

Regulatory Impacts of 6S pRNA transcripts

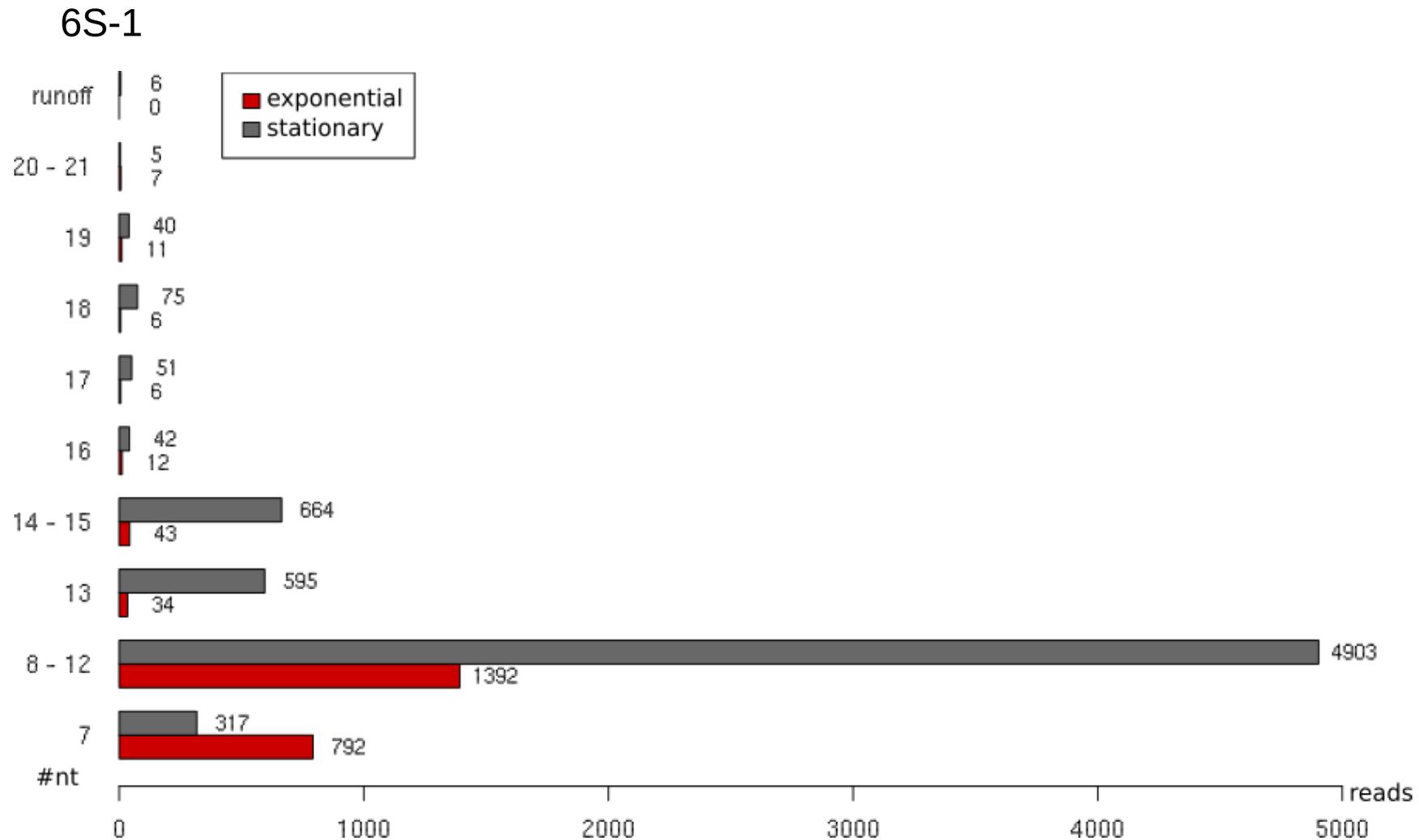
