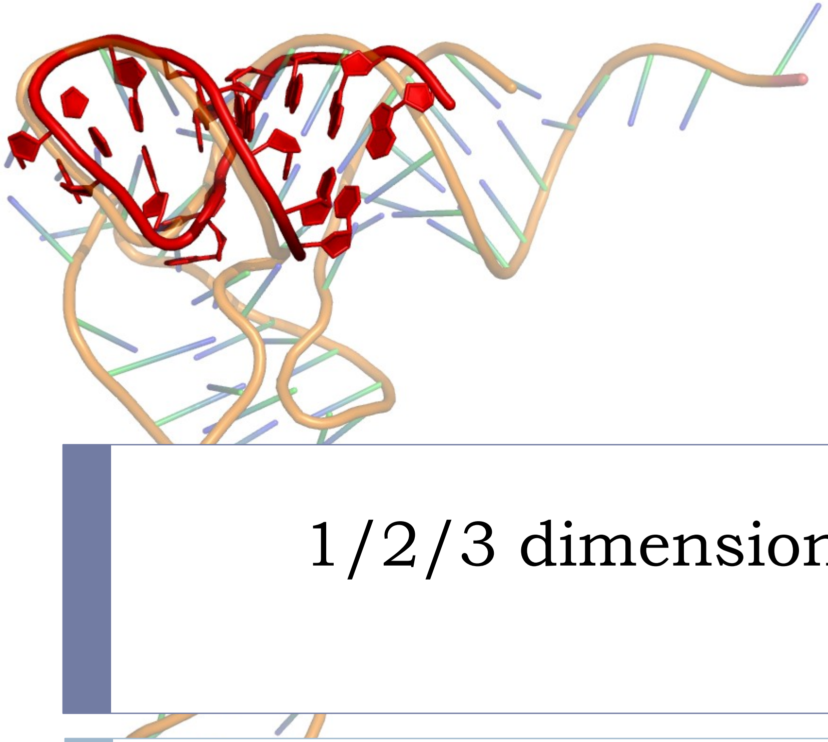
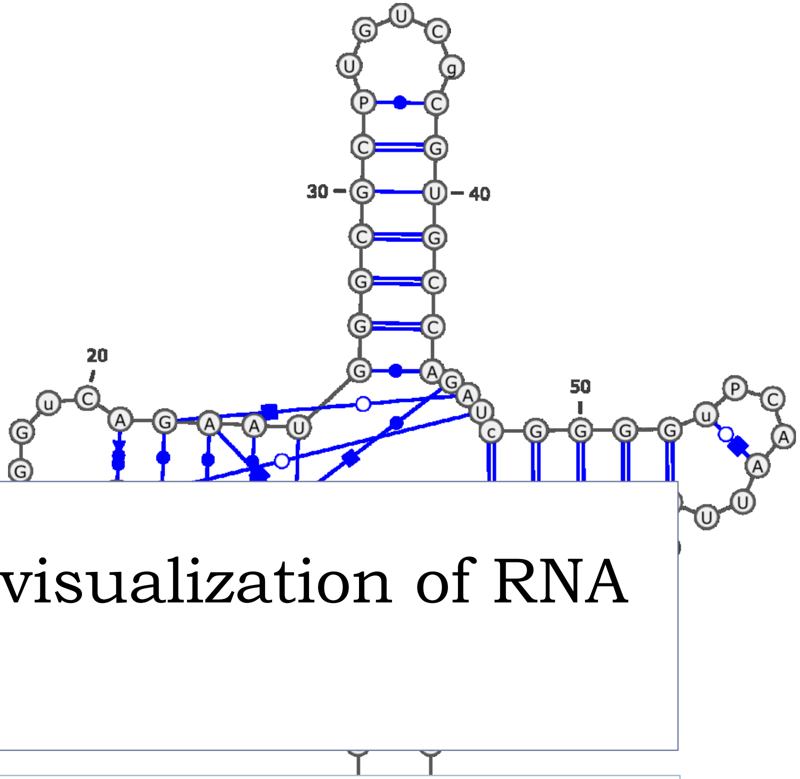




1/2/3 dimensional visualization of RNA

Yann Ponty (VARNA), CNRS/Ecole Polytechnique, France
Jim Procter (JalView), University of Dundee, UK

Goals

- To help your survive the *RNA data jungle*.
- To conceptually and practically connect the three levels of RNA structural information.
- To introduce mature prediction and annotation tools.
- To illustrate the structure-informed curation RNA alignments.
- To keep this fun and interactive.

