The quest for conserved RNAs in viral genomes

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The World's Deadliest Animals

Number of people killed by animals, 2015

gates notes

830,000
Mosquito

580,000
Human

- 60,000 Snake
- 24,200 Sandfly
- 17,400 Dog
- 8,000 Kissing bug
- 4,400 Freshwater snail
- 3,500 Scorpion
- 3,500 Tsetse fly
- 1,600 Tapeworm
- 500 Hippopotamus
- 100 Elephant
- 60 Bee
- 40 Jellyfish
- 6 Shark

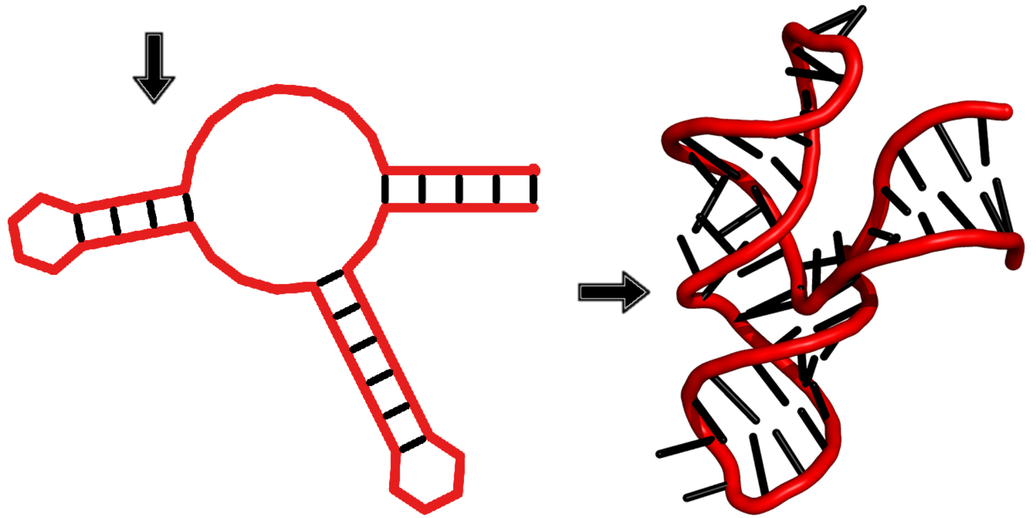
Sources: IHME, WHO, CrocBITE, FAO, Norwegian Institute for Nature Research, International Shark Attack File, National Geographic, PBS, National Science Foundation, CDC, WWF, *Wilderness & Environmental Medicine*, *Nature*, French Institute of Research for Development. All calculations have wide error margins.

Part I:

RNA structure prediction

The RNA Folding Problem

GCGCUCUGAUGAGGCCGCAAGGCCGAAACUGCCGCAAGGCAGUCAGCGC

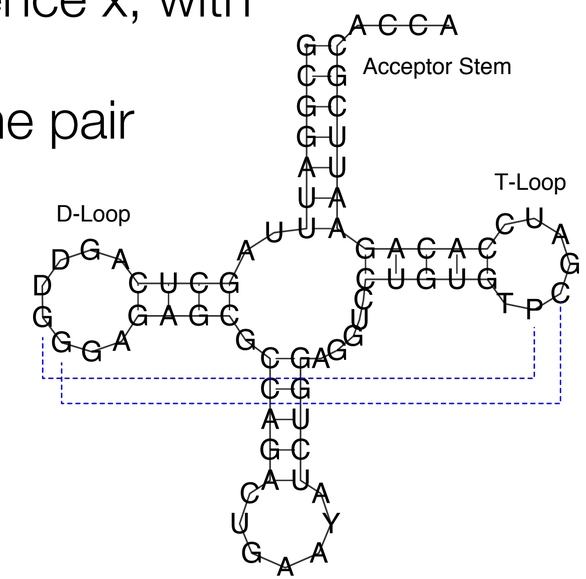


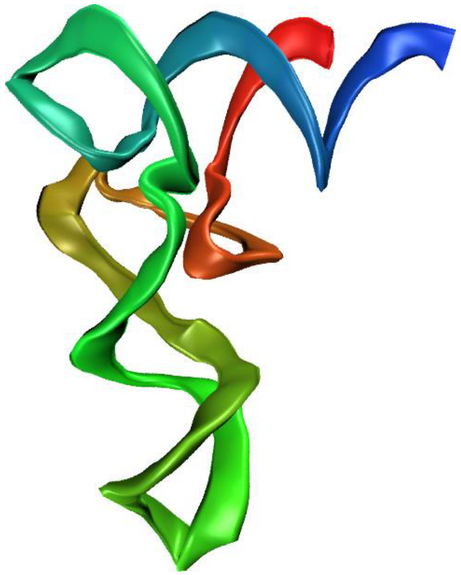
- Hierarchical folding: Secondary structure forms first then helices arrange to form tertiary structure
- Secondary structures cover most most of the folding energy
- Convenient and biologically useful description
- Computationally easy to handle
- Tertiary structure prediction needs knowledge of secondary structure

Secondary Structures

A secondary structure is a list of base pairs (i,j) on a sequence x, with

- Any nucleotide (sequence position) can form at most one pair
- No pseudo-knots: No pairs (i,j) and (k,l) with $i < k < j < l$
- If (i,j) is a pair then $x_i x_j \in \{GC,CG,AU,UA,GU,UG\}$
- If (i,j) is a base pair, then $j - i > 3$





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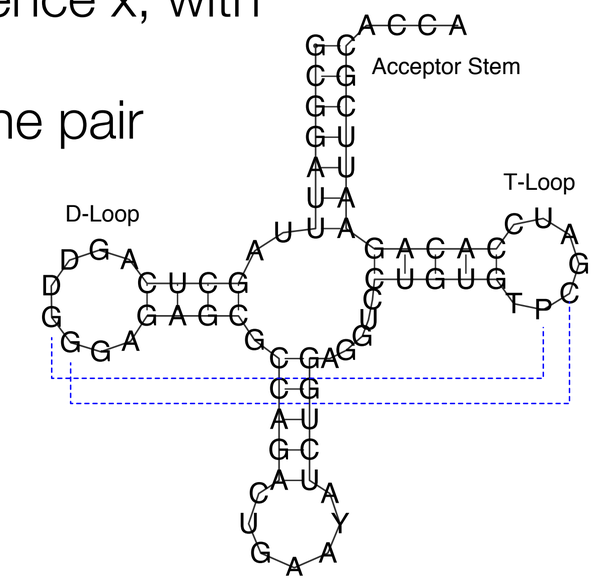

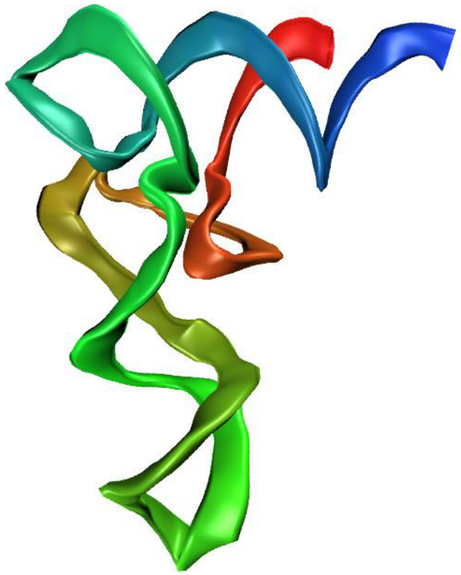

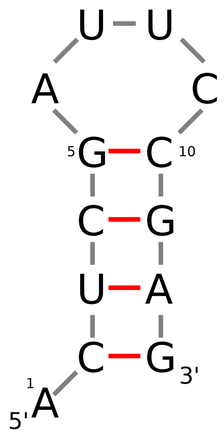

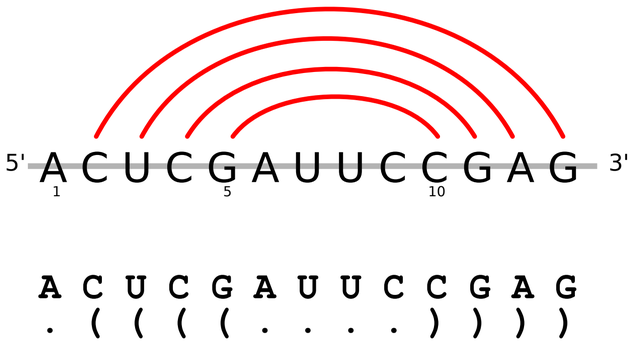

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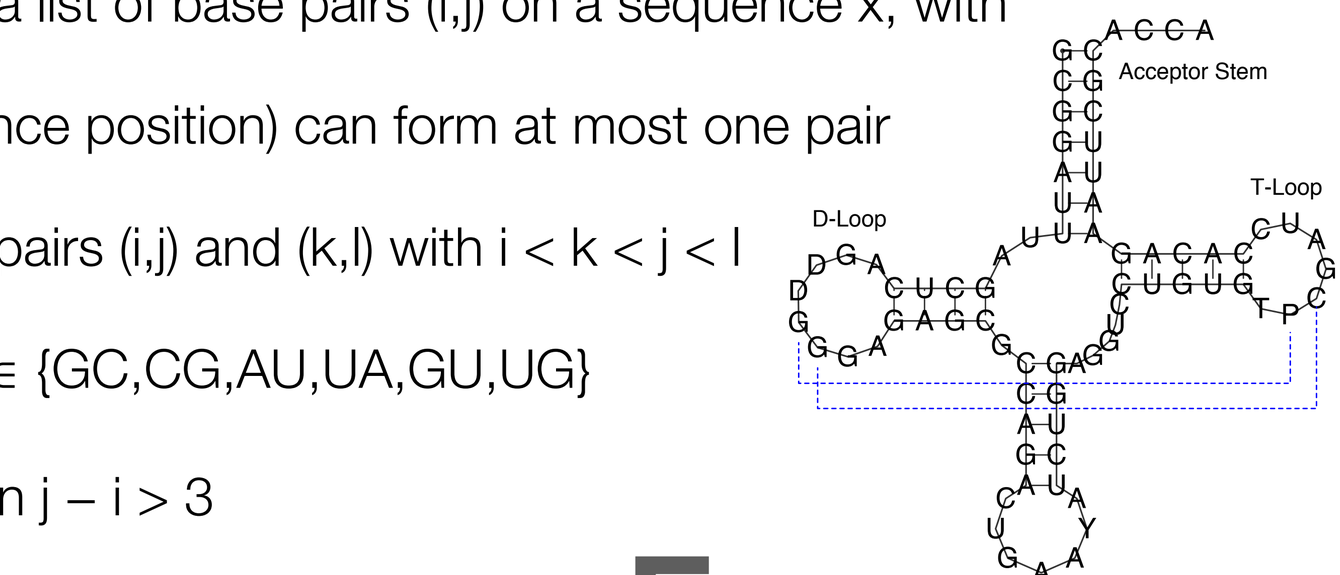
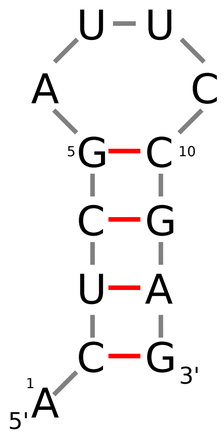
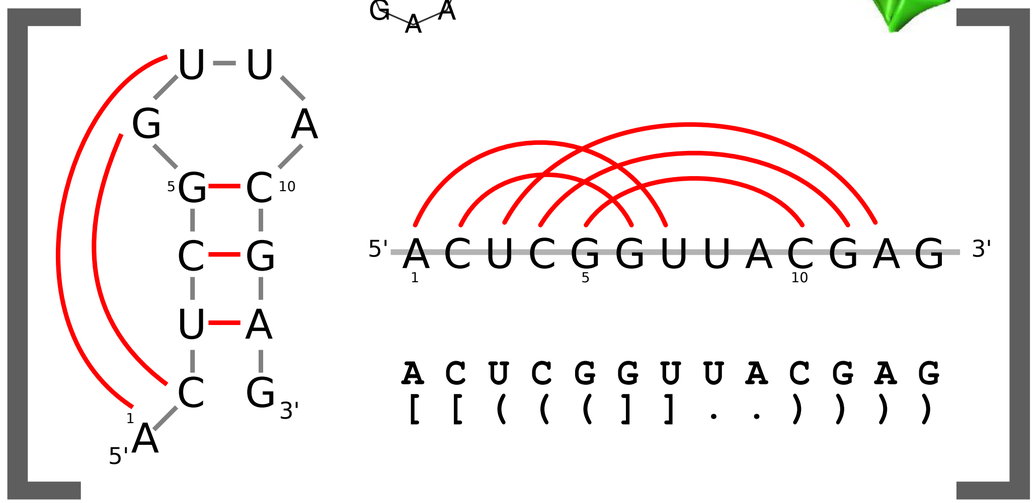
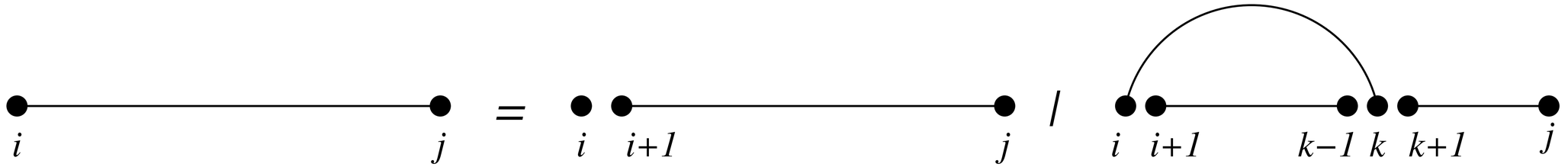






Conformation Space

The number of secondary structures for a sequence $x = x_1 \ldots x_n$ can be computed recursively



$$S_{ij} = S_{i+1,j} + \sum_{k=i+m}^{j} S_{i+1,k-1} S_{k+1,j} \Pi_{kj}$$

$\Pi_{ik} = 1$ if $x_i x_k \in \{GC, CG, AU, UA, GU, UG\}$, otherwise $\Pi_{ik} = 0$

For sequences with equal {A,U,G,C} content, the number of conformations grows asymptotically with sequence length

$$\bar{S}(n) \sim n^{-\frac{3}{2}} 1.85^n$$

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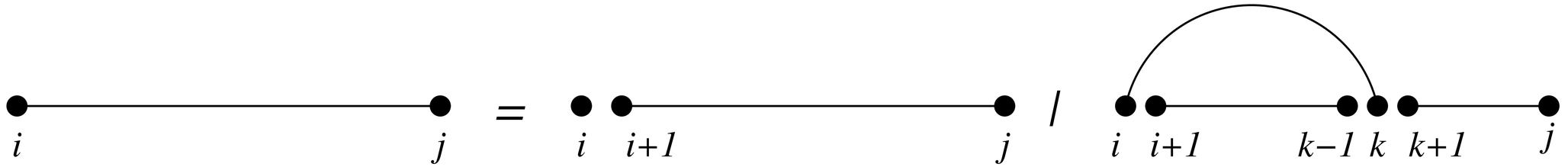

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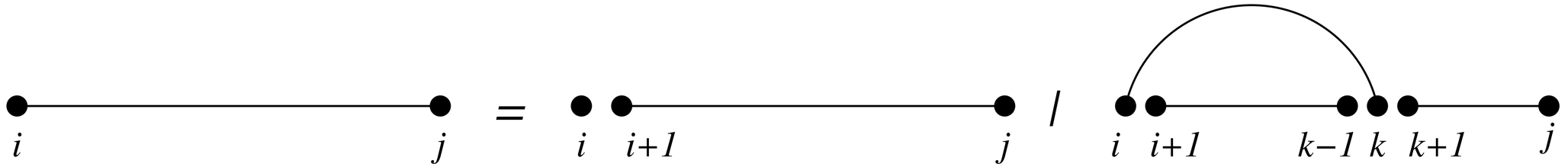

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Nature ‘exploits’ this property

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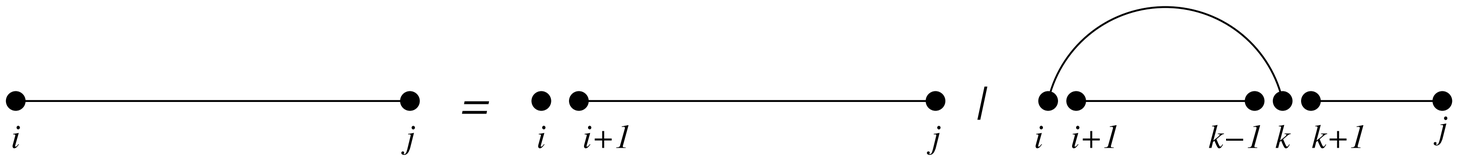
Solving the RNA Folding Problem

Toy model for RNA folding: assign energies to base pairs $\varepsilon(x, y)$

Easily solved by Dynamic Programming: recursive computation with tabulation of intermediate results



$$E_{ij} = \min_{i<k\leq j} \left\{ E_{i+1,j} \; ; \quad \left(E_{i+1,k-1} + E_{k+1,j} \; + \varepsilon(x_i, x_k) \right) \right\}$$

- E_{1n} is the best possible energy for our sequence
- Backtracing through the E table yields the corresponding structure
- The algorithms requires $\mathcal{O}(n^2)$ memory and $\mathcal{O}(n^3)$ CPU time

In practice this toy model is not good enough !
We need loop-dependent energies for serious predictions

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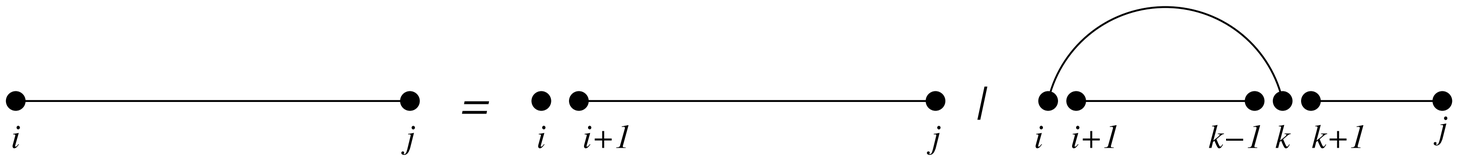

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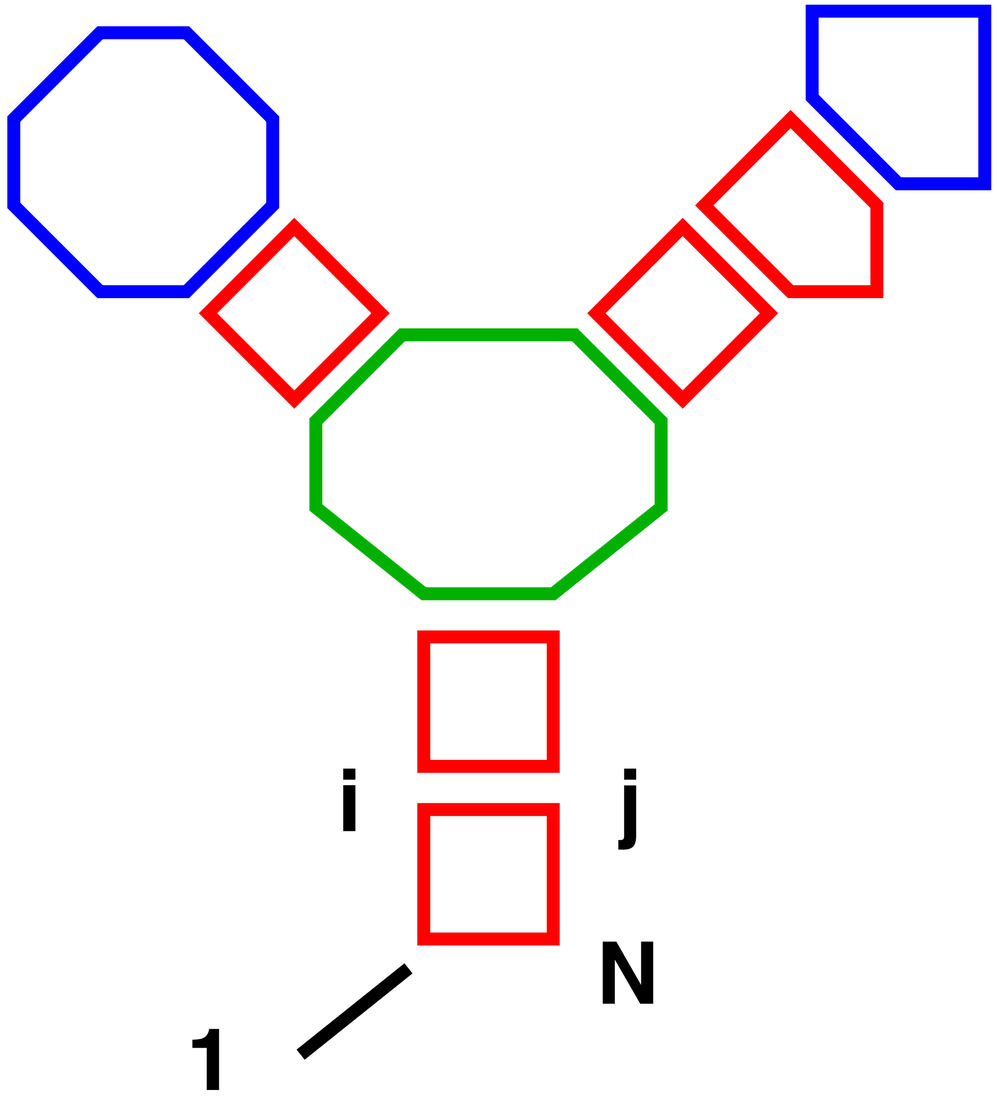
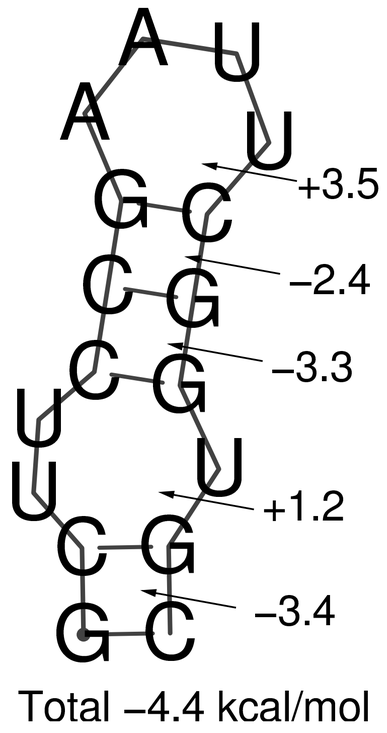
Loop Decomposition



Nearest Neighbour Model

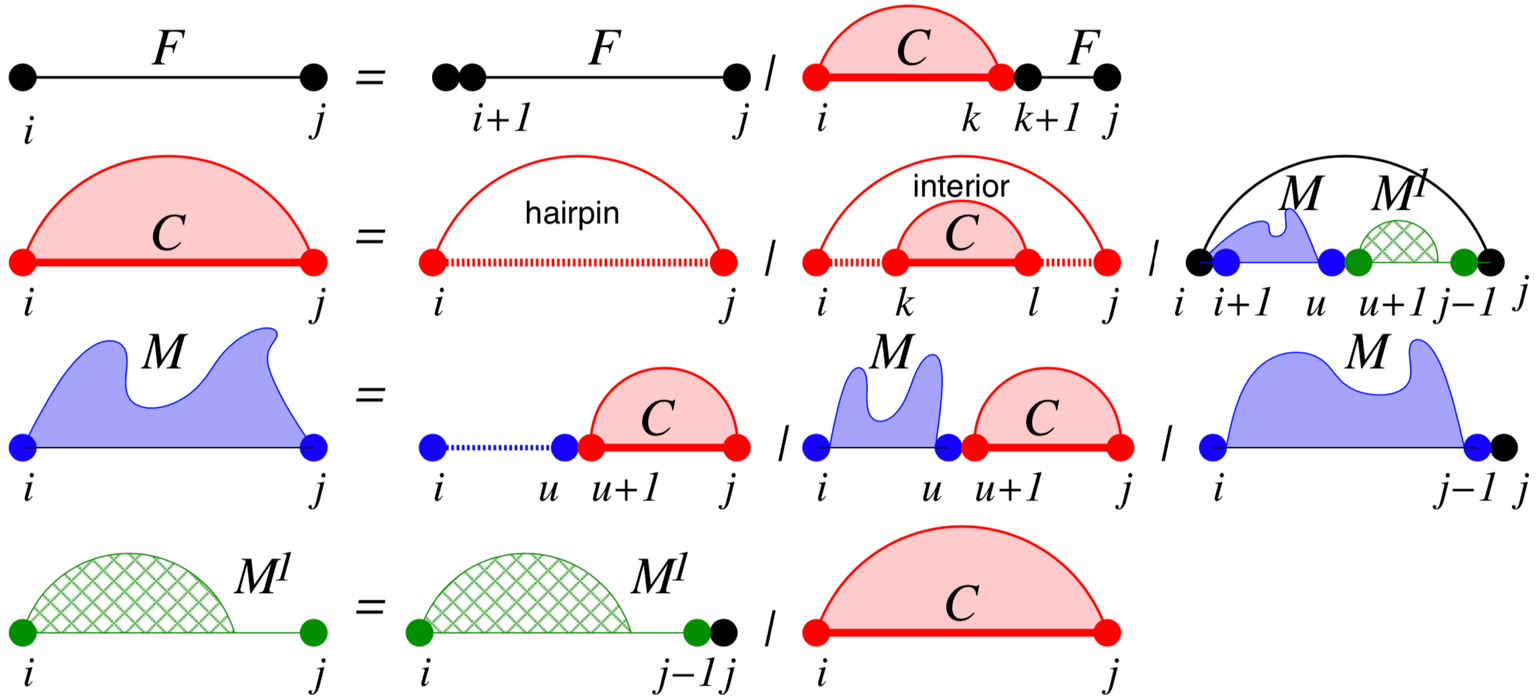
The standard energy model expresses the free energy of a structure as the sum over its loop energies

$$E(S) = \sum_{l \in S} E(l)$$



- Good approximation for most oligonucleotides
- Loop energies depend on loop type/size and some sequence dependence
- Most relevant parameters are experimentally measured; some still guesswork
- Secondary structures are macro-states, hence energies are temperature-dependent free energies
- Training parameters is becoming a viable alternative to experiment

Folding with Loop Based Energies



F_{ij} free energy of the optimal substructure on the subsequence $x[i..j]$.

