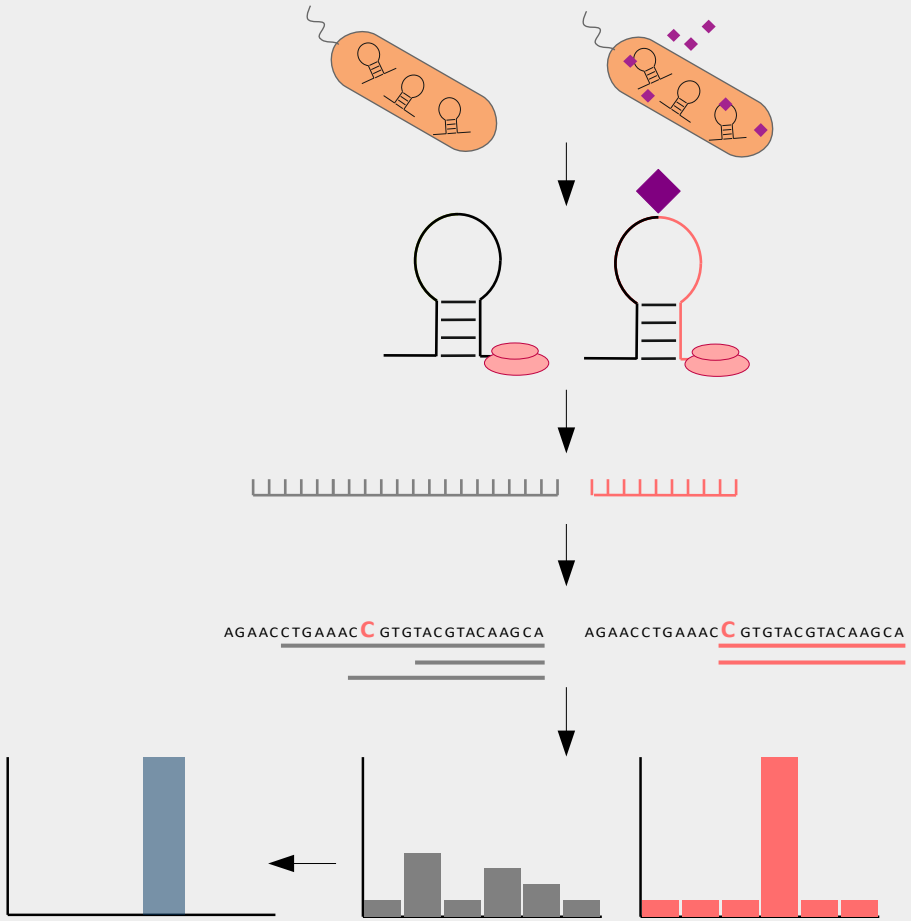

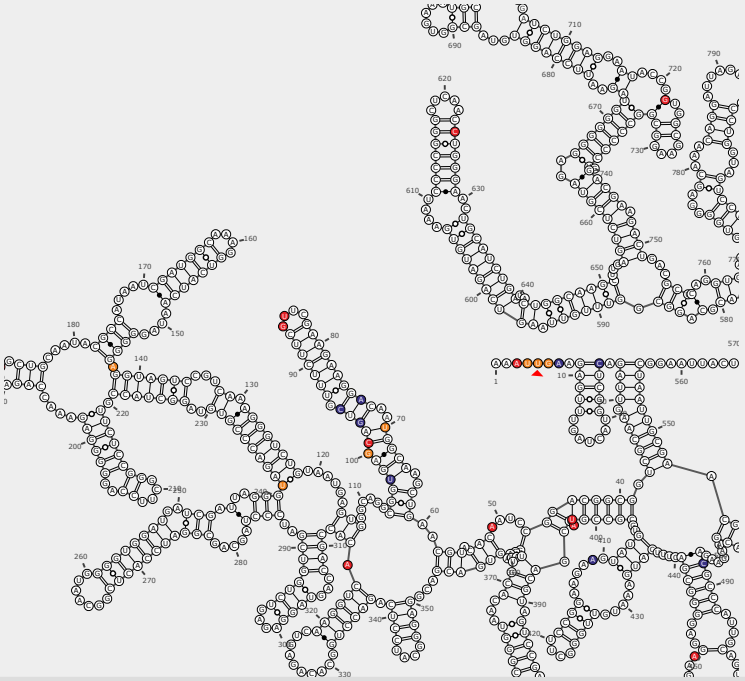

RUB

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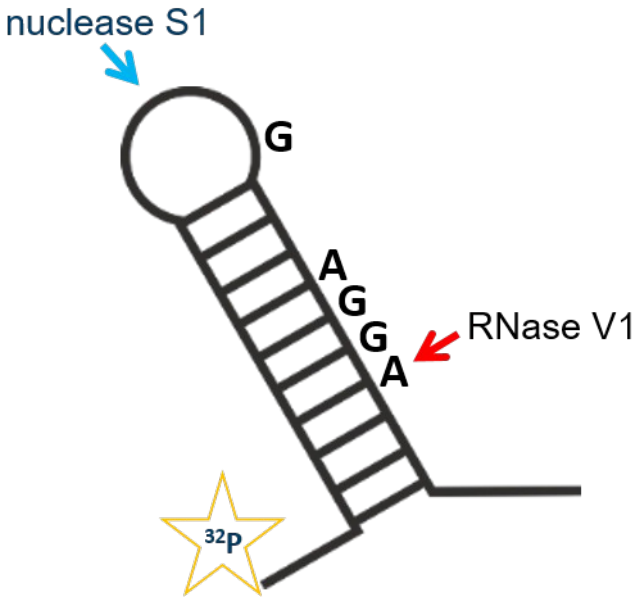
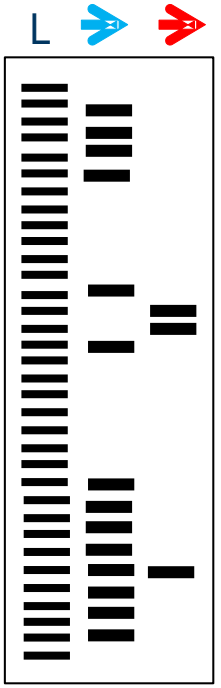
Transcriptome-wide Structure Probing with Lead-Seq

