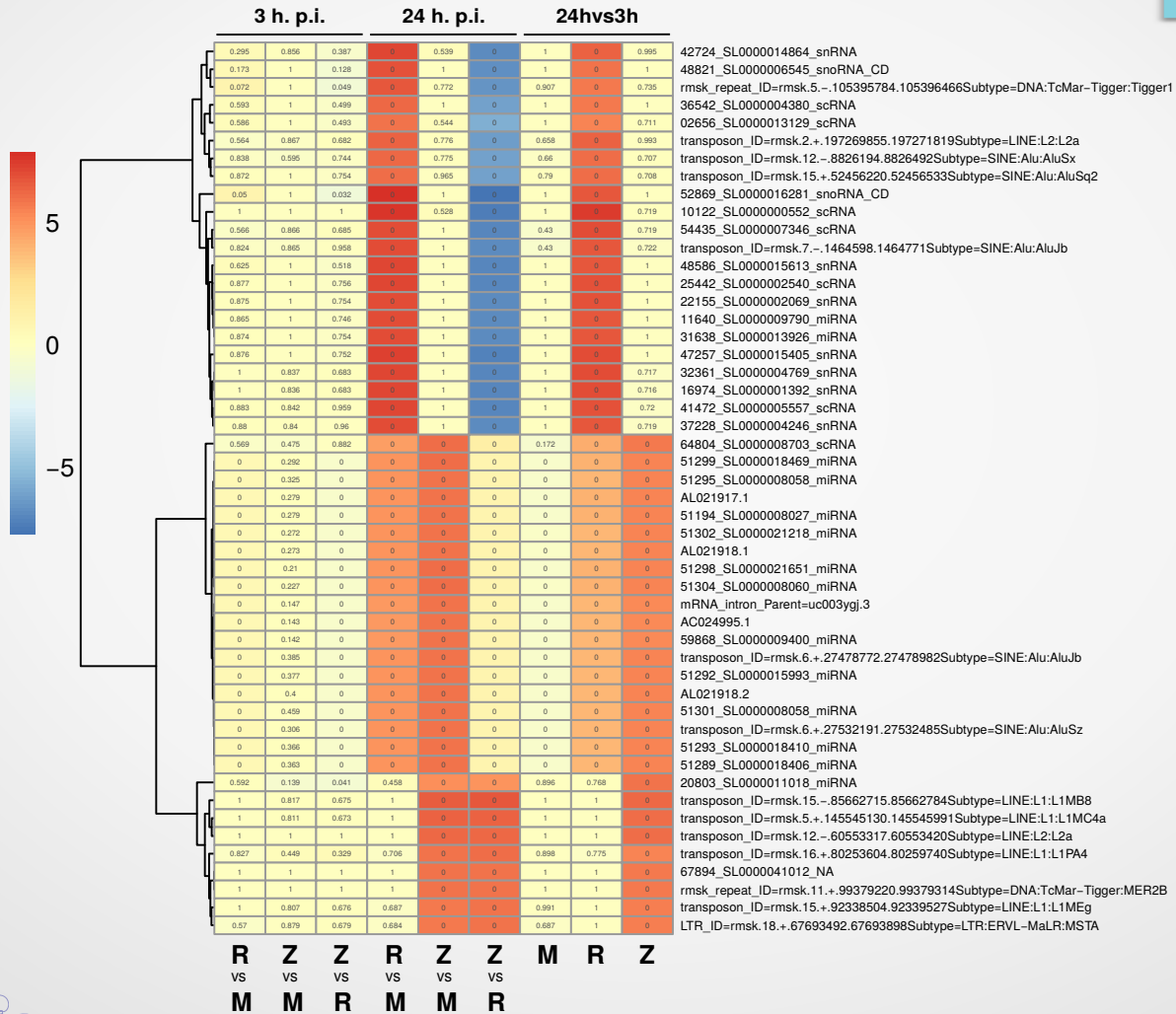
- *De novo* prediction of genes was performed with BlockClust tool, CoRal, miRDeep2
- Genes included current human annotation, potential new genes and rnsk.
- Counting tool: FeatureCounts → ~98% to ~99%
- Differential expression analysis was performed using DeSeq2
- Heatmaps were prepared, based on highest fold change (FC) and p-value $0,01 >$