C_{ij} optimal free energy on $x[i..j]$, where (i, j) pair.

M_{ij} $x[i..j]$ is part of a multiloop and contains at least one pair.

M^1_{ij} same as M_{ij} but contains exactly one component closed by (i, h).

Partition Function

Recall: $\bar{S}(n) \sim n^{-\frac{3}{2}} 1.85^n$

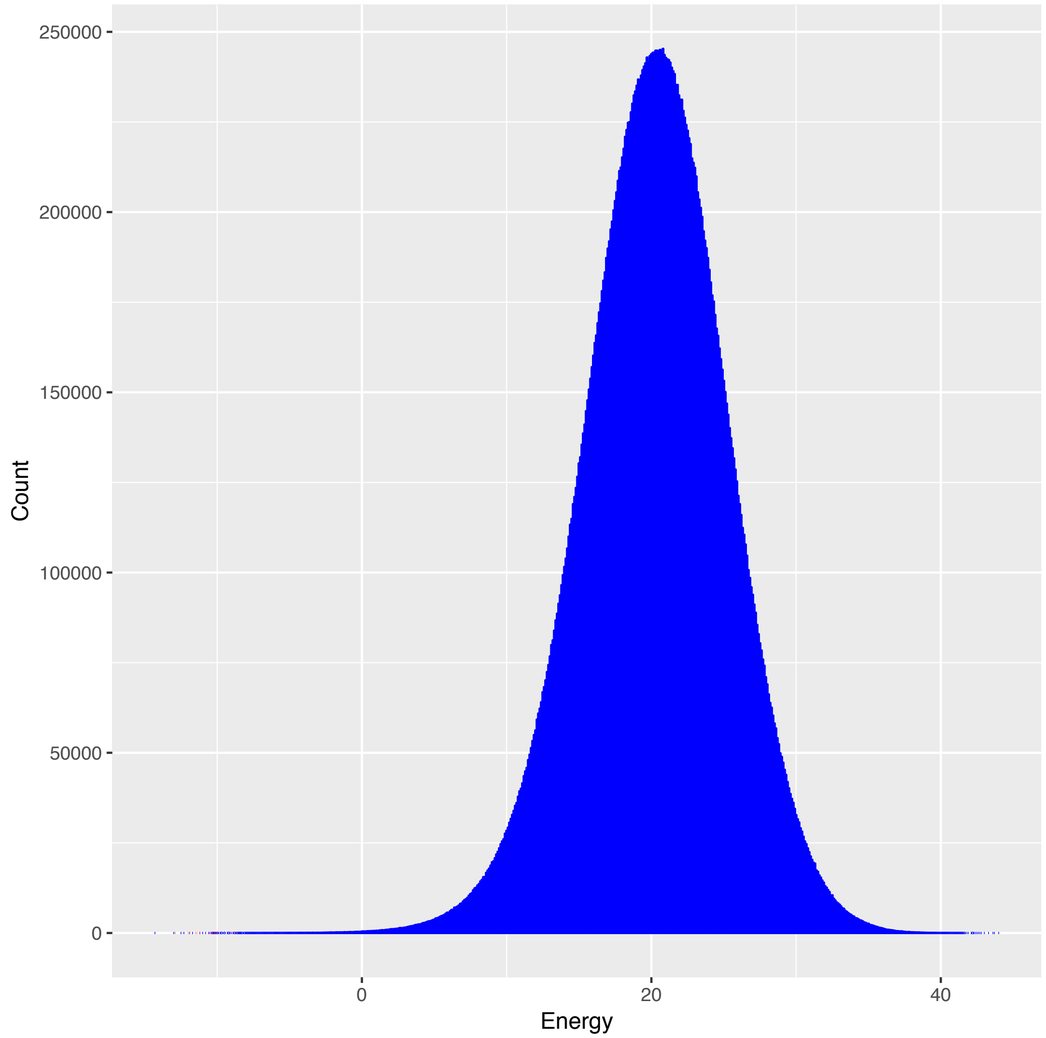
RNA is a biopolymer and ruled by thermodynamics

The partition function is the fundamental quantity of statistical mechanics and all thermodynamic properties can be derived from it

$$Z = \sum_{\Psi} exp\left(-\frac{E(\Psi)}{RT}\right)$$

E.g. the free energy of formation is given by

$$\Delta G = -RT \ln Z$$



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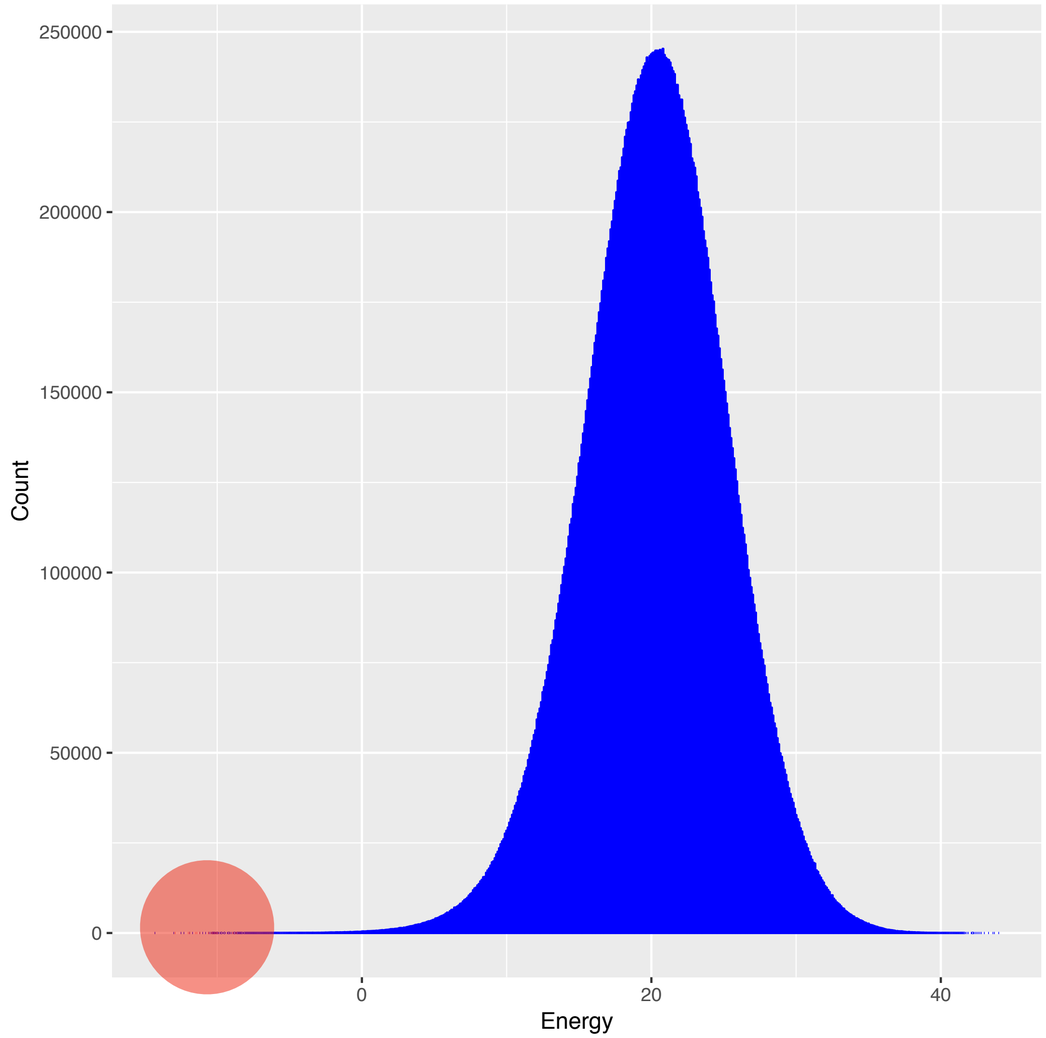


Computing the Partition Function

The recursion has the same structure as for energy minimisation, with two differences

- replace minimum operation by sums
- addition of energies by products of partition functions

$$E_{ij} = \min_{i<k\leq j} \left\{ E_{i+1,j}\,;\ \ \left(E_{i+1,k-1} + E_{k+1,j} + \varepsilon(x_i, x_k) \right) \right\}$$

$$Z_{ij} = Z_{i+1,j} + \sum_{k,\,(i,k)\ \text{pairs}} Z_{i+1,k-1} Z_{k+1,j} \exp(-\varepsilon(x_i, x_k)/RT)$$

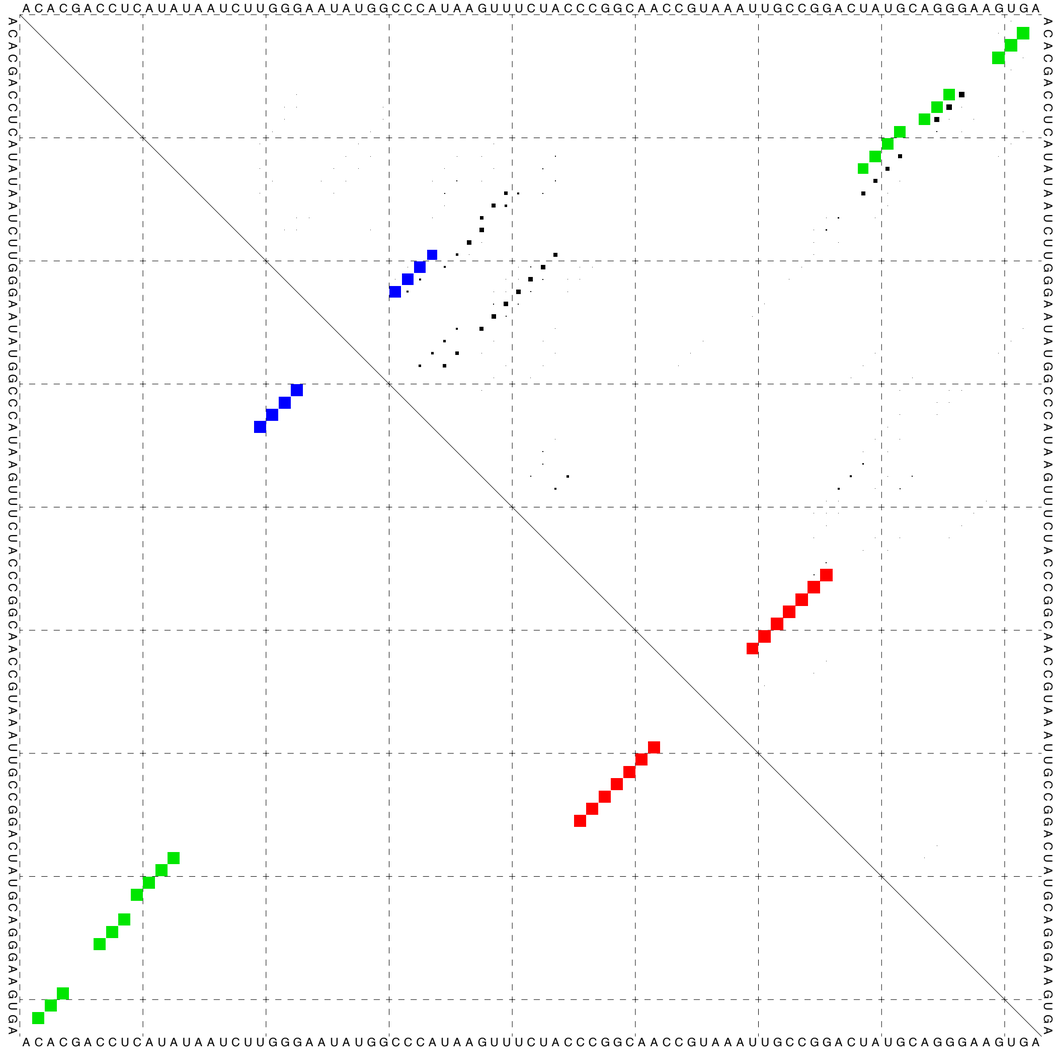
The probability of structure features can be computed from Z, e.g. the probability that a pair is formed

$$p_{ij} = \sum_{\Psi,(i,j)\in\Psi} p(S)$$

For efficient computation define the partition function $\widehat{Z}_{ij}$ for structures outside the subsequence x[i..j]

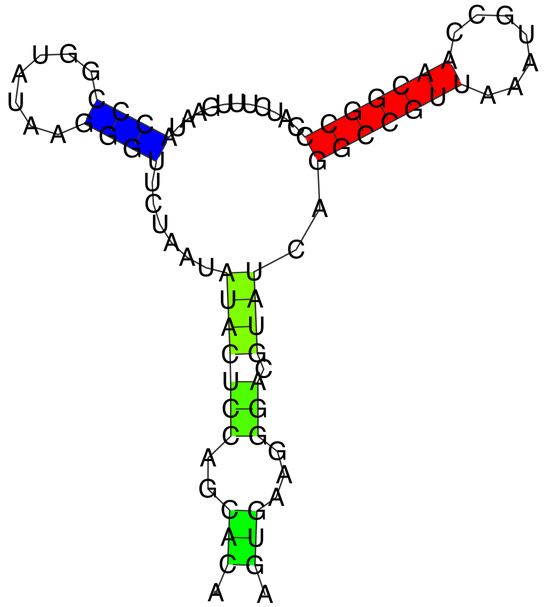
$$p_{ij} = \widehat{Z}_{ij} Z_{i+1,j-1} \exp(-\varepsilon_{ij}/RT)/Z$$

Representing Ensembles of RNA Structures



Ensembles of structures (thermodynamic equilibrium) are best represented by base pair probabilities.

A pair (i , j) with probability p is represented by a square in row i and column j with area p.



The Vienna RNA Package

- Minimum free energy and partition function folding
- Complete suboptimal folding
- Inverse folding / RNA design
- Comparison of secondary structures
- Specific heat curves
- Inclusion of structure probing data
- Analysis of folding kinetics / co-transcriptional folding
- Utilities for plotting and annotation structures
- 2.5D prediction: G-quadruplexes and pseudo-knots
- Prediction of consensus structures

For the programmer:

- A *C* library to link against your programs
- Python/Perl scripting language interface

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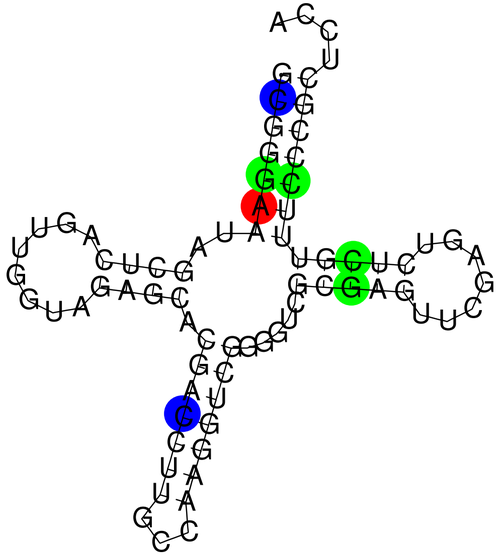
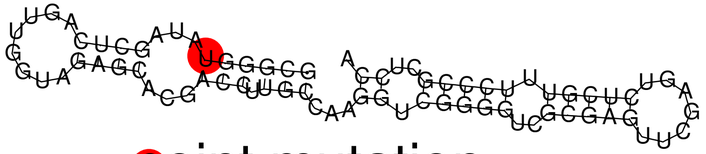
Free software, C source code and fold server available at

http://www.tbi.univie.ac.at/RNA/

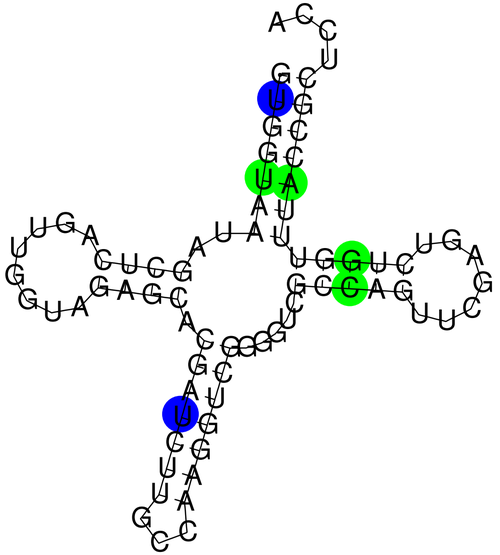
Functional Structures: Point Mutations



original structure

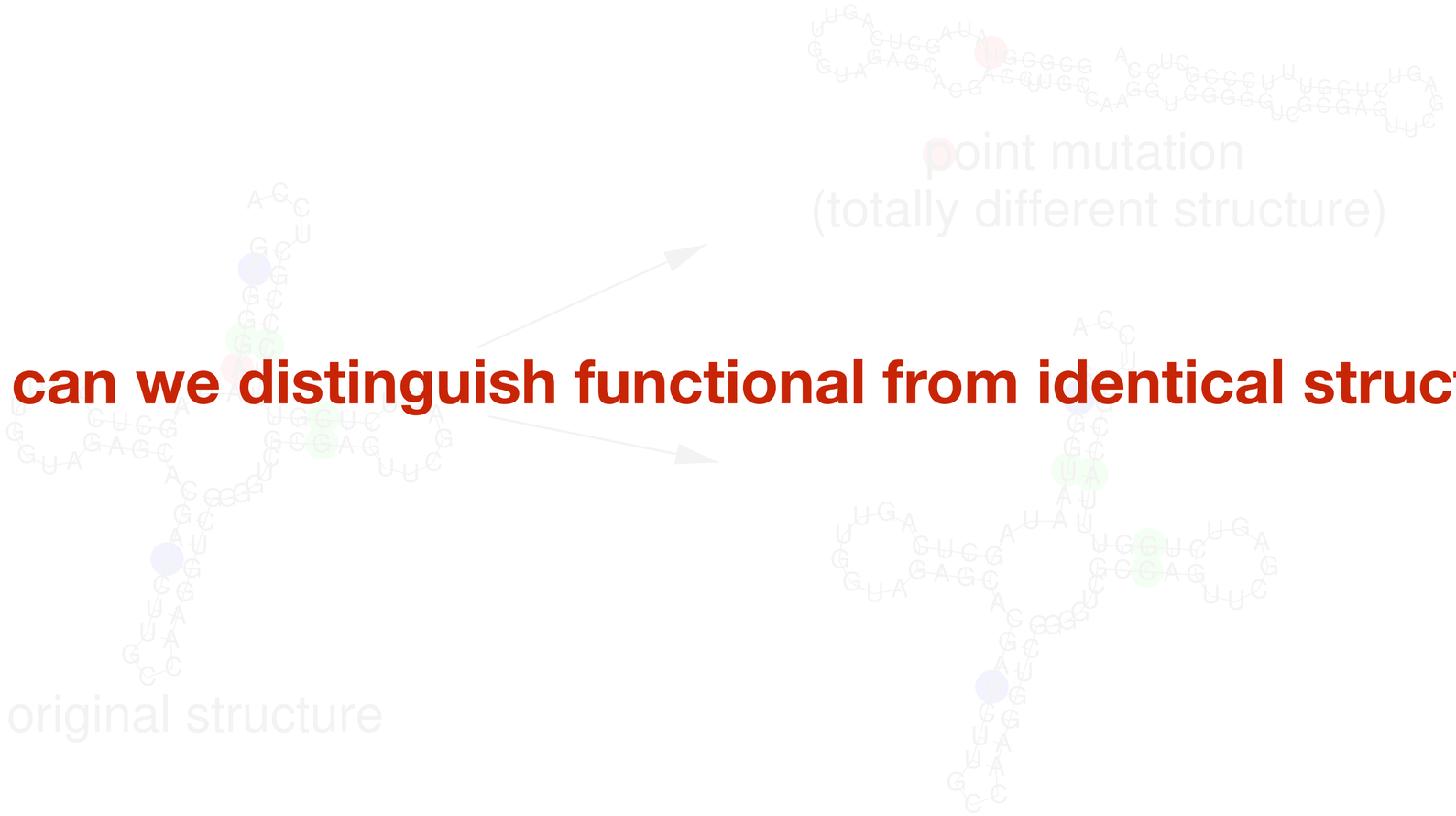


point mutation
(totally different structure)



compensatory and consistent mutations
(no structural change)

Functional Structures: Point Mutations



How can we distinguish functional from identical structures?

