Identifying unknown sRNAs in Listeria monocytogenes

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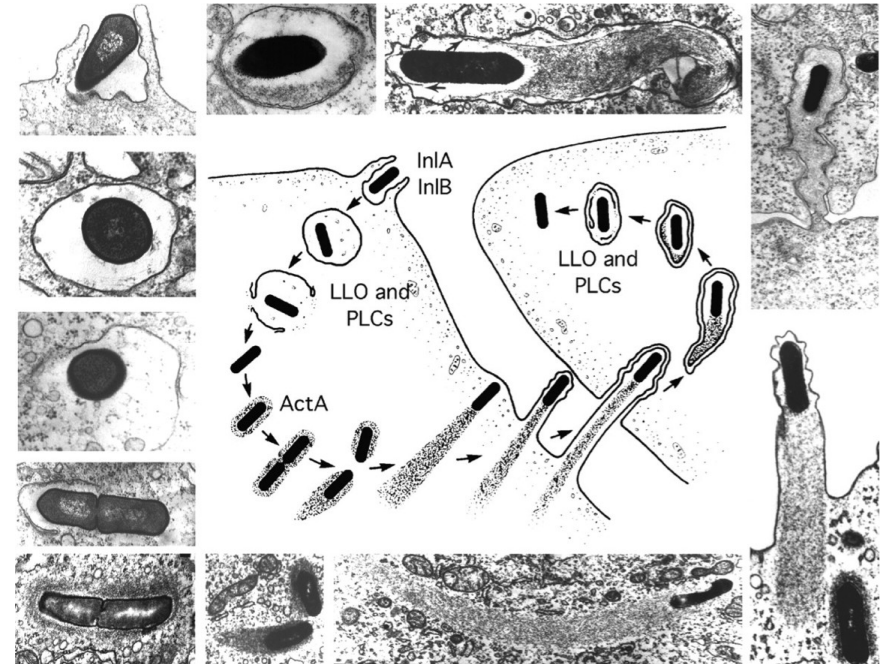
15.02.2012 - Winterseminar Bled

Overview

▶ Listeria & small RNAs
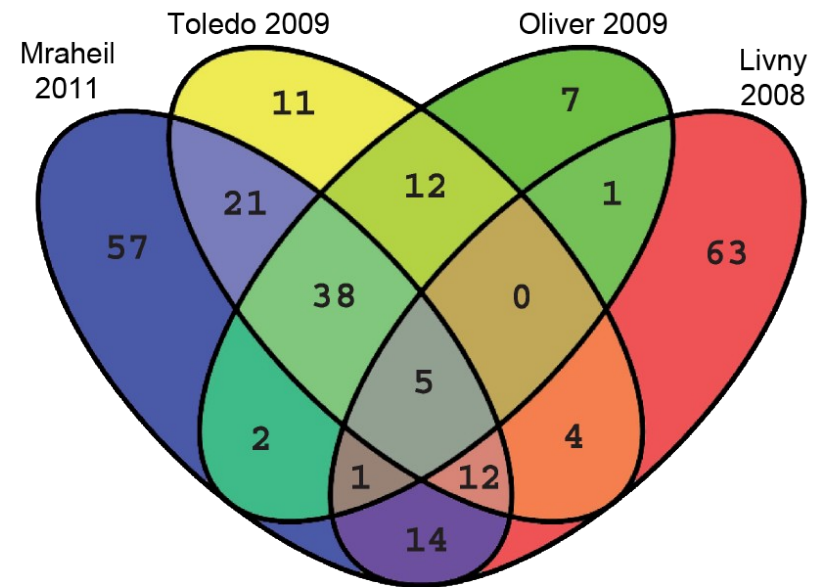
▶ Data & methods