Vivian Brandenburg
35th TBI Winterseminar in Bled
12/02/20

RNA Structure Probing





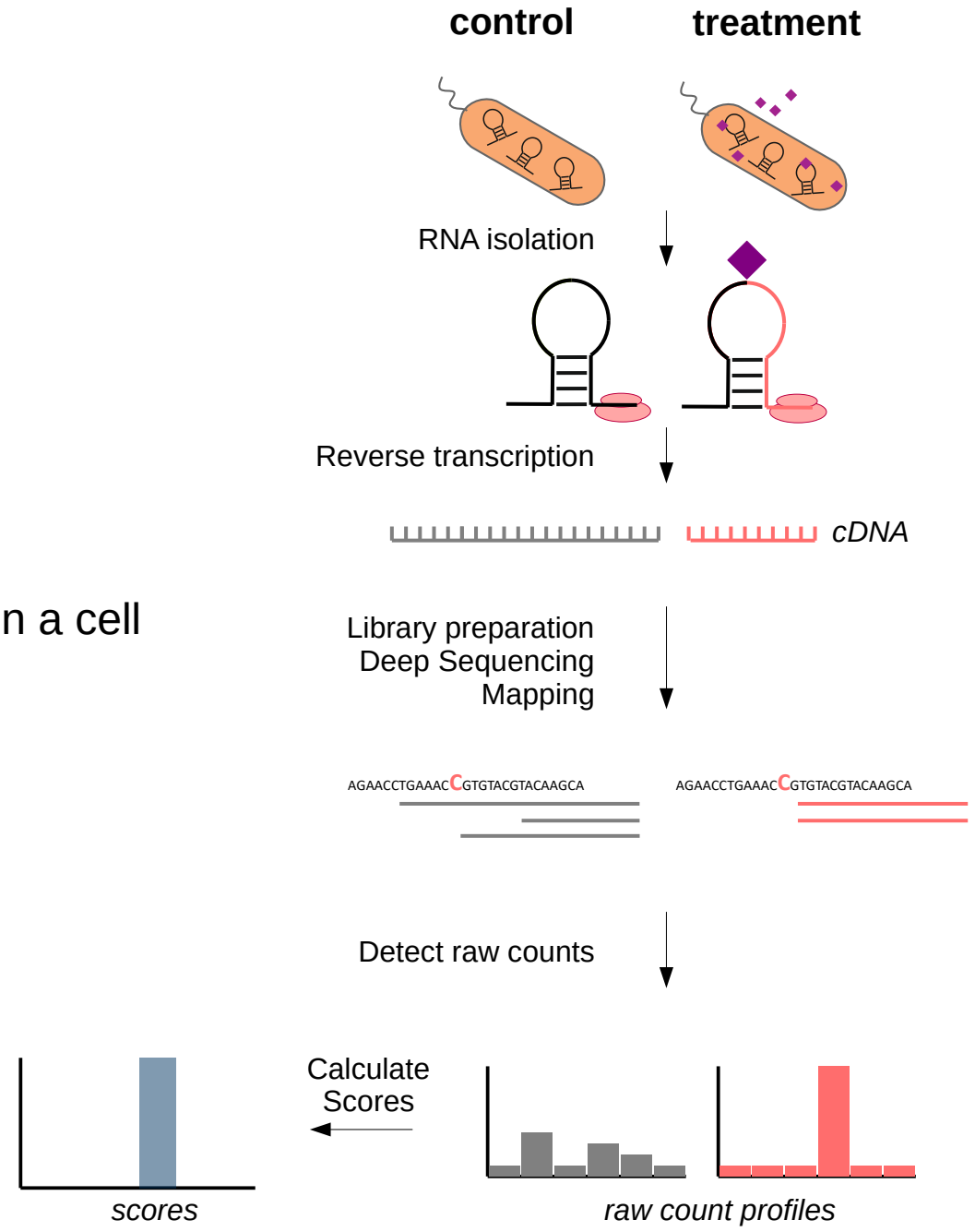
S1 → cuts single-stranded nt
V1 → cuts double-stranded nt

Polyacrylamide urea gel
→ separation of fragments by size
→ detection via radioactive P-32

Ehresmann *et al.*, 1987

Global RNA Structure Probing

- Structure Probing + NGS
- **Structurome** = Structure of all RNAs in a cell
- *In vitro:* enzymes
- *In vivo:* chemical agents



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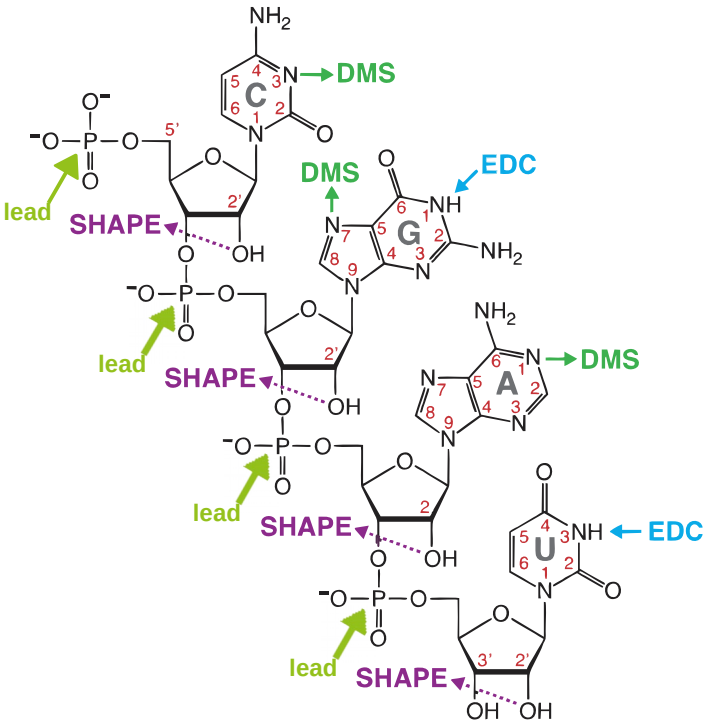
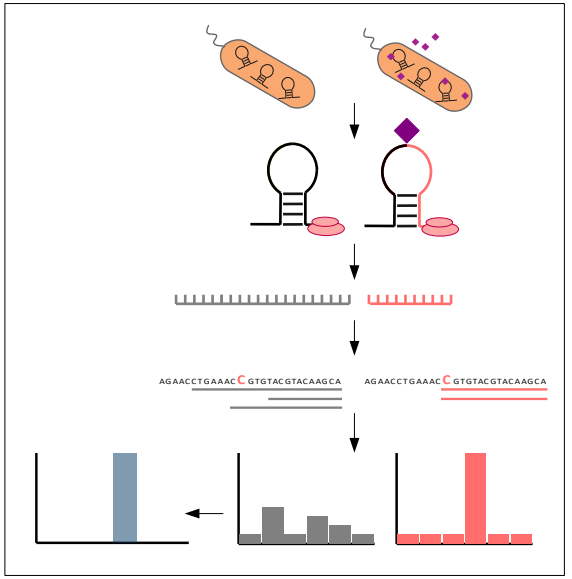
Chemical Agents for *in vivo* probing