Consensus Structures: Alignment Folding

Combine covariance analysis and folding into one DP algorithm

- Apply conventional folding algorithm to alignment
- Use a modified energy function that includes covariance score

$$E_c(A, \Psi) = \sum_k E(A_k, \Psi) + cv \cdot \sum_{(i,j) \in \Psi} B_{ij}$$

- Can be used for all variants: MFE, partition function, …
- Efficient: $\mathcal{O}(N \cdot n^2 + n^3)$ CPU and $\mathcal{O}(n^2)$ memory for alignment length n and N sequences
- Same results as RNAfold for single sequences

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RNAalifold

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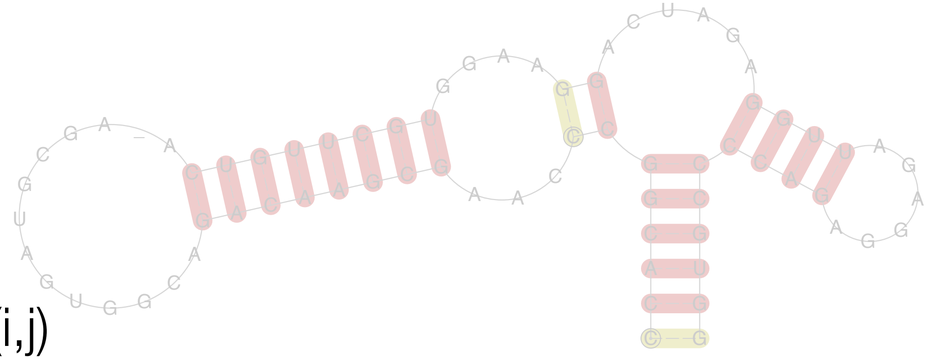
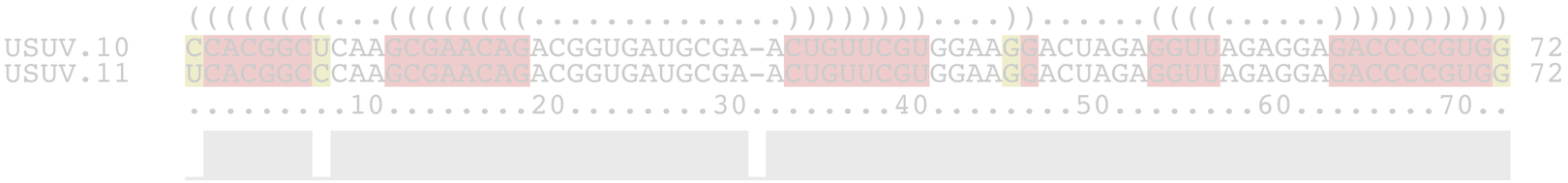
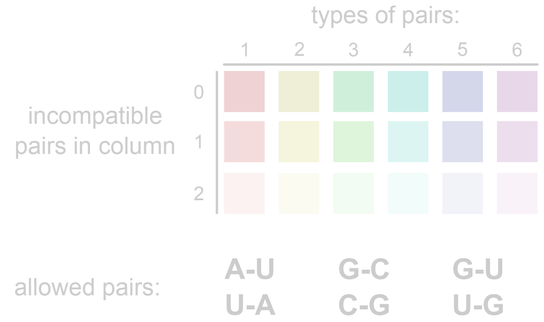
```
GGGCCGGUAGCUCAUUUAGGCAGAGCGUCUGACUCUUAAUCAGACGGUCGCGUGUUCGAAUCGCGUCCGGCCCA
GCCGAAAUAGCUCAGUU-GGGAGAGCGUUAGACUGAAGAUCUAAAGGUCCCCGGUUCAAUCCCGGGUUUCGGCA
GCGGAUUUAGCUCAGUU-GGGAGAGCGCCAGACUGAAGAUUUGGAGGUCCUGUGUUCGAUCCACAGAAUUCGCA
CAGAGUGUAGCUUA---ACACAAAGCACCCAACUUACACUUAGGAGAU-UUCAACU-UAACUUGACCGCUCUGA

(((((((..((((.........)))).(((((.......)))))).....(((((.......)))))))))))).
mfe: -29 kcal/mol = (-21.3 +  -7.7) kcal/mol
```

RNA Covariation as Evolutionary Trait

- High mutation rate in RNA viruses due to error-prone RdRP
- For base pair (i,j): GC/CG/AU/UA/GU/UG
- Consistent mutation: different standard combinations
- Compensatory mutation: both positions are mutated
- Presence of both strongly supports predicted base pair (i,j)







