Differential analysis and *de novo* annotation of RNA-RNA interactions with RNAswarm

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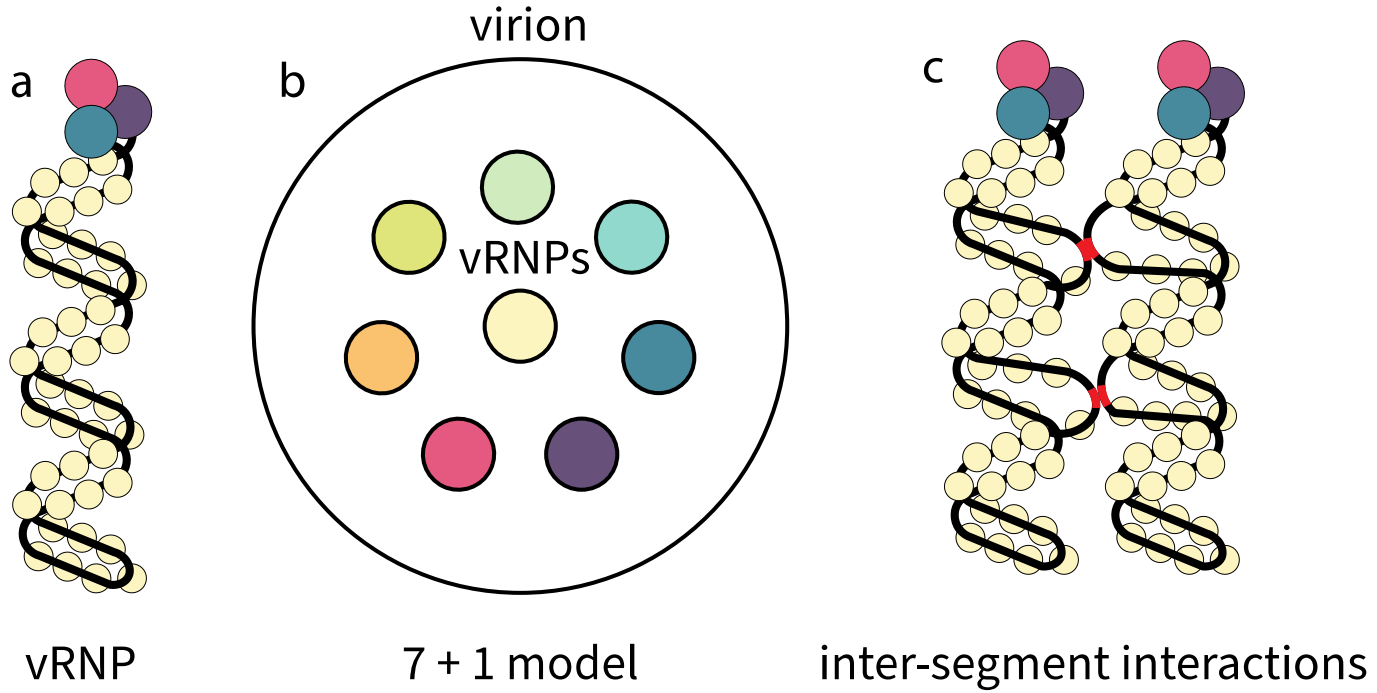
Understanding virus-host interactions

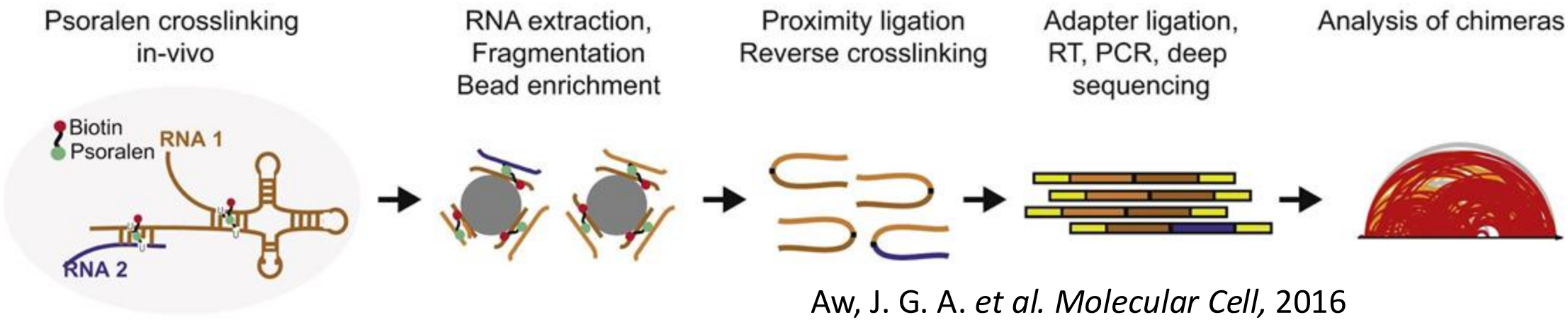
FRIEDRICH-SCHILLER-UNIVERSITÄT JENA

Our guiding thread: The Influenza A Virus (IAV) genome packaging problem



a. IAV's genome is organized in viral ribonucleoproteins (vRNPs)
b. vRNPs are thought to be organized in a 7 +1 model
c. Exposed RNA are thought to play a crucial role in IAV genome packaging via RNA-RNA interactions

RNA-crosslinking methodologies can probe RNA-RNA interactions (RRIs)



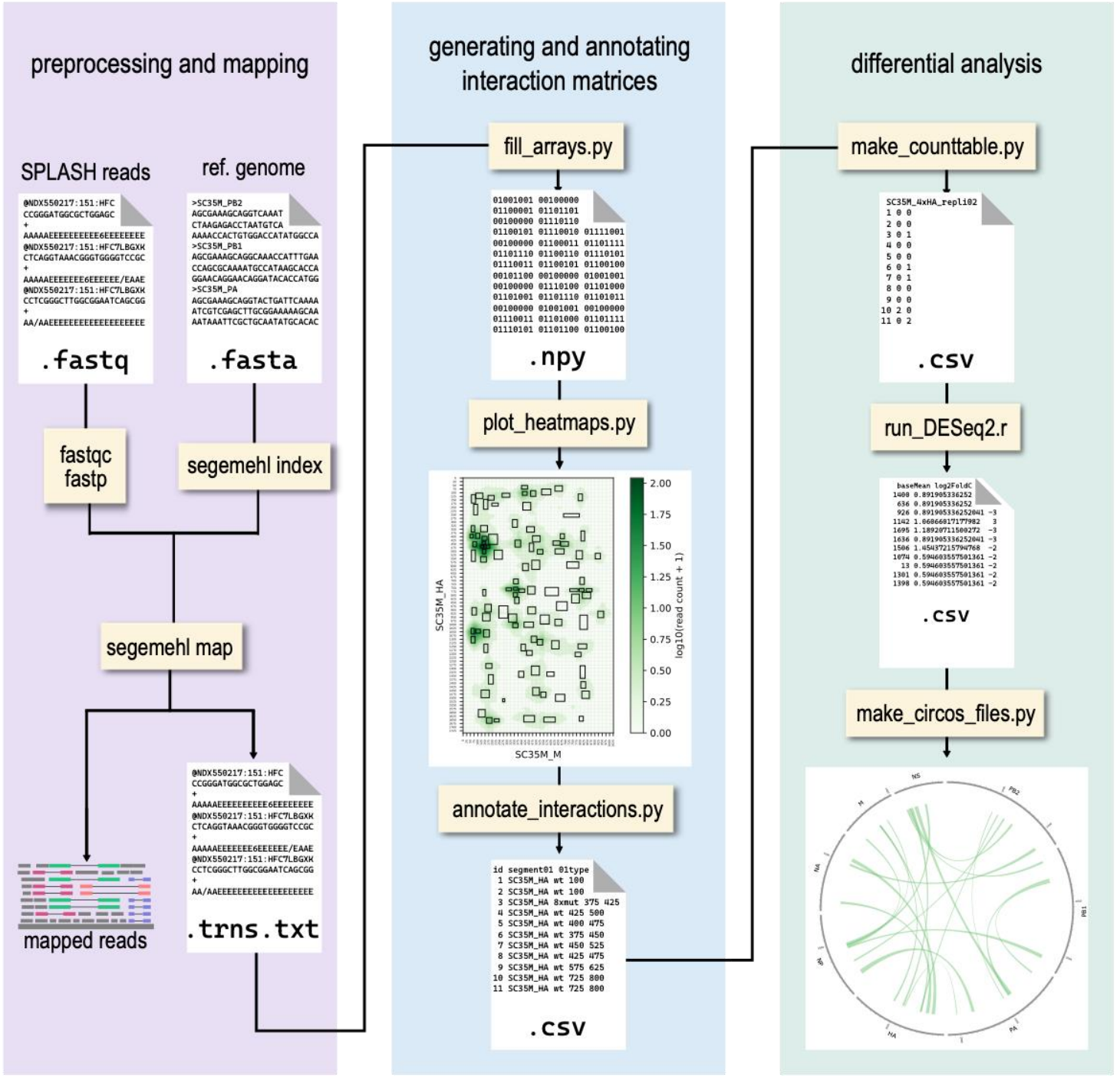
Aw, J. G. A. *et al. Molecular Cell,* 2016

Implementing a reproducible analysis pipeline

- Automate analysis of chimeras from read trimming to interaction mapping.