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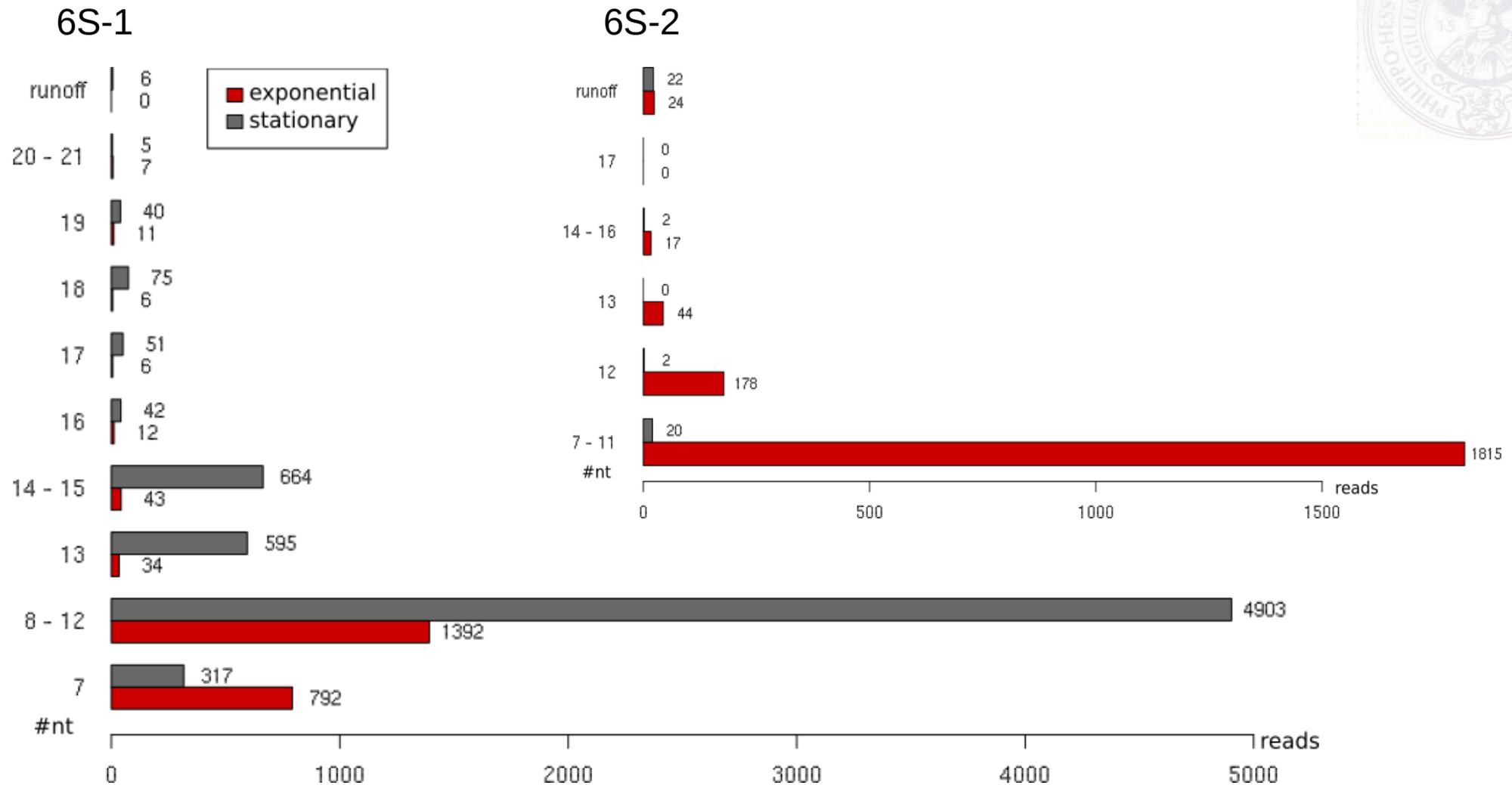
Universität