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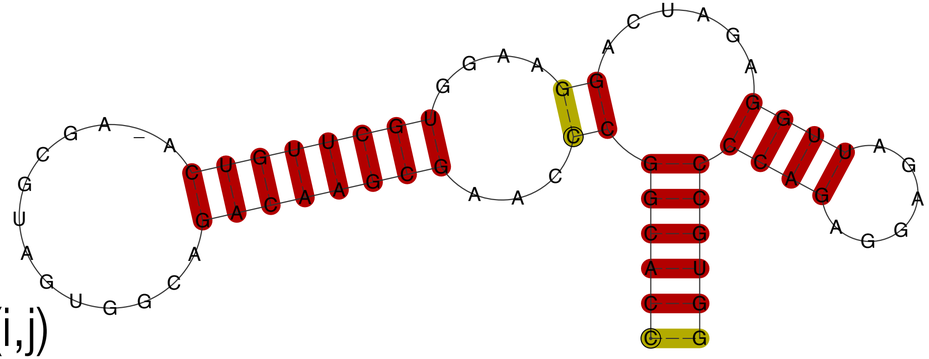

```
                 (((((((((...(((((((((..............)))))))))....)).....(((((.....)))))))))))
USUV.10          CCACGGCUCAAGCGAACAGACGGUGAUGCGA-ACUGUUCGUGGAAGGACUAGAGGUUAGAGGAGACCCCGUGG 72
USUV.11          UCACGGCCCAAGCGAACAGACGGUGAUGCGA-ACUGUUCGUGGAAGGACUAGAGGUUAGAGGAGACCCCGUGG 72
                 .........10........20........30........40........50........60........70..
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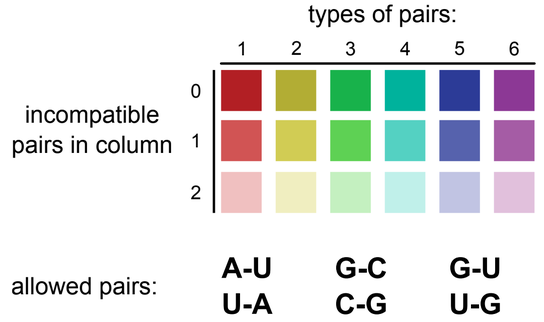

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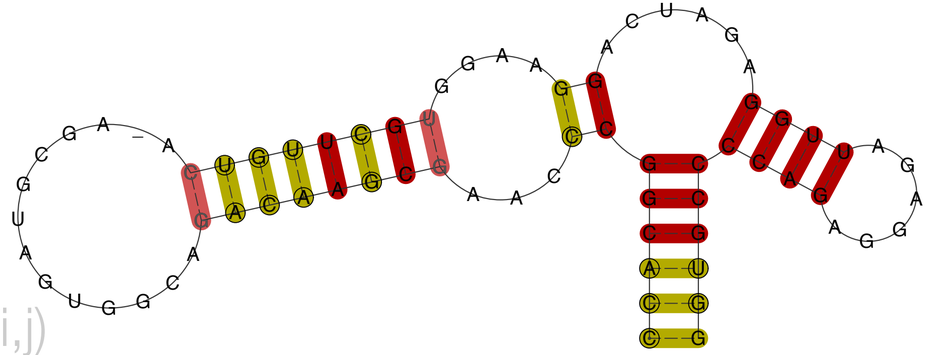

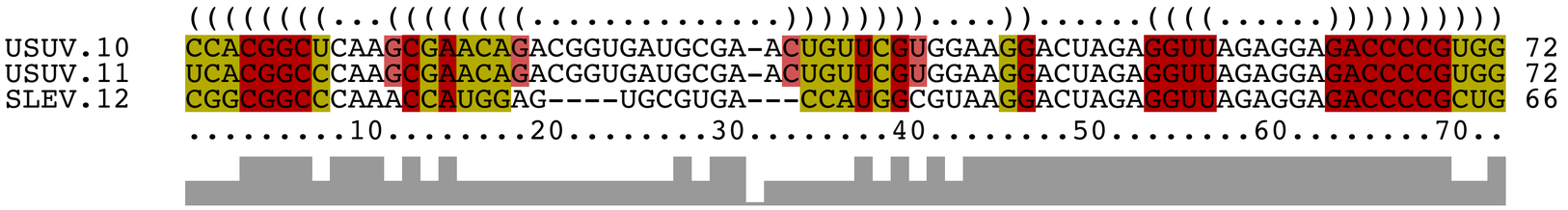

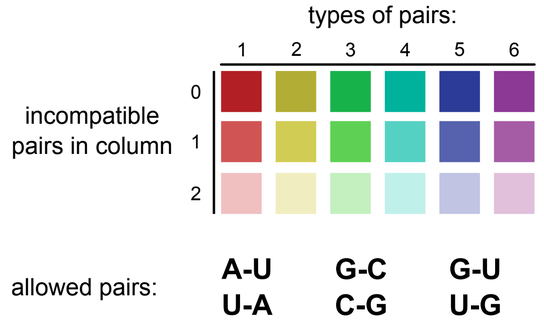

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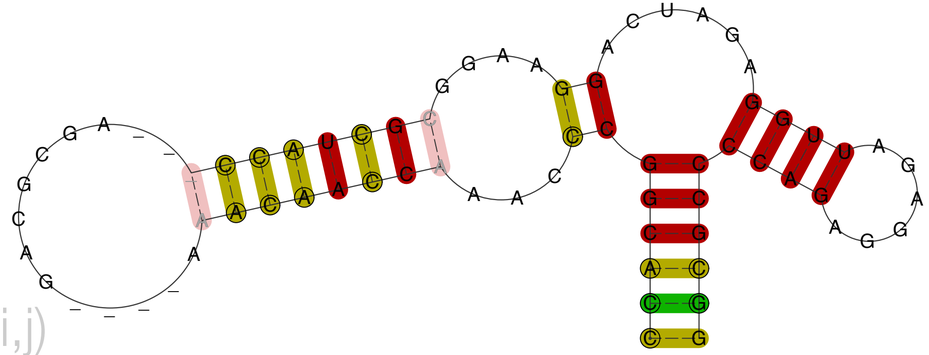

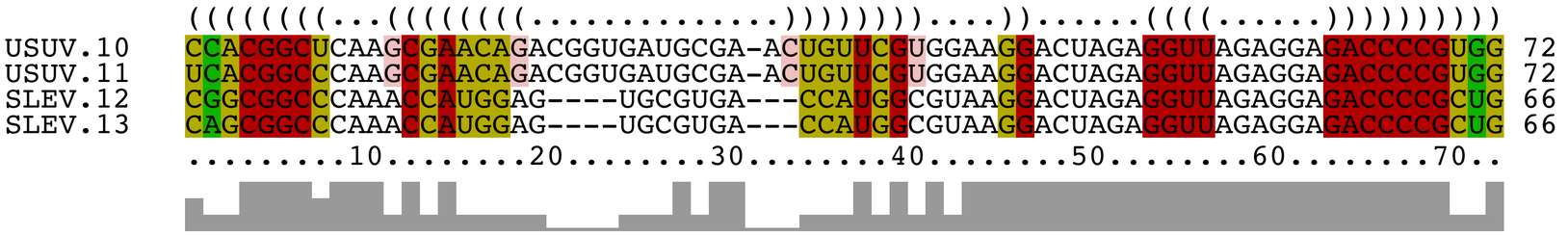

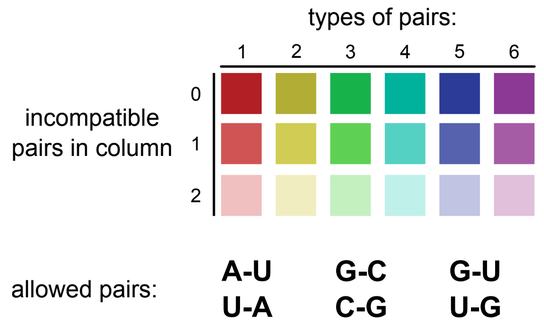

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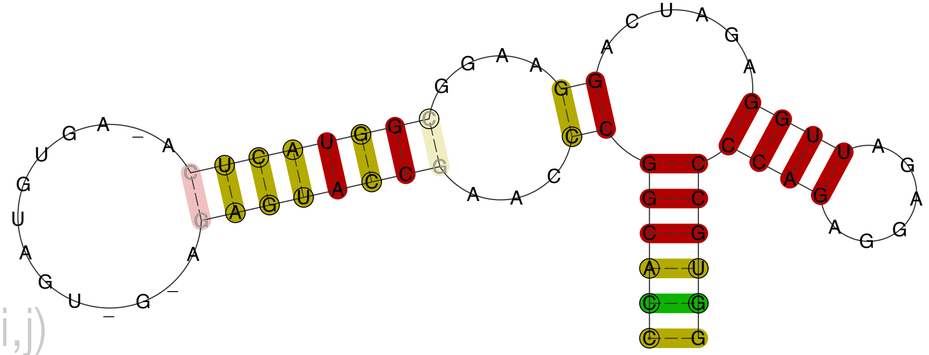

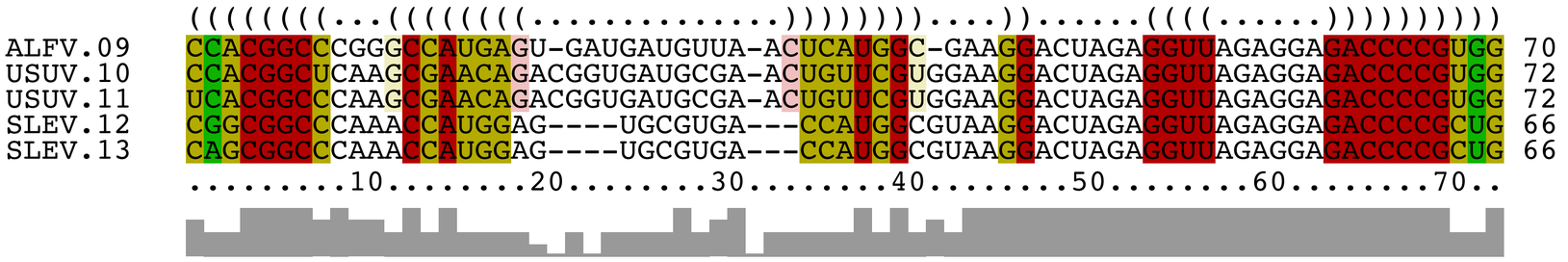

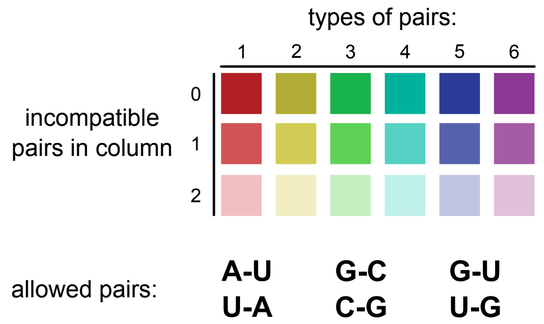

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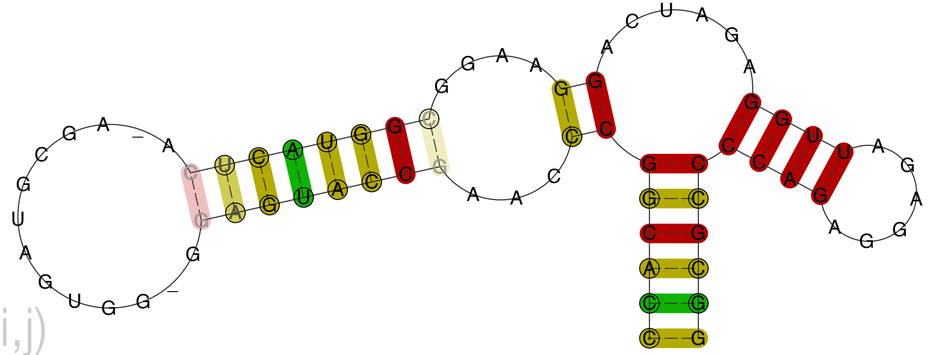

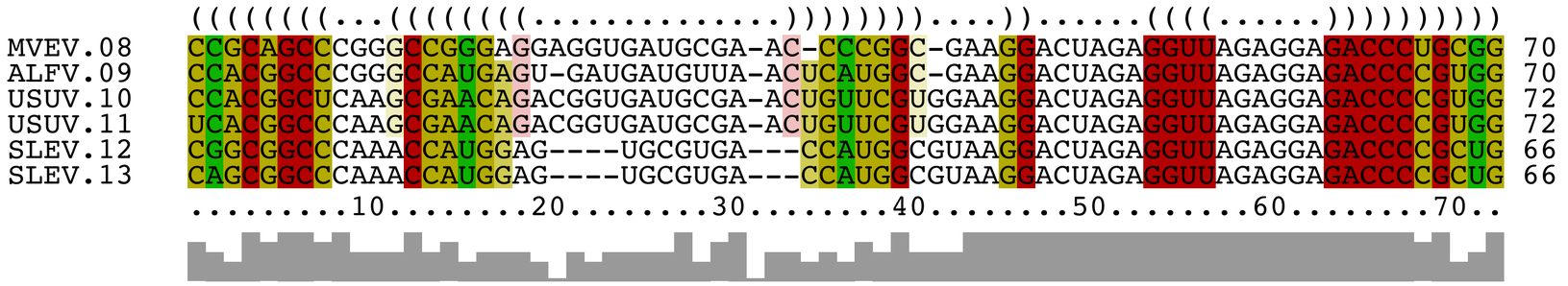

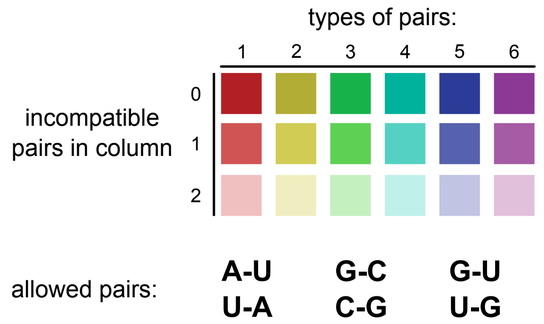

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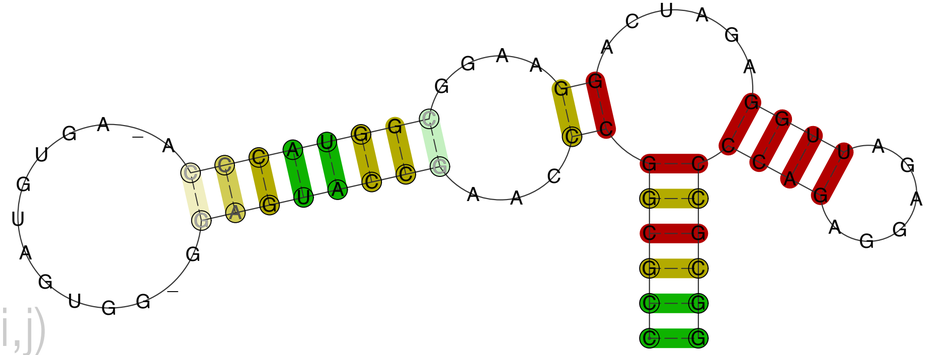

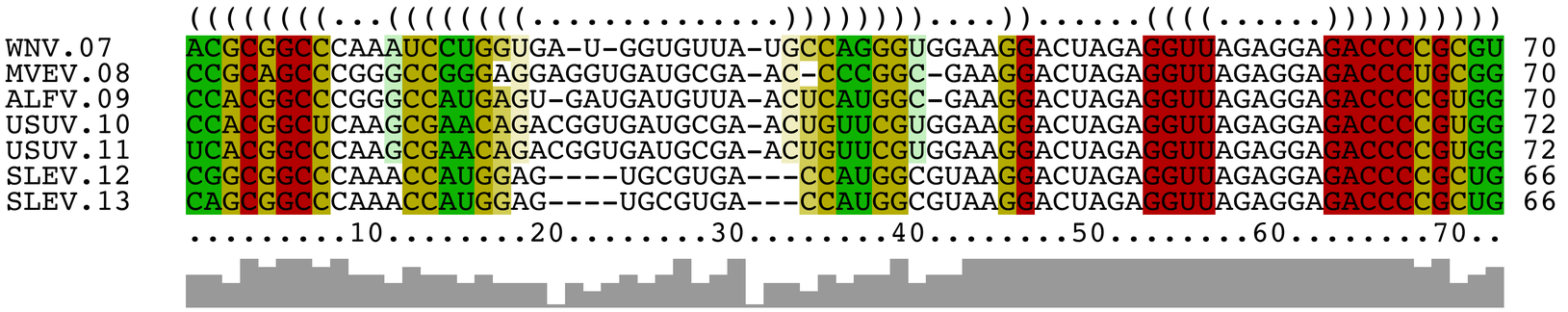

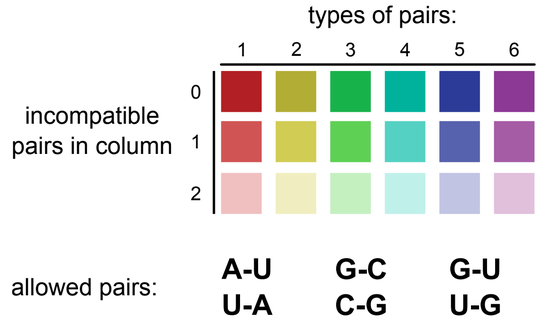

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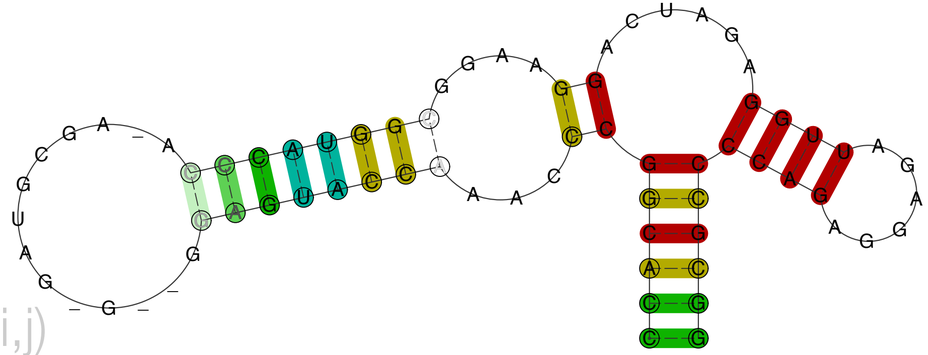

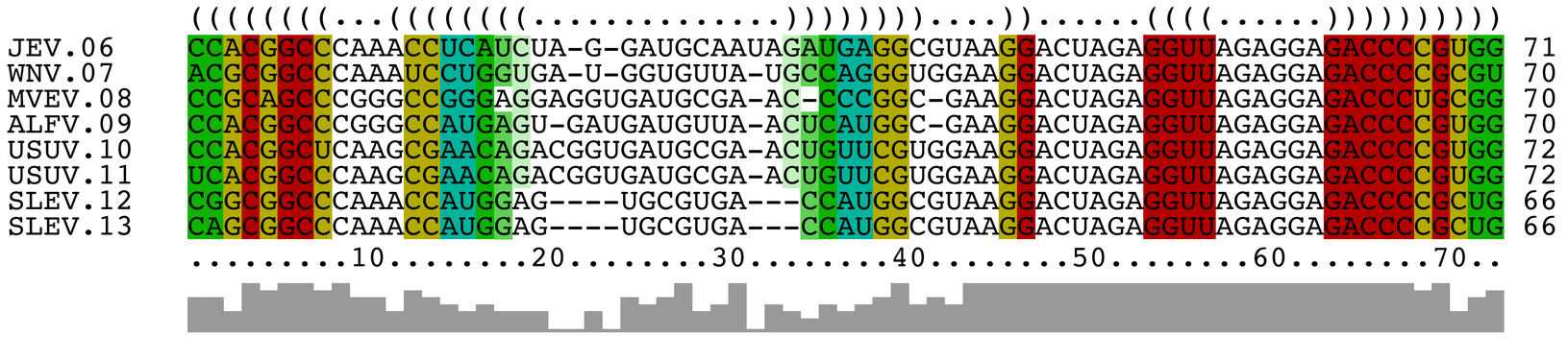

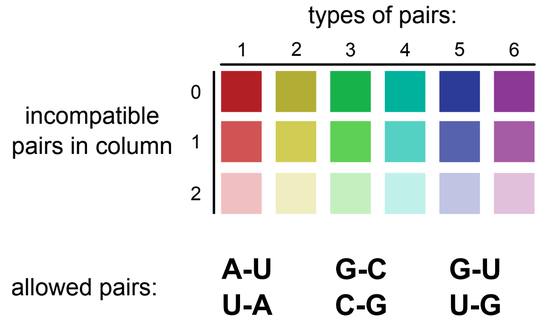

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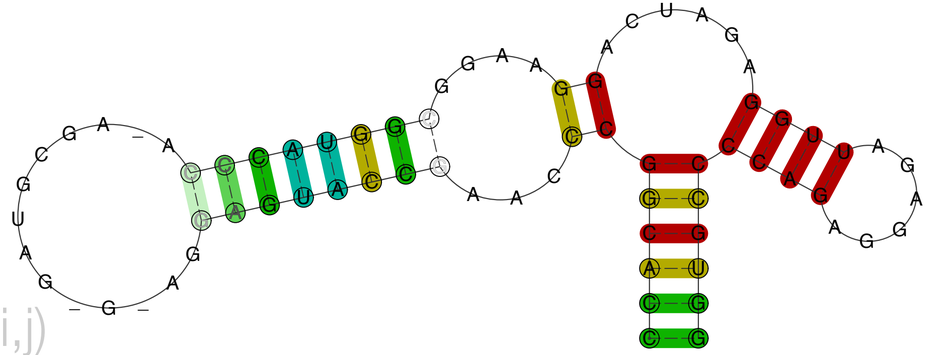

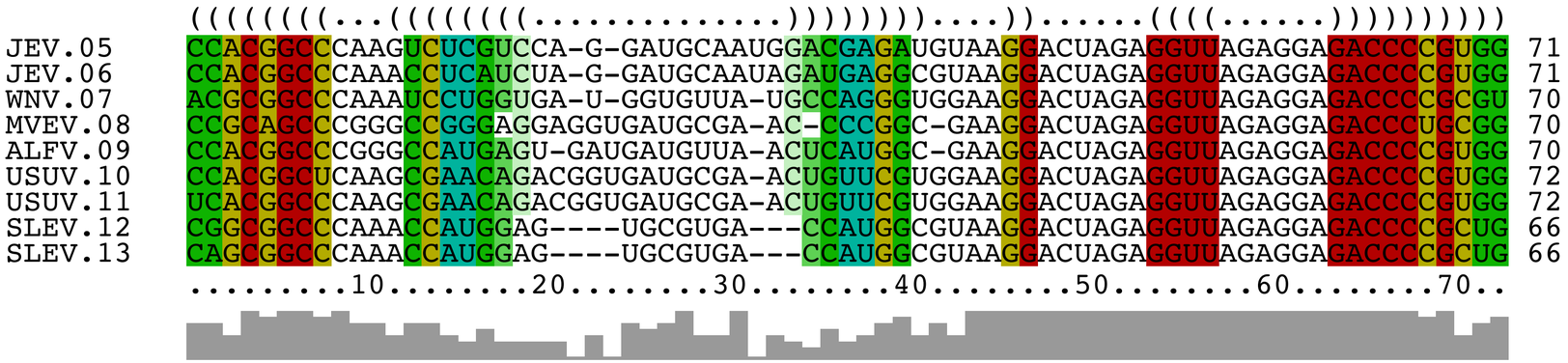

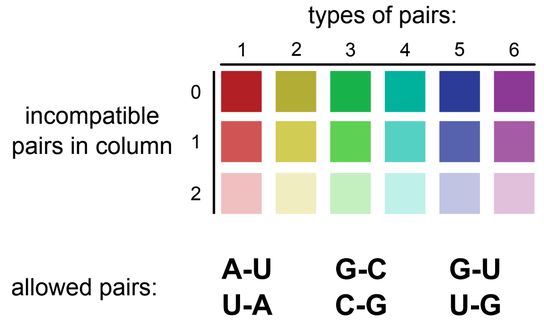

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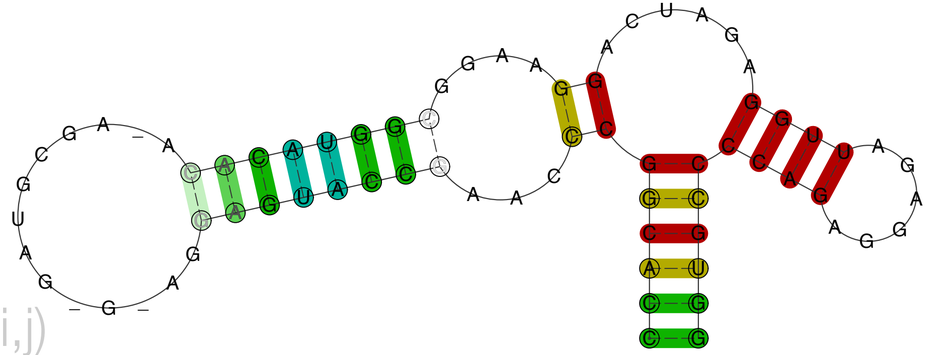

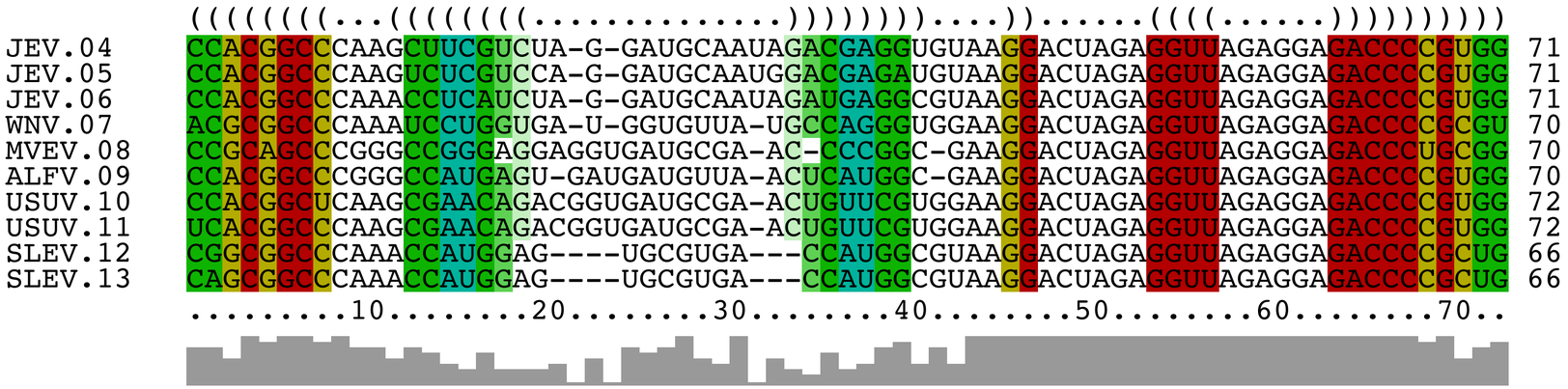

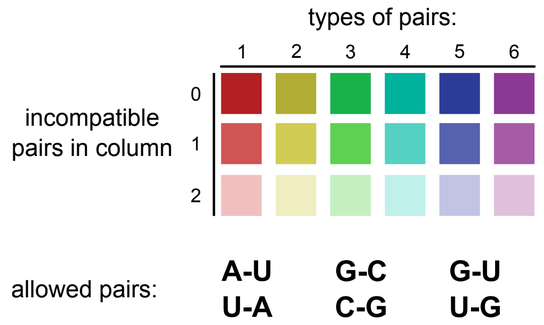

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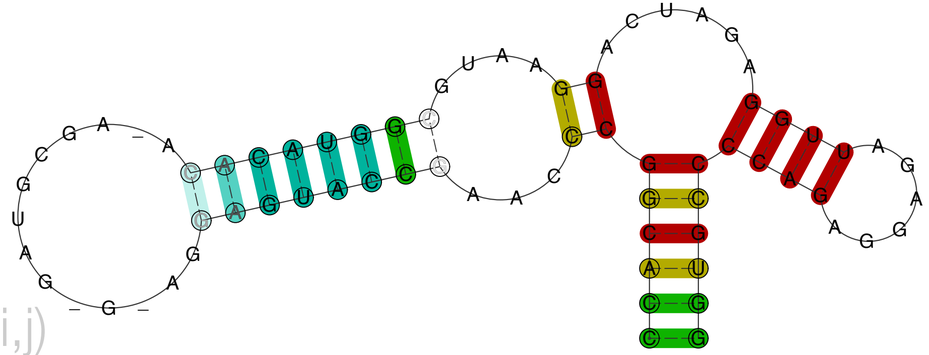

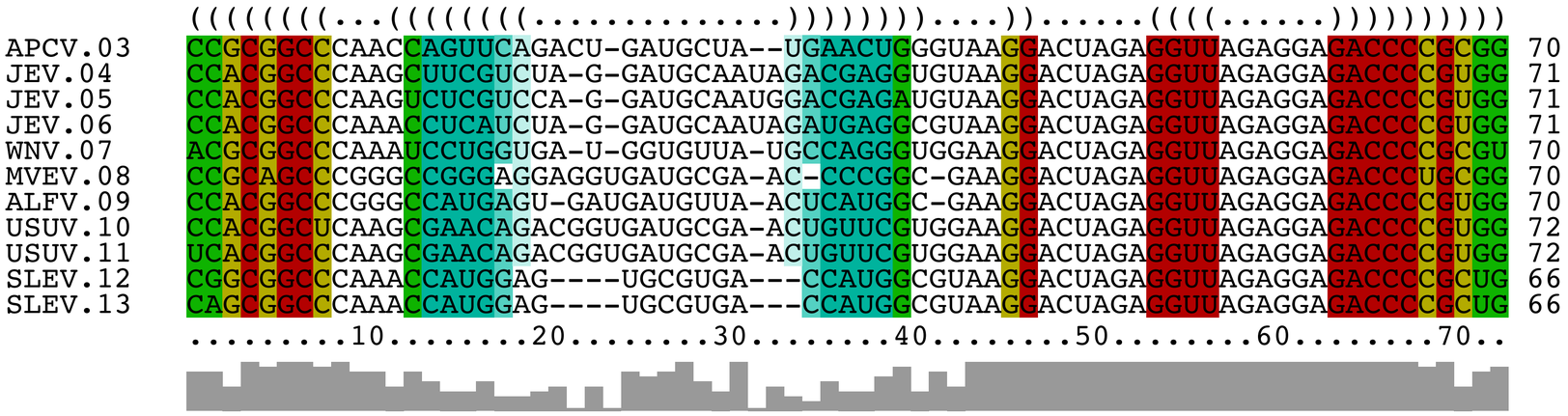

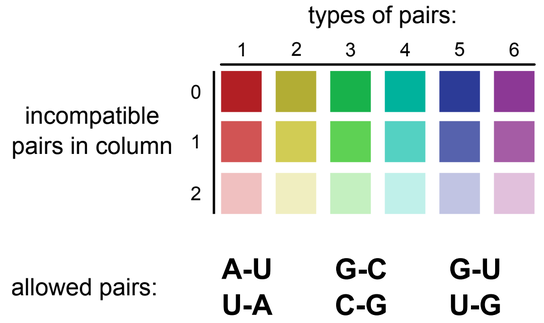

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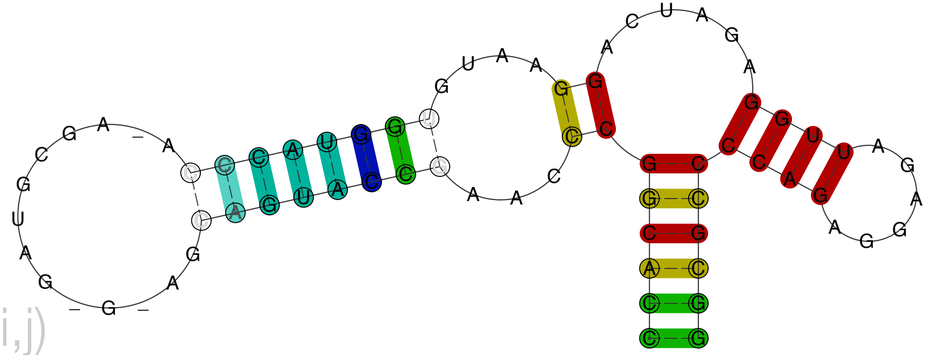

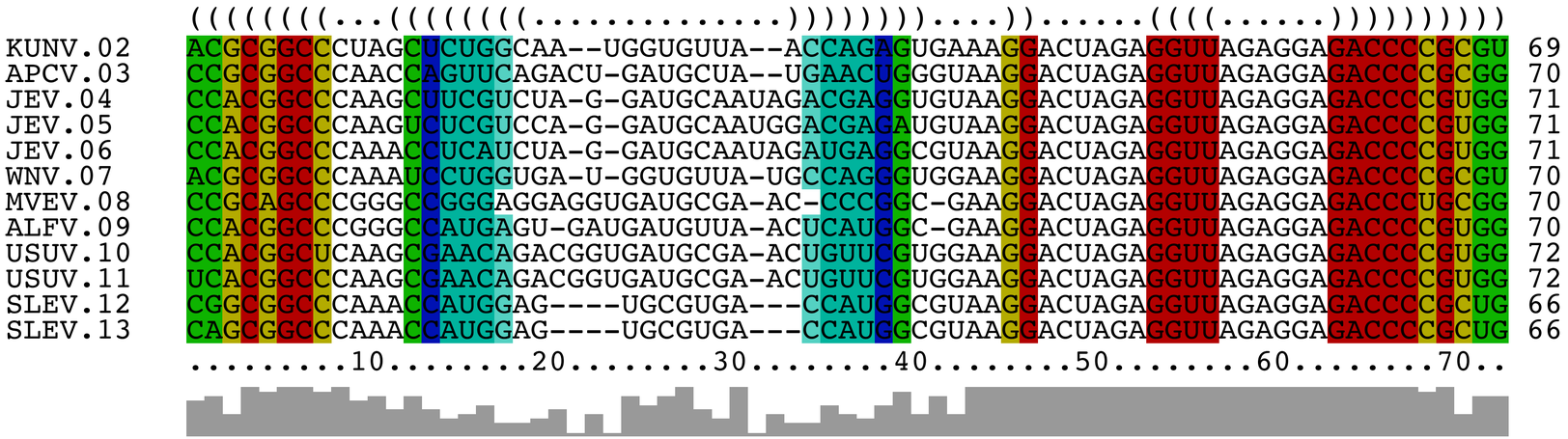

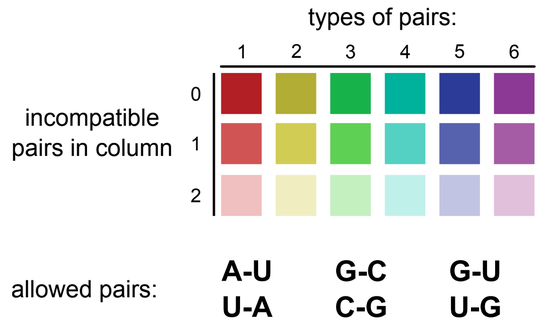

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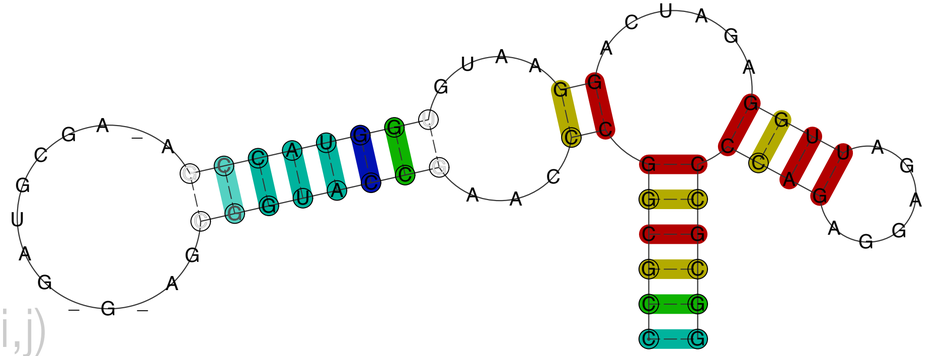