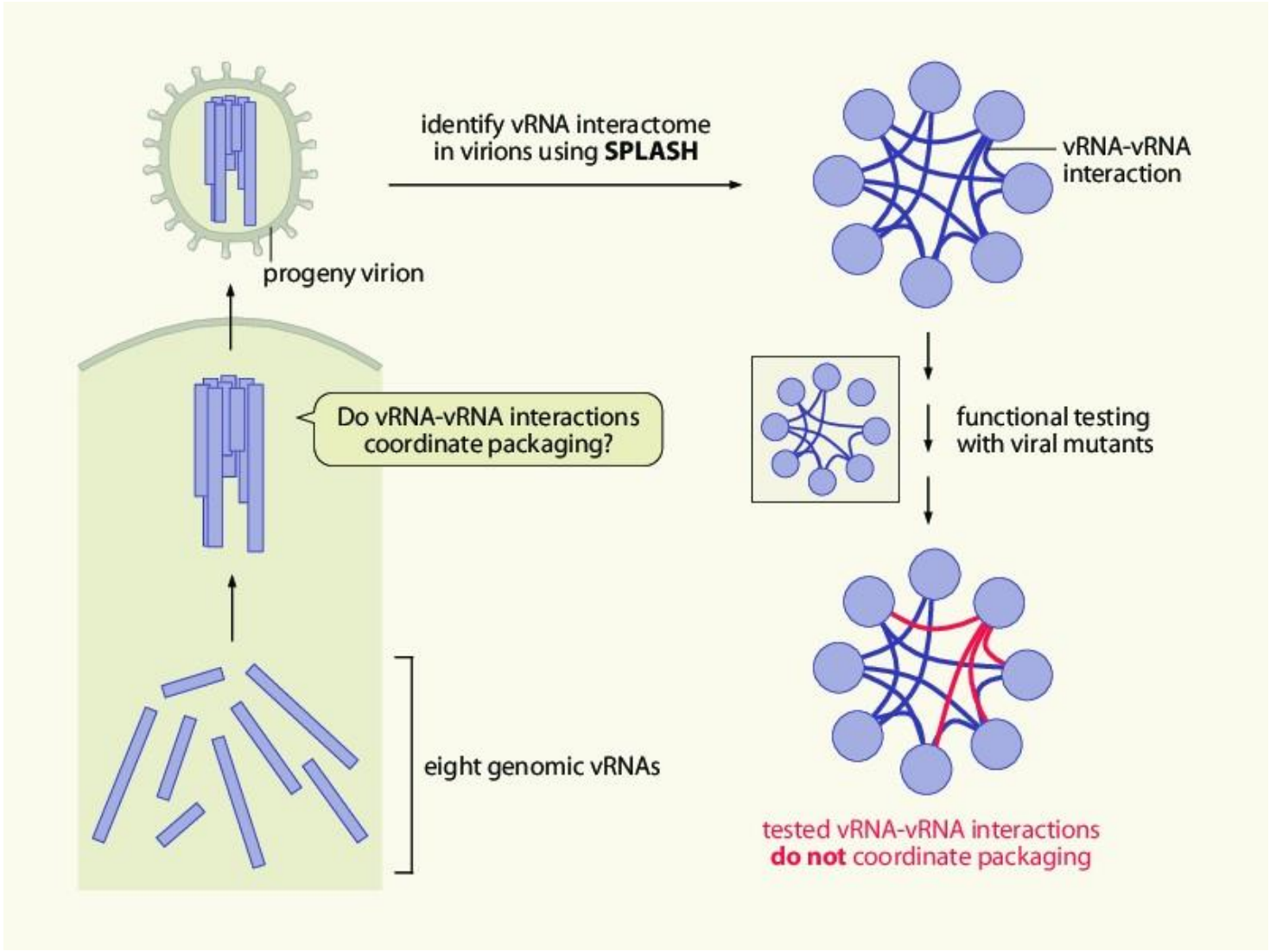
Differential analysis

- Evaluating significance of interactions.
- Comparing mutants and wild-type sequences.



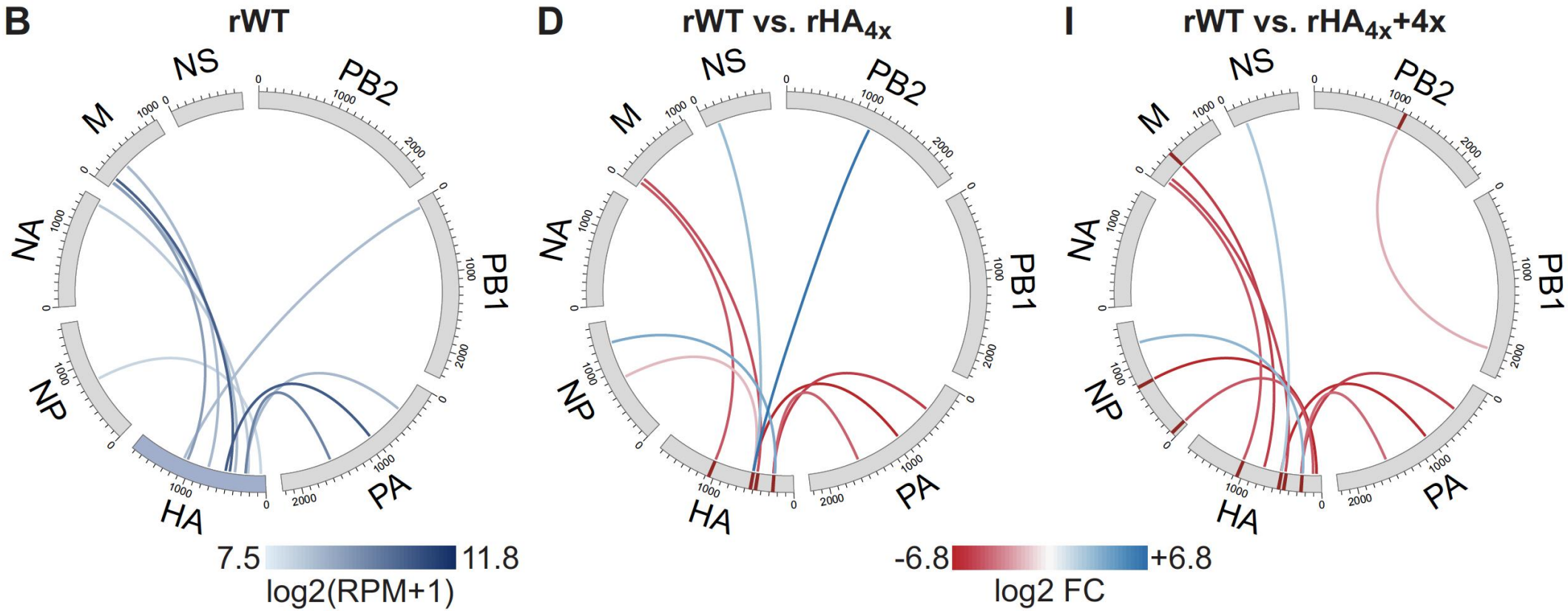
RNAswarm performs differential analyzes of SPLASH datasets

Are high-high frequency cross-linked reads packaging signals?