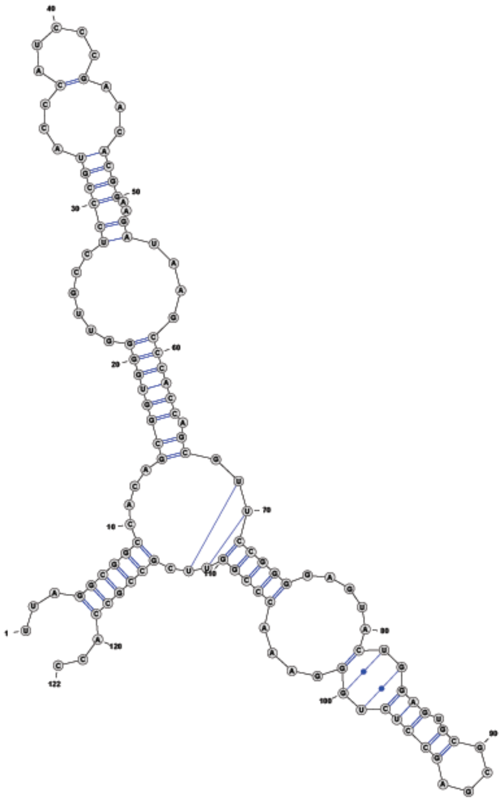
Schedule (French)

When?	What?
9:30	Introduction
9:45	First session: Databases, 2D structure prediction tools, 3D annotations tools, hands on.
10:30	Interactive coffee break
10:45	Second session: Ensemble approaches, comparative methods, further refinement of alignments, assessment.
12:30	Discussion
13:00	Lunch

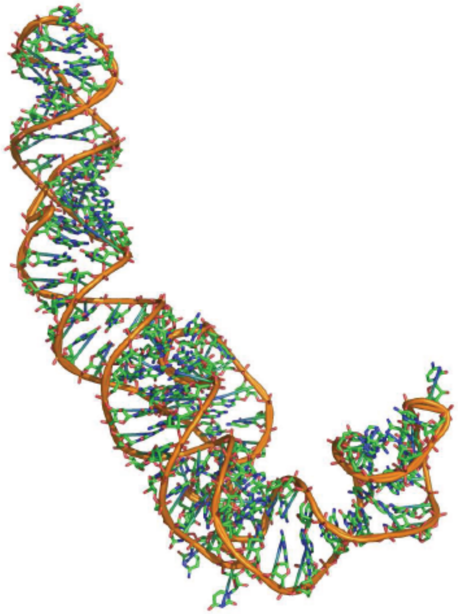
RNA structure(s)

```
UUAGGCGGCCACAGC
GGUGGGGUUGCCUCC
CGUACCCAUCCCGAA
CACGGAAGAUAAGCC
CACCAGCGUUCCGGG
GAGUACUGGAGUGCG
CGAGCCUCUGGGAAA
CCCGGUUCGCCGCCA
CC
```