Marburg

The role of short pRNAs in *B. subtilis*



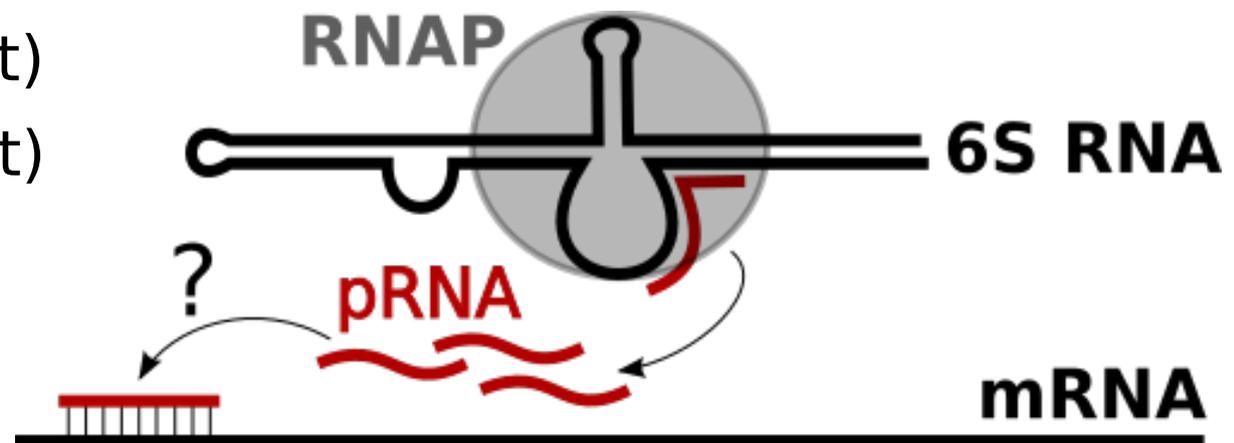
The role of short pRNAs in *B. subtilis*



Could pRNAs play a regulatory role?