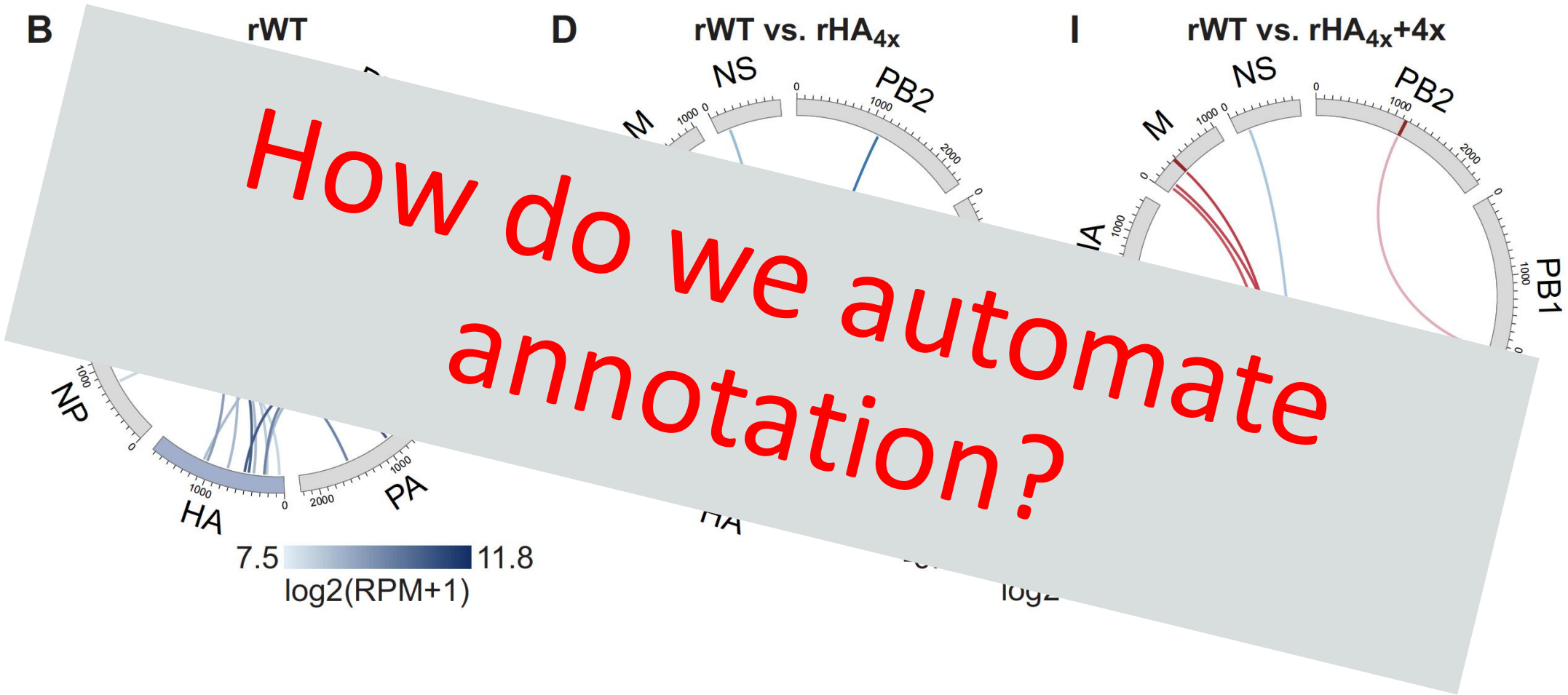
Jakob et al. *Nucleic Acids Research*, 2023

Mutations do not impair packaging of SC35M



Jakob et al. *Nucleic Acids Research*, 2023

Mutations do not impair packaging of SC35M



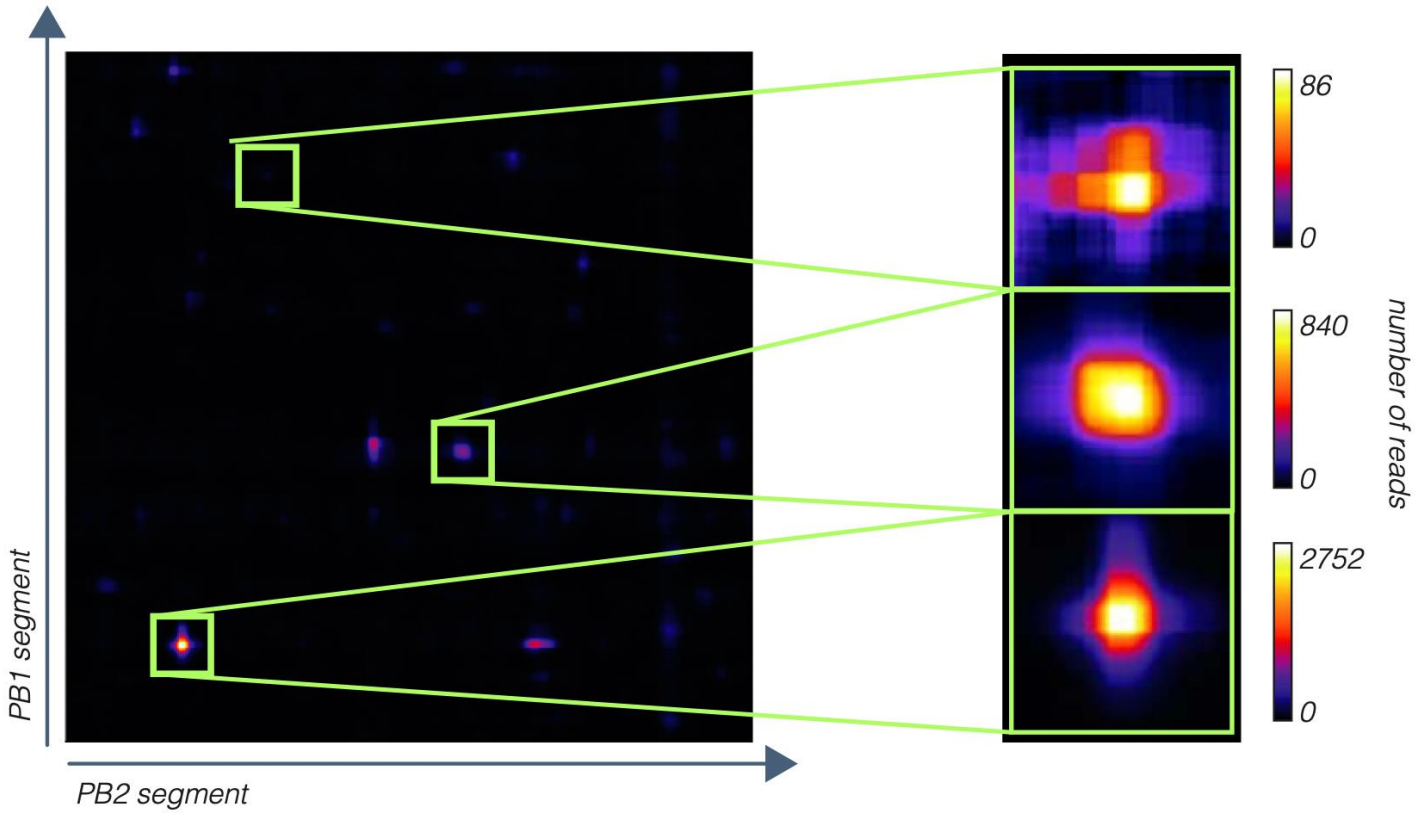
How do we automate annotation?

Jakob et al. *Nucleic Acids Research*, 2023

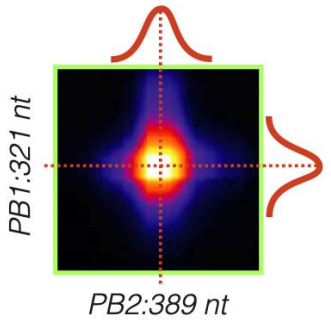
Interaction matrices can be annotated by fitting a 2d gaussian function to hand-picked quadrants