Primary structure



Secondary structure

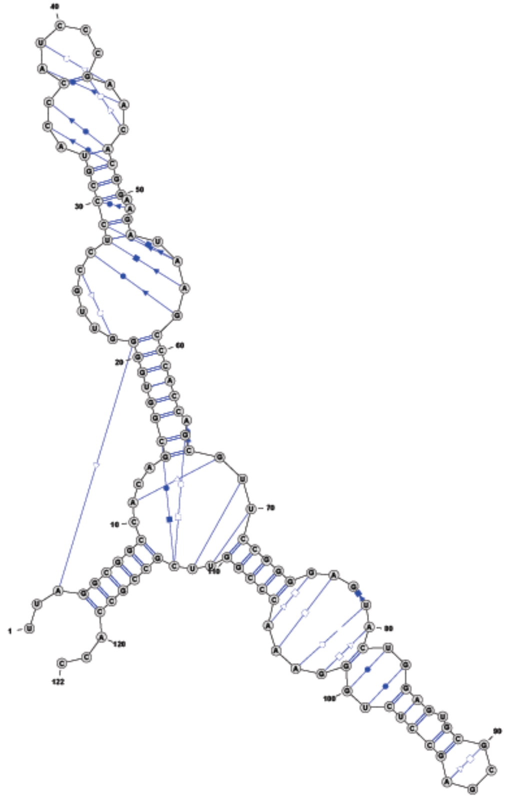


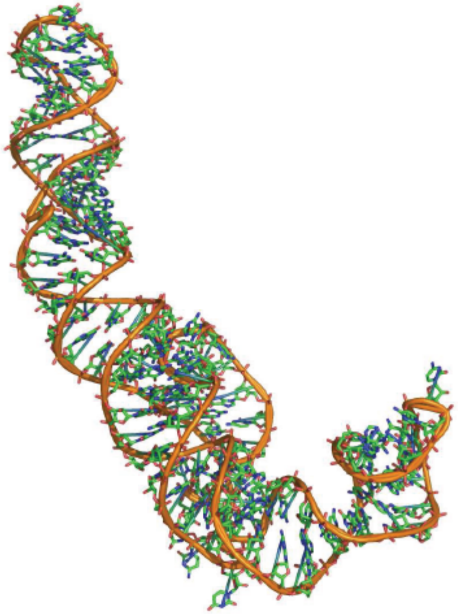
Tertiary structure

Source: 5s rRNA (PDBID: 1K73:B)

RNA structure(s)

UUAGGCGGCCACAGC
GGUGGGGUUGCCUCC
CGUACCCAUCCCGAA
CACGGAAGAUAAGCC
CACCAGCGUUCCGGG
GAGUACUGGAGUGCG
CGAGCCUCUGGGAAA
CCCGGUUCGCCGCCA
CC