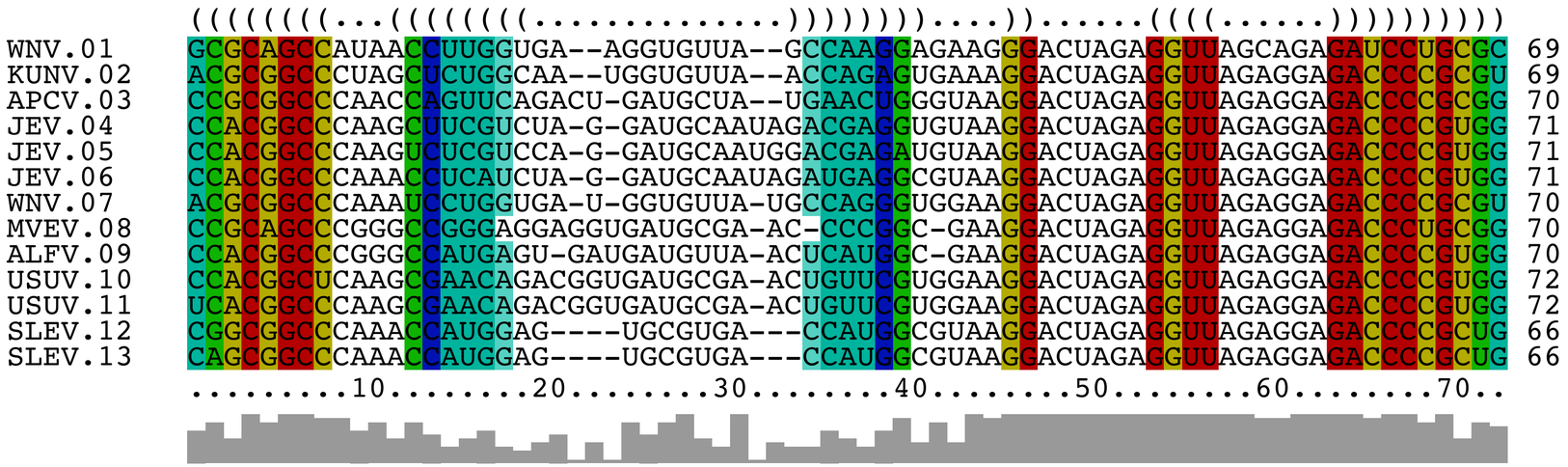

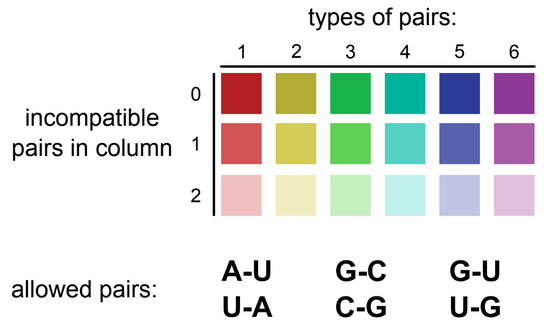

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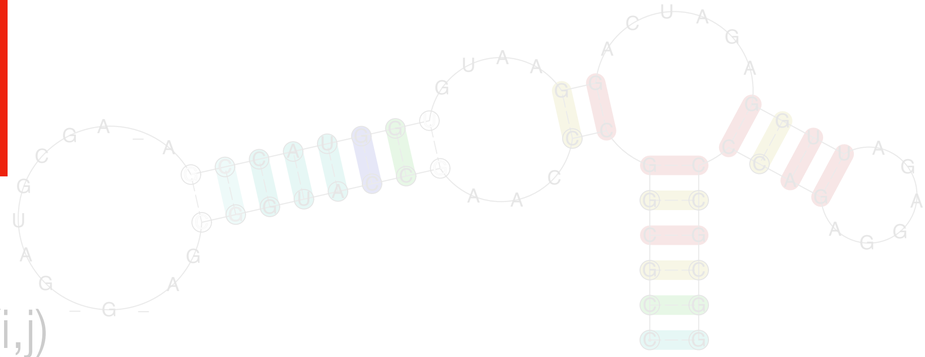

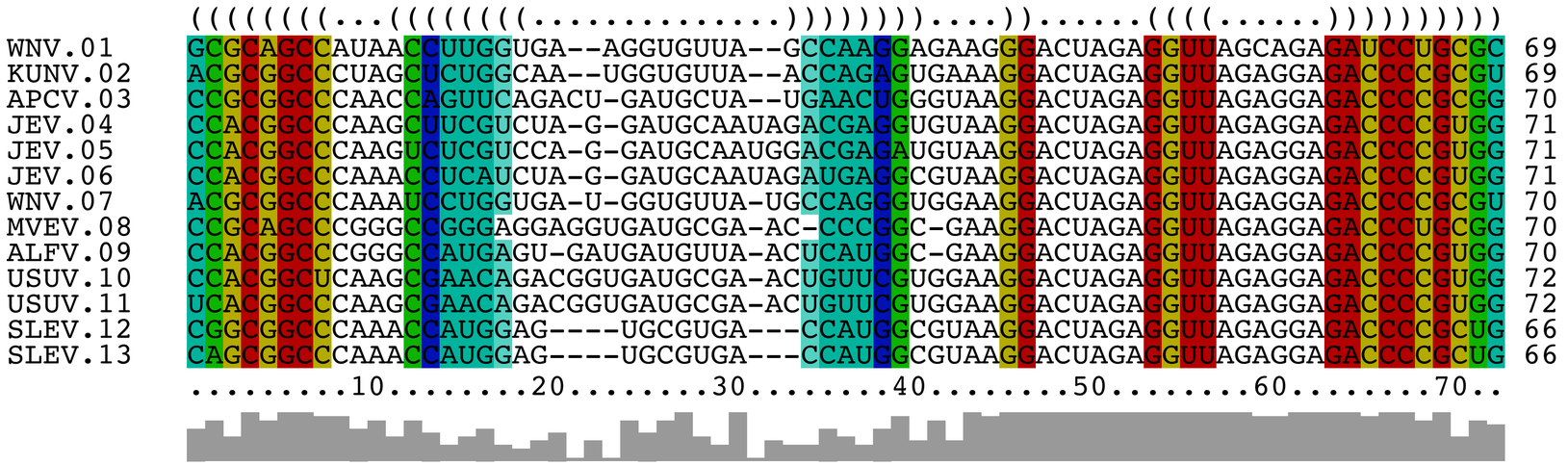

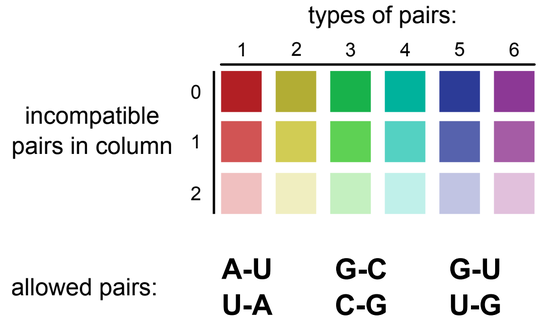

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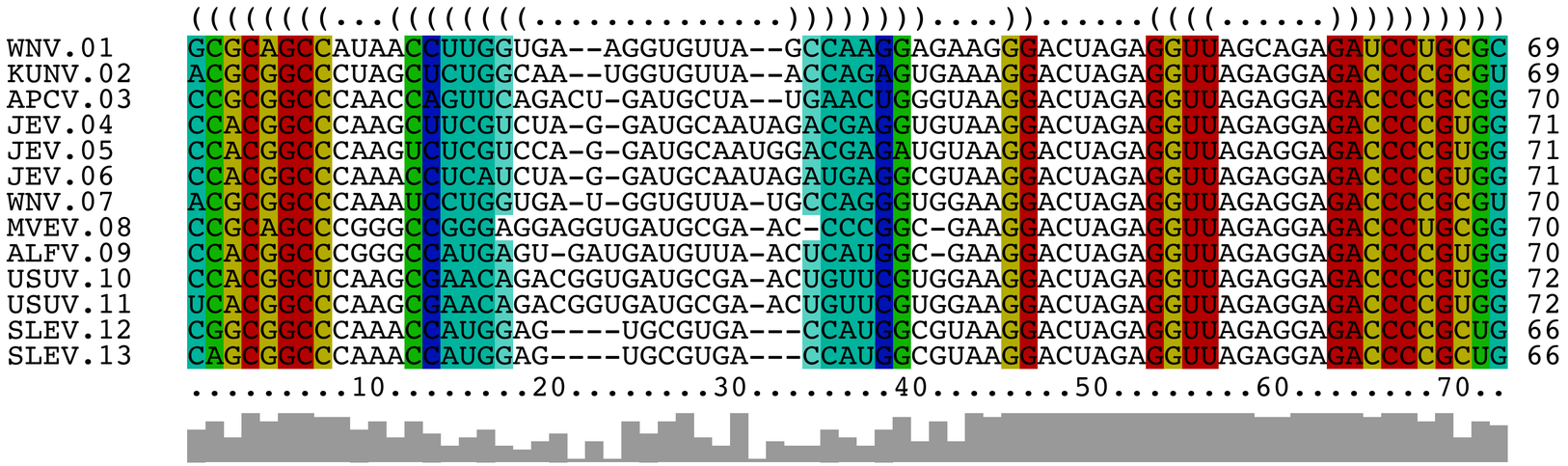
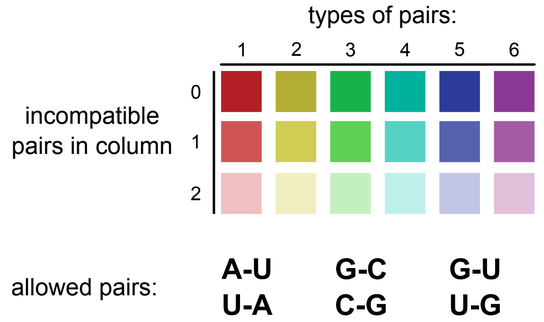


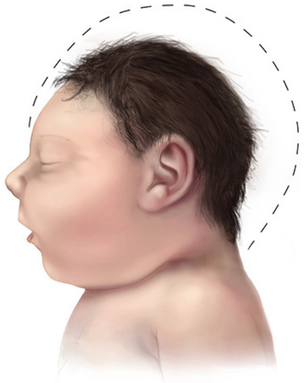


Part II:

Conserved RNA structures in flaviviruses

Flaviviruses as Global Health Threat

- Family *Flaviviridae* / genus *Flavivirus*; arthropod-borne (mainly mosquitoes & ticks)
- FV include (re-)emerging human pathogens like YFV, DENV, JEV, WNV, TBEV
- Clinical manifestations: headache, rash, (hemorrhagic) fever, meningitis, encephalitis
- FV neurotropism reported for YFV, WNV, TBEV, DENV, ZIKV
- ZIKV outbreak in the Americas 2015-2017
- Congenital neurotropism: high increase in microcephaly cases in newborns



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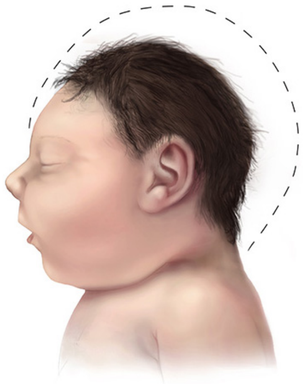
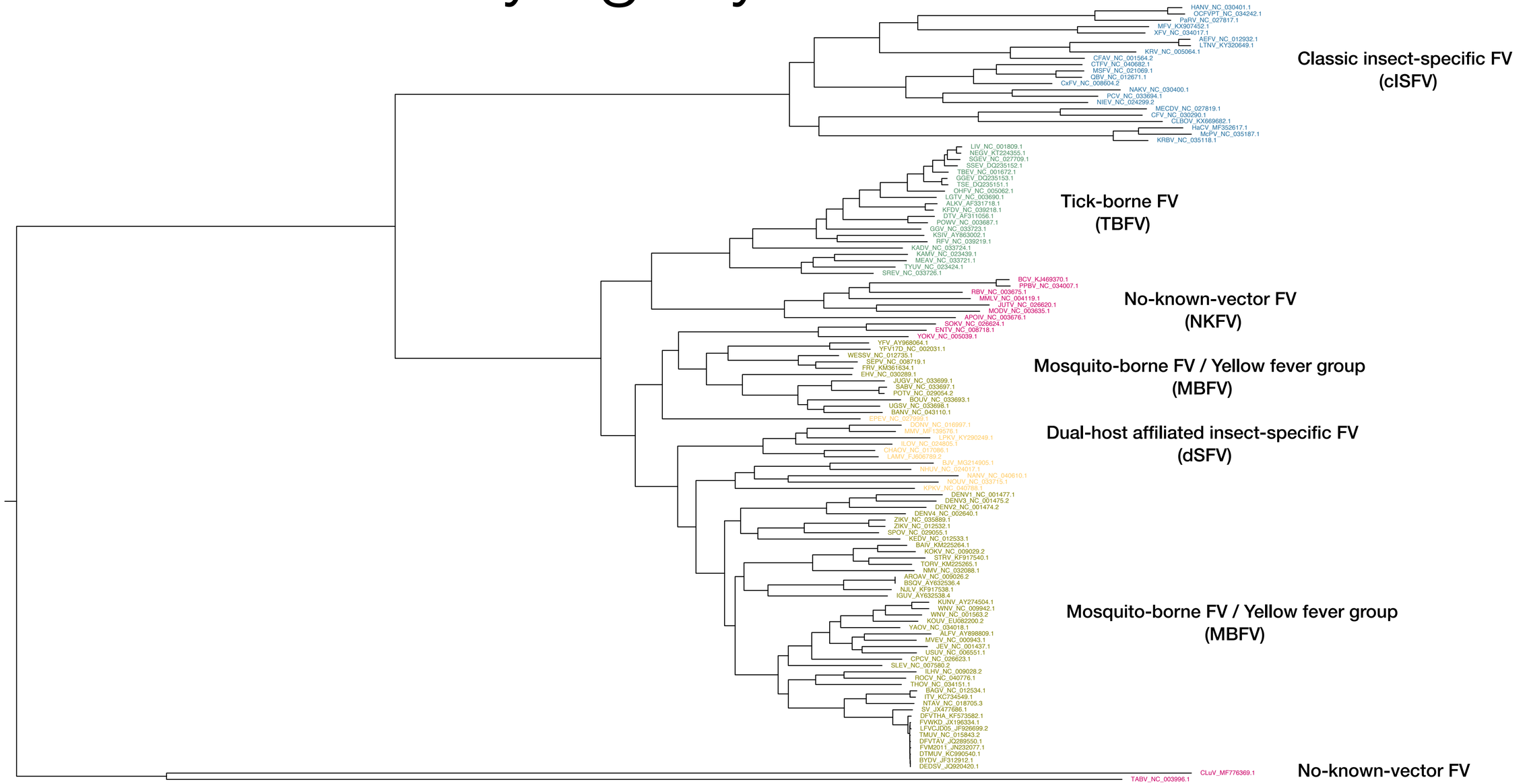


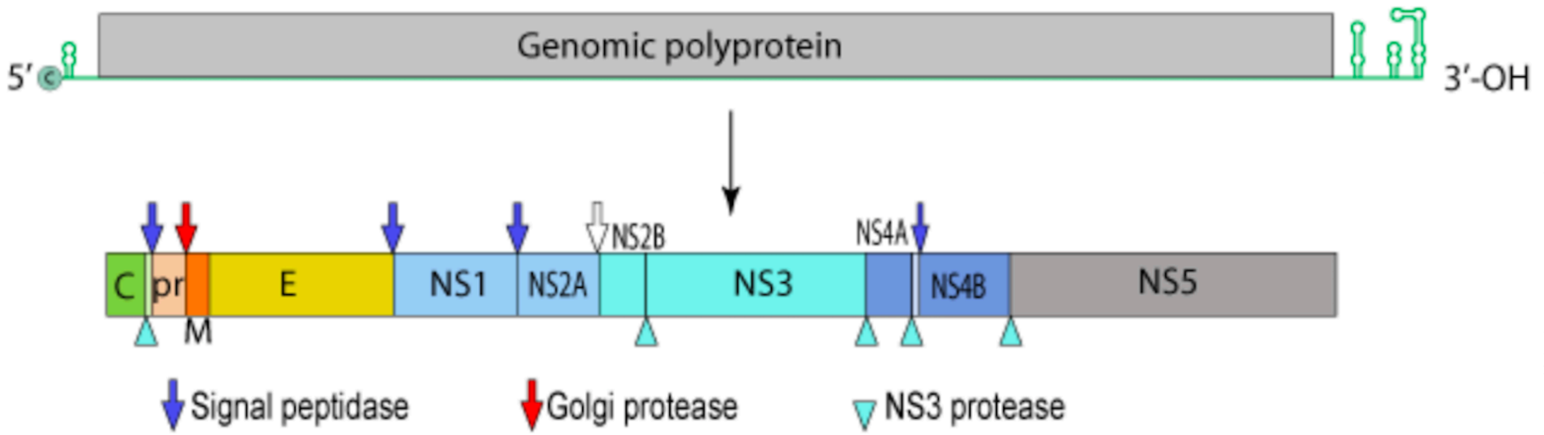
Source: Wikipedia

Vaccine only available for YFV

Flavivirus Phylogeny



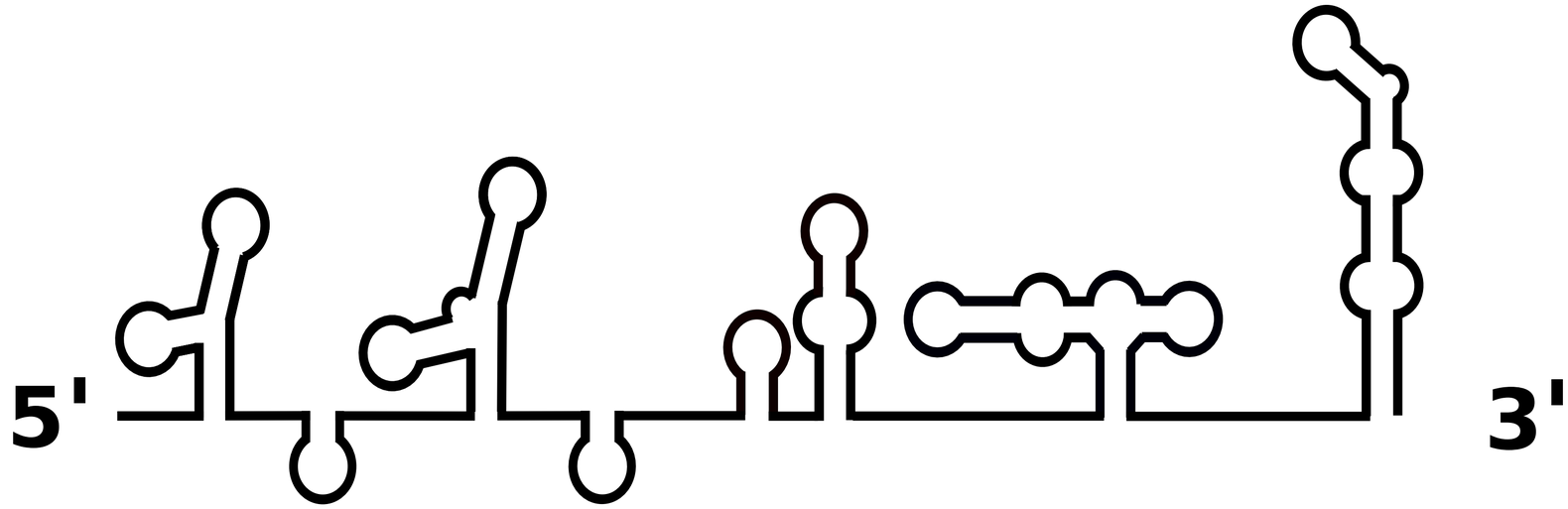
Flavivirus Genome Organization



https://viralzone.expasy.org/

- Non-segmented, single-stranded, (+)-sense RNA genomes of 10-12kB length
- Capped, non-polyadenylated
- Encode a single ORF, flanked by highly structures 5’UTR and 3’UTR

Conserved RNAs in Viral 3’UTR: ZIKV



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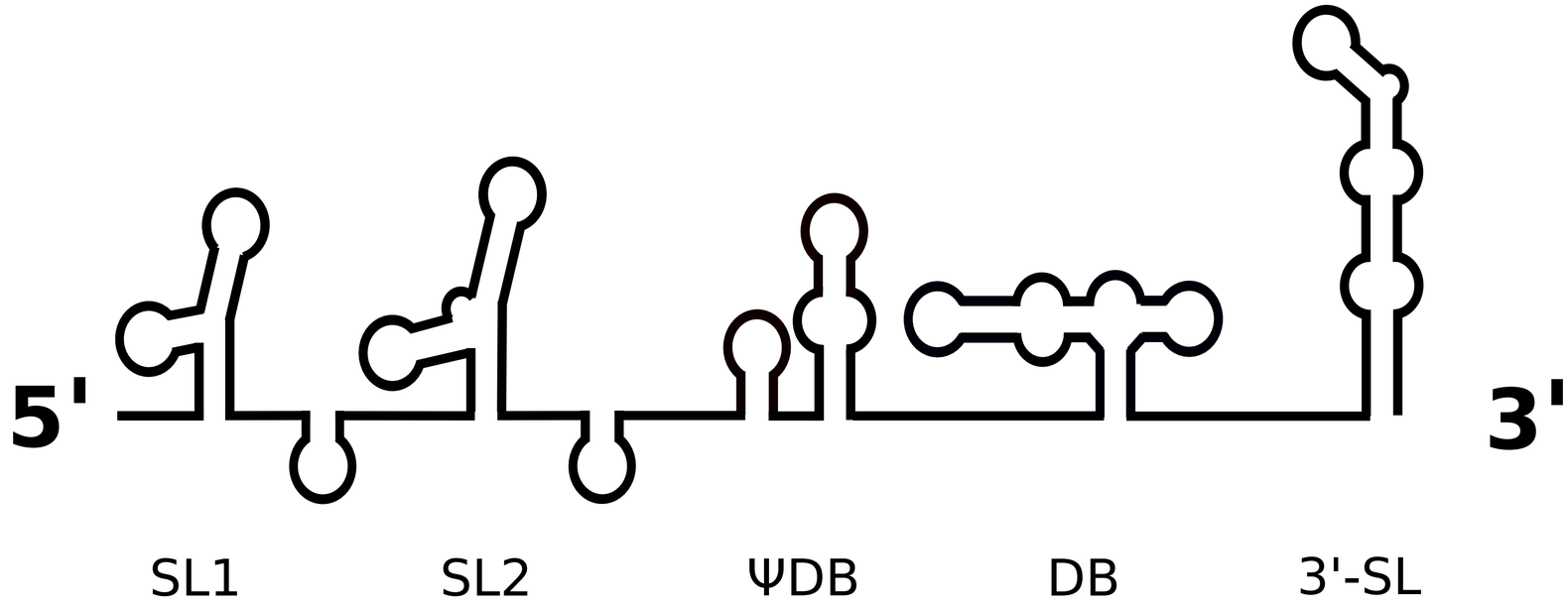

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- Stable decay intermediates produced by partial exoribonuclease degradation
- Xrn1 is efficiently stalled at conserved xrRNA structures

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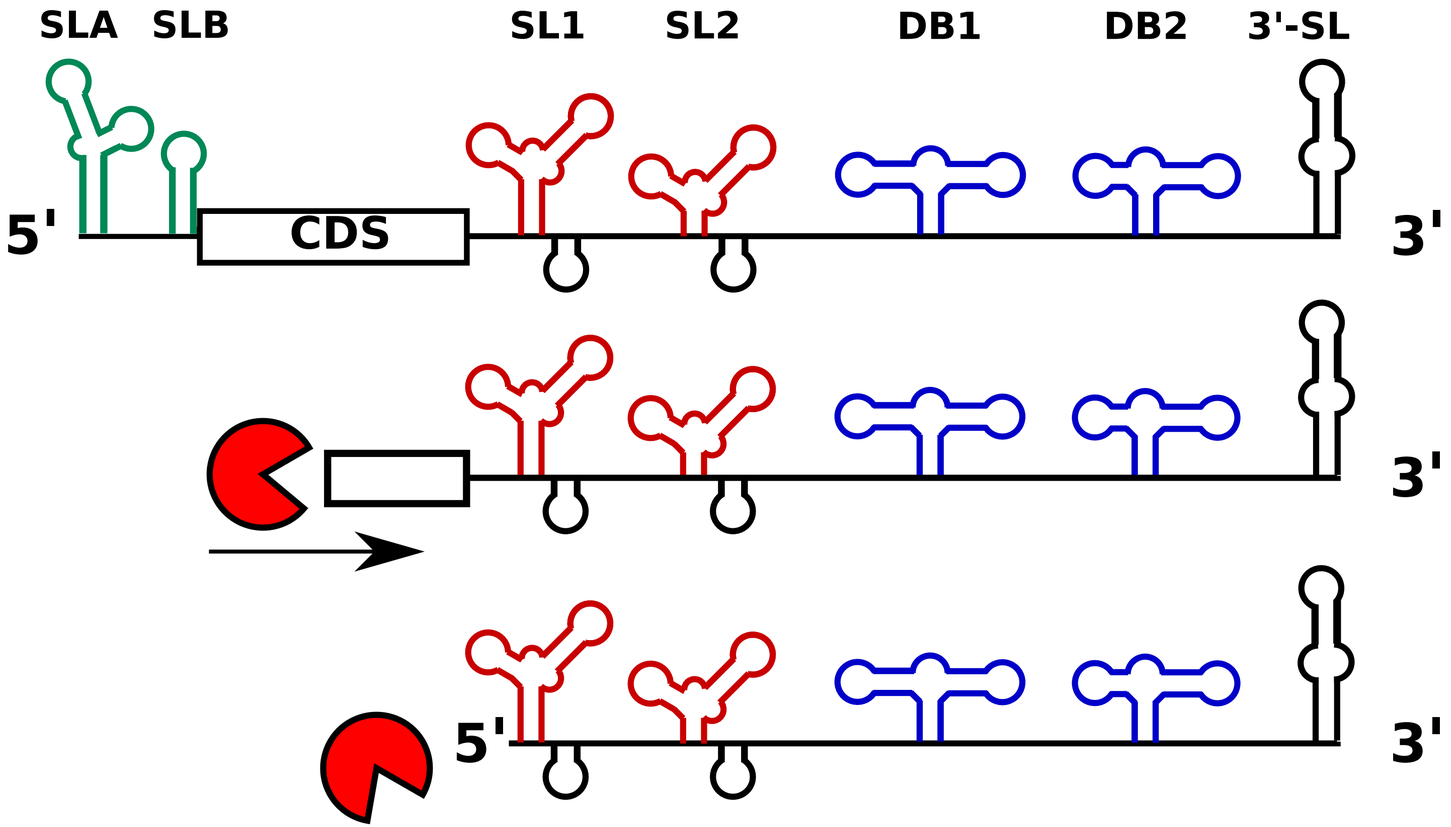

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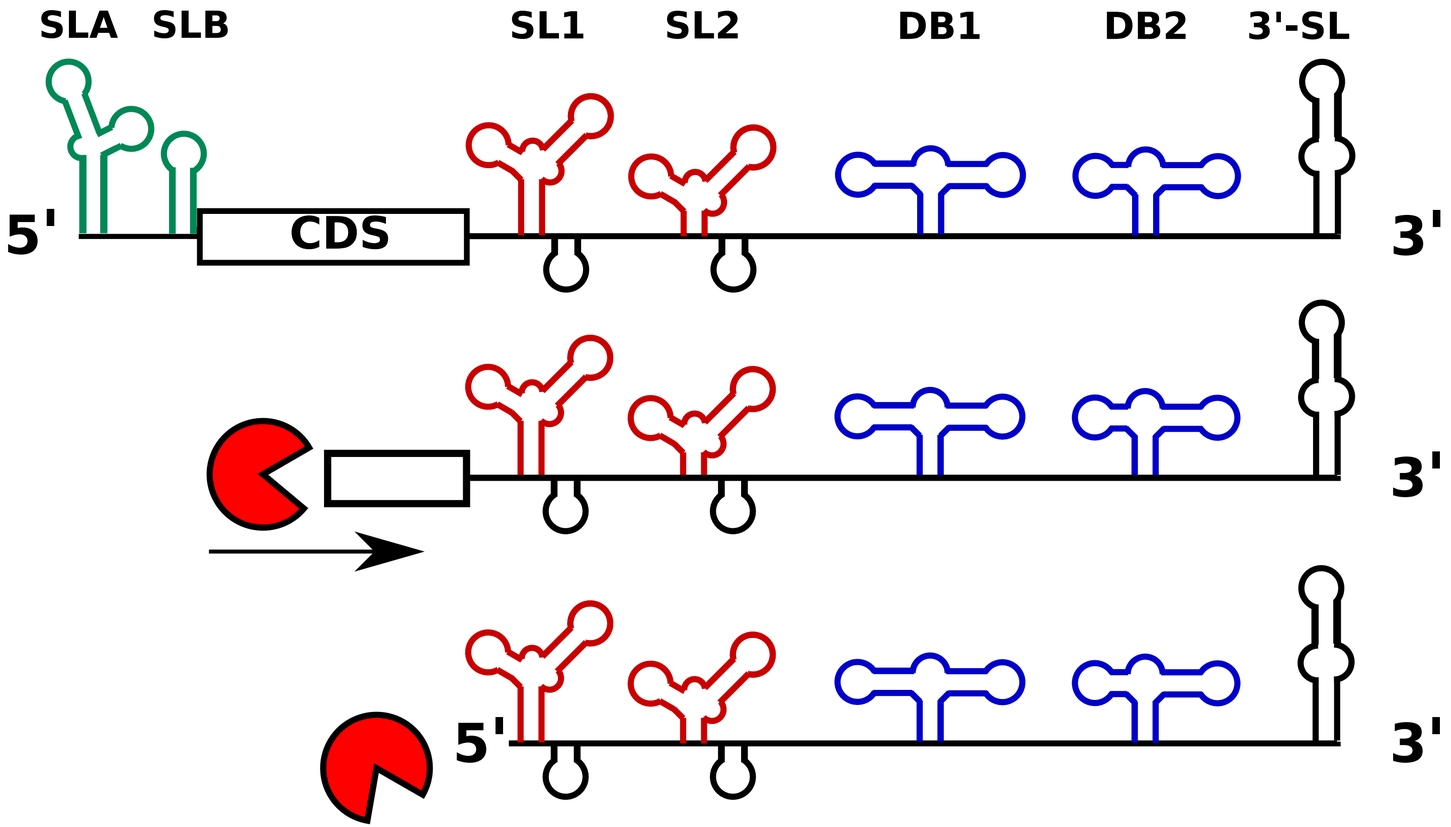

sfRNA mediates pathogenicity !

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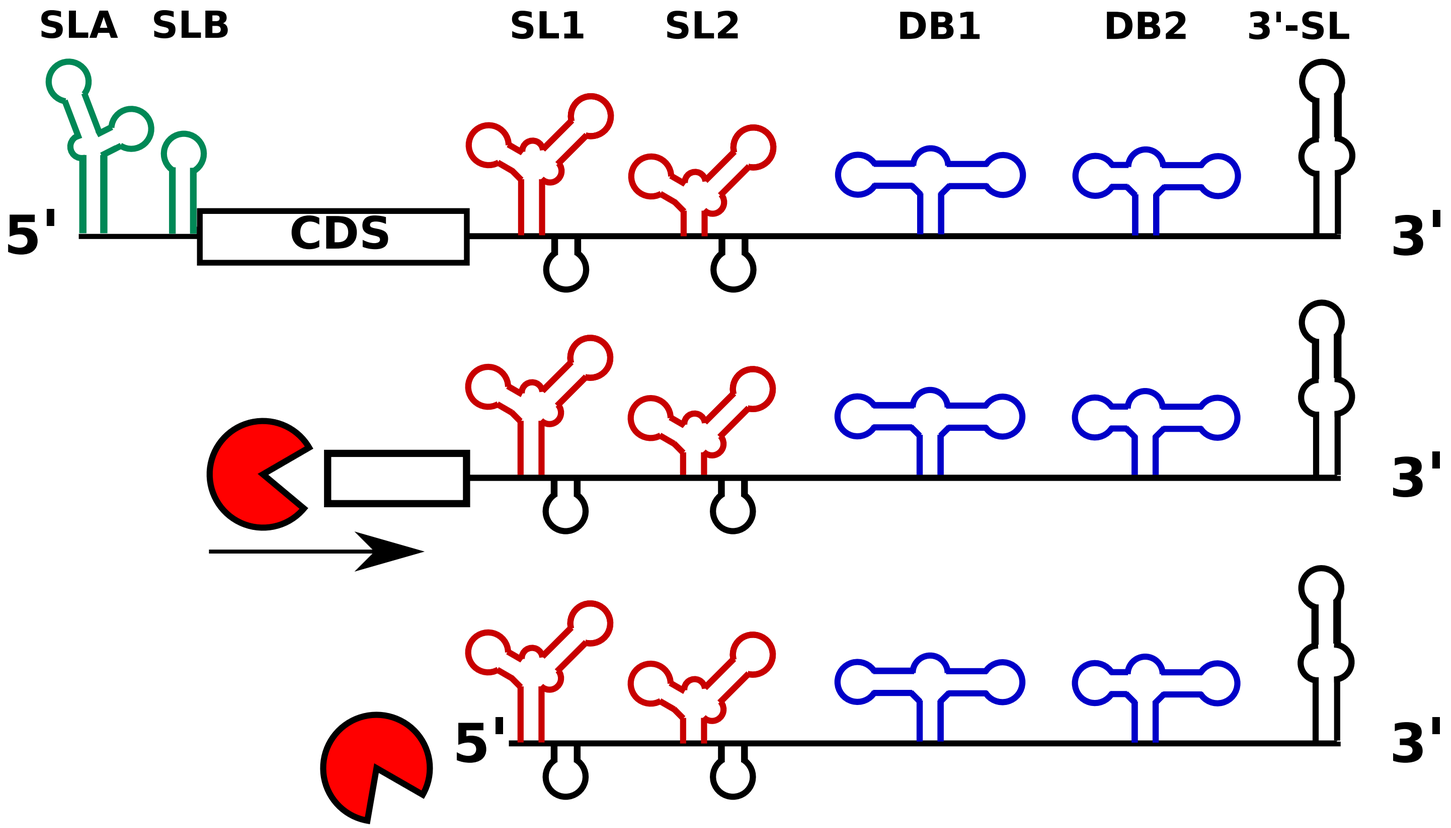

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How to find them?

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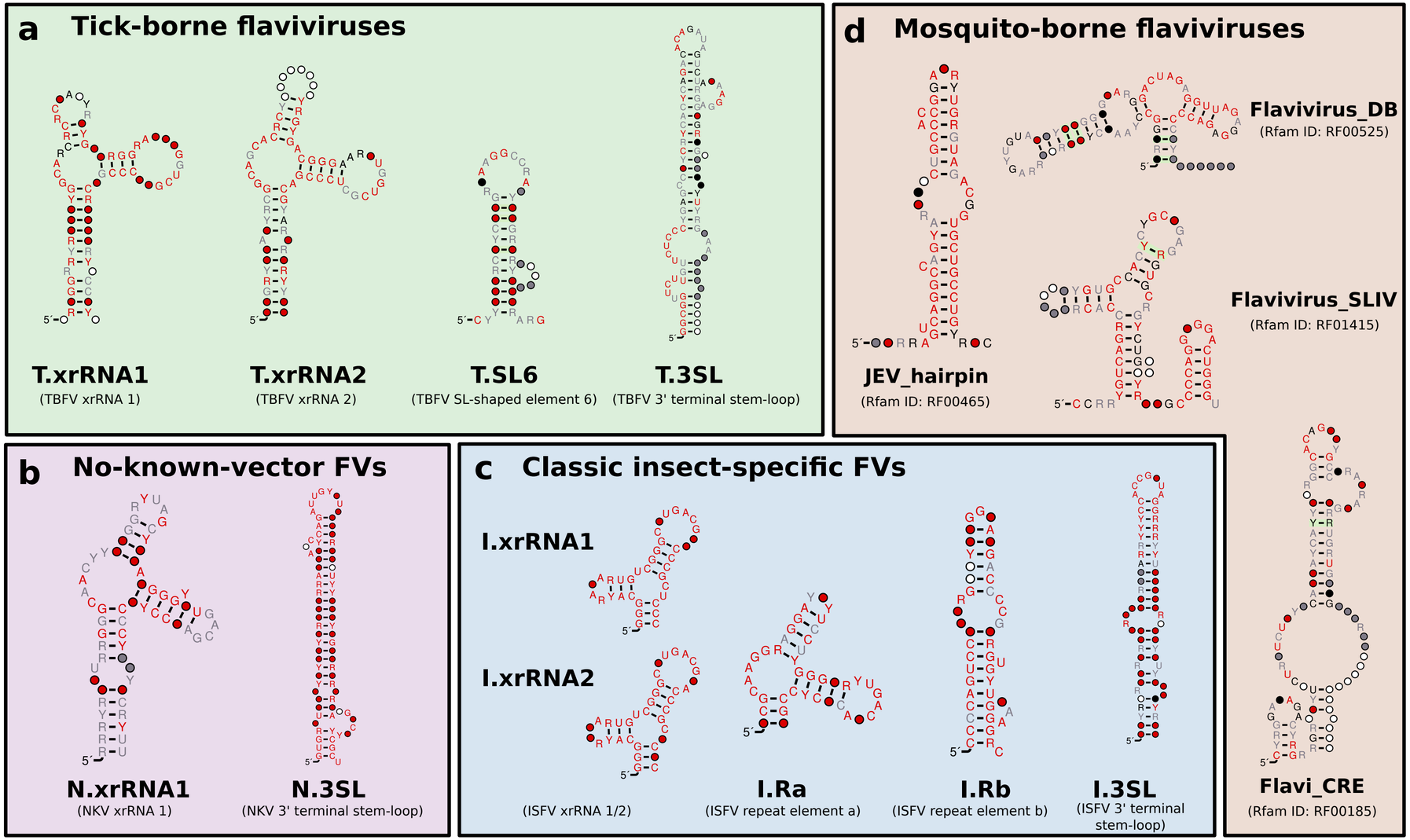
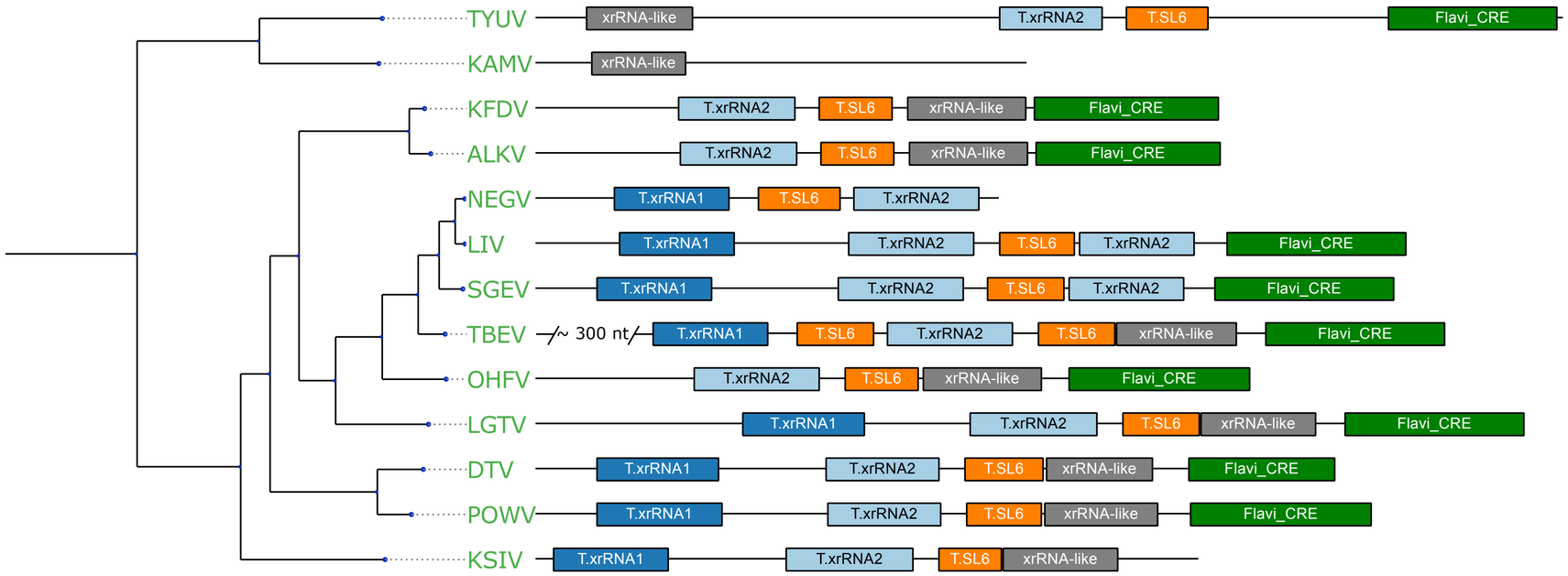
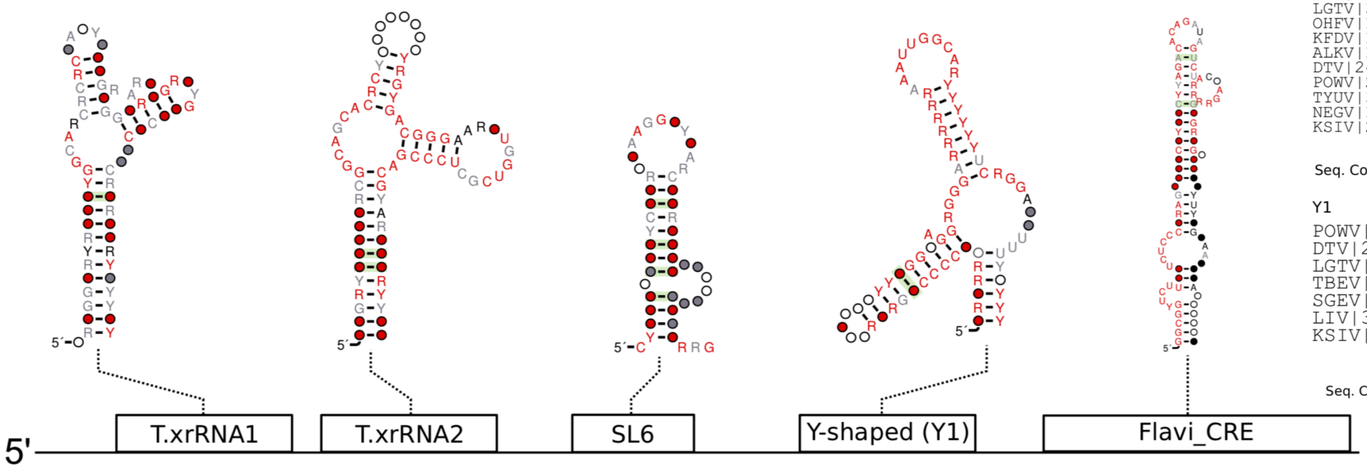
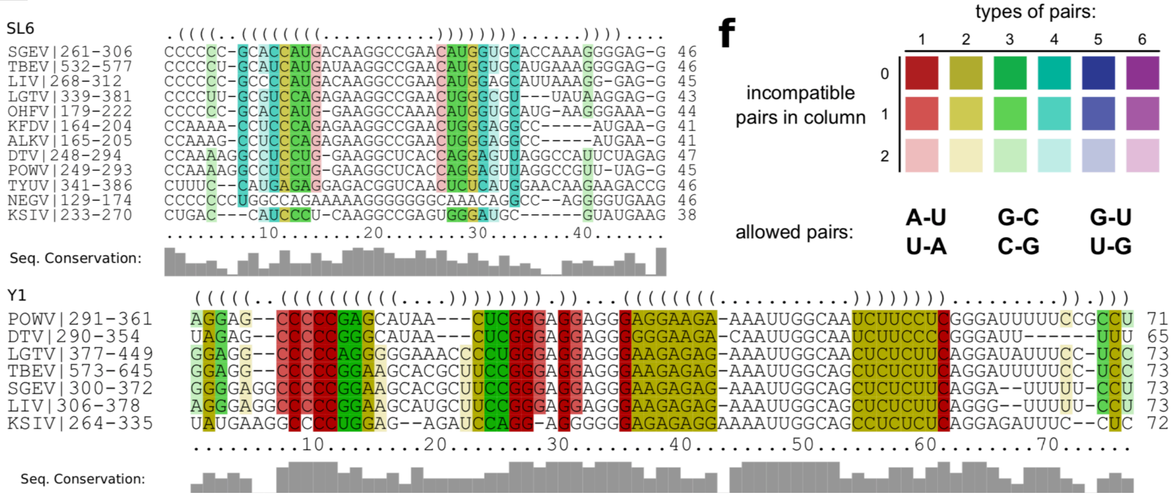