SPLASH identification of interaction loci

1. Visualise interaction matrices between each pair of segments as a heatmap and select loci of interaction



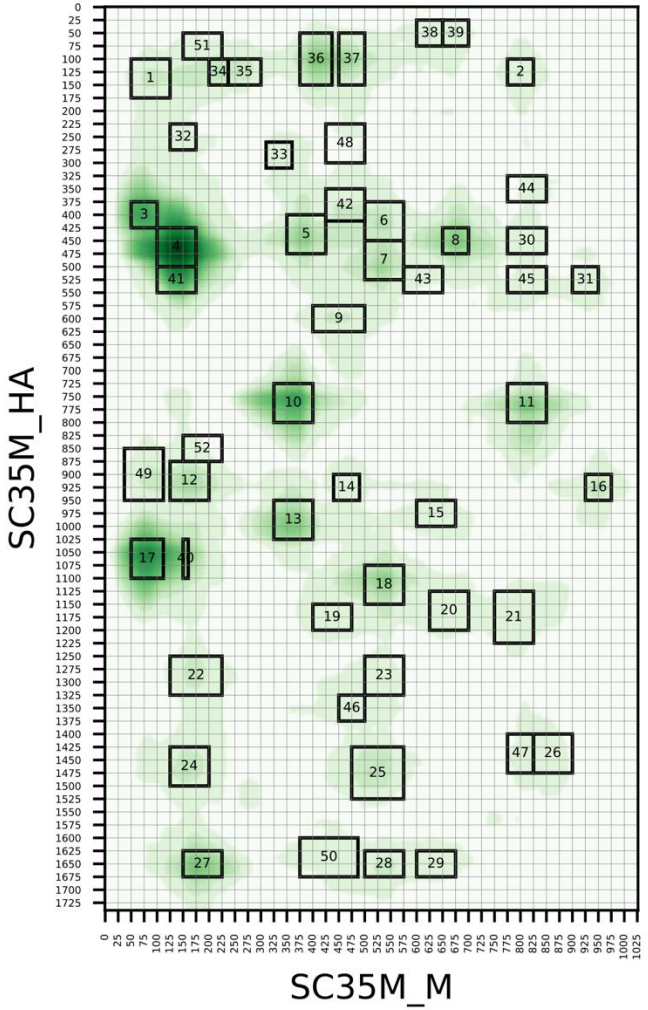
2. Fit a 2D Gaussian function to each locus to extract coordinates of interaction



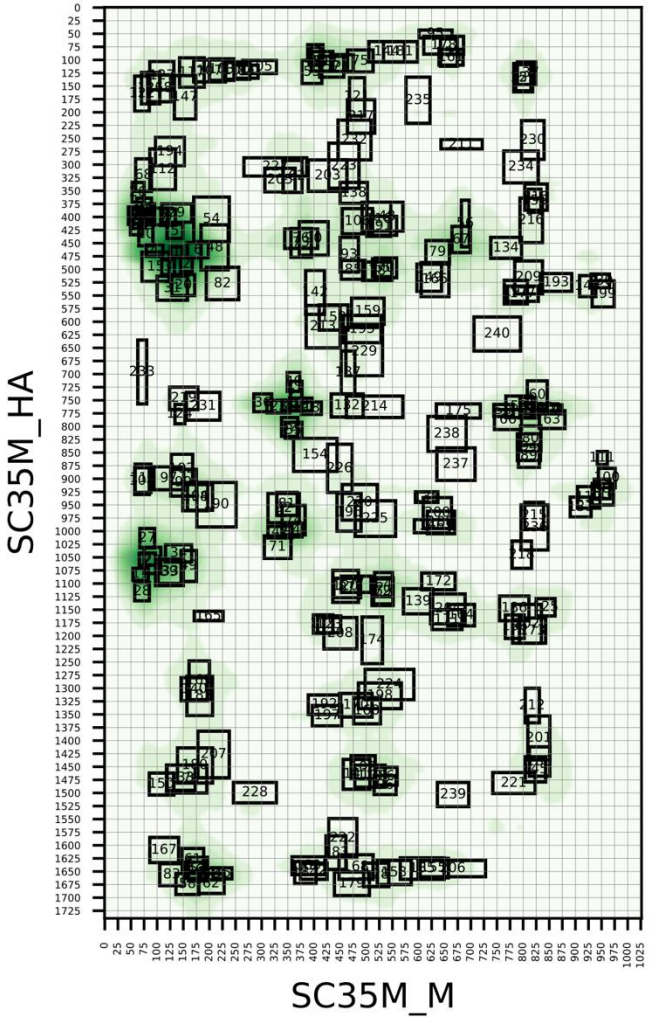
Dadonaite et al. *Nat. Microbiol* (2019)

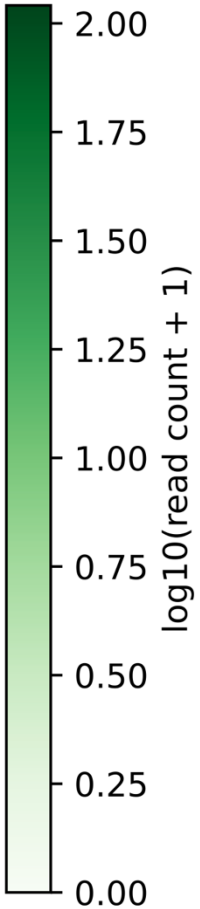
We can use heuristics to deduplicate the annotations