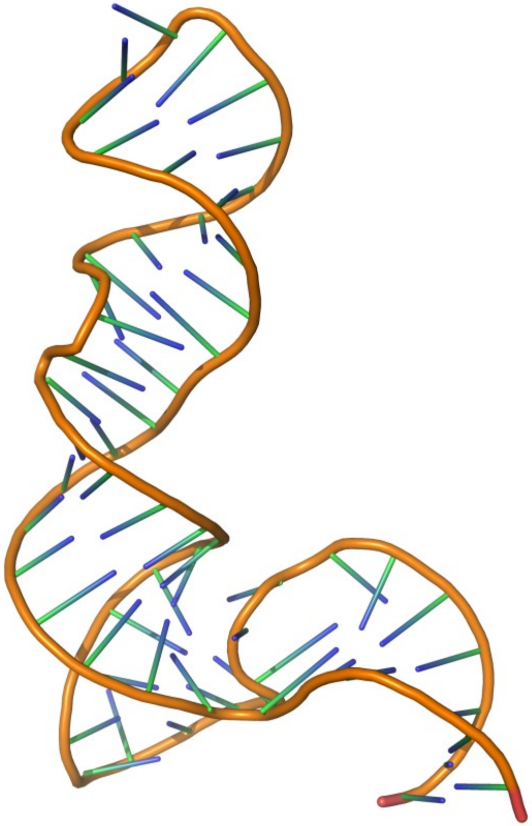
Primary structure

Secondary^{+} structure

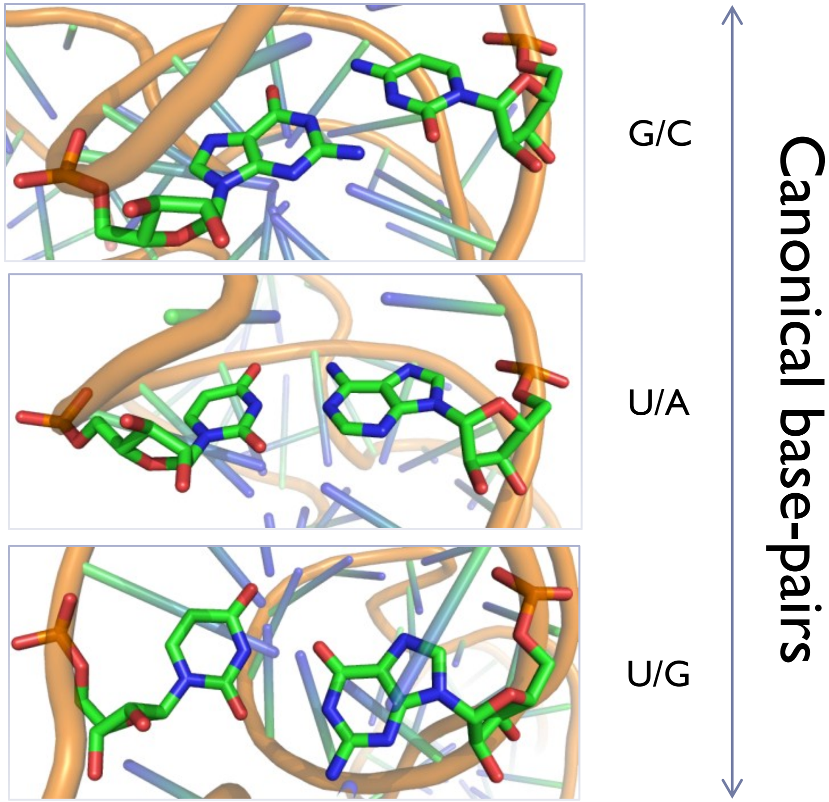
Tertiary structure

Source: 5s rRNA (PDBID: 1K73:B)

How RNA folds



5s rRNA (PDB ID: 1UN6)

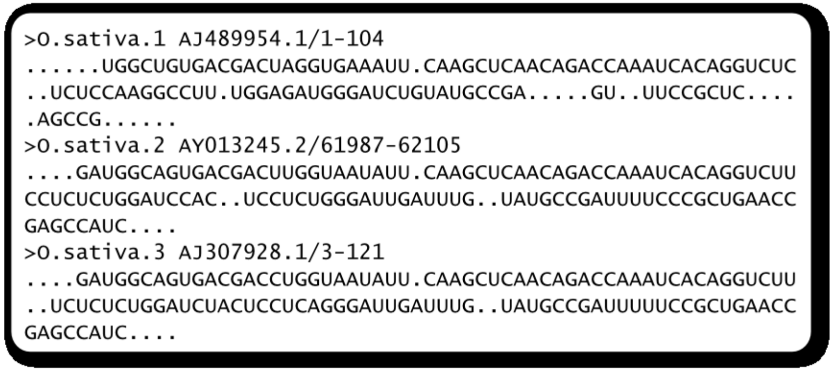


RNA folding = Hierarchical stochastic process driven by/resulting in the pairing (hydrogen bonds) of a subset of its bases.