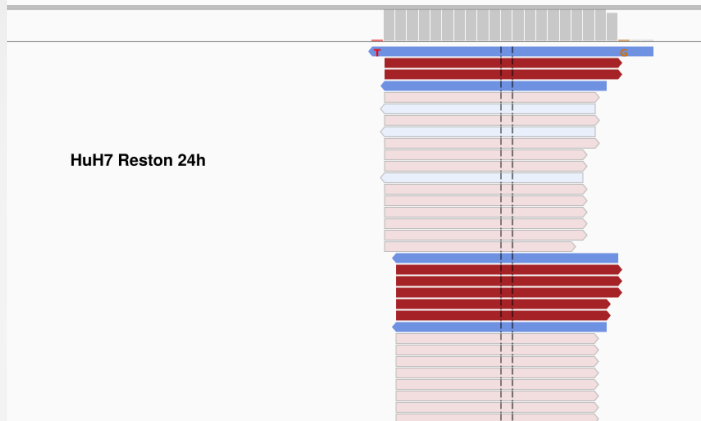
RESULTS: Top50 DEG THP1



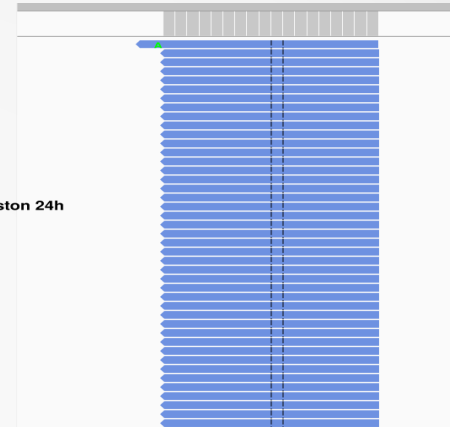
RESULTS: Top50 DEG HuH7



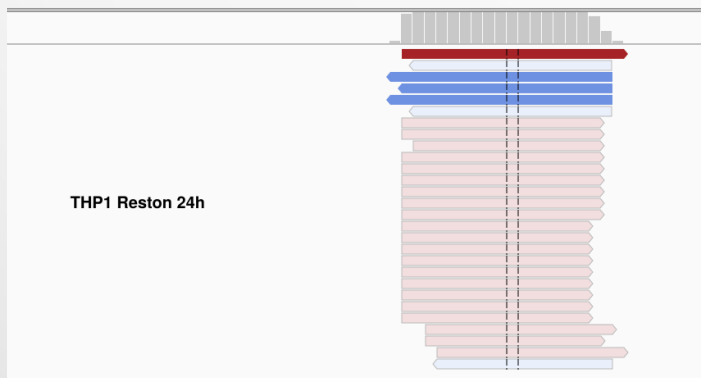
Exploring the candidates:



47257_SL0000015405_snRNA



48821_SL000006545_snoRNACD



THP1 Reston 3h

THP1 Reston 24h



Same gene covered by both strands?



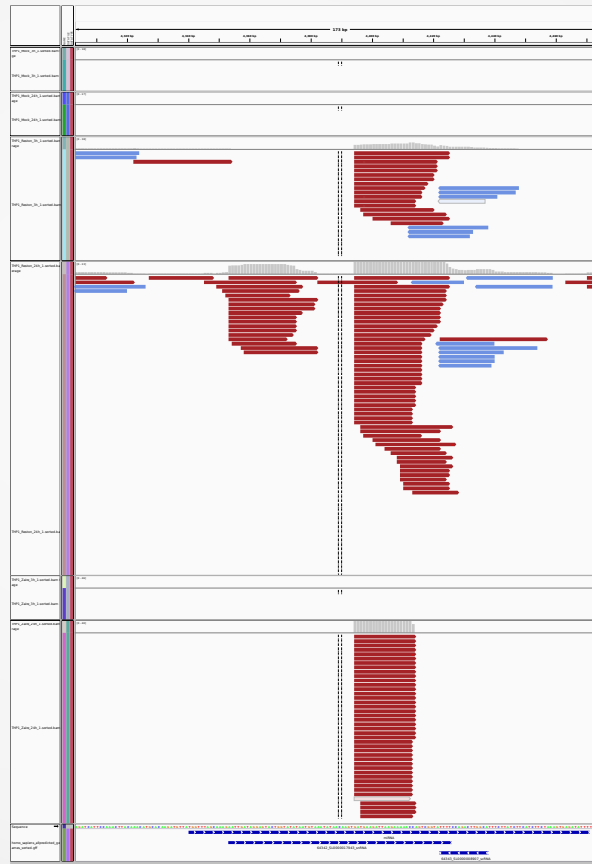
- Most genes were observed as strand specific
- ← However, some showed this pattern
- The prediction tools annotates them as different molecules
- Could it be a secondary product of miRNA biogenesis?

