

Theoretical and practical metagenomic approaches to viral discovery

Practical Session: LRIsScan for viral long-range RNA-RNA Interactions

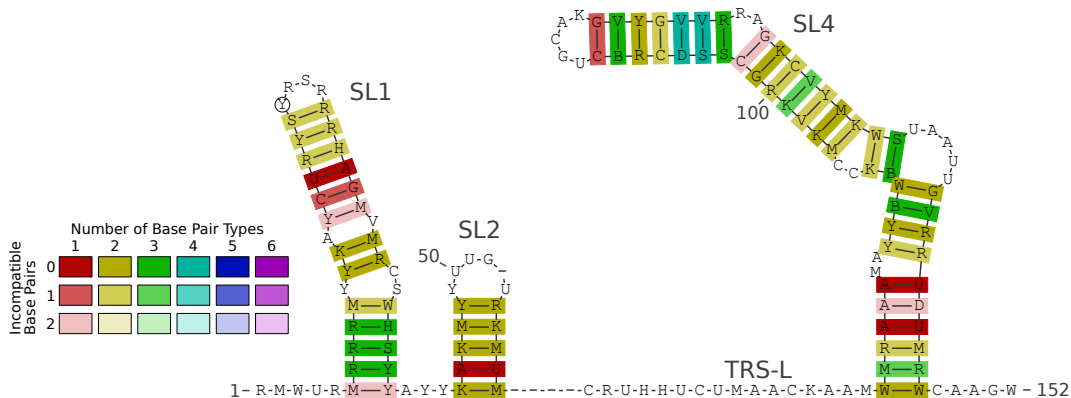
Kevin Lamkiewicz, Manja Marz

25.10.2019

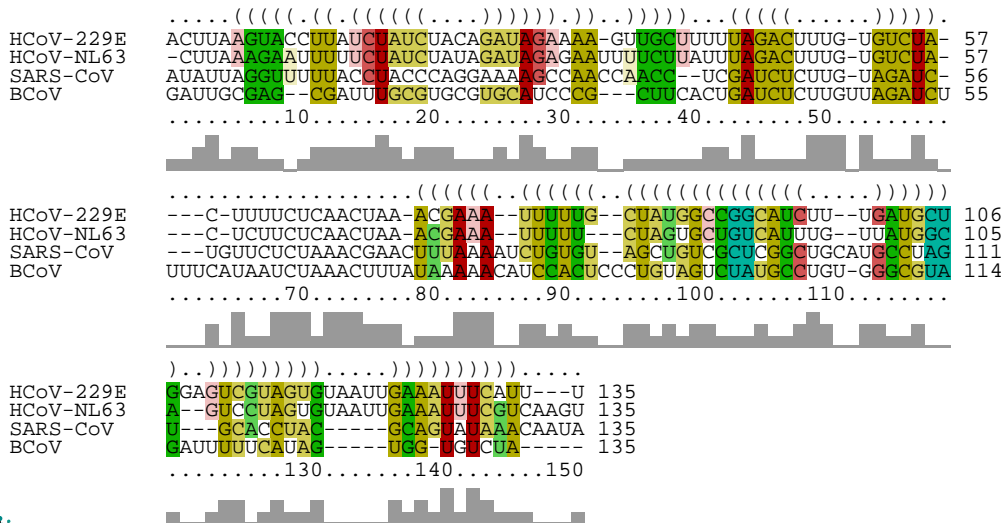
European Virus Bioinformatics Center

Alignments and compensatory mutations

UNCONSERVED SEQUENCE, CONSERVED STRUCTURE



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Compensatory mutations underline the importance of a specific secondary structure.

But be careful!

If we're assuming a uniform mutation rate, every third pair of mutations is a compensatory mutation.

A	U
A	A
A	C
A	G
C	A
C	C
C	G
C	U

A	U
G	A
G	C
G	G
G	U
U	A
U	C
U	G
U	U

RNA-RNA Long-Range Interactions

WHY LRIs?

- ▶ Interaction spans distances between a few hundred and several thousands of nucleotides
- ▶ few are described in positive stranded RNA viruses
- ▶ often located in loop regions (bulges, hairpins, ...)

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- ▶ few are described in positive stranded RNA viruses
- ▶ often located in loop regions (bulges, hairpins, ...)
⇒ pseudo-knots!
- ▶ LRIs may play a very important role in viral replication

HOW TO CALCULATE LRIs

Approach I

- ▶ RNAduplex
- ▶ RNAplex
- ▶ RNAhybrid

HOW TO CALCULATE LRIs

Approach I

- ▶ RNAduplex
- ▶ RNApplex
- ▶ RNAhybrid

Approach II

- ▶ RNAcofold

HOW TO CALCULATE LRIs

Approach I

- ▶ RNAduplex
- ▶ RNApflex
- ▶ RNAhybrid

Approach II

- ▶ RNAcofold

Approach III

- ▶ RNAup
- ▶ IntaRNA

HOW TO CALCULATE LRIs

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- ▶ IntaRNA

Approach IV

- ▶ interRNA
- ▶ inRNAs

HOW TO CALCULATE LRIs

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Approach IV

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- ▶ inRNAs

Approach V

- ▶ PETcofold
- ▶ RNAaliduplex

LRISCAN

Prediction of conserved long-range RNA-RNA interactions in full viral genomes, 2016. M. Fricke, M. Marz

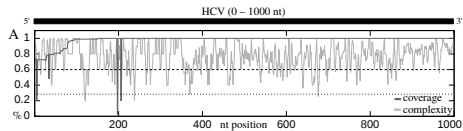
LRIsCan

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⇒ LRIsCan

How does LRIsScan work and how do I use it?