▶ Results

Listeria moncytogenes

- **Facultative intracellular bacterium**

- **Causative agent of listeriosis**
  - high lethality (30%-70%)
  - food born

- **Growth in harsh conditions**
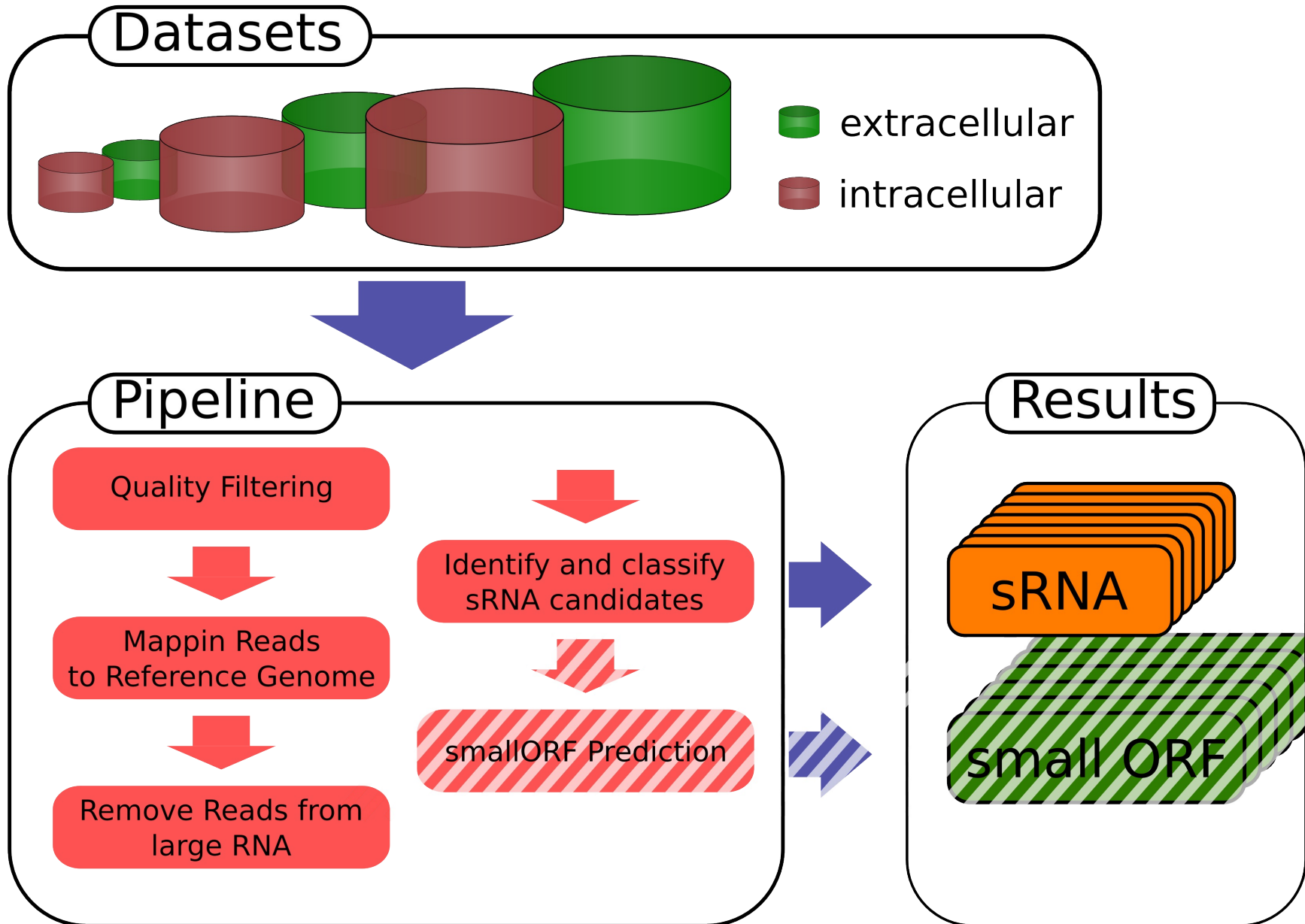 - fridge temperatures
  - high salt
  - low pH

[5] Portnoy et al. 2002

Small RNA in Listeria

▶ Switch to pathogenicity proposed to be regulated by sRNAs.

▶ Several studies found a total number of ~250 sRNAs in Listeria species

Methods - Overview

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Data - Overview

▶ Reinsequencing of Listeria transcriptome [mraheil et al.]

▶ SOLiD platform (previously 454)

▶ Transcriptome under two growth conditions

 ▶ Intracellular (pathogenic) growth in mouse MΦ

 ▶ Extracellular (saprophytic) growth in BHI medium

▶ RNA split into three fractions

 ▶ $x > 150$

 ▶ $150nt > x > 40nt$

 ▶ $40nt > x$

+ Total of six data sets

Mapping

- ► Shrimp and BWA for mapping

- ► ~50% of reads could be mapped to reference

	>150	<150;>40	<40		Extra	Intra
Bwa	57.56%	9.66%	11.10%		34.41%	17.79%
Shrimp	89.71%	51.74%	68.88%		82.77%	57.45%