Adapted from Mitchell *et al.*, 2019



- DMS: alkylation of Watson-Crick-face of A and C
  → can be combined with e.g. EDC
- SHAPE: 2'-OH acylation of ribose sugar
- Lead: nucleophilic attack of phosphorus group

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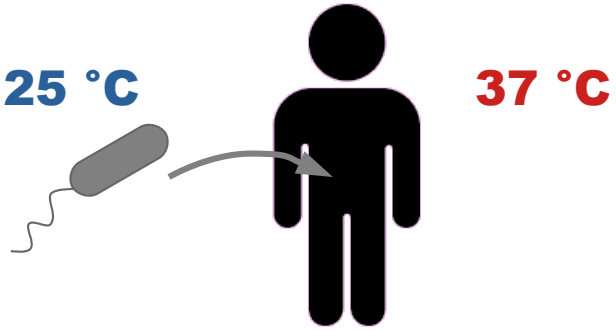
Y. pseudotuberculosis

- Gram-negative *y*-proteobacterium
- Closely related to *Y. pestis* / plague
- Mammalian pathogen
- Gut-associated diseases

Extensive regulatory response to temp. changes

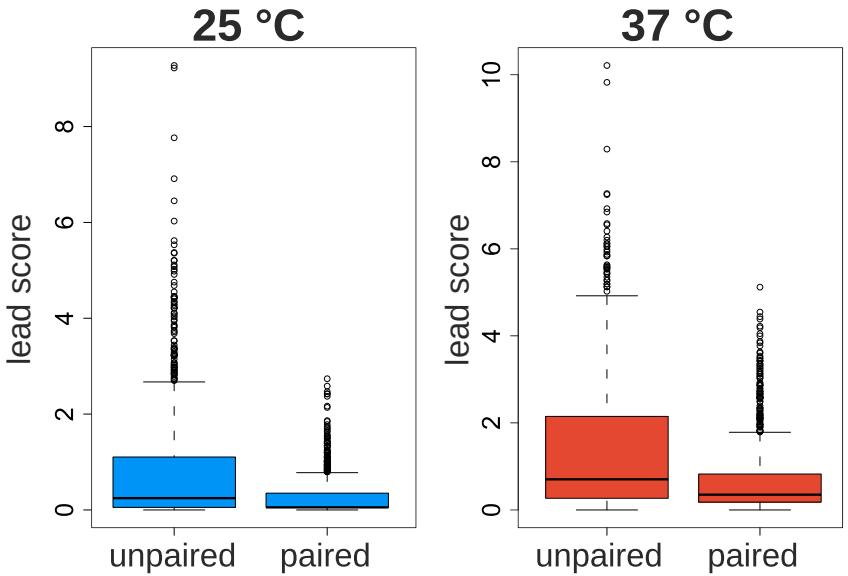
- > 300 genes differentially expressed
  (Nuss *et al*., 2015)
  - virulence, metabolism, diverse stress response

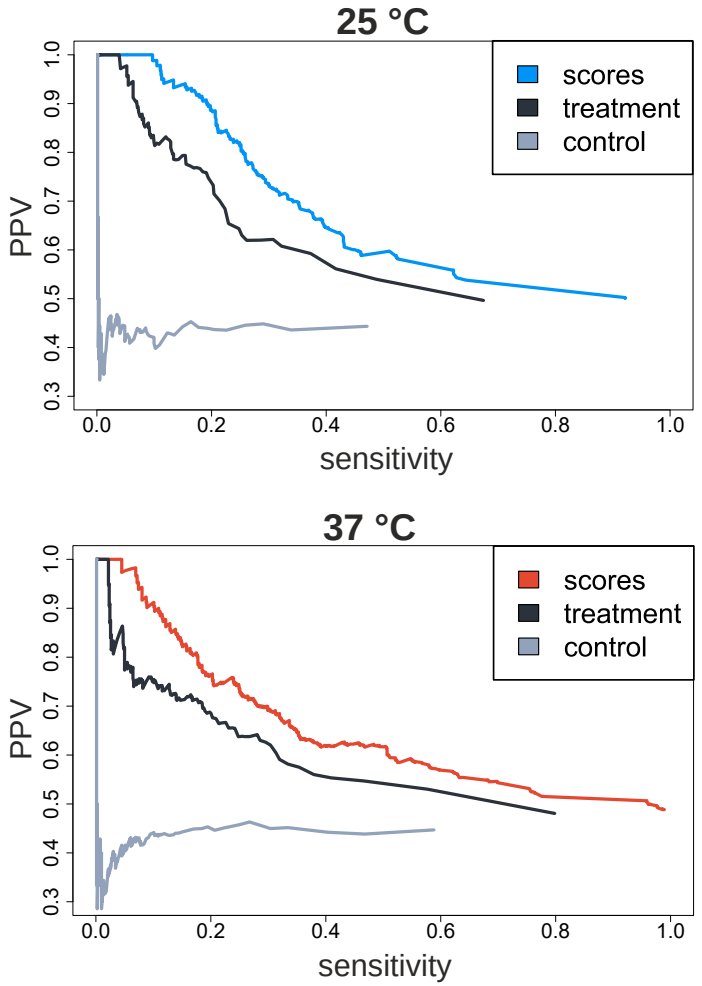


Lead-Seq at 25 °C and 37 °C

Reliability of Lead Scores: tRNAs

Test data: 32 tRNAs (764 paired + 665 unpaired nt)





PPV: How many of the nt with scores are actually unpaired?

Sensitivity: how many of all unpaired nt can we detect?