Consensus Structures & Covariance Models



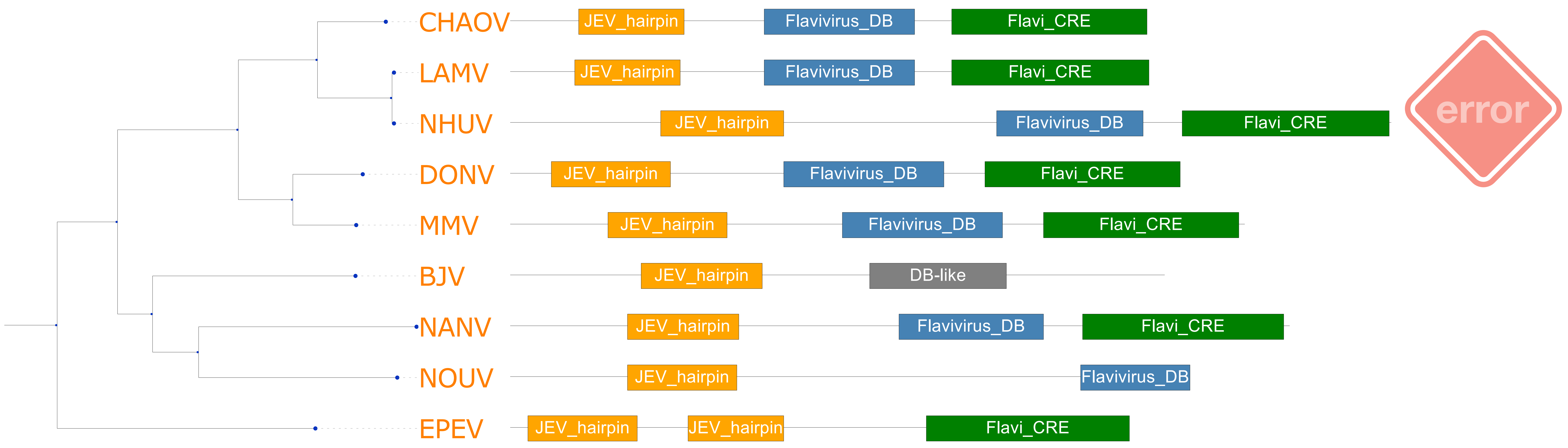
Tick-Borne Flaviviruses



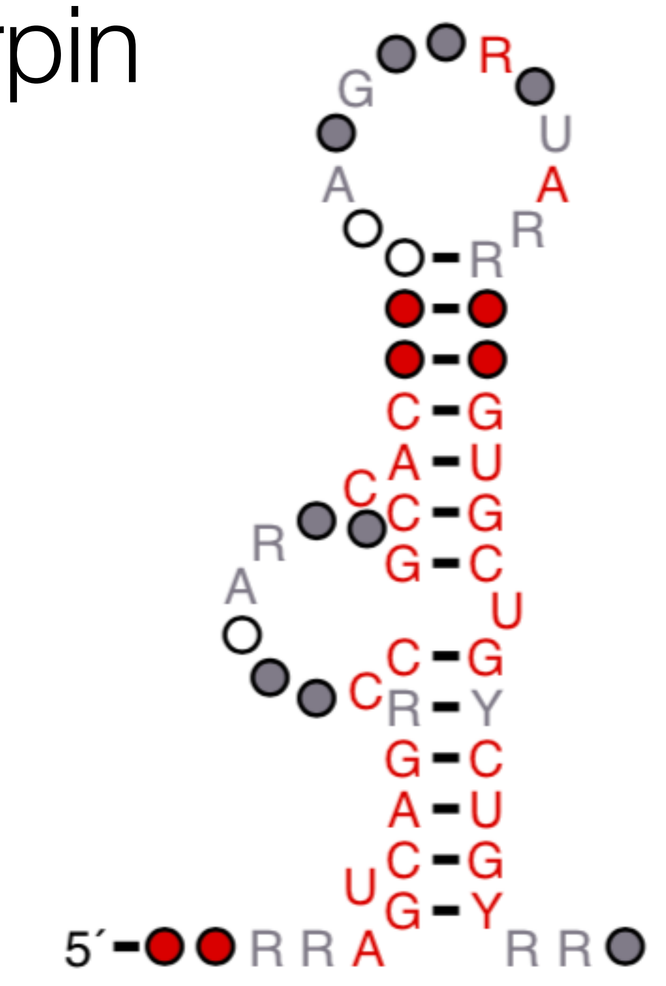


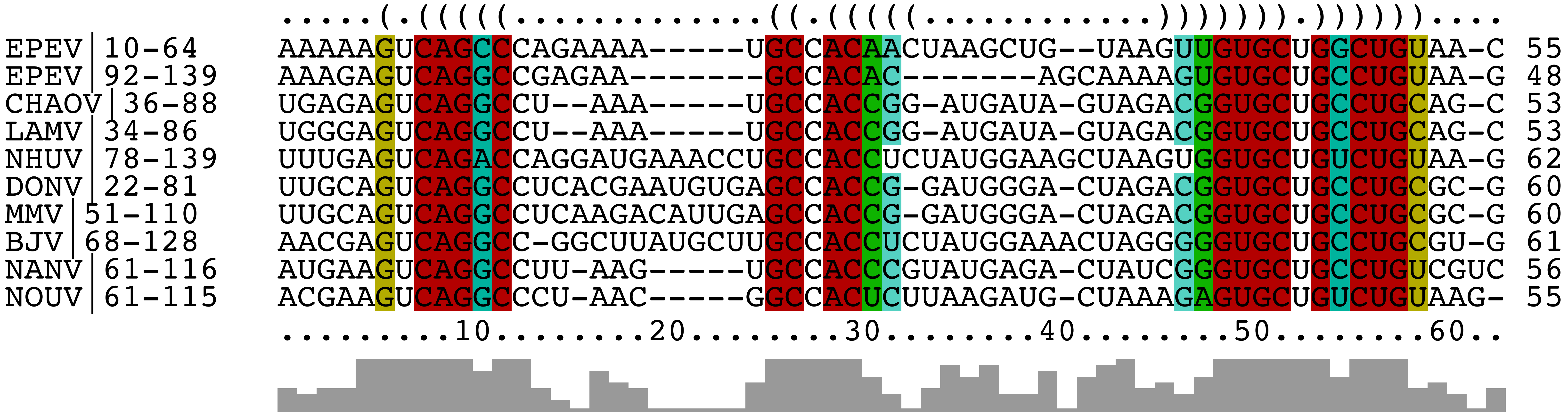


Dual-Host Affiliated Insect-Specific FVs

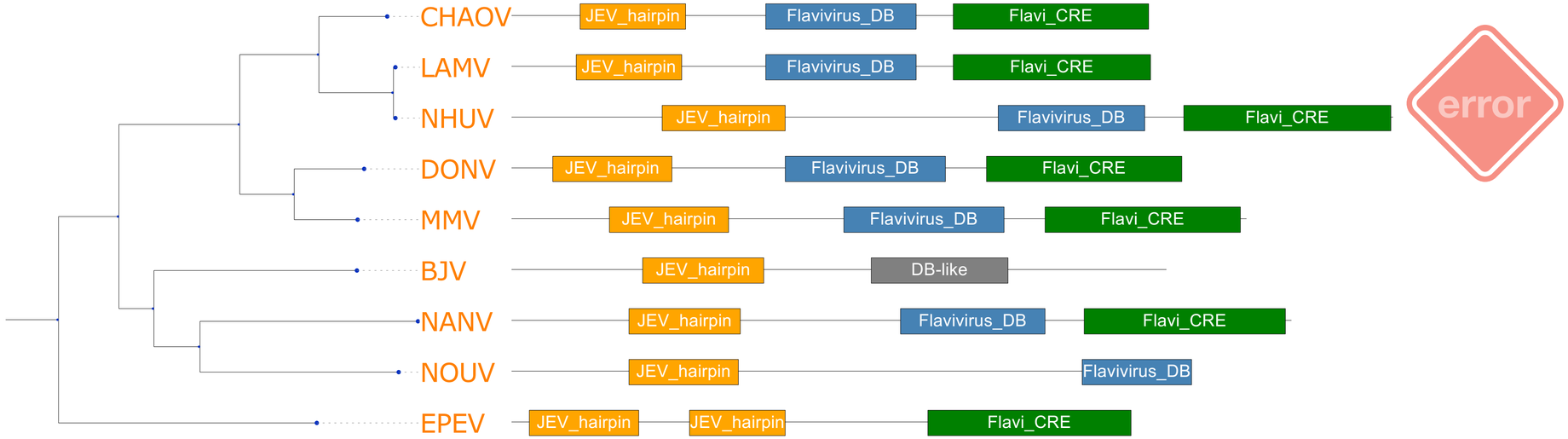


JEV_hairpin

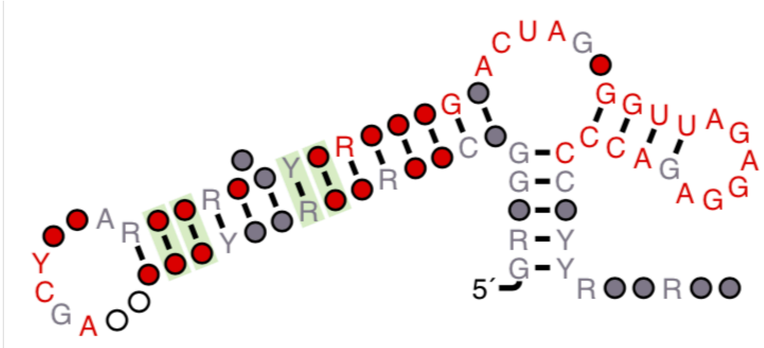


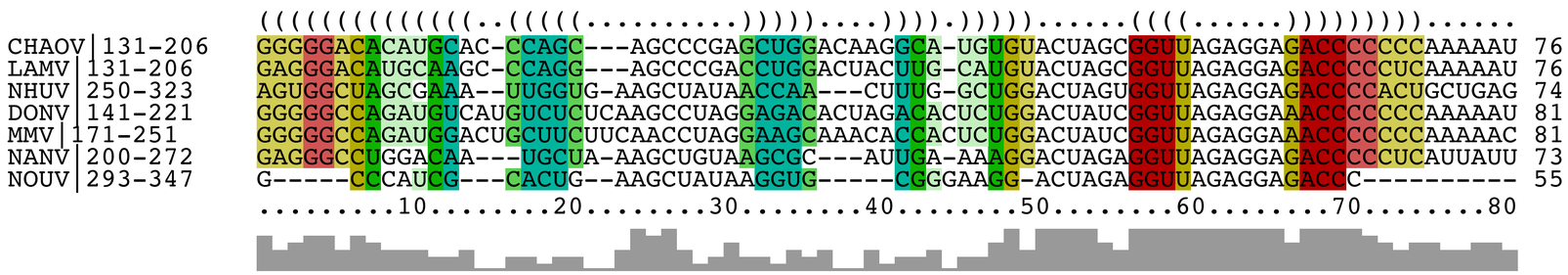


Dual-Host Affiliated Insect-Specific FVs

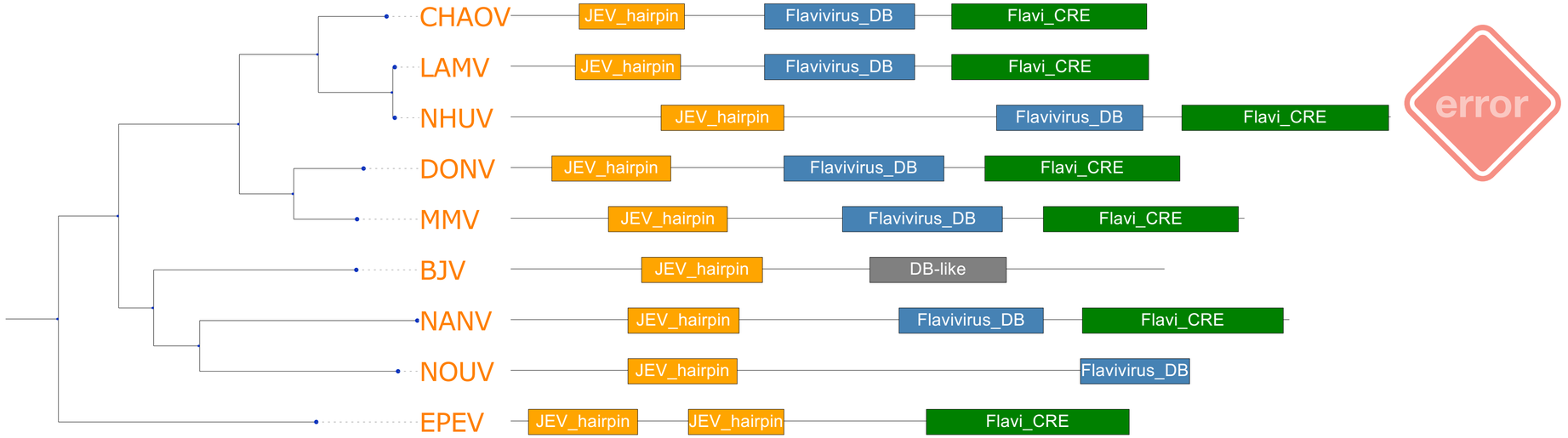


Flavivirus_DB

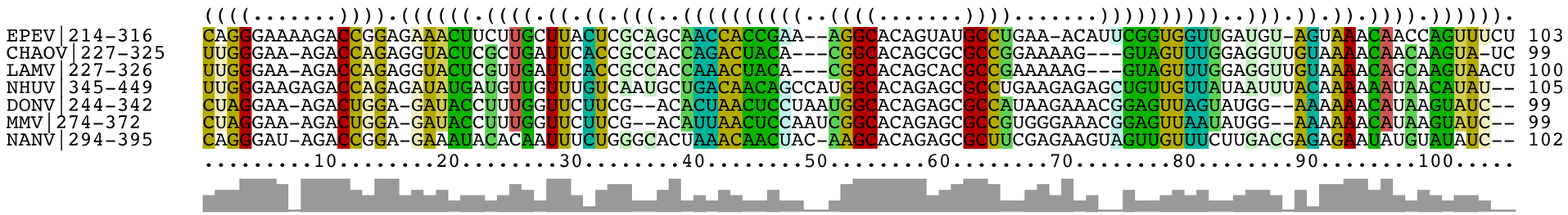


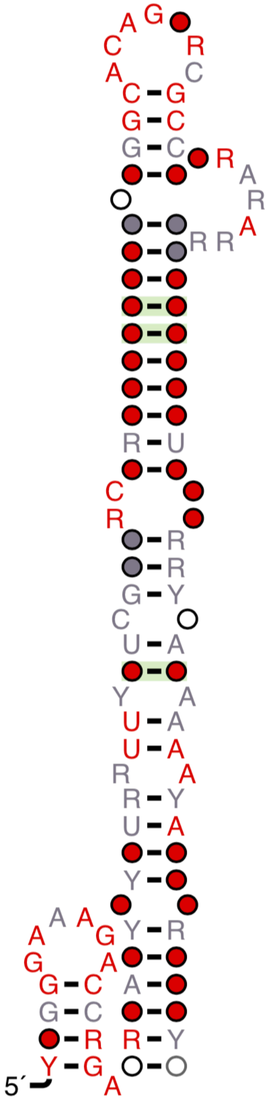


Dual-Host Affiliated Insect-Specific FVs

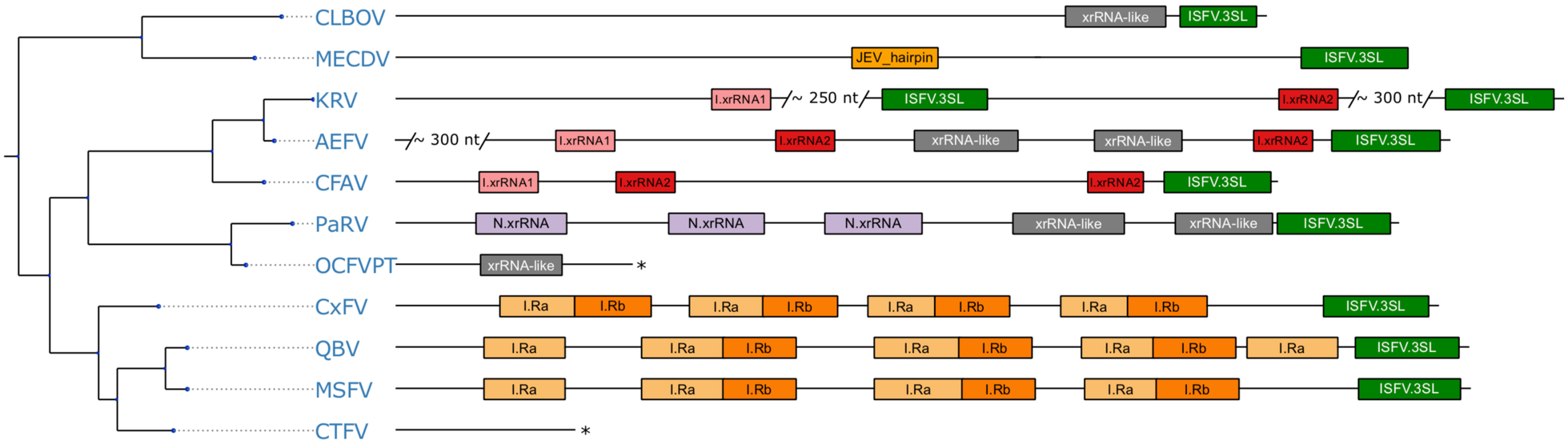


Flavi_CRE

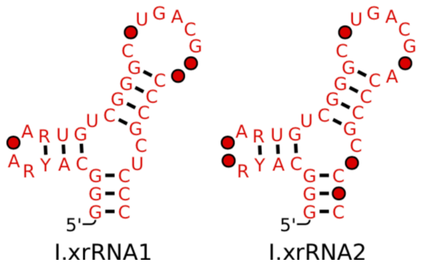


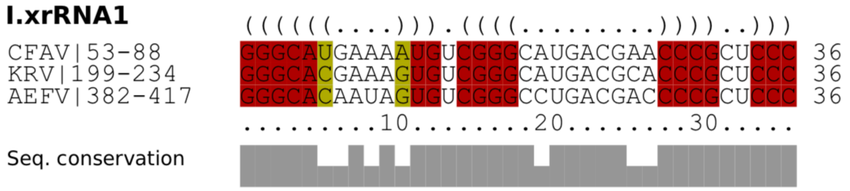


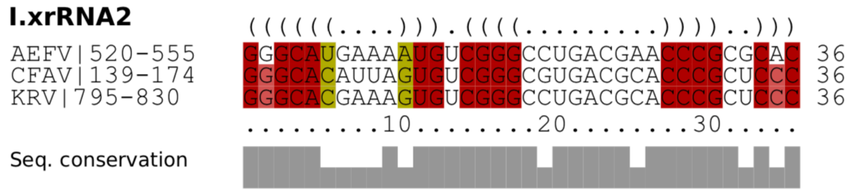
Classic Insect-Specific FVs



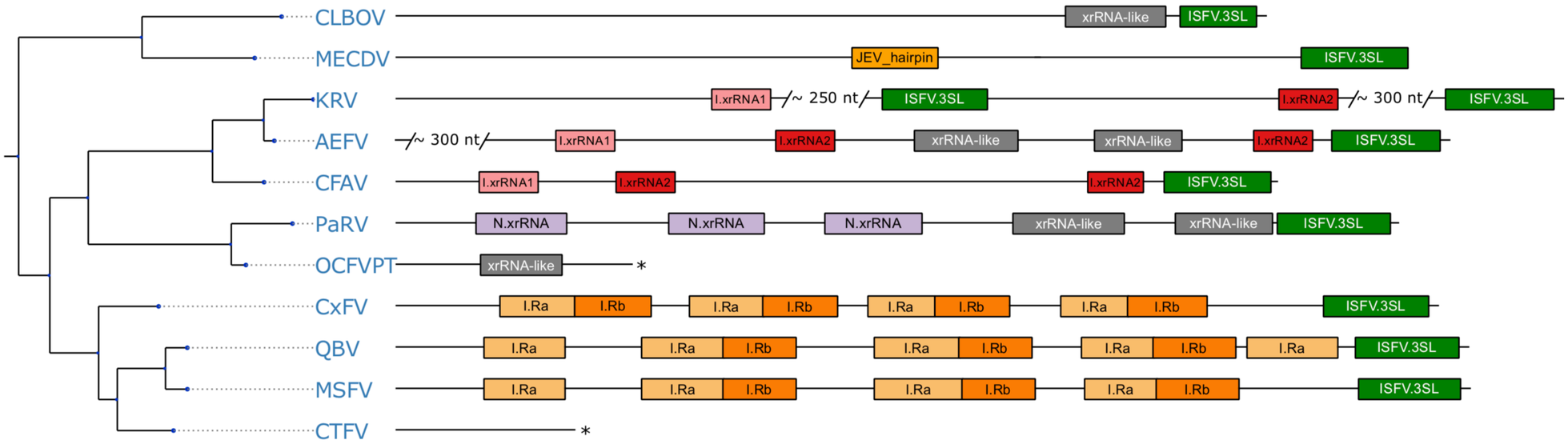
KRV / AEFV / CFAV



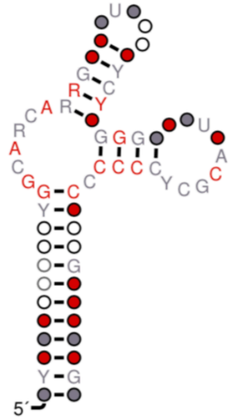


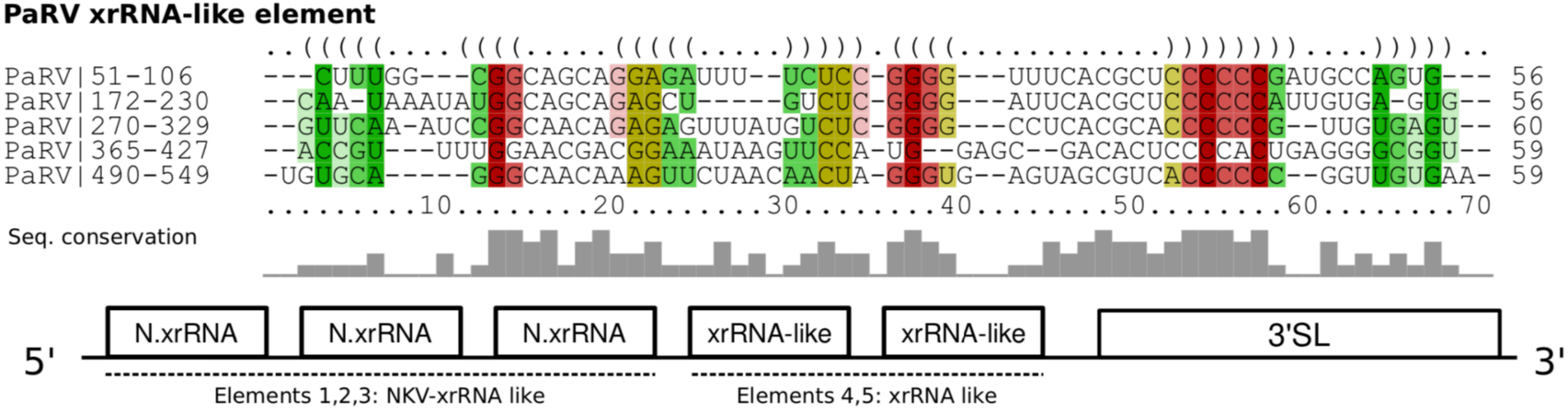


Classic Insect-Specific FVs

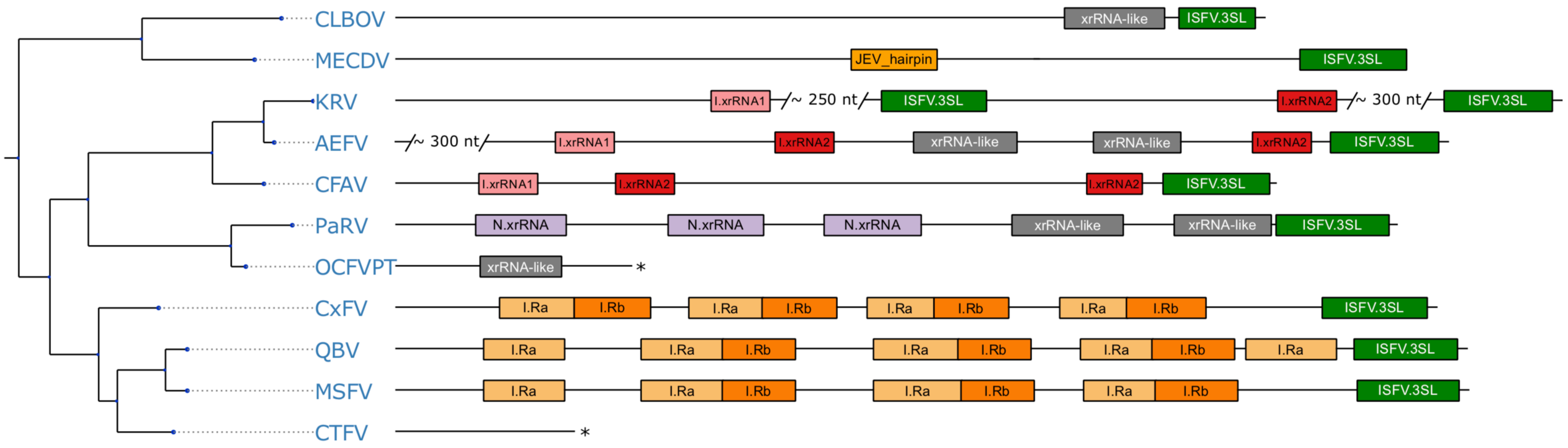


PaRV

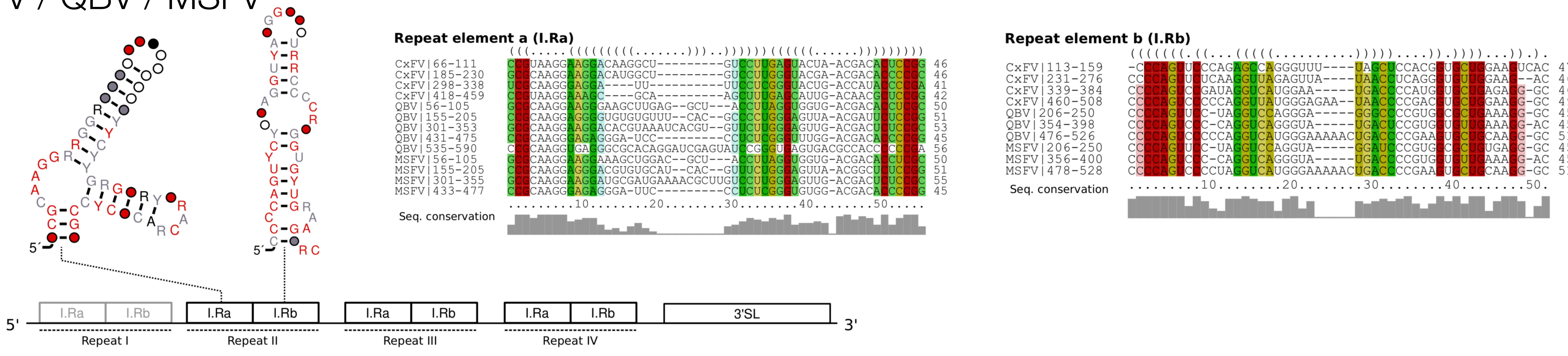




Classic Insect-Specific FVs



CxFV / QBV / MSFV

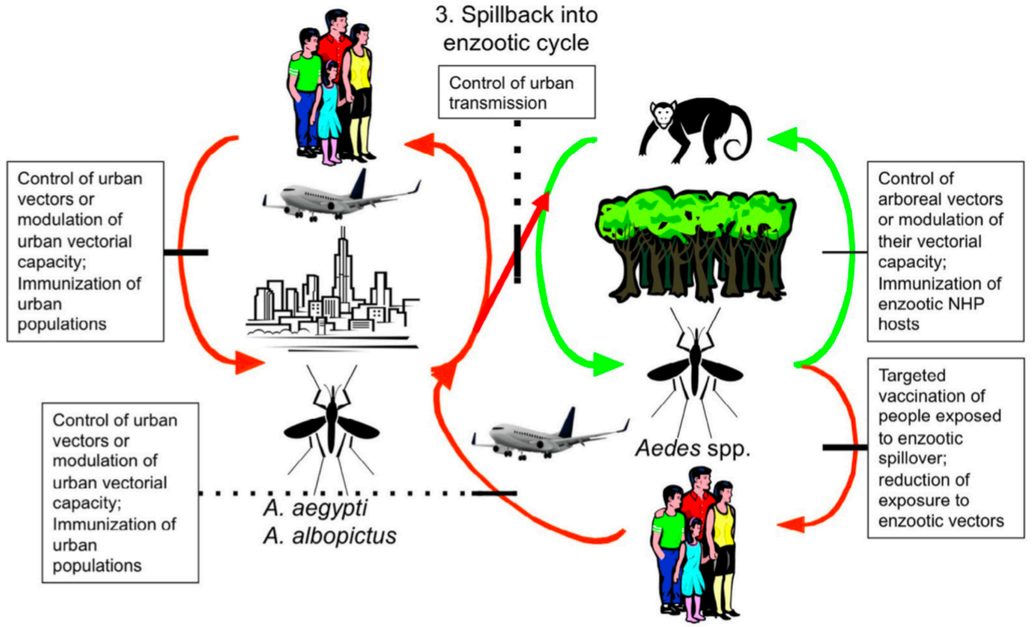


Part II:

Conserved RNA structures in alphaviruses

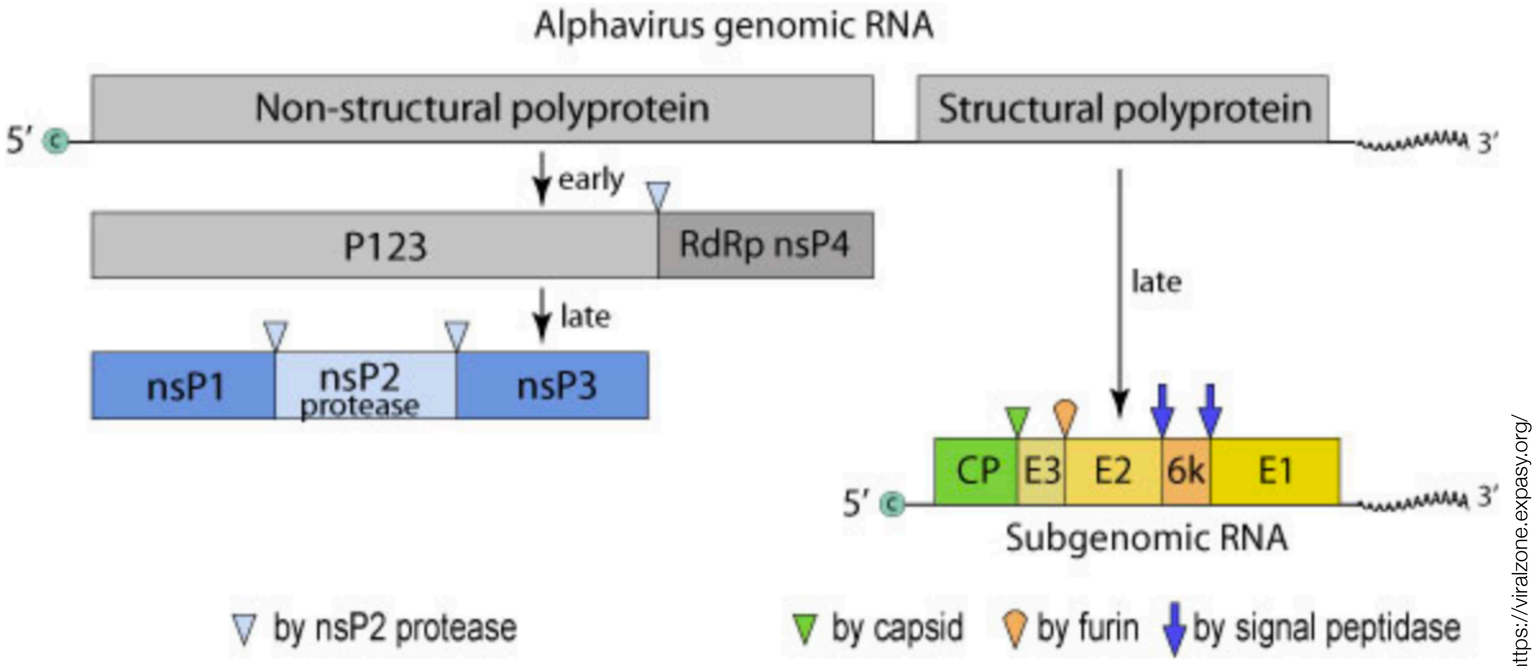
Chikungunya Virus (CHIKV)

- Family *Togaviridae* / genus *Alphavirus*; mosquito-borne (*Aedes* spp.)
- Single-stranded (+) sense RNA virus
- Chikungunya fever: febrile illness, arthralgia, rash, rarely causes hemorrhagic complications
- Enzootic in tropical and subtropical regions of Africa
- First outbreak described 1952 in Tanzania
- No vaccine available



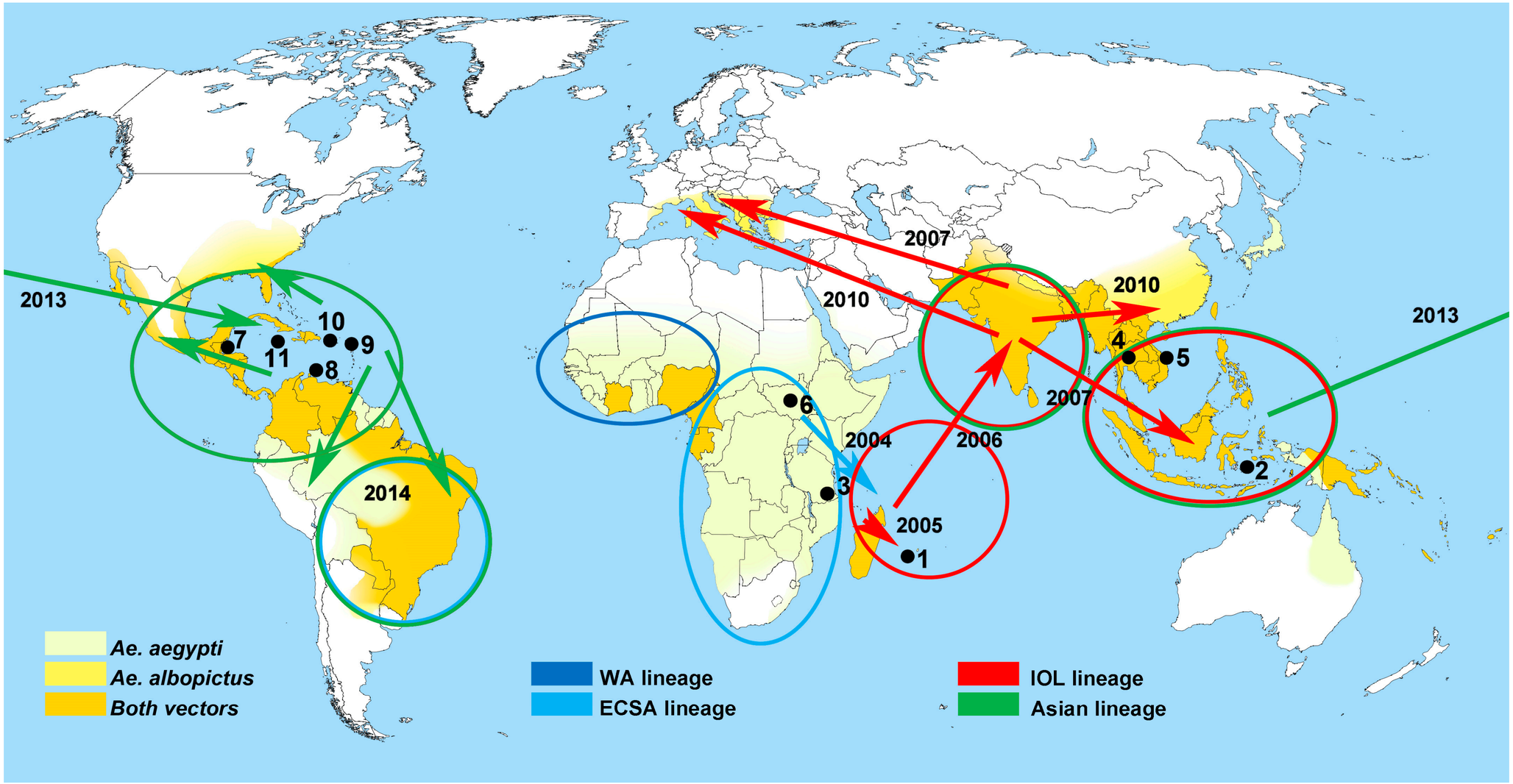
Weaver and Forrester, Antivir. Res. (2015)

Alphavirus Genome Organization



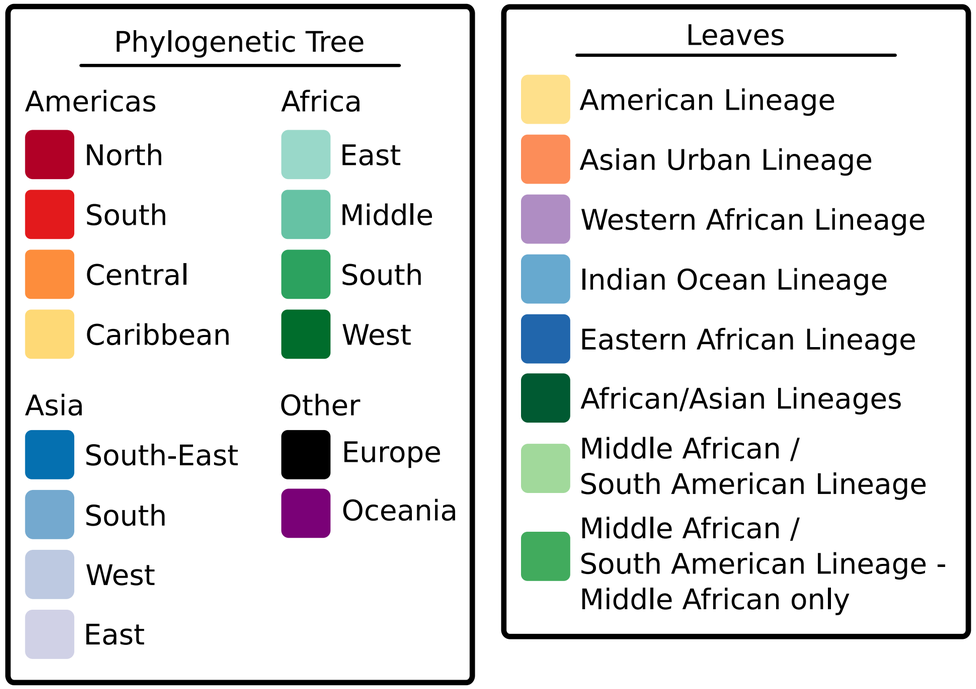
- Non-segmented, single-stranded, (+)-sense RNA genomes of 11-12kB length
- Capped and polyadenylated
- AV genomes appear to host cells as mRNA for immediate translation upon entry into the cytoplasm

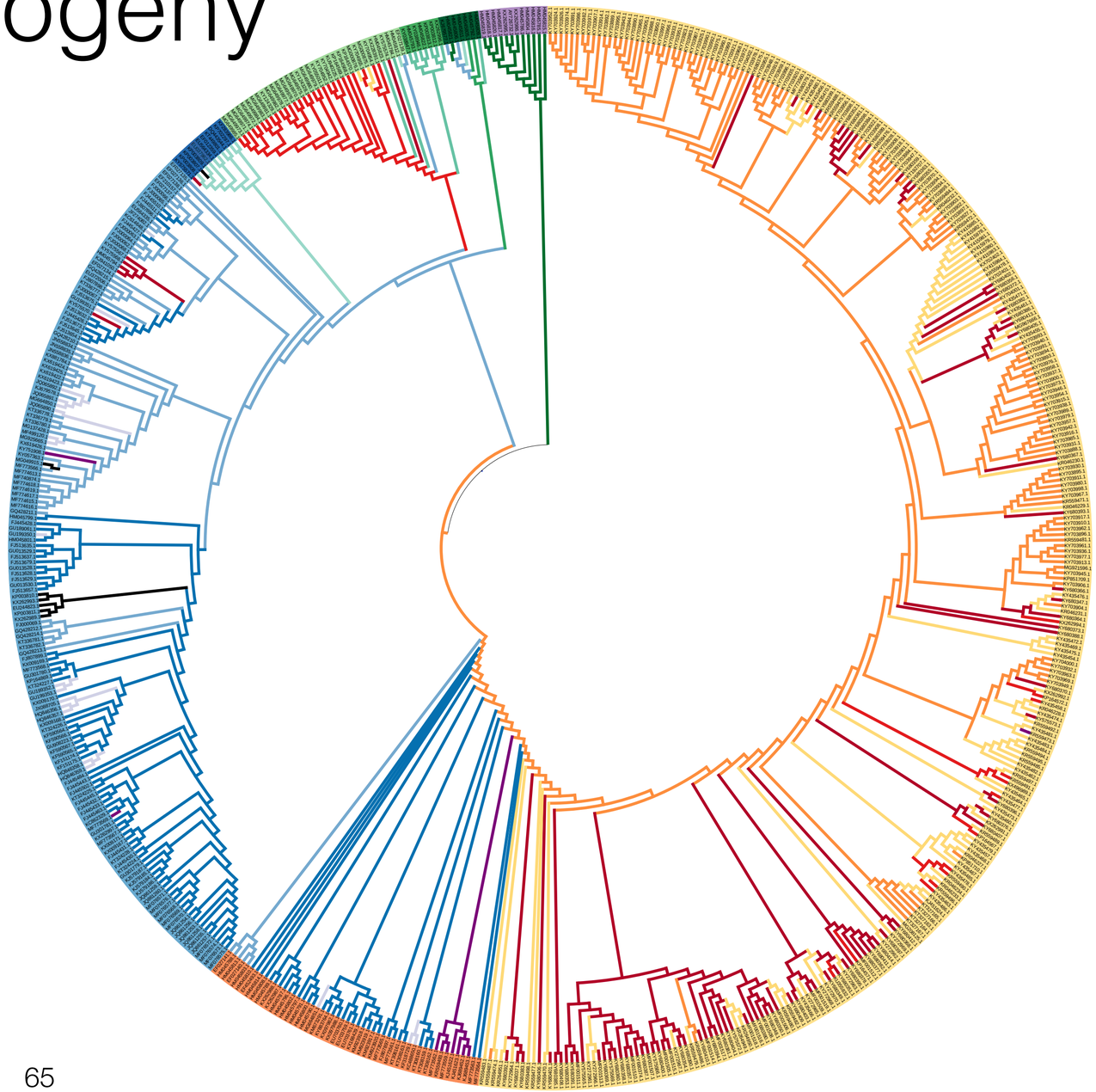
CHIKV Epidemic Spread



Frickmann et al., Viruses (2019)

Updated CHIKV Phylogeny





- 590 CHIKV genomes
- iq-tree / SH-aLRT

CHIKV 3’UTR

- 110 full length CHIKV 3’UTRs
- Variable length (510nt — 930nt)
- Structural alignments + Covariance models
- Thermodynamic modelling based on ViennaRNA Package

CHIKV 3’UTR: Conserved RNA Structures

Conserved Sequence Element (CSE)