- ► Mapping correlates with fraction

- ► Mapping correlates with growth condition

- ► Bwa and Shrimp have different sensitivity

Filtering for Candidates

▶ Using two filter approaches

  ▶ Annotation-dependent: Masking regions within annotated CDS

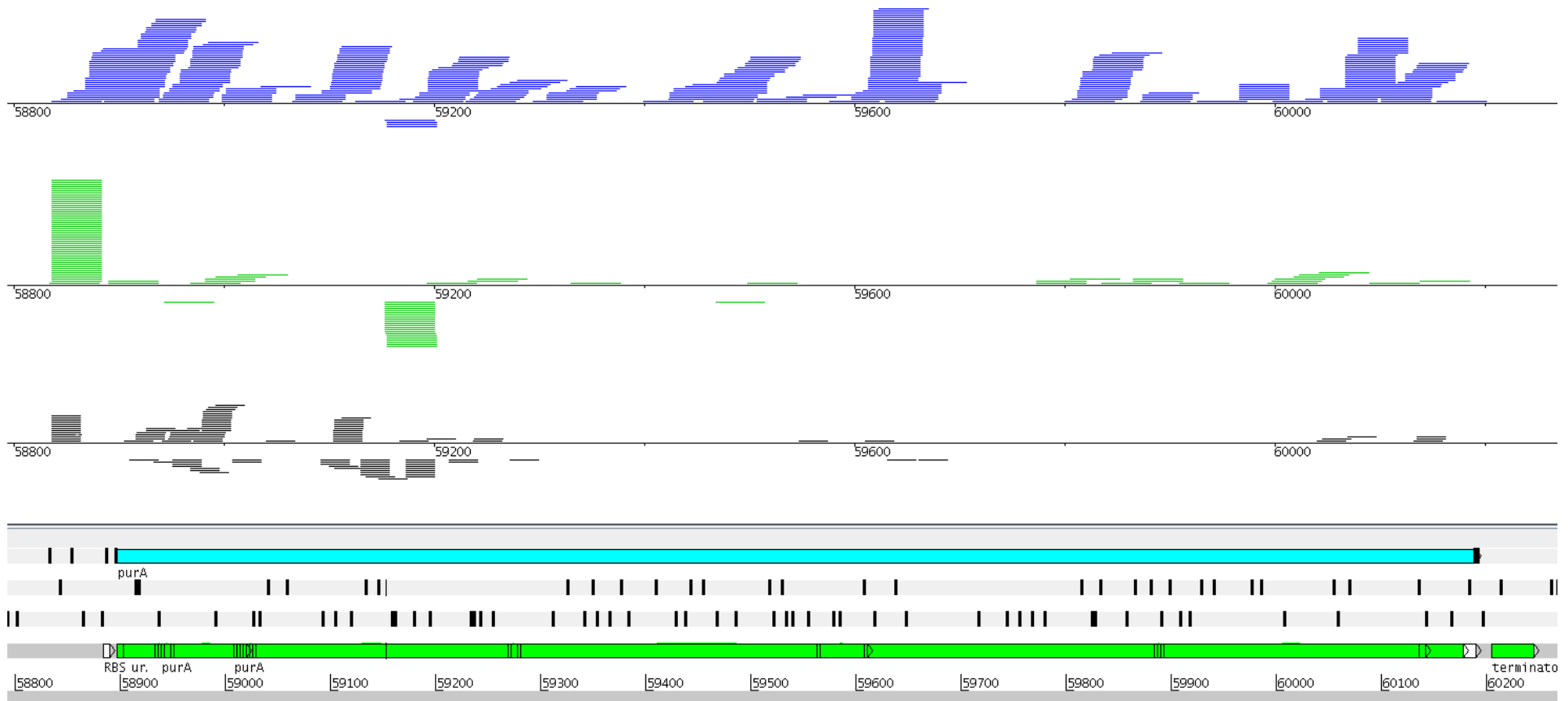 ▶ Annotation-independent: Making use of the size fractioning of samples to mask regions
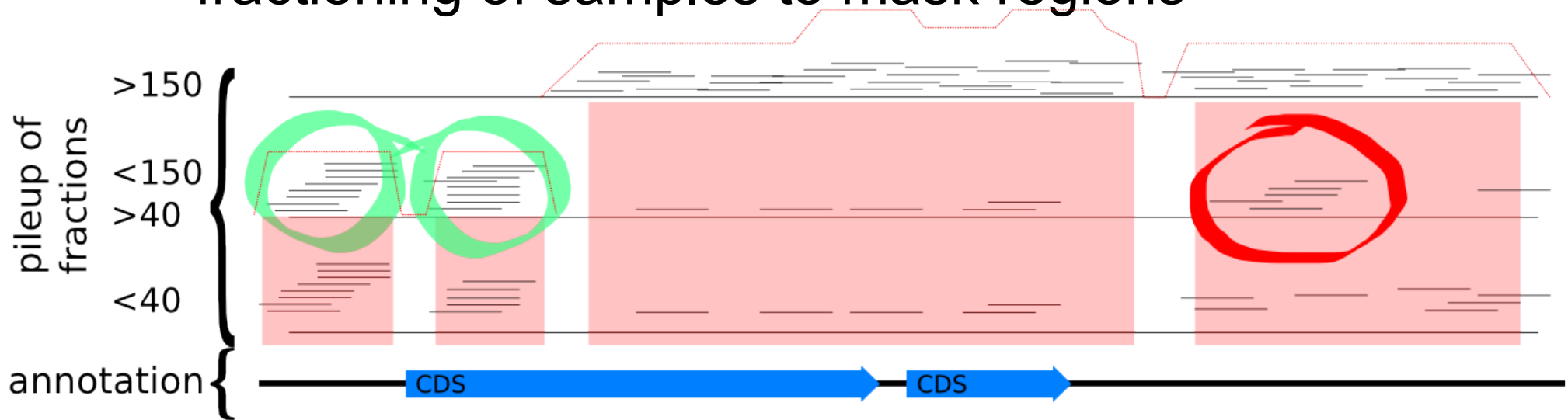
Classification of Candidates