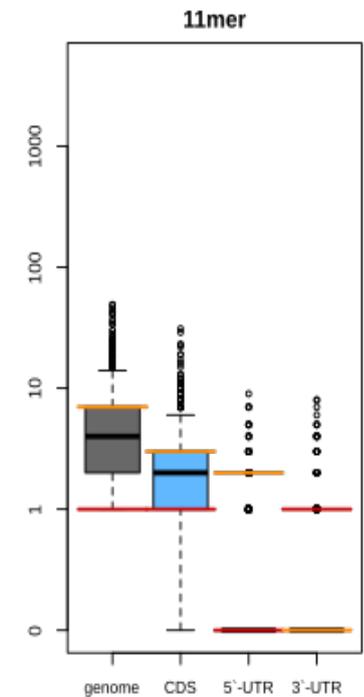
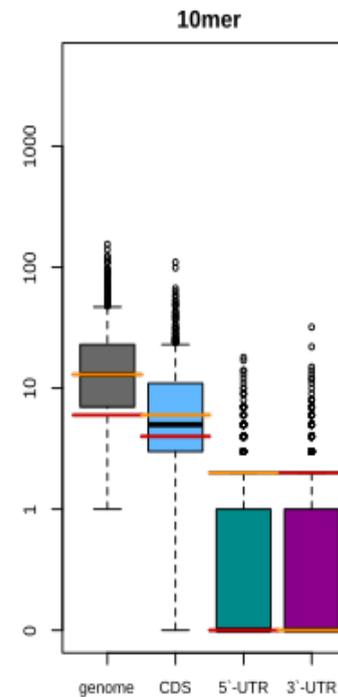
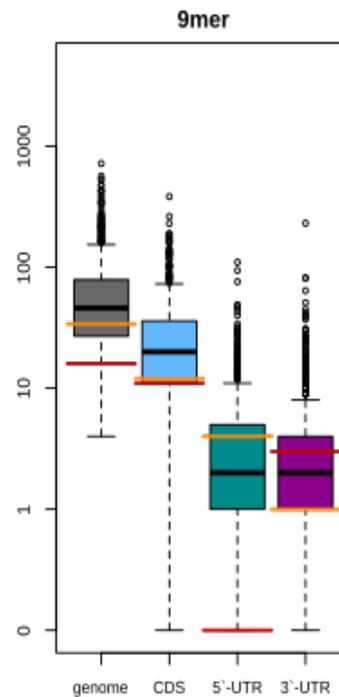
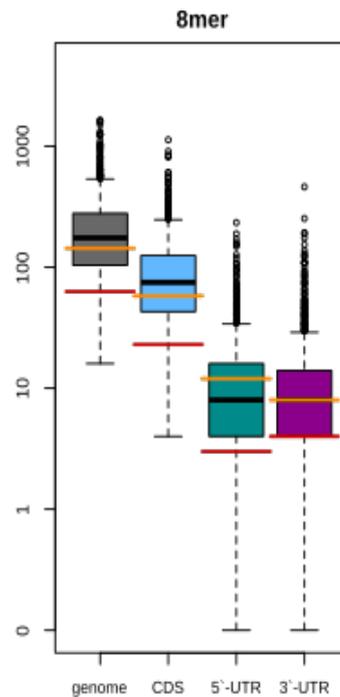
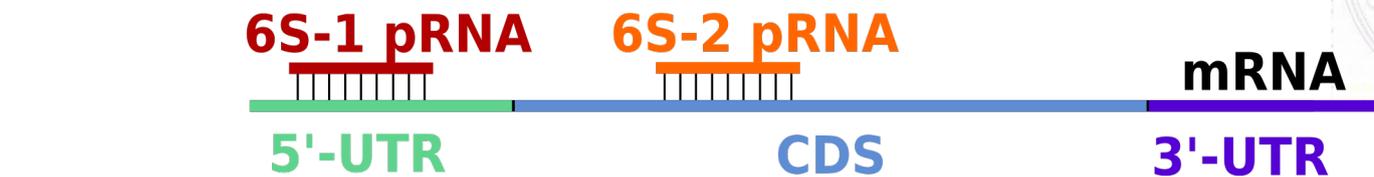
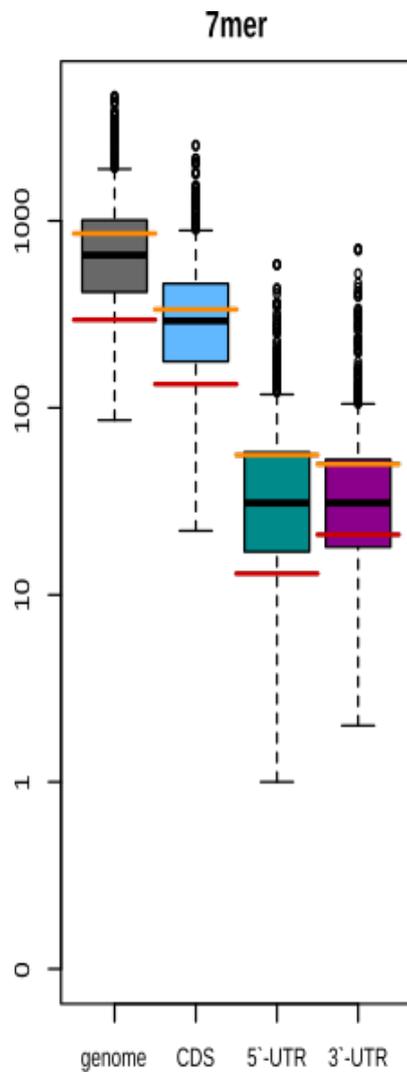
Genome-wide search for antisense sequences:

- oligonucleotides of length 7-14nt
- differentiating:
 - genome-wide
 - CDS
 - 5'-UTR (-100nt)
 - 3'-UTR (+100nt)



pRNA targets are underrepresented

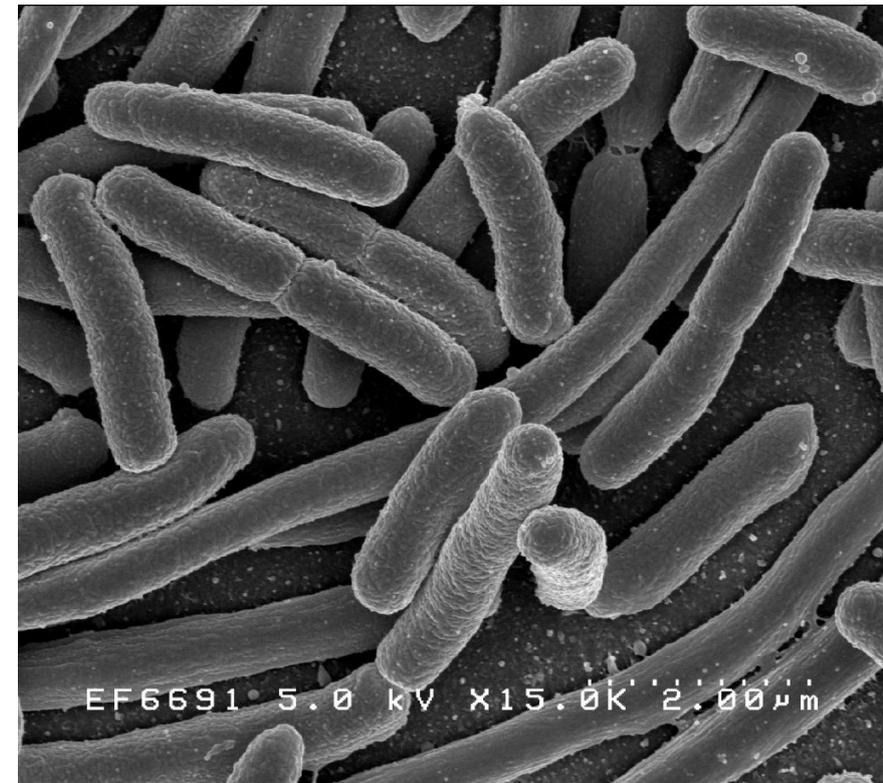
6S pRNA target frequencies vs. random oligomer distribution



Comparison with other organisms

- pRNA detected in:
 - *E. coli* (Proteobacteria) ¹
 - *B. subtilis* (Firmicutes) ²
 - *R. sphaeroides* (Proteobacteria) *
 - *A. aeolicus* (Aquificae) ³
 - *H. pylori* (Proteobacteria) ⁴
 - ...

¹ Wassermann (2007)