manual curation



automated



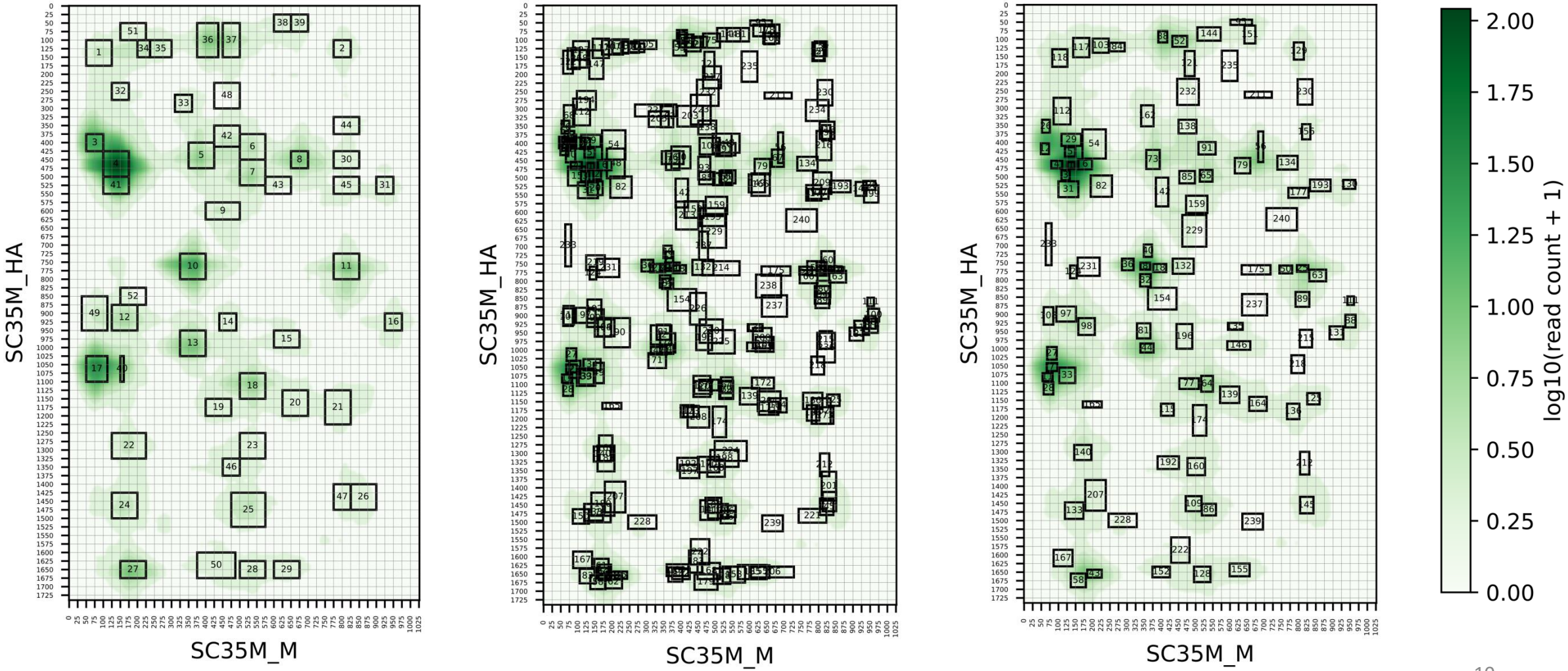


We can use heuristics to deduplicate the annotations

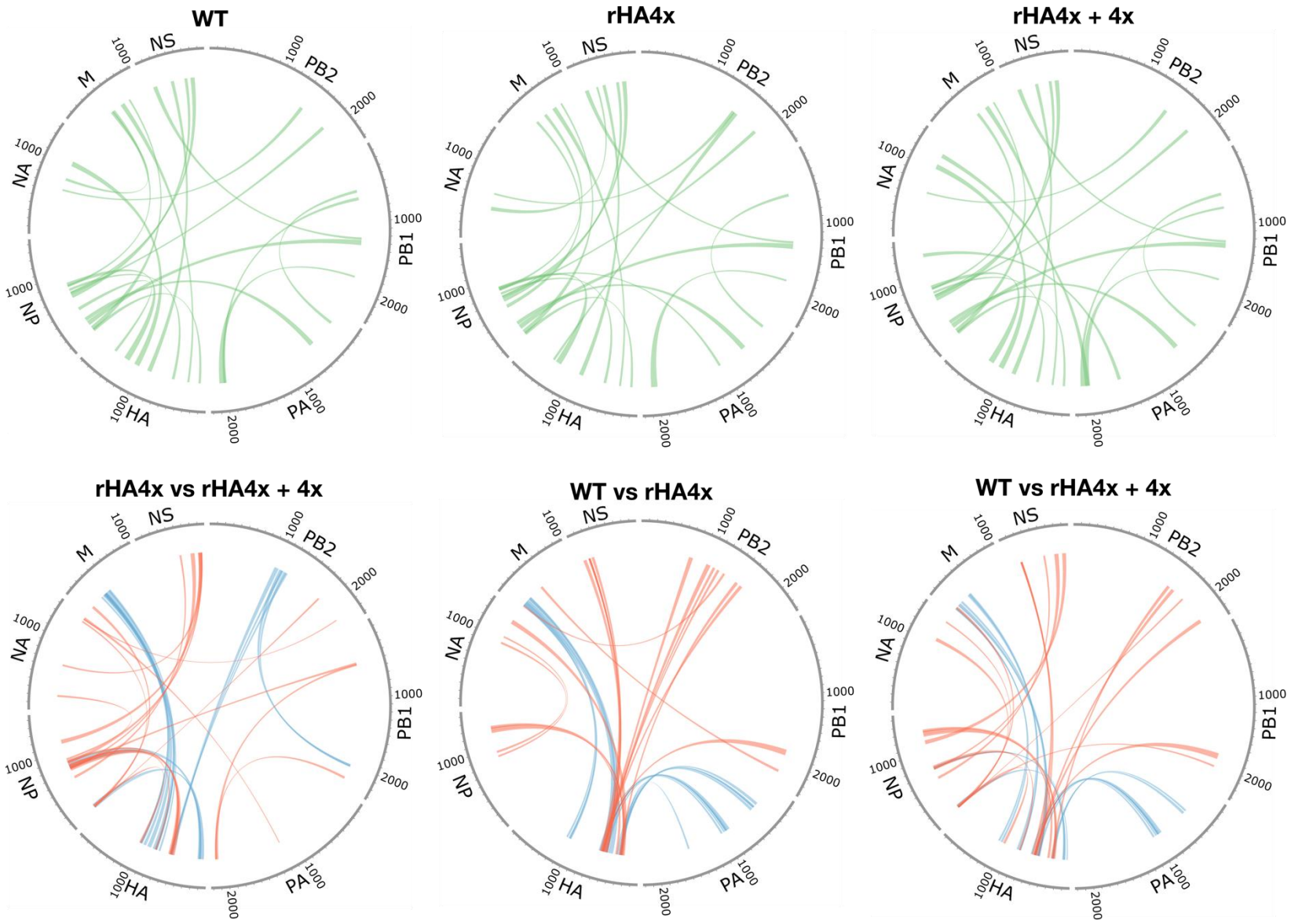
manual curation

automated

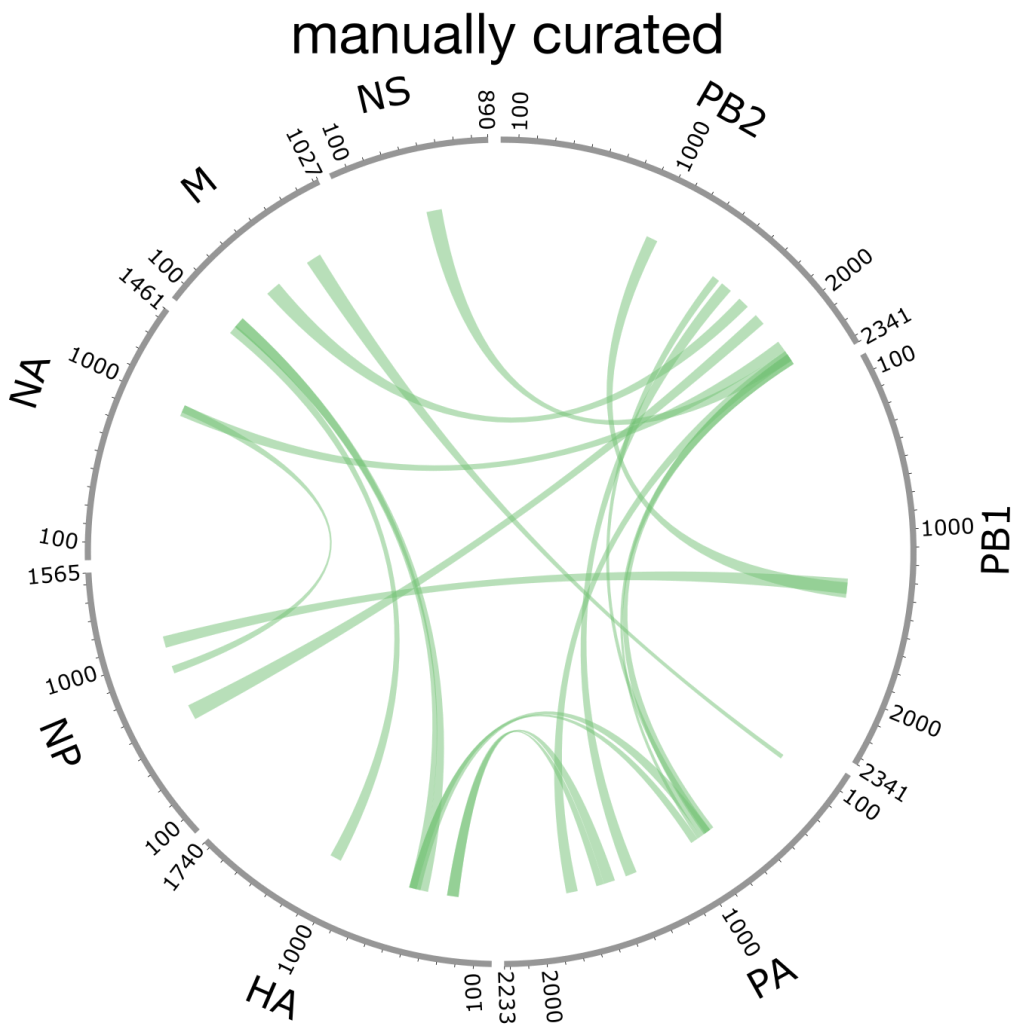
automated w/ deduplication

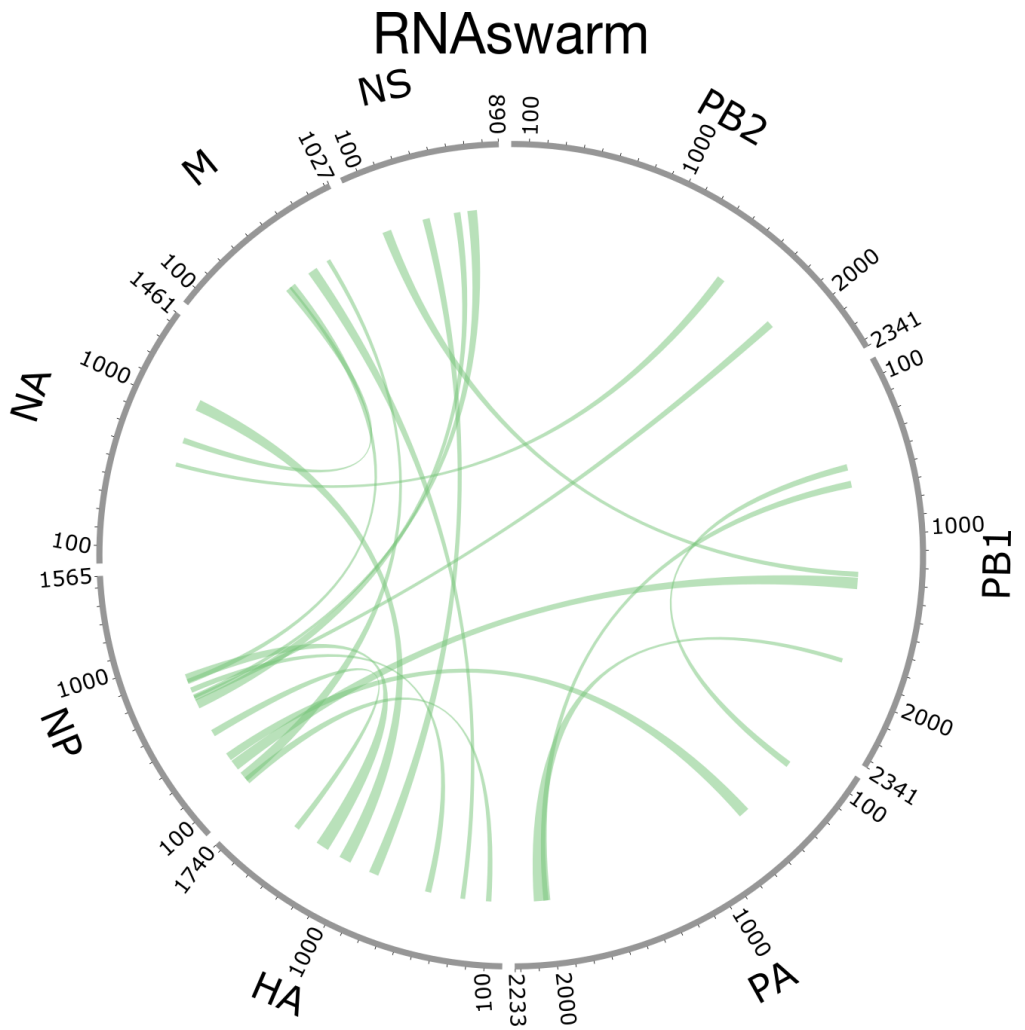


RNAswarm *de novo annotations*

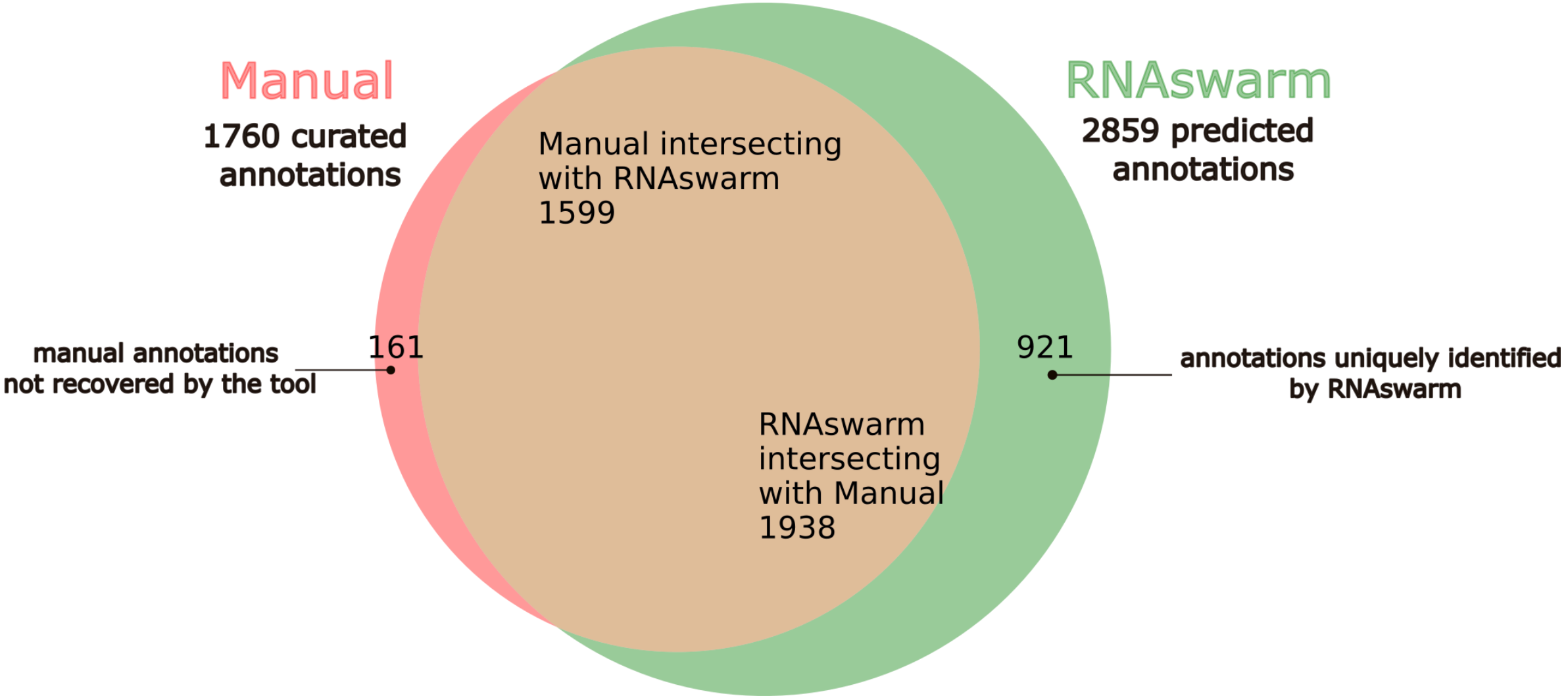


Manual vs RNAswarm





Manual vs RNAswarm



So what do I need to use RNAswarm?

- Input: fasta and fastq files
- Output: Interaction matrices, heatmaps, differential interaction table, and circos plots