- Manual inspection of randomly selected candidates to identify classes of sRNAs in the data

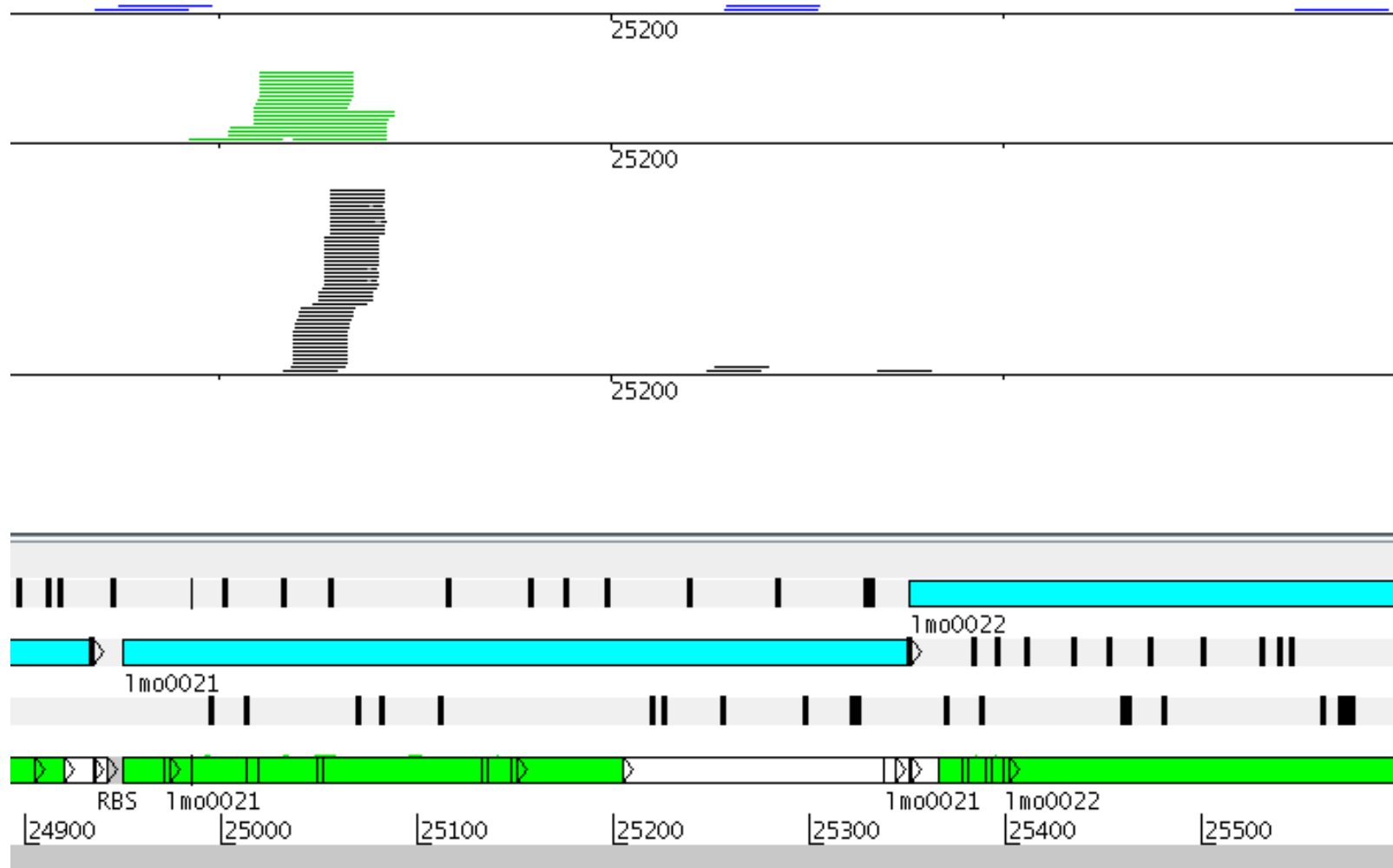