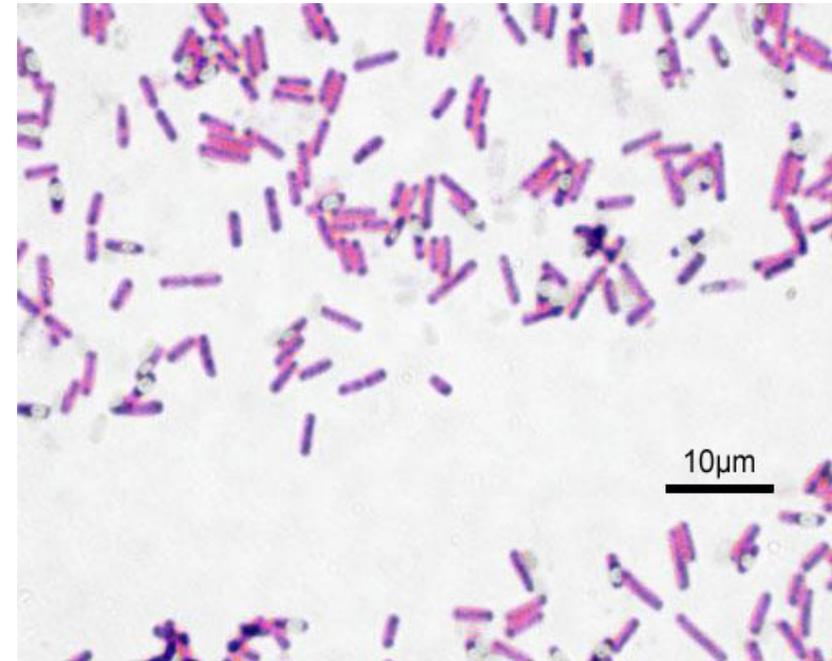


Credit: Rocky Mountain Laboratories, NIAID, NIH [Public domain], via Wikimedia Commons

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² Beckmann et al. (2012)



Credit: Y tambe [CC-BY-SA-3.0], via Wikimedia Commons

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* not published



Credit: http://genome.jgi-psf.org/finished_microbes/rhosp/rhosp.home.html

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³ Lechner et al. (2014)

Credit: <http://www.lpi.usra.edu/education/EPO/yellowstone2002/workshop/octopus1/index.html#aquifex>

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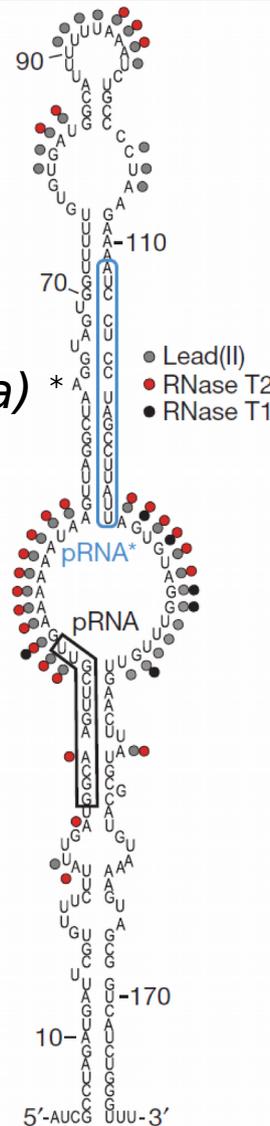
⁴ Sharma et al. (2010)

Credit: Yutaka Tsutsumi, M.D. Professor Department of Pathology Fujita Health University School of Medicine [Copyrighted free use], via Wikimedia Commons

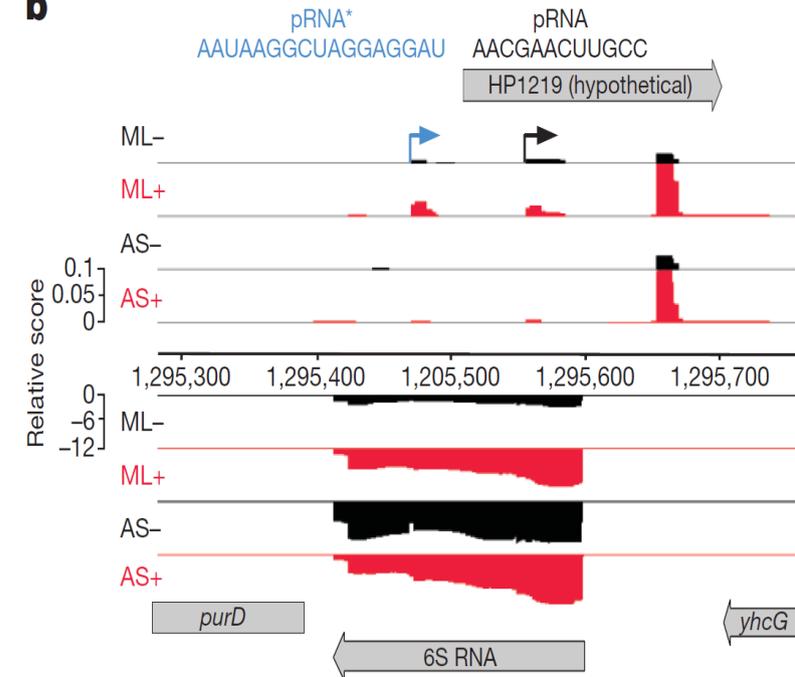
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- ...