Sources of RNA data

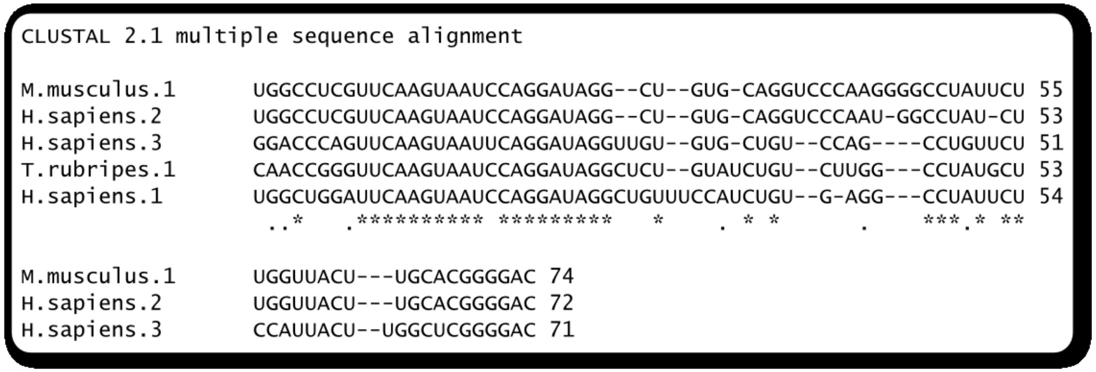
Name	Data type	Scope	Description	File formats	#Entries	URL
PDB	All-atoms	General	RCSB Protein Data Bank – Global repository for 3D molecular models	PDB	~1,900 models	http://www.pdb.org
NDB	All-atoms, Secondary structures	General	Nucleic Acids Database – Nucleic acids models and structural annotations.	PDB, RNAML	~2,000 models	http://bit.ly/rna-ndb
RFAM	Alignments, Secondary structures[3]	General	RNA FAMilies – Multiple alignments of RNA as functional families. Features consensus secondary structures, either predicted and/or manually curated.	STOCKHOLM, FASTA	~1,973 Alignments/ structures, 2,756,313 sequences	http://bit.ly/rfam-db
STRAND	Secondary structures	General	The RNA secondary STRucture and statistical ANalysis Database – Curated aggregation of several databases	CT, BPSEQ, RNAML, FASTA, Vienna	4,666 structures	http://bit.ly/sstrand
PseudoBase	Secondary structures	Pseudoknotted RNAs	PseudoBase – Secondary structure of known pseudonotted RNAs.	Extended Vienna RNA	359 structures	http://bit.ly/pkbase
CRW	Sequence alignments, Secondary structures	Ribosomal RNAs, Introns	Comparative RNA Web Site – Manually curated alignments and statistics of ribosomal RNAs.	FASTA, ALN, BPSEQ	1,109 structures, 91,877 sequences	http://bit.ly/crw-rna

RNA file formats: Sequences (alignments)



```
>O.sativa.1 AJ489954.1/1-104
......UGGCUGUGACGACUAGGUGAAAUU.CAAGCUCAACAGACCAAAUCACAGGUCUC
..UCUCCAAGGCCUU.UGGAGAUGGGAUCUGUAUGCCGA.....GU..UUCCGCUC....
.AGCCG......
>O.sativa.2 AY013245.2/61987-62105
....GAUGGCAGUGACGACUUGGUAAUAUU.CAAGCUCAACAGACCAAAUCACAGGUCUU
CCUCUCUGGAUCCAC..UCCUCUGGGAUUGAUUUG..UAUGCCGAUUUUCCCGCUGAACC
GAGCCAUC....
>O.sativa.3 AJ307928.1/3-121
....GAUGGCAGUGACGACCUGGUAAUAUU.CAAGCUCAACAGACCAAAUCACAGGUCUU
..UCUCUCUGGAUCUACUCCUCAGGGAUUGAUUUG..UAUGCCGAUUUUUCCGCUGAACC
GAGCCAUC....
```