- We built classifiers for the four most common classes found
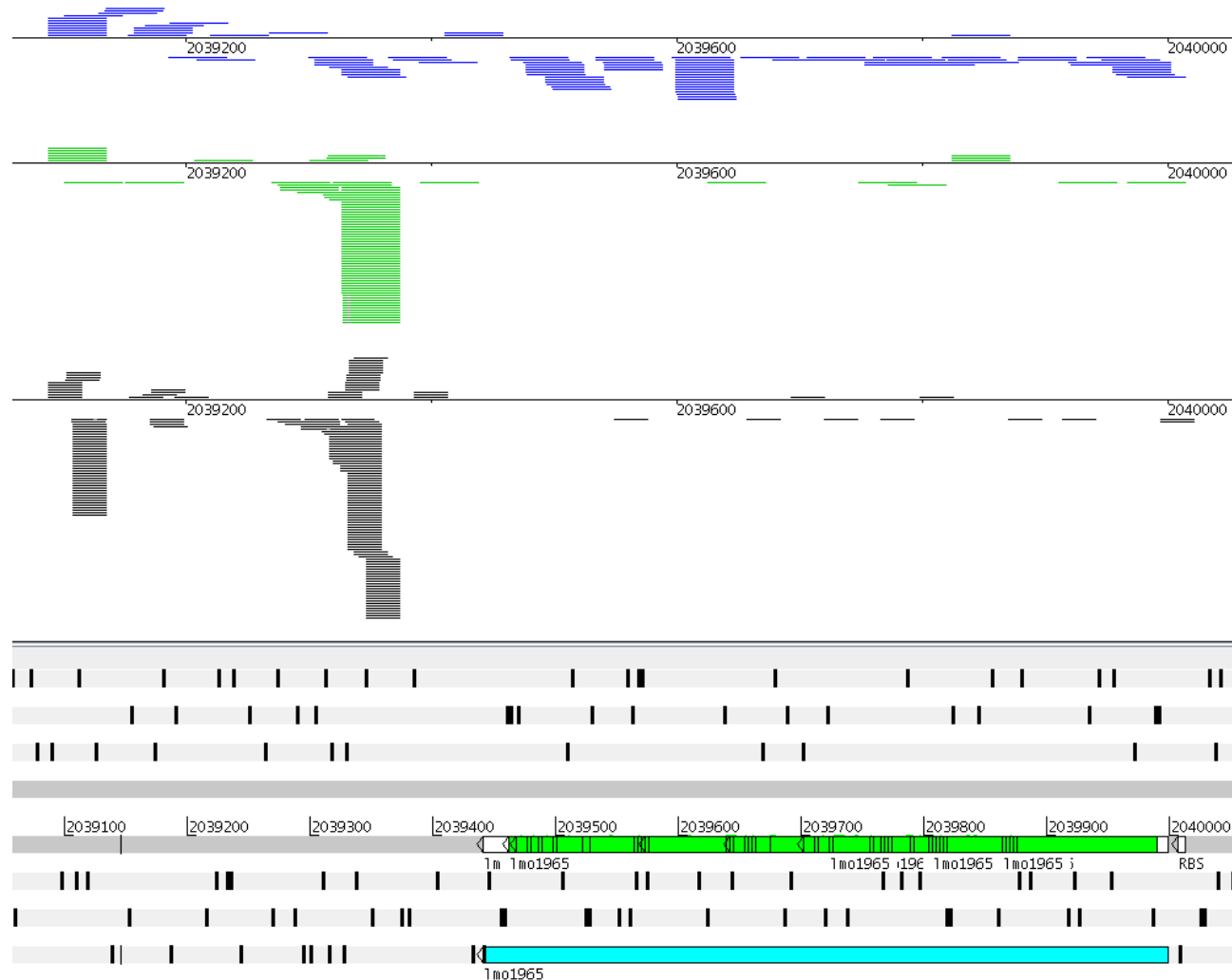