```
                                 (((((((((.........)))))))))
KF318729.1/688-714   GAGAUGUUAUUUUGUUUUUAAUAUUUC 27
KT327163.2/862-888   GAGAUGUUAUUUUGUUUUUAAUAUUUC 27
JF274082.1/472-498   GAGAUGUUAUUUUGUUUUUAAUAUUUC 27
KY038946.1/472-498   GAGAUGUUAUUUUGUUUUUAAUAUUUC 27
AY726732.1/540-566   GAGAUGUUAUUUUGUUUUUAAUAUUUC 27
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                     .........10........20......
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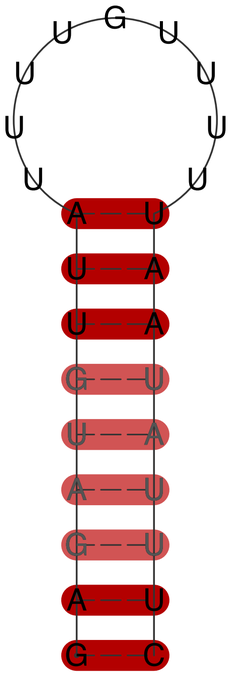
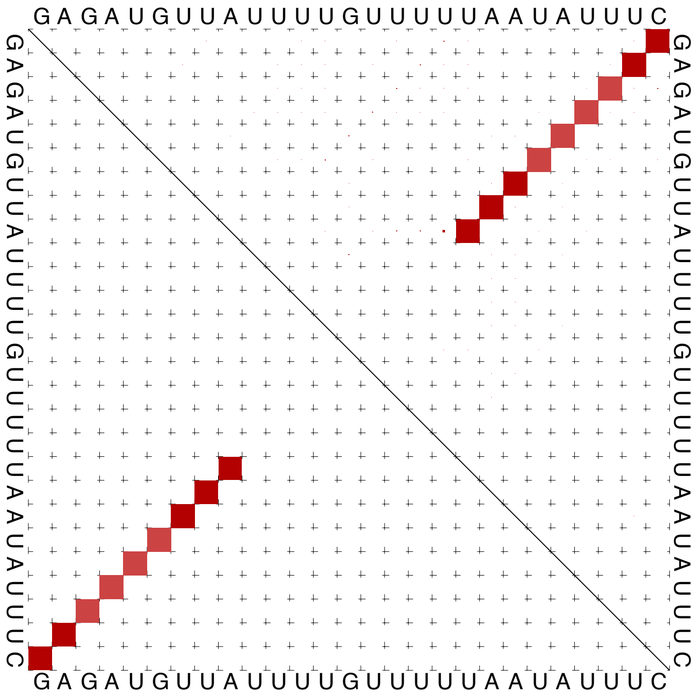
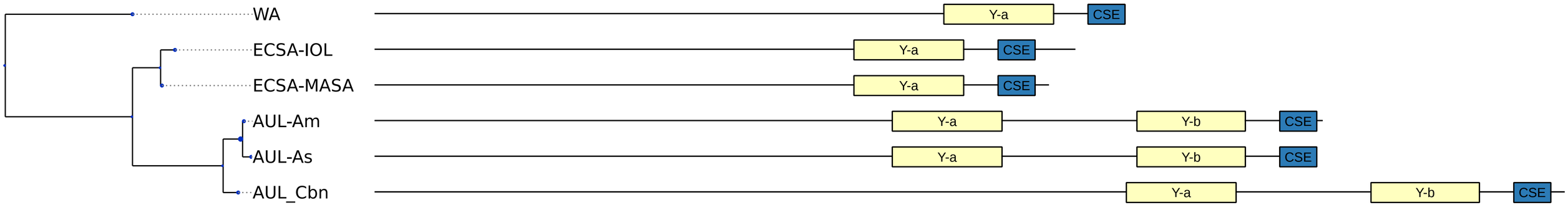
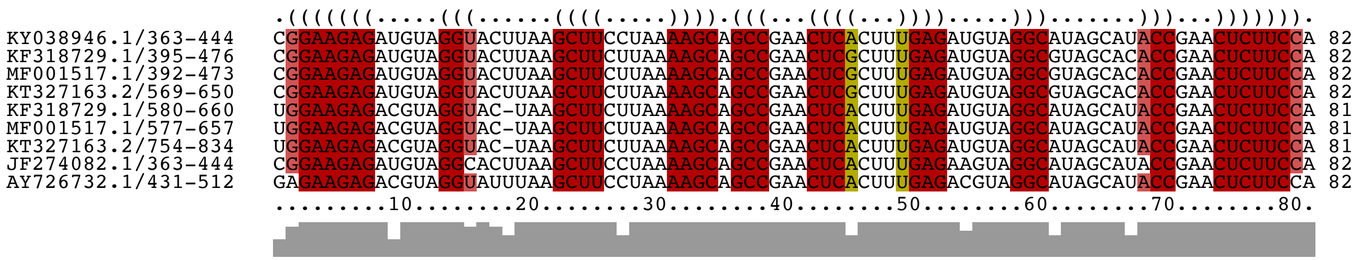