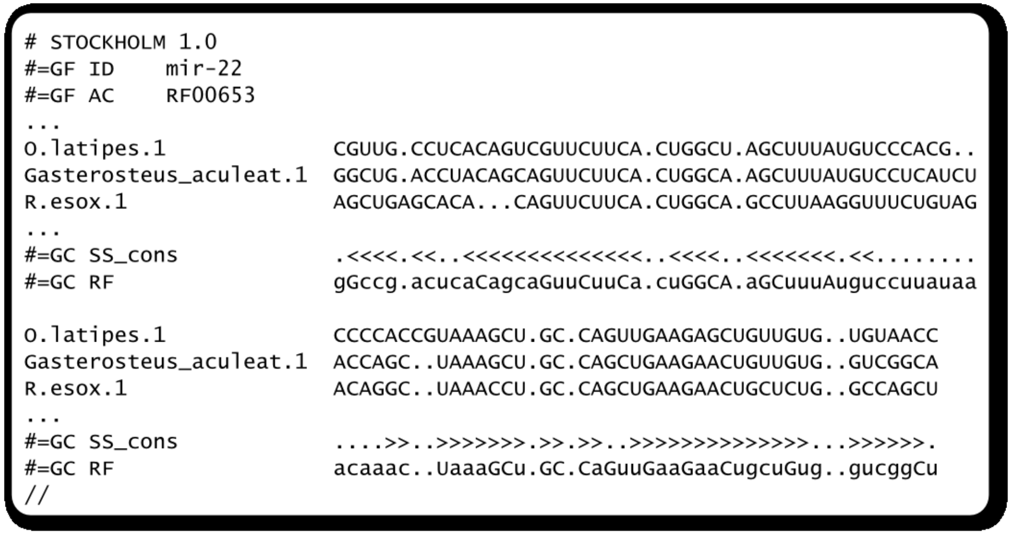
Fasta



```
CLUSTAL 2.1 multiple sequence alignment

M.musculus.1      UGGCCUCGUUCAAGUAAUCCAGGAUAGG--CU--GUG-CAGGUCCCAAGGGGCCUAUUCU 55
H.sapiens.2       UGGCCUCGUUCAAGUAAUCCAGGAUAGG--CU--GUG-CAGGUCCCAAU-GGCCUAU-CU 53
H.sapiens.3       GGACCCAGUUCAAGUAAUUCAGGAUAGGUUGU--GUG-CUGU--CCAG----CCUGUUCU 51
T.rubripes.1      CAACCGGGUUCAAGUAAUCCAGGAUAGGCUCU--GUAUCUGU--CUUGG---CCUAUGCU 53
H.sapiens.1       UGGCUGGAUUCAAGUAAUCCAGGAUAGGCUGUUUCCAUCUGU--G-AGG---CCUAUUCU 54
                   ..*   .********** *********   *    . * *      .    ***.* **

M.musculus.1      UGGUUACU---UGCACGGGGAC 74
H.sapiens.2       UGGUUACU---UGCACGGGGAC 72
H.sapiens.3       CCAUUACU--UGGCUCGGGGAC 71
```

Aln

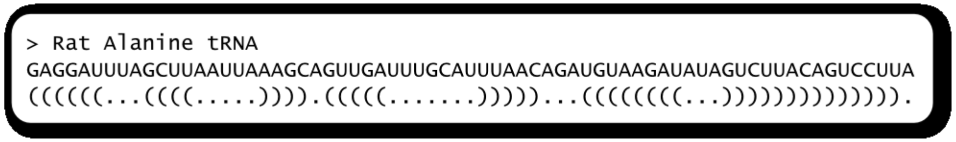
RNA file formats: Sequences (alignments)



```
# STOCKHOLM 1.0
#=GF ID    mir-22
#=GF AC    RF00653
...
O.latipes.1              CGUUG.CCUCACAGUCGUUCUUCA.CUGGCU.AGCUUUAUGUCCCACG..
Gasterosteus_aculeat.1  GGCUG.ACCUACAGCAGUUCUUCA.CUGGCA.AGCUUUAUGUCCUCAUCU
R.esox.1                 AGCUGAGCACA...CAGUUCUUCA.CUGGCA.GCCUUAAGGUUUCUGUAG
...
#=GC SS_cons             .<<<<.<<..<<<<<<<<<<<<<<..<<<<..<<<<<<<.<<........
#=GC RF                  gGccg.acucaCagcaGuuCuuCa.cuGGCA.aGCuuuAuguccuuauaa

O.latipes.1              CCCCACCGUAAAGCU.GC.CAGUUGAAGAGCUGUUGUG..UGUAACC
Gasterosteus_aculeat.1  ACCAGC..UAAAGCU.GC.CAGCUGAAGAACUGUUGUG..GUCGGCA
R.esox.1                 ACAGGC..UAAACCU.GC.CAGCUGAAGAACUGCUCUG..GCCAGCU
...
#=GC SS_cons             ....>>..>>>>>>>.>>.>>..>>>>>>>>>>>>>>...>>>>>>.
#=GC RF                  acaaac..UaaaGCu.GC.CaGuuGaaGaaCugcuGug..gucggCu
//
```