Example 1 - putative transcriptions starts
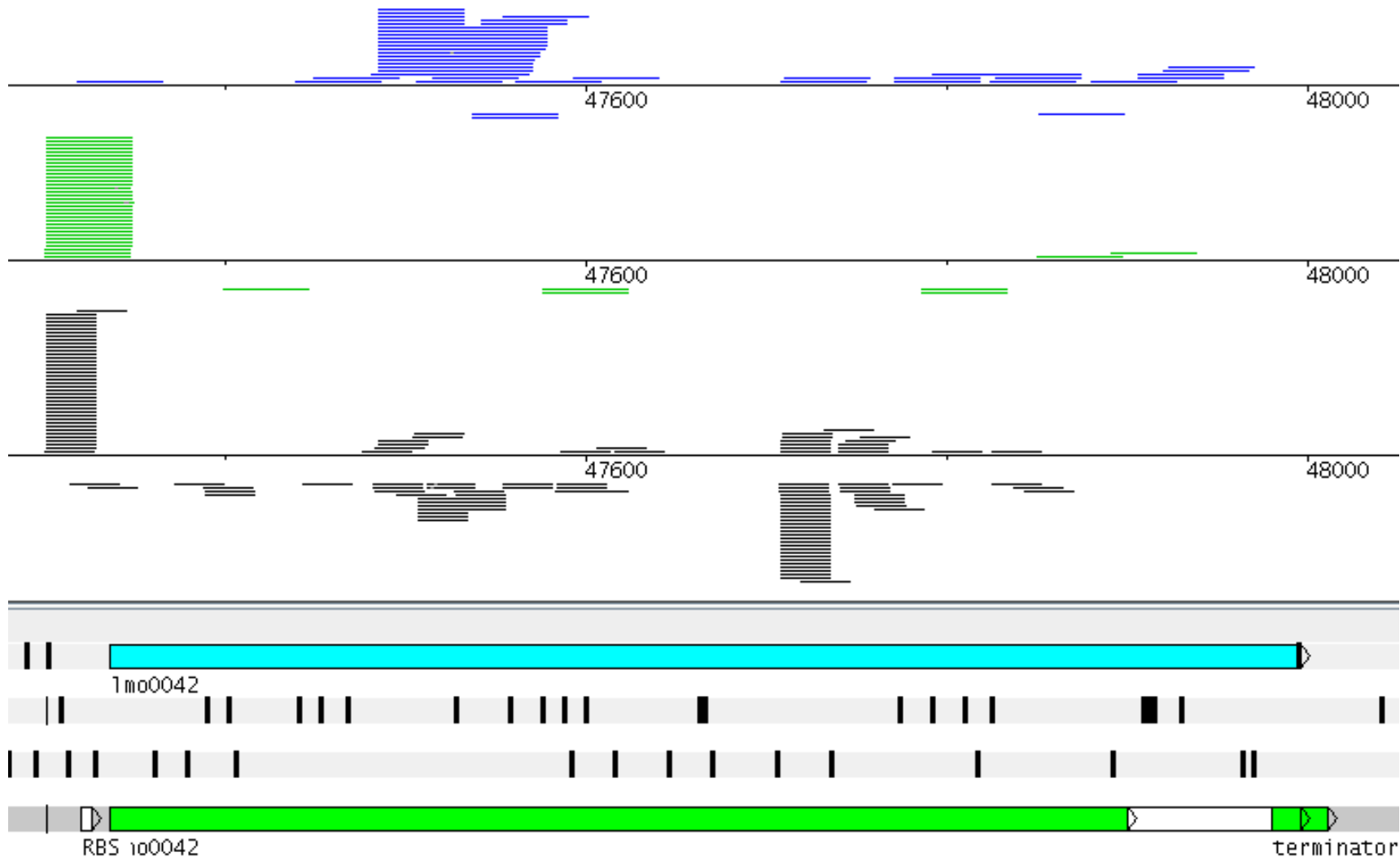
Example 2 - antisense RNA