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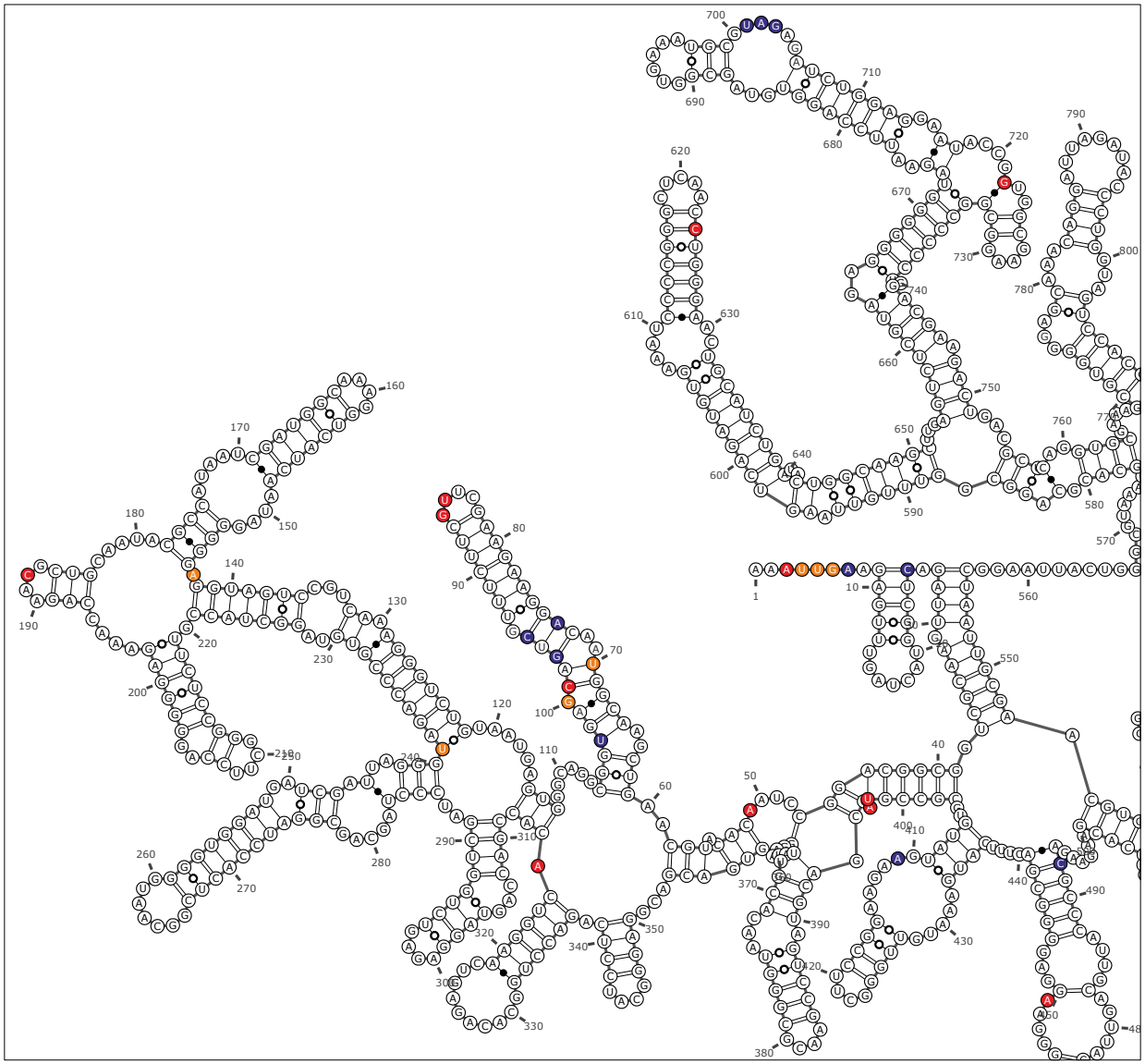
Reliability of Lead Scores: 16S-rRNA



lead cleavage at
- 25 °C
- 37 °C
- 25/37 °C

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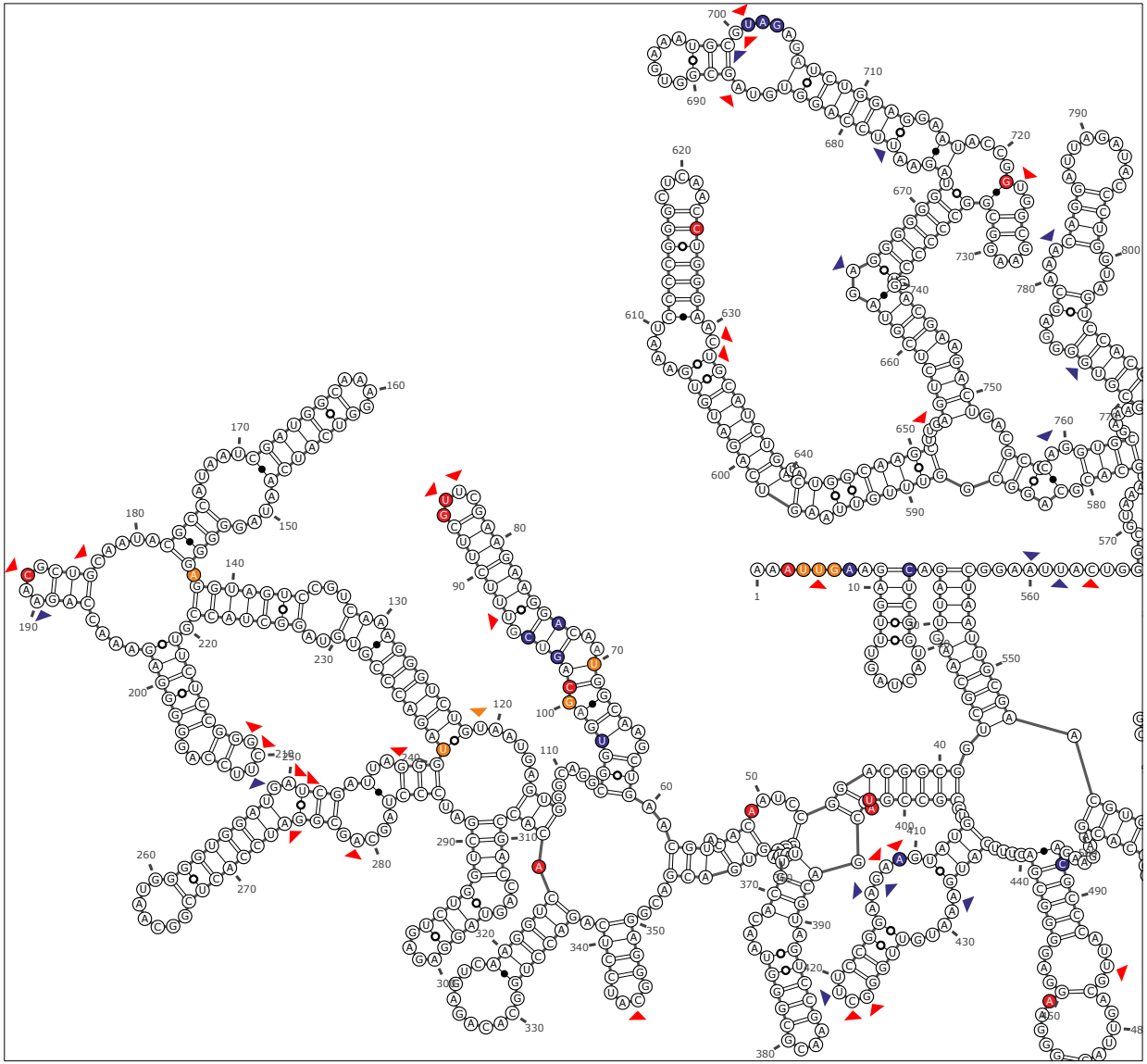
Reliability of Lead Scores: 16S-rRNA



lead cleavage at
- 25 °C
- 37 °C
- 25/37 °C

strong reactivity
- DMS
  Mustoe *et al.*, 2019
- SHAPE
  McGinnis *et al.*, 2015