WORKFLOW OF LRISCAN



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Coverage of an alignment

Relative number of sequences that do not have a gap on a specific position.

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$$C_i = \frac{1}{m} \sum_{k=1}^m \frac{|\delta(a_{i \dots i+s-1}^k)|}{|(a_{i \dots i+s-1}^k)|}$$

COVERAGE AND COMPLEXITY

Coverage of an alignment

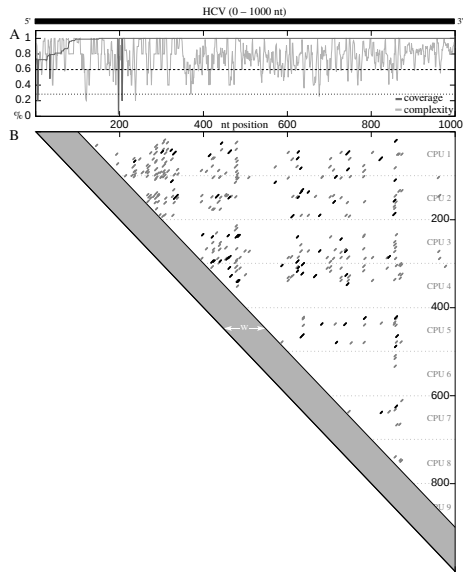
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Complexity of the alignment

$$C_i = \frac{1}{m} \sum_{k=1}^m \frac{|\delta(a_{i \dots i+s-1}^k)|}{|(a_{i \dots i+s-1}^k)|}$$

$$\delta(\text{CCUUUGGAAA}) = \text{CUGA}$$

WORKFLOW OF LRISCAN – STEP 2

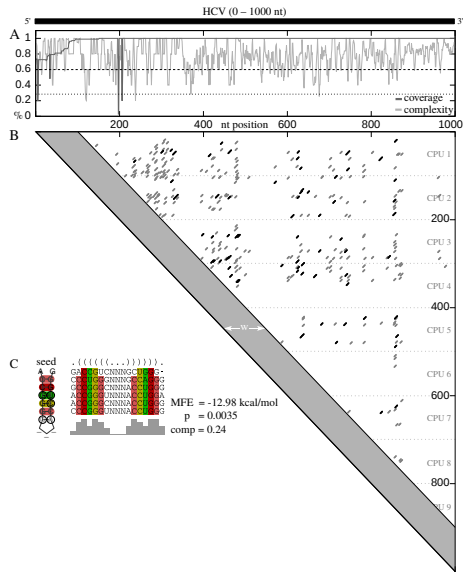


FINDING SEEDS

$$S_{i,j} = (S_{i-1,j+1} + 1) \cdot \Pi_{ij} \cdot \Phi_{ij}$$

- ▶ Π_{ij} : do at least t percent of the input sequence form the basepair (i, j) ?
- ▶ Φ_{ij} : do both alignment columns A_i and A_j meet the coverage threshold?

WORKFLOW OF LRISCAN – STEP 3



SEED SCORING

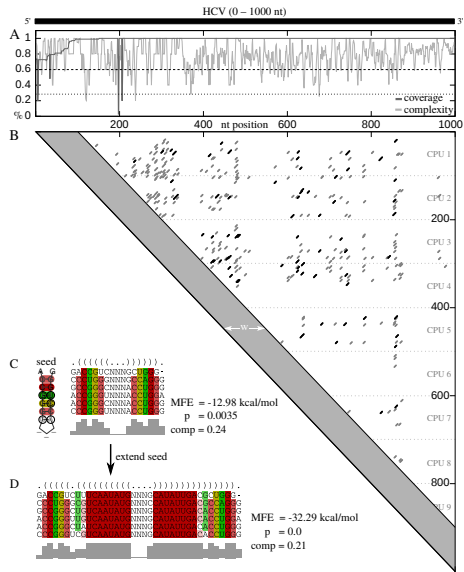
- ▶ z-Score analysis for each seed to measure reliability
- ▶ compensatory score τ

$$\tau = \frac{\sum_b (u \cdot h)}{6 \cdot |b| \cdot k}$$

with:

- ▶ u : number of different base-pair types
- ▶ h : number of incompatible base-pairs

WORKFLOW OF LRISCAN – STEP 4



SEED EXTENSION

- ▶ each seed is extended 10 nts at the 5' (and 3' respectively)
- ▶ calculate MFE with RNAalifold
 - ▶ hard constraints for seed region
 - ▶ soft constraints for extension, such that intermolecular interactions are formed

LRIsScan Results and Output

LRISCAN USAGE

```
1 $> ./LRIsScan.rb -c 2 -f <ALIGNMENT> -o <OUTPUT>
```

- ▶ tabular output in .tsv format
- ▶ table and figures in .html
- ▶ all figures are also stored in the `ps/` directory

LRISCAN HANDS-ON

Exercise:

Go to <https://www.rna.uni-jena.de/supplements/lriscan/>

1. Download the MSA of the Flaviviruses
2. Apply LRIsCan
3. Do not look at the results on the webpage (yet)

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If you have your own dataset, roughly of the same size as the Flavivirus MSA, feel free to use it.

COFFEE BREAK