And what can I do with RNAswarm?

- RNAswarm can deal with data resulting from a broad range of proximity ligation methods (not only SPLASH)
- We can identify discrete interactions in an unsupervised fashion using GMMs
- We can compare the prevalence of discrete interactions across conditions using DESeq2

With a Little Help from My Friends

Manja Marz's Group
Christian Höner zu Siederdissen
Kevin Lamkiewicz

Martin Schwemmle's group
Hardin Bolte
Celia Jakob

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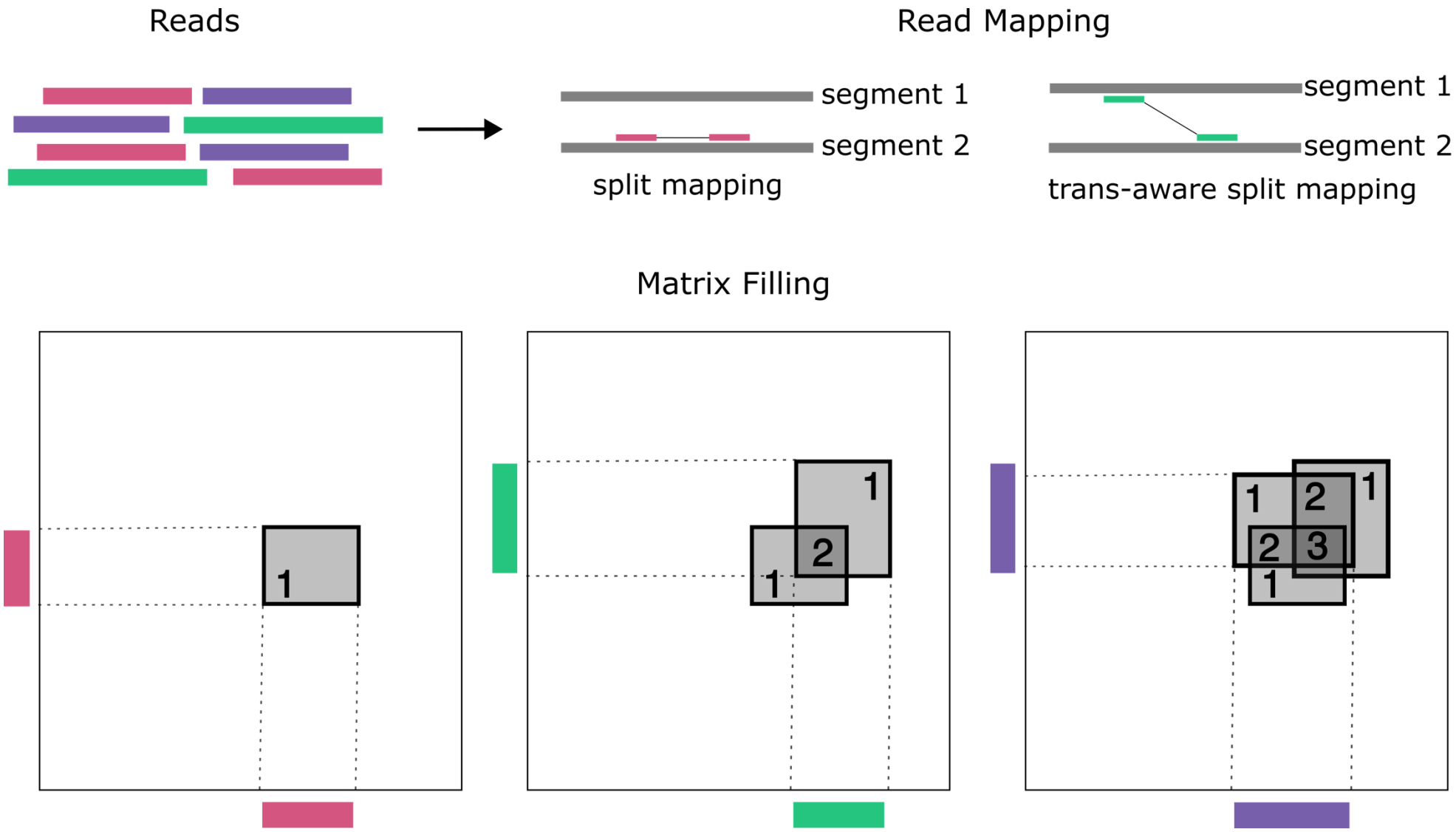
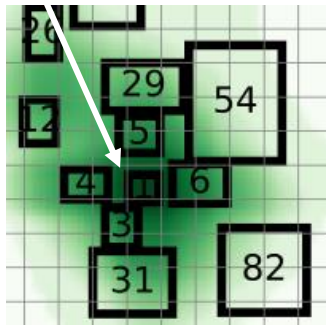
The lost slides