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Secondary Structure Prediction

Problem: No information about pairing partners

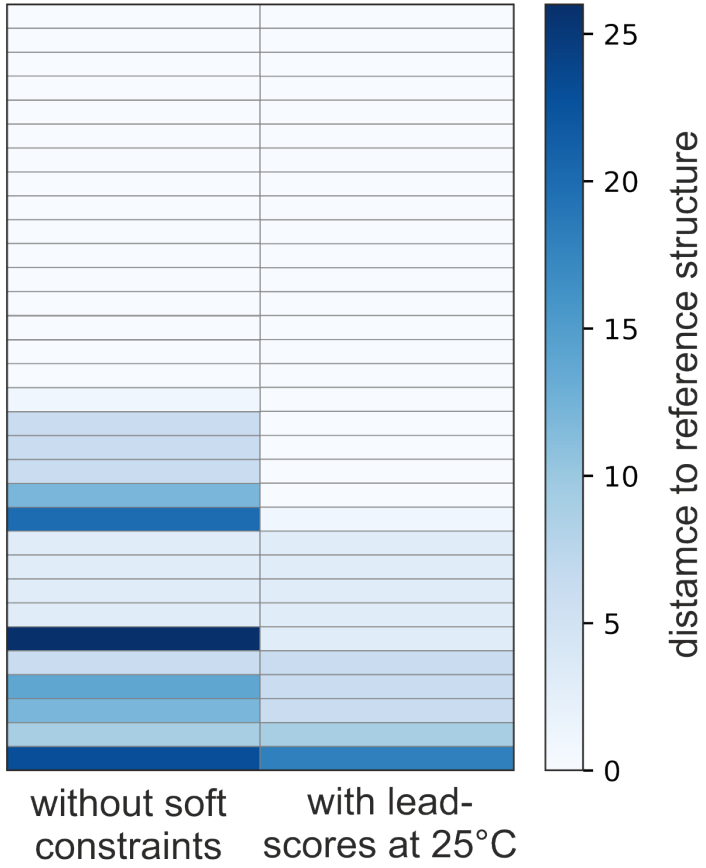
Solution: RNA structure prediction with soft constraints with ViennaRNA 2.0

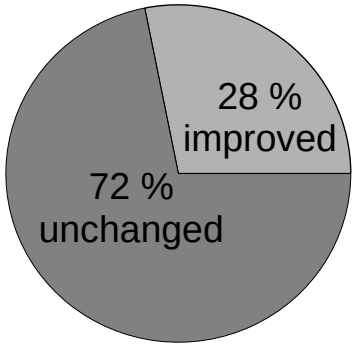
approach from Washietl *et al.*, 2012 (RNApvmin: tauSigmaRatio=0.6)

Test data: 32 tRNAs

Pre-processing:

- cap at 90 %
- rescale to range 0, 2
- exclude all < 1





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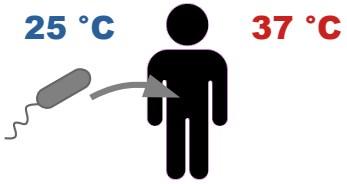
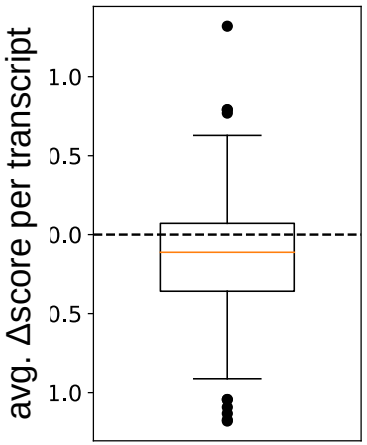
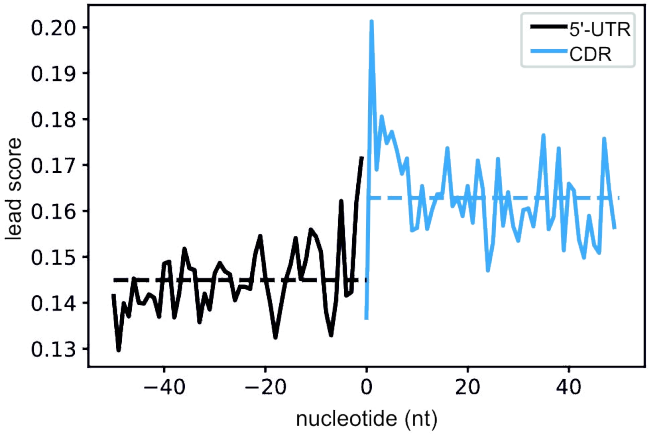
Temp. comparisons