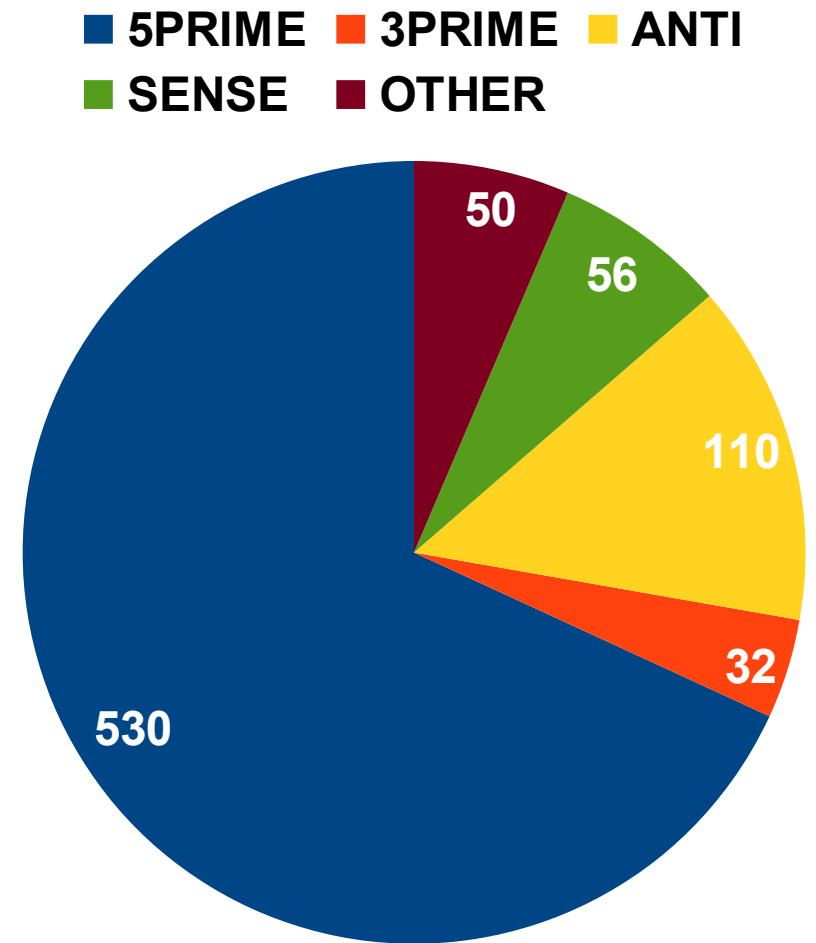# Example 3 - sense RNA

Example 4 - 3' end of long transcript

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Classification of sRNA candidates