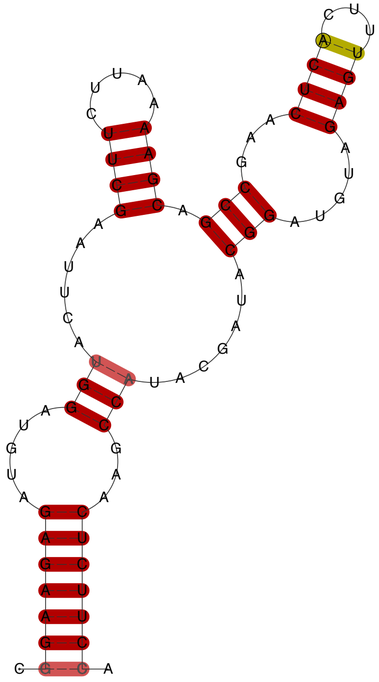


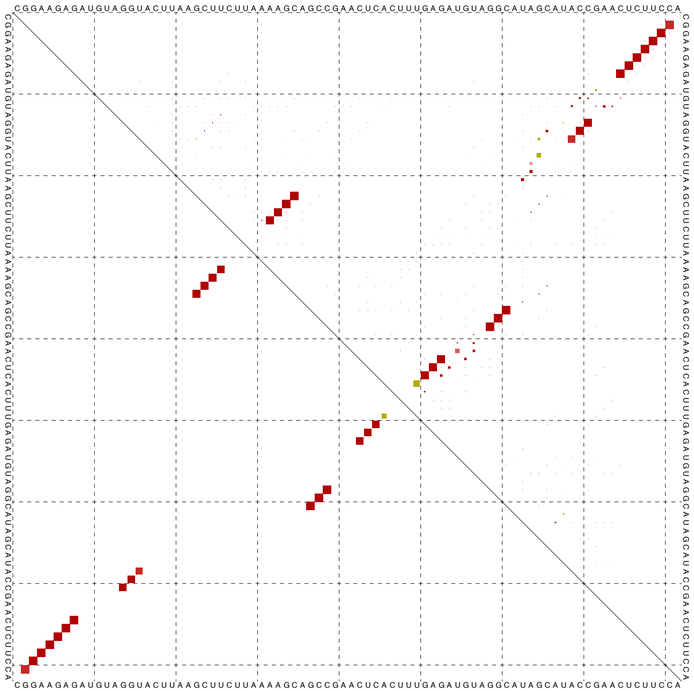
CHIKV 3’UTR: Conserved RNA Structures



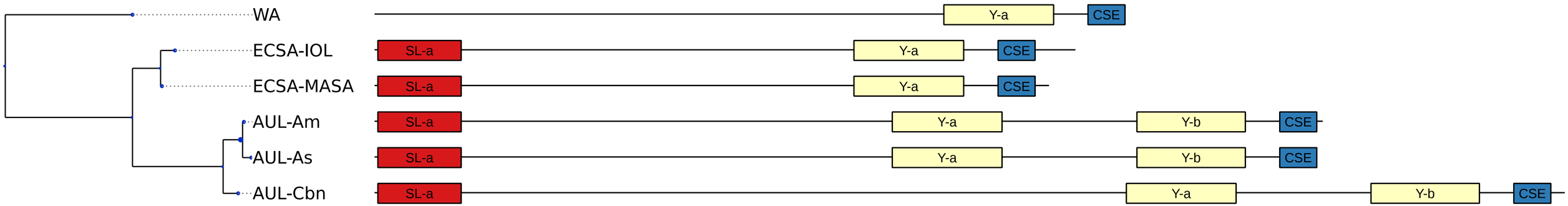
Y-shaped Element (SL-Y)



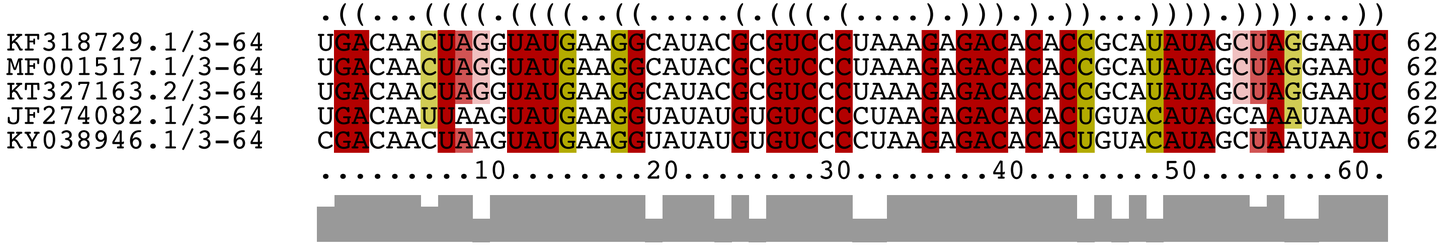


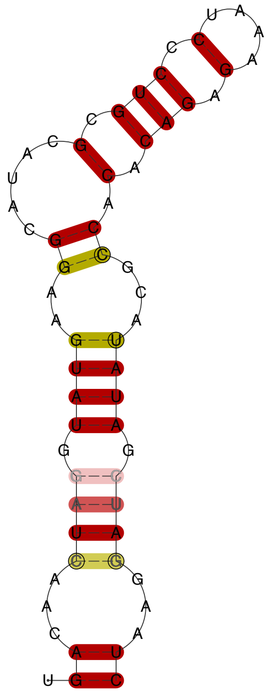


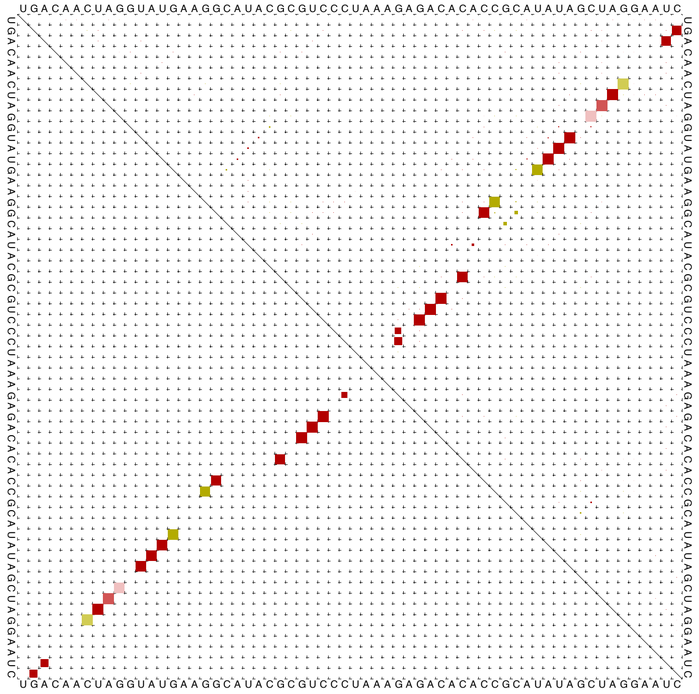
CHIKV 3’UTR: Conserved RNA Structures



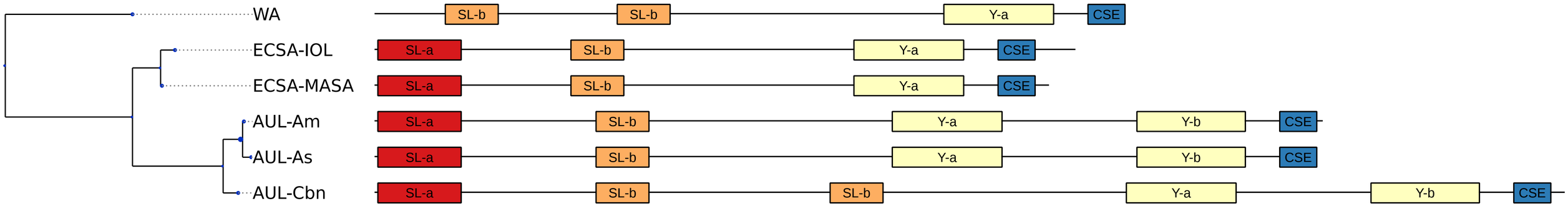
Stem-loop a (SL-a)



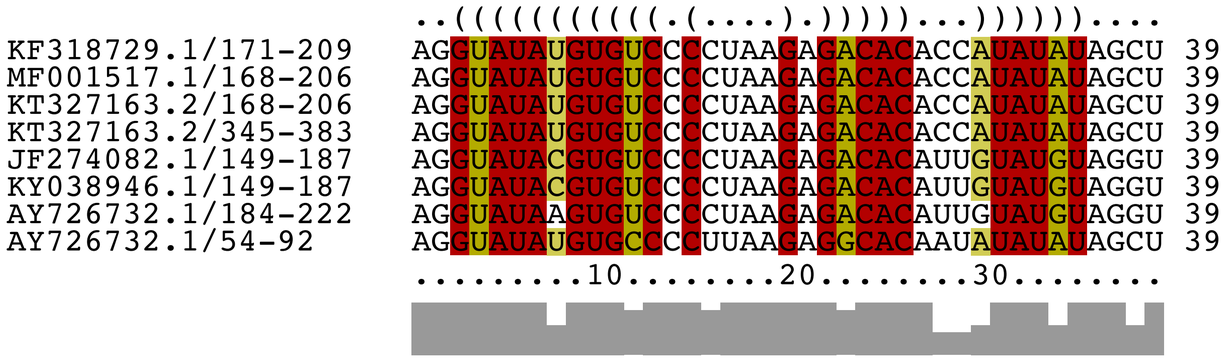


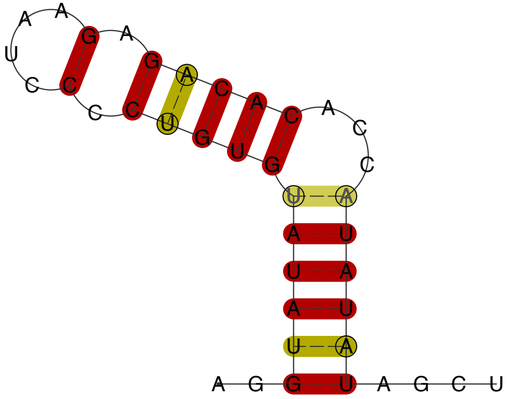


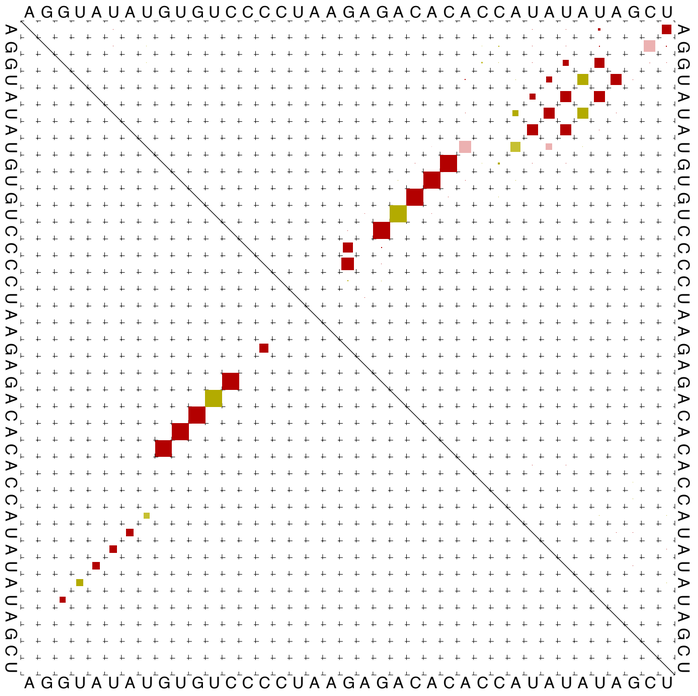
CHIKV 3’UTR: Conserved RNA Structures



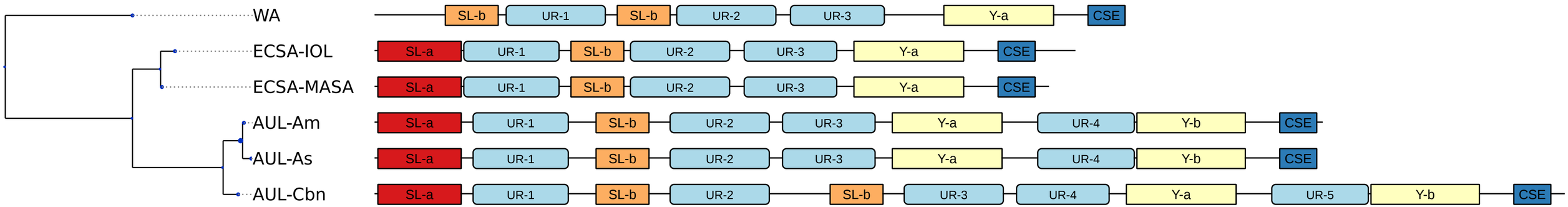
Stem-loop b (SL-b)





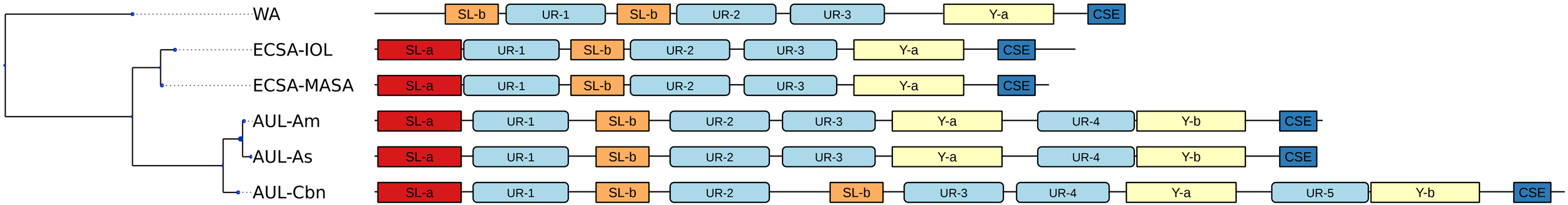


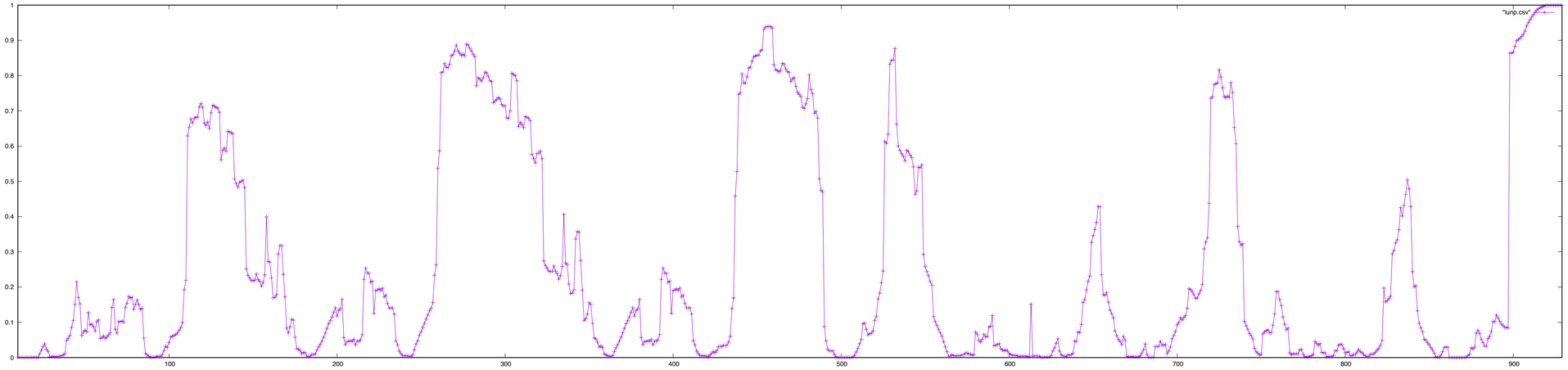
CHIKV 3’UTR: Conserved RNA Elements



Unstructured repeat (UR)

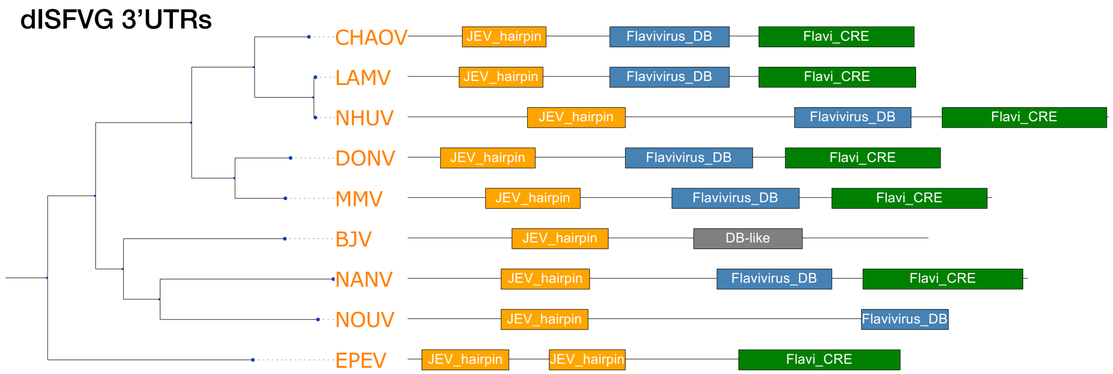
CHIKV-Cbn Accessibility

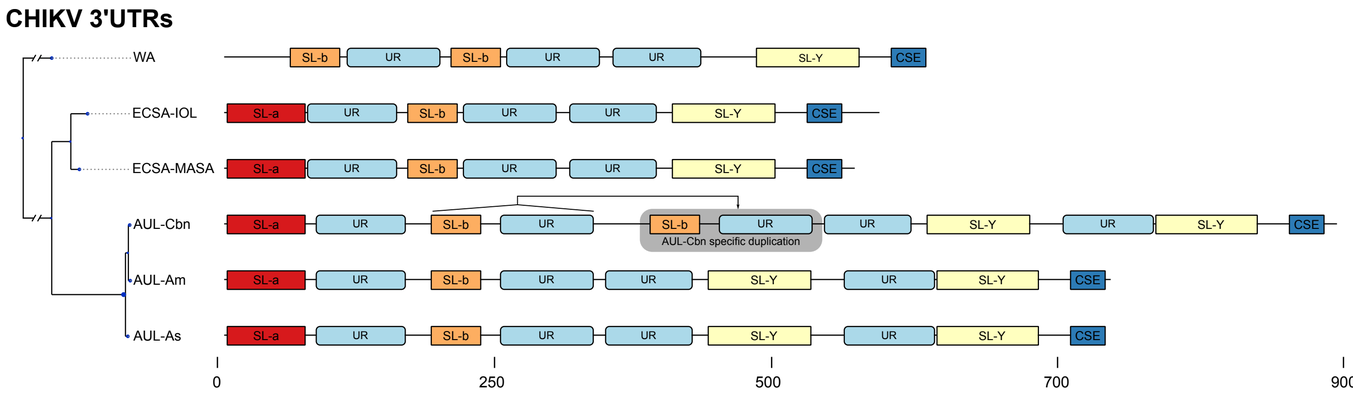




Summary

- Functional RNA structures are evolutionarily conserved
- Structural alignments and covariance models
- Varied architecture of conserved RNAs in flavivirus UTRs
- Lineage-specific non-coding RNA architecture in Alphaviruses
- Tools freely available through ViennaRNA Package





Acknowledgements

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Adriano de Bernardi Schneider

Functional RNA Structures in the 3′ UTR of Tick-Borne, Insect-Specific and No-Known-Vector Flaviviruses. *Viruses* 11.3 (2019): 298. **https://doi.org/10.3390/v11030298**

Updated phylogeny of Chikungunya virus suggests lineage-specific RNA architecture. *Viruses* 11.9 (2019): 798. **https://doi.org/10.3390/v11090798**

Musashi binding elements in Zika and related Flavivirus 3′ UTRs: A comparative study in silico. *Scientific reports* 9.1 (2019): 6911. **https://doi.org/10.1038/s41598-019-43390-5**

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