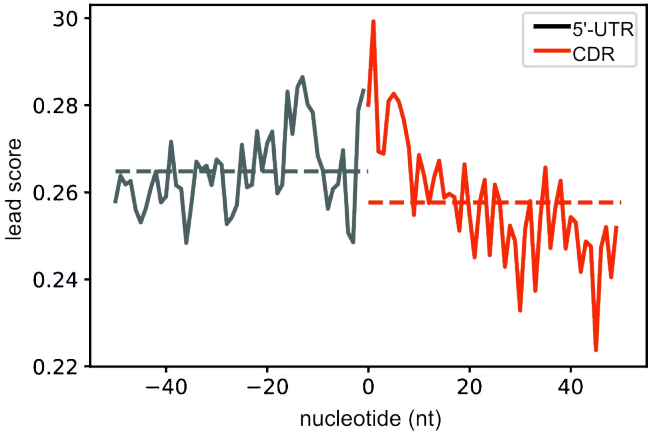




25 °C



37 °C



Same results: HIV-1 virions, Fruit flies, humans

Different: Arabidopsis th., rice, yeast

Same result: rice

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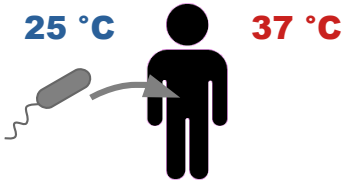
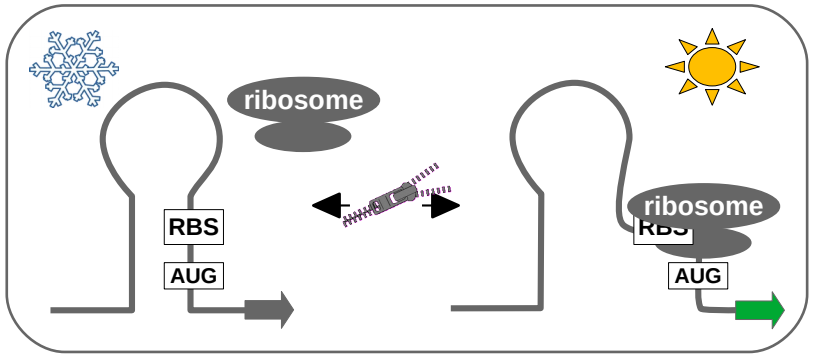
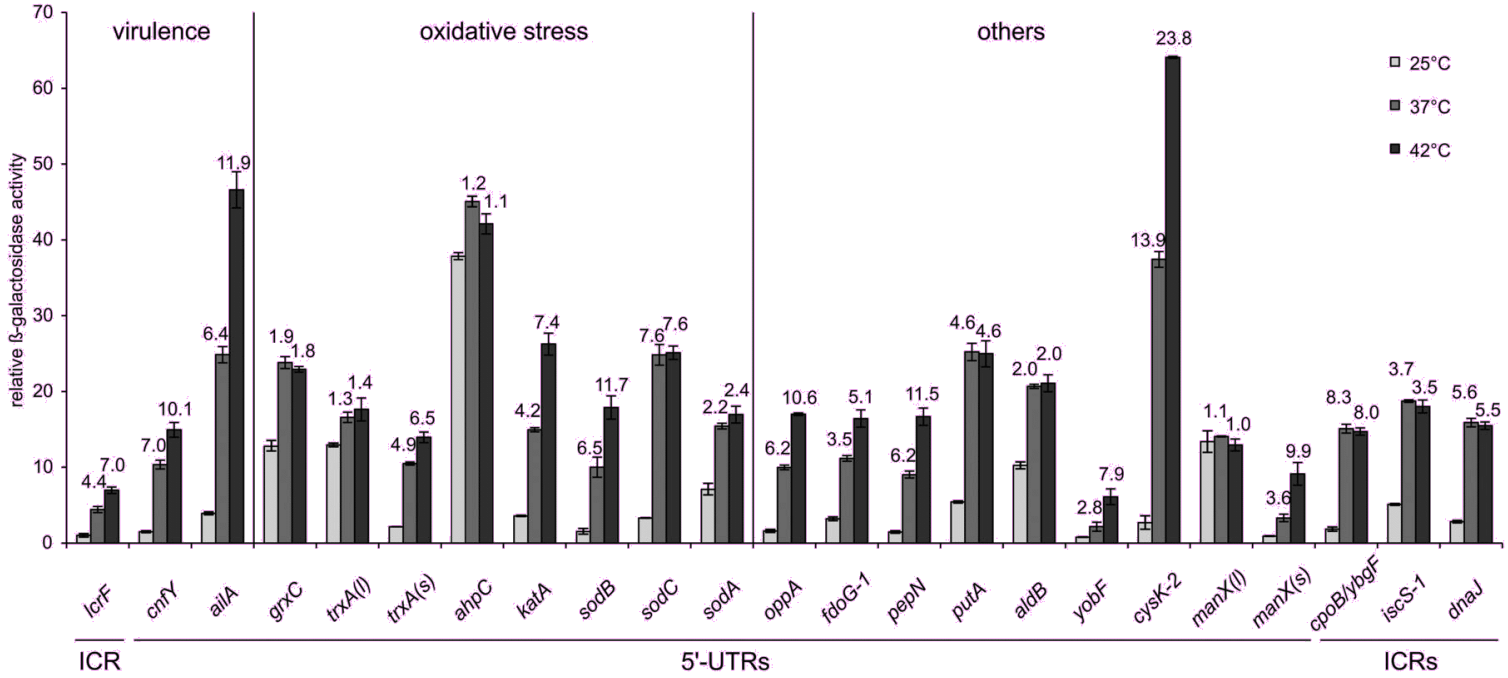
RNA Thermometers





- Diverse examples in bact. pathogenes
- *Yersinia pseudotuberculosis*
  - LcrF: major virulence regulator (Böhme *et al.*, 2012)



Righetti *et al.*, 2016

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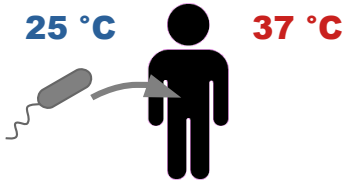
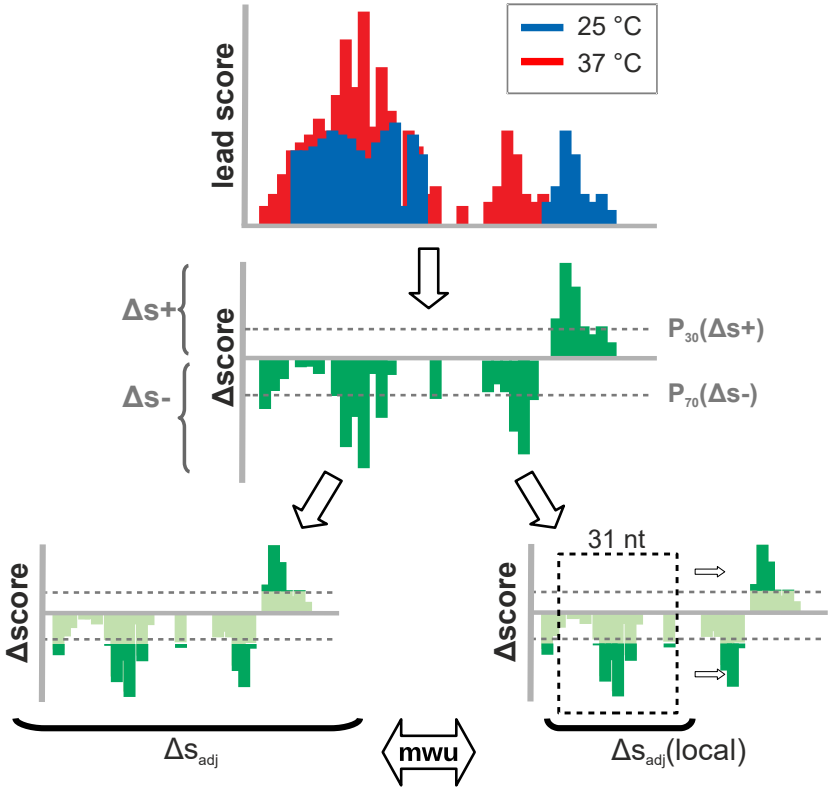
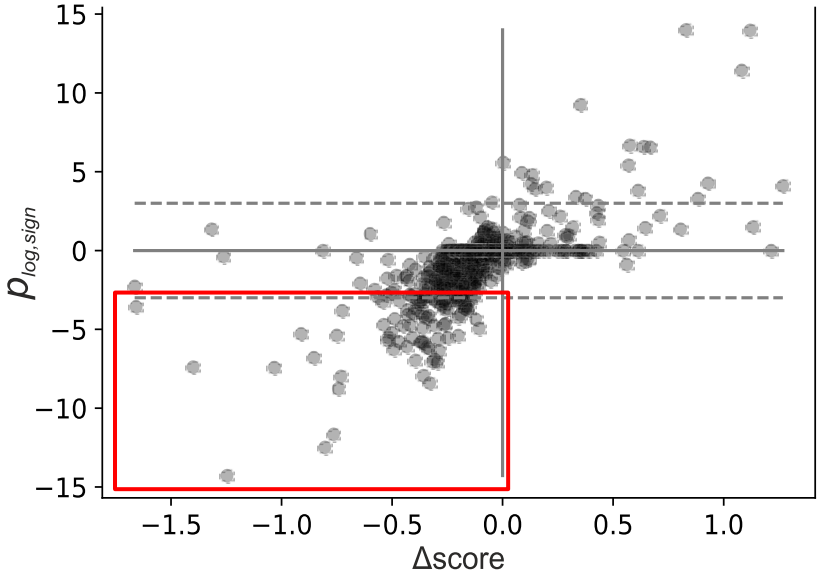
RNA Thermometers