Viral ncRNAs?

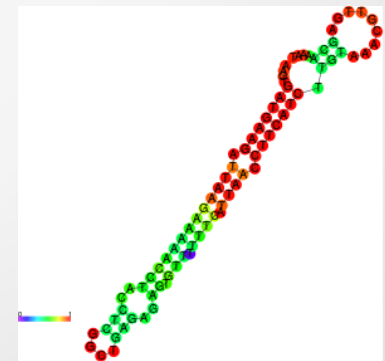
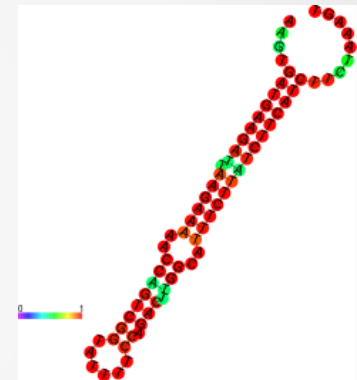
HuH7



THP1

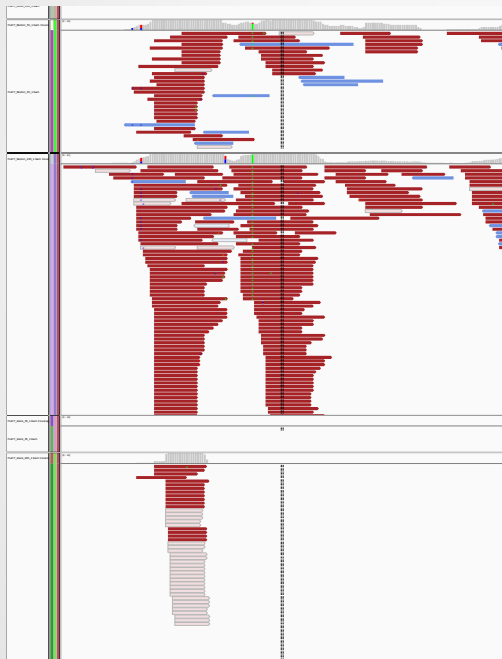


Between: VP35
and VP40

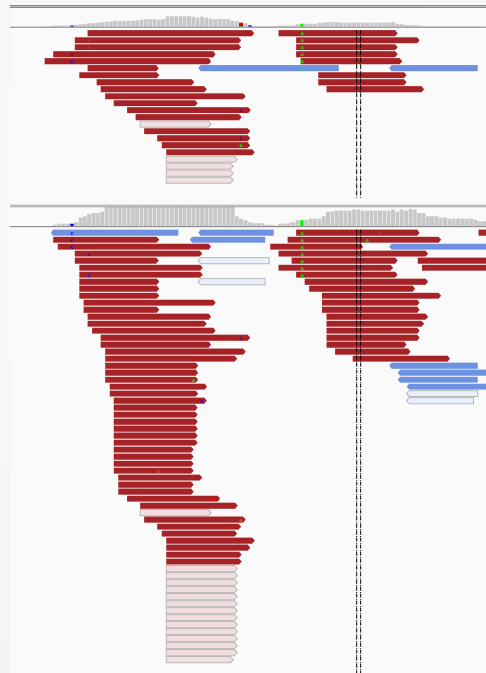


Are all processed ncRNAs?

HuH7



THP1



Position:
Leader hairpin
(e.g. Reston)



Summary

- More unknown genes related to *Ebolavirus* infection --> “new” ncRNAs?
- Promising targets for Reston vs Zaire differences
- Not many “miRNAs” as candidates
- Both viruses present possible ncRNAs, but how are they processed?



Thank you



AG Becker:
Nadine Biedenkopf
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Manja Marz

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Jochen Wilhelm
Uni Giessen (CRC1021)