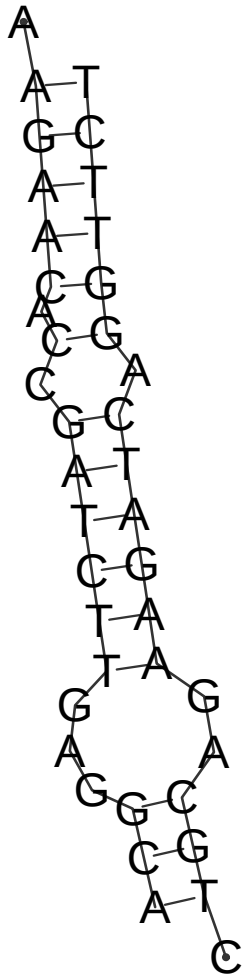
Matrix filling



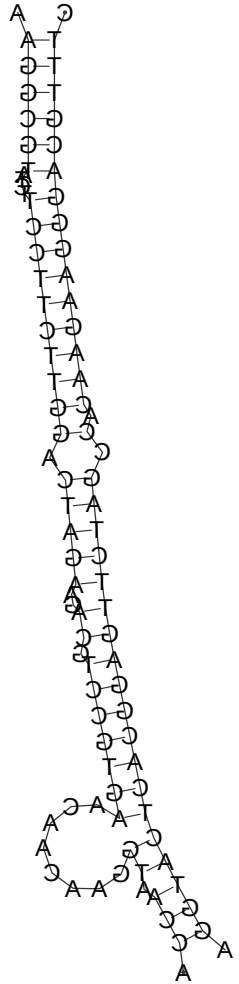
Structure prediction using RNAduplex (or RNAcofold)



Identified region



25-nt extended



Refining annotations with structure predictions

```
Raw                    .(((((.(.((((((...(((          &                     .))).)).))))).((((((
Extend  5 nt       .((((((((((.(.((((((((((((((.      &               .)))))))..))..))))).))))))))))
Extend 10 nt       .((((((((((.(.((((((((((((((.      &               .)))))))..))..))))).))))))))))).
Extend 15 nt  .((((((((((((((.(.((((((((((((((.      &               .)))))))..))..))))).))))))))))....))))))).
Extend 20 nt  .((((((((((((((.(.((((((((((((((((((. & .))).))........)))))))..))..))))).))))))))))....))))))).
Sequence      CTTTGCAGGGAAGAACACCGATCTTGAGGCACTCATGGA & ACCAATGGAACAACAAGTGCCTGCAGAAGATCAGGTTCTTCCTTCTATGCGGAA
```

Gaussian Mixture Models (GMMs) can be used to identify discrete interactions

Independent from user intervention

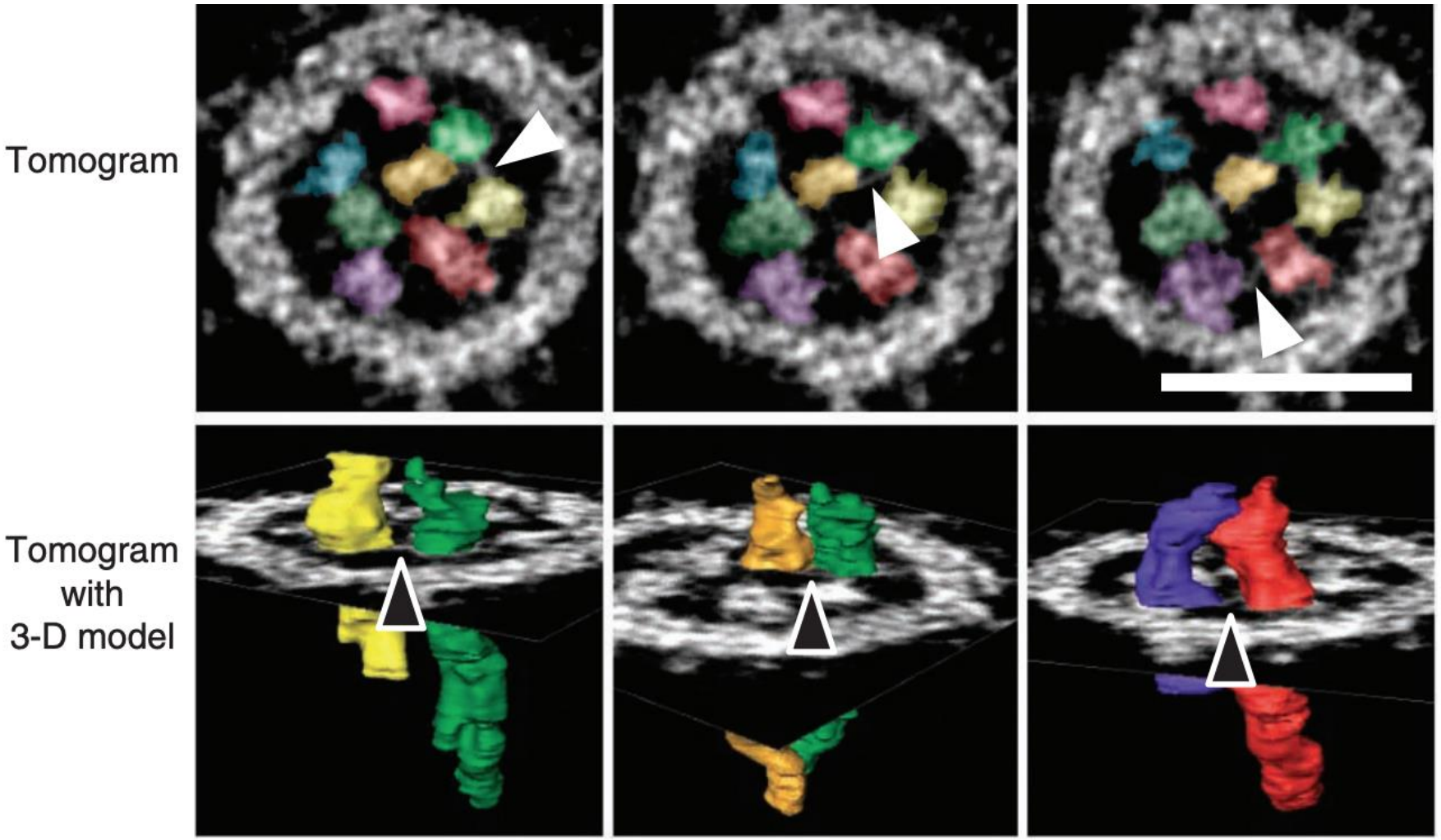
Can be fit to the data using an expectation-maximization algorithm:

1. Initialization: Assume random components.
2. Expectation Step (E-step): Compute for each point a probability of being generated by each component of the model.
3. Maximization Step (M-step): Update the parameters to maximize the likelihood of the data given those assignments.
4. Repeat 2 and 3 until convergence.