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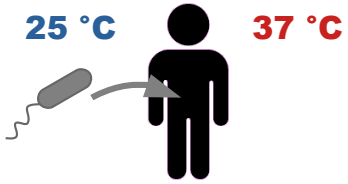
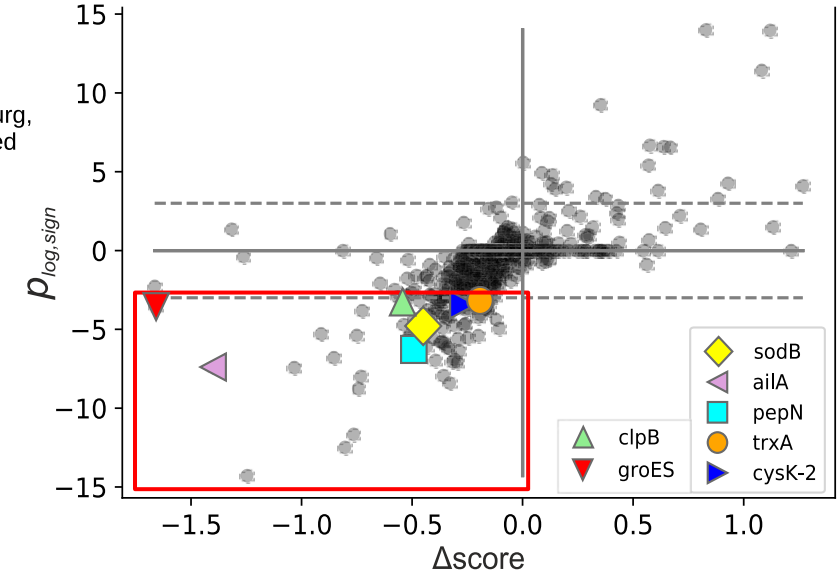
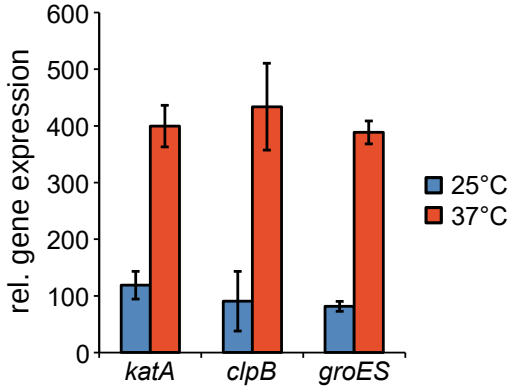
RNA thermometer



gene	gene description	Δscore	$p_{log,sign}$	
ailA	outer membrane protein	-1.4	-7.4	Righetti *et al.*, 2016
pepN	aminopeptidase	-0.5	-6.3	
sodB	superoxide dismutase	-0.5	-4.8	
cysK-2	cysteine synthetase	-0.3	-3.4	
trxA	thioredoxin	-0.2	-3.2	
groES	chaperonin	-1.7	-3.6	Brandenburg, unpublished
clpB	ATP-dependent chaperone	-0.5	-3.3	





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Summary

- Lead-Seq
  - Reliable information about tRNAs + larger molecules
  - Improve secondary structure predictions with soft constraints

- *Y. pseudotuberculosis'* structurome at 25 °C + 37 °C
  - General melting at higher temperature
  - 5'-UTRs more structured than CDS at 25 °C
  - Known + unknown RNA thermometers detectable

Microbial Biology, Ruhr-University Bochum

Prof. Dr. Franz Narberhaus

Dr. Christian Twittenhoff

Dr. Francesco Righetti

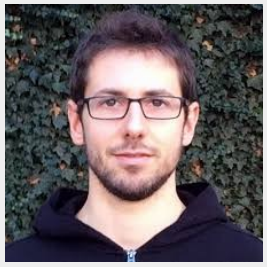
Bioinformatics, Ruhr-University Bochum

Prof. Dr. Axel Mosig

Molecular Infection Biology, HZI

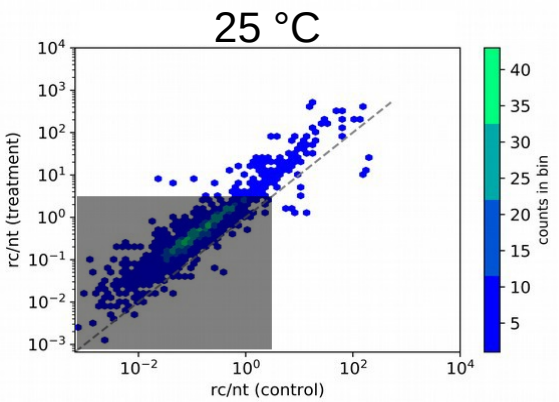
Prof. Dr. Petra Dersch

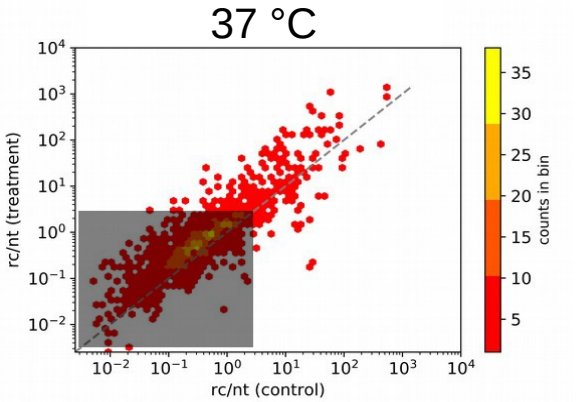
Dr. Aaron M. Nuss



Thank you.
Questions?