- ▶ Majority of candidates are putative transcription start sites

- ▶ 50 candidates need to be categorized manually

Legend:
- ■ 5PRIME
- ■ 3PRIME
- ■ ANTI
- ■ SENSE
- ■ OTHER

- 50
- 56
- 110
- 32
- 530

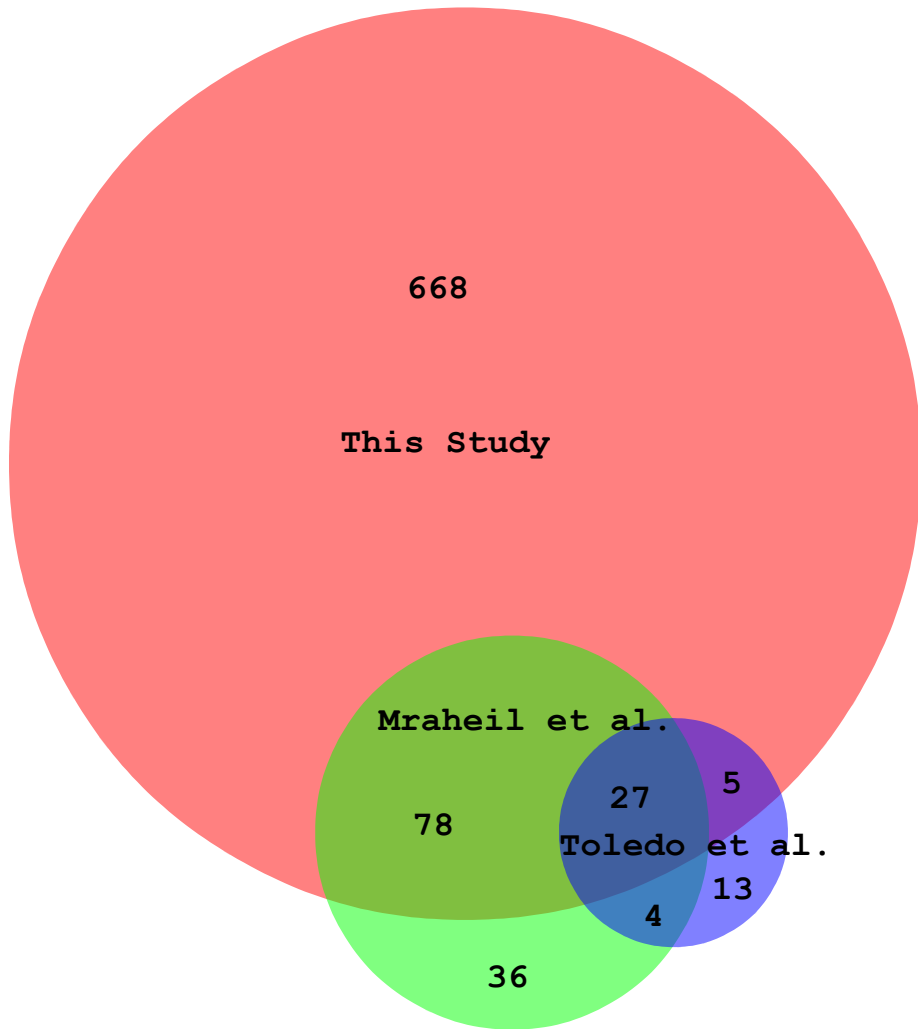# Differential Expression

- Majority of candidates show differential expression with regard to growth condition

- Indication for role in transition from saprophytic and pathogenic

Intracellular

245

197

336

Extracellular
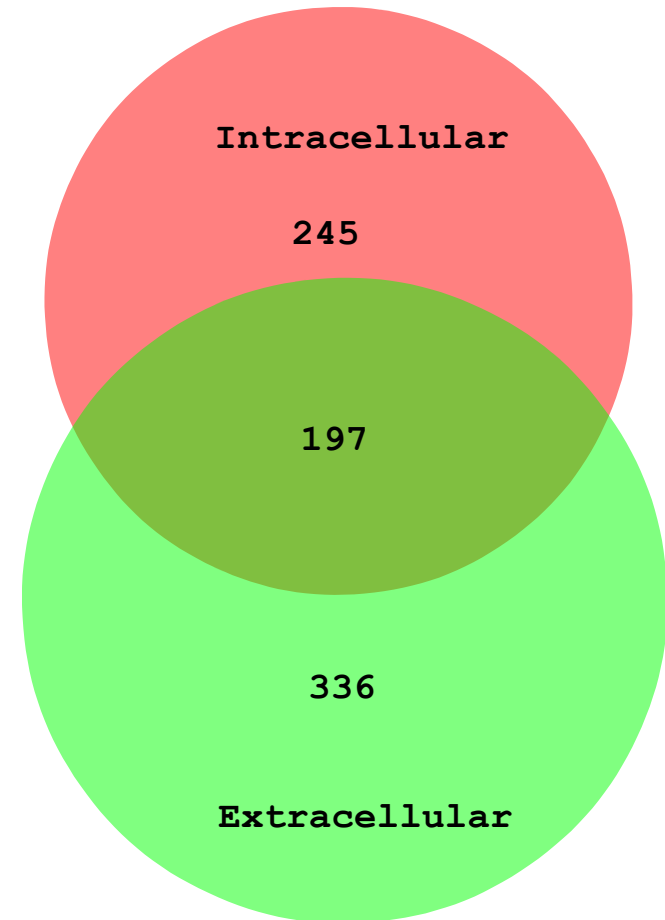
Overlap with previous studies

▶ 66% of candidates found in previous studies were confirmed.

▶ Total of 668 previously undescribed sRNAs

Conclusion

▶ We constituted a list of 778 sRNAs, 668 of which were previously unknown.

▶ We propose 581of to have a possible role in regulation of pathogenicity

▶ The SOLiD platform is suitable to detect sRNAs from bacterial transcriptomes

▶ We find increased frequency of sRNAs at the putative transcription start of larger RNAs

Perspective

- Small-ORF prediction

- Target prediction and functional analysis of possible sRNA targets

- Functional analysis of differentially expressed sRNAs

I would like to thank...

Stefanie Widder

Thomas Rattei

Torsten Hain

You

[1] A. Toledo-Arana et al. The listeria transcriptional landscape from saprophytism to virulence. Nature, 459(7249):950–956, 2009.

[2] H. Oliver et al. Deep rna sequencing of L.monocytogenes reveals overlapping and extensive stationary phase and sigma b-dependent transcriptomes, including multiple highly transcribed noncoding rnas. BMC genomics, 10(1):641, 2009.

[3] M.A. Mraheil et al. The intracellular srna transcriptome of listeria monocytogenes during growth in macrophages. Nucleic acids research, 39(10):4235, 2011.

[4] J. Livny, H. Teonadi, M. Livny, and M.K. Waldor. High-throughput, kingdom-wide prediction and annotation of bacterial non-coding rnas. PLoS One, 3(9):e3197, 2008.

[5] Portnoy, D.; Auerbuch, V. & Glomski, I. The cell biology of Listeria monocytogenes infection The Journal of cell biology, Rockefeller Univ Press, 2002, 158, 409