b



⁴ Sharma et al. (2010)

Comparison with other organisms

- pRNA detected in:

E. coli 6S pRNA

B. subtilis 6S-1 pRNA

B. subtilis 6S-2 pRNA

R. sphaeroides 6S pRNA

A. aeolicus 6S pRNA

H. pylori 6S pRNA

H. pylori 6S pRNA*

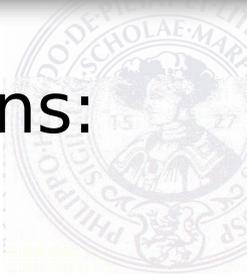


| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | U | C | G | G | C | U | C | A | G | G | G | G | A | C | U | G |
| G | U | U | C | G | G | U | C | A | A | A | A | C | U | A | G | G |
| A | A | A | G | G | U | U | A | A | A | A | C | U | U | A | A | U |
| A | U | C | G | G | C | C | A | C | U | G | G | A | A | A | A | G |
| G | U | A | G | G | C | C | C | C | A | U | U | G | A | C | A | A |
| A | A | C | G | A | A | C | U | U | G | C | C | A | U | C | A | A |
| A | A | U | A | A | G | G | C | U | A | G | G | A | G | G | A | U |

Transcriptome analysis

Deep sequencing data of *B. sub.* **deletion** strains:

- **wt**
 - **Δ6S-1**
 - **Δ6S-2**
 - **Δ6S-1/-2** (both deleted)
-
- Illumina MiSeq 50nt reads
 - three biological replicates each



Differential gene expression

- Mapping of reads with segemehl¹
- Statistical evaluation with edgeR²
- Estimating common dispersion
- correct gene-wise dispersion estimation

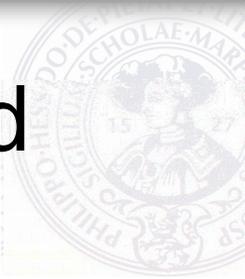
¹ Hoffmann et al. (2009)

² Robinson et al. (2010)



Evaluation of candidates

- Correlate antisense targets & regulated genes



6S-1

| | | |
|------|-------------|----------------------------------|
| up | vpr | minor extracellular protease vpr |
| down | ppsB | plipastatin synthase subunit B |

6S-2

| | | |
|------|-------------|--|
| up | ywfA | MFS transporter |
| up | nasB | assimilatory nitrate reductase electron transfer subunit |
| down | rapK | response regulator aspartate phosphatase K |

Outlook

- Annealing probability:
 - secondary structure model
 - free energy of target RNA:pRNA hybridization (RNAup)
- more deep sequencing data with short RNAs
 - determine other pRNA sequences
- target sequence comparison across organisms
 - on protein level
- confirmation with experimental results
 - test hybridization with pLNAs in gel
 - control with pRNA mutations



Acknowledgement

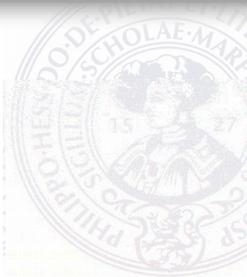
Thanks to:

Marburg

- Philipp Hoch
- Maria Schmidt
- Roland K. Hartmann
- Marcus Lechner

Moscow

- Olga Burenina
- Elena Kubareva



... and thank you for your attention!