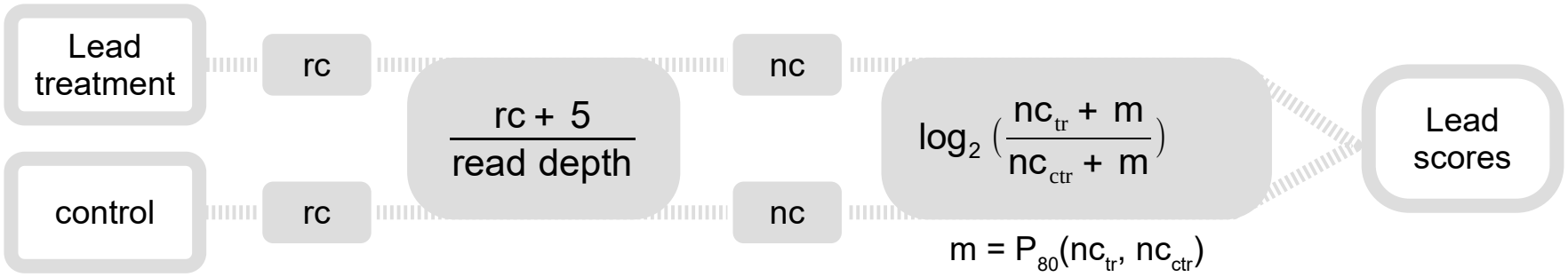
Score calculation





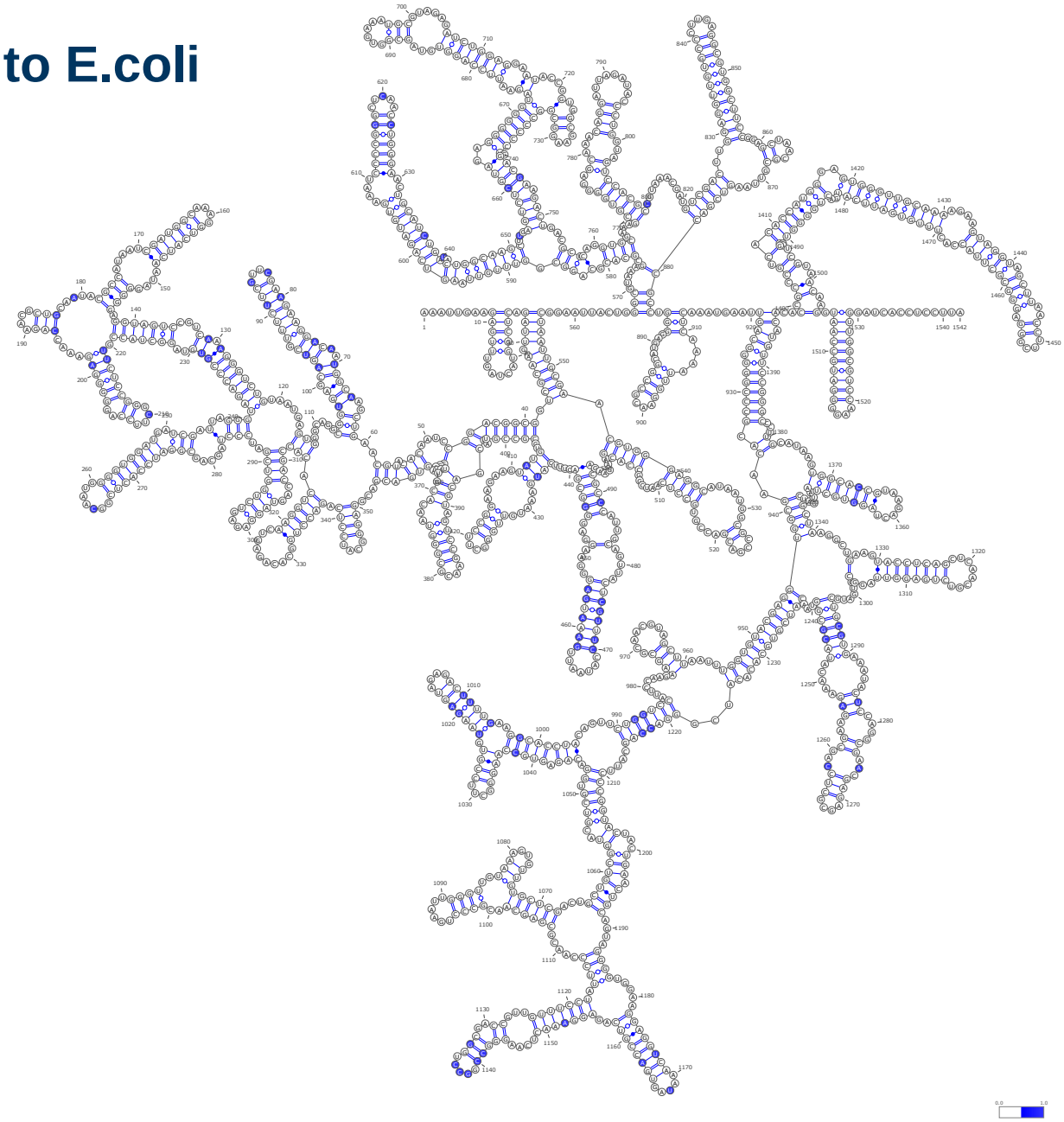
Avg. reads: 10005328 rc
Transcriptome: 3009815 nt
3.3 rc/nt

→ high signal-to-noise ratio at low-rawcount sites



Library ID	Temperature	Treatment	Total number of read counts	Number of reads after trimming	Number of mapped reads t
BC_01	25 °C	control	10475715	9249830	529702
BC_02	25 °C	+lead(II) acetate	8853413	8441722	808248
BC_03	37 °C	control	17473908	15623283	815170
BC_04	37 °C	+lead(II) acetate	10069470	9952848	1093248

16S rRNA diff. to E.coli



User file #6