Pseudocode: deduplicate the annotations

```
Initialize bestRegions as an empty map

For each targetRegion in regions:
    Set highestRatio to 0
    Set bestRegion to None

    For each otherRegion in regions:
        If targetRegion intersects with otherRegion:
            Calculate ratio as readCount of otherRegion divided by area of otherRegion

                If ratio is greater than highestRatio:
                    Update highestRatio to this new ratio
                    Update bestRegion to otherRegion

     Assign bestRegion as the optimal choice for targetRegion in bestRegions map

 Return bestRegions
```

The IAV genome packaging problem

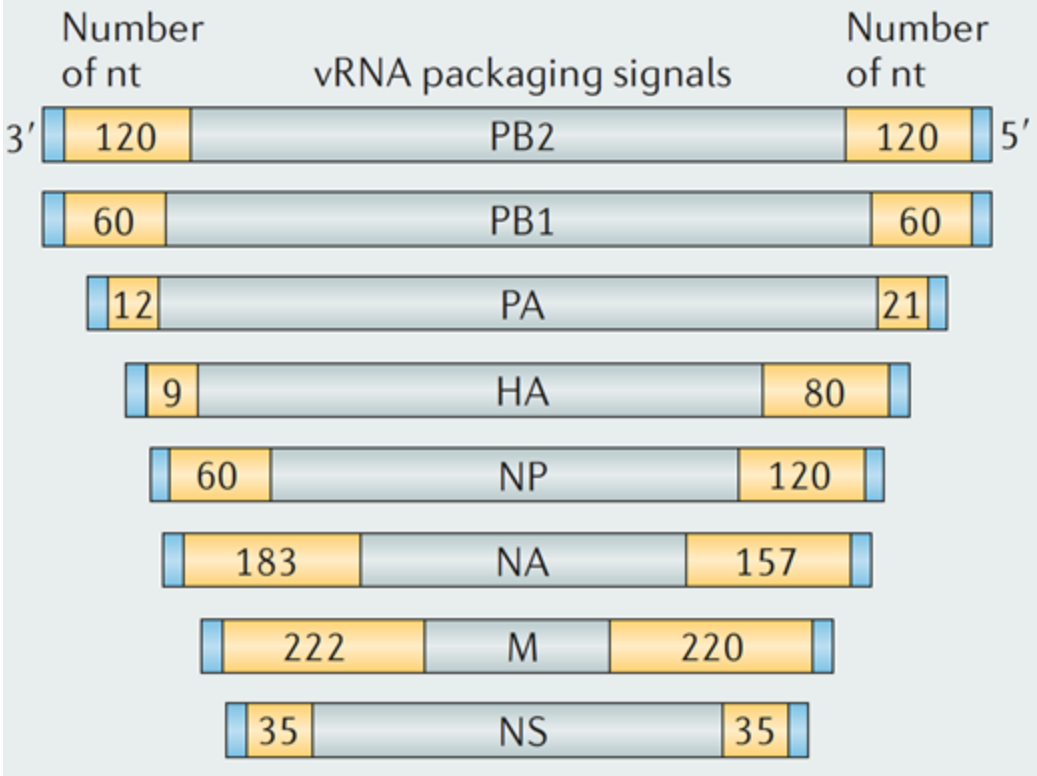


- Genome topology of IAV supports the 7 + 1 model
- vRNPs connected by a string-like structure

Noda, T. et al. Nat Commun 2012, 3 (1), 639.

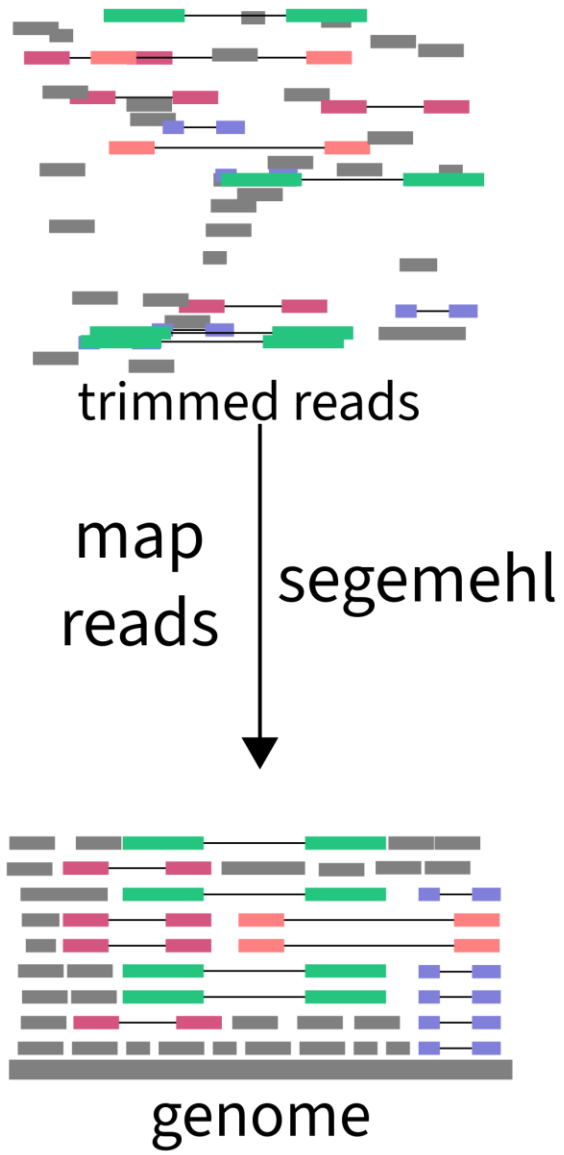
Known vRNA packaging signals

- Concentrated on 3’ and 5’
- Internal signals are not the same in all strains



Eisfeld, Amie J. et al. Nature Reviews Microbiology 13, no. 1 (January 2015): 28–41.

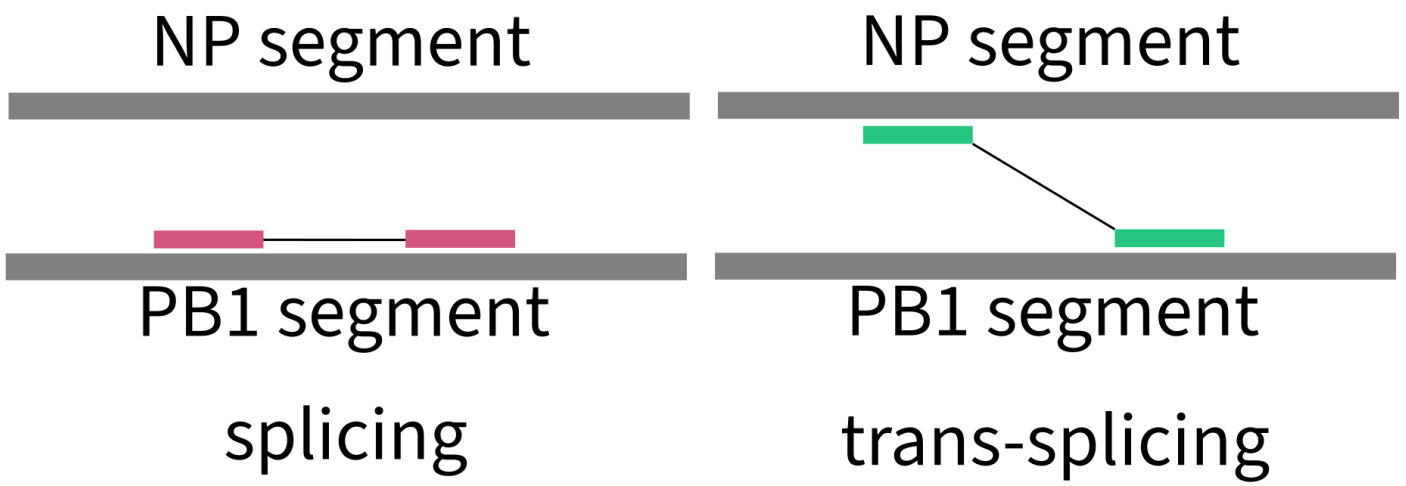
Split-read mapping



- Reads are aligned to the viral genome
- Chimeric reads are splitted during mapping
- segemehl as default mapper

Finding chimeric reads

- We rely on tools built for mapping splicing-product RNAs
- Segemehl classify spliced reads in 3 categories:
  - BED files for single and multi splicing
  - Custom file for trans-spliced reads



Interaction matrices are processed to identify differentially structured regions in the genome.