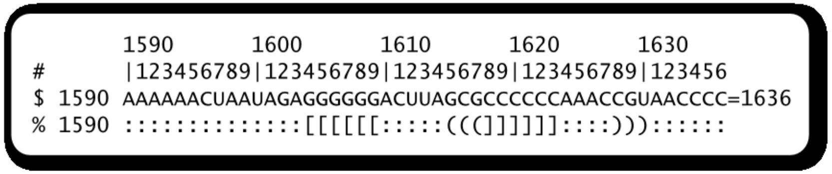
Stockholm (RFAM)

RNA file formats: Secondary Structures



```
> Rat Alanine tRNA
GAGGAUUUAGCUUAAUUAAAGCAGUUGAUUUGCAUUUAACAGAUGUAAGAUAUAGUCUUACAGUCCUUA
((((((...((((.....)))).(((((.......)))))...((((((((...)))))))))))))).
```

Vienna (RNAFold)

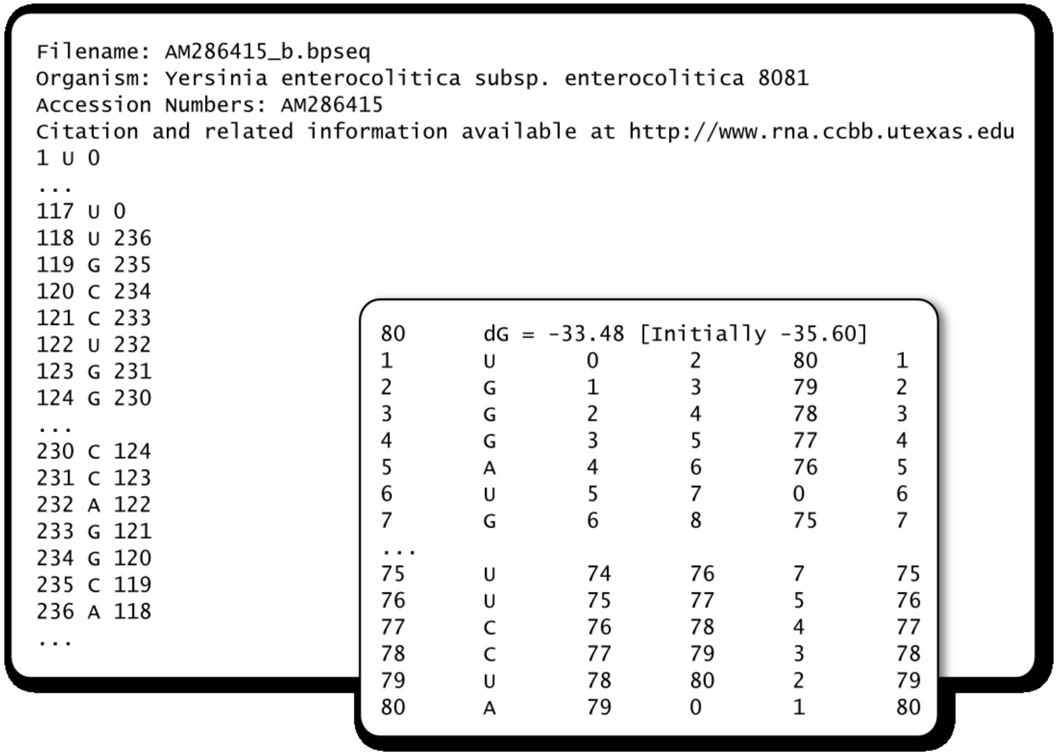


```
         1590      1600      1610      1620      1630
#        |123456789|123456789|123456789|123456789|123456
$ 1590   AAAAAACUAAUAGAGGGGGACUUAGCGCCCCCCAAACCGUAACCCC=1636
% 1590   ::::::::::::::[[[[[[:::::(((]]]]]]::::))):::::: 
```

PseudoBase

RNA file formats: Secondary Structures

BPSeq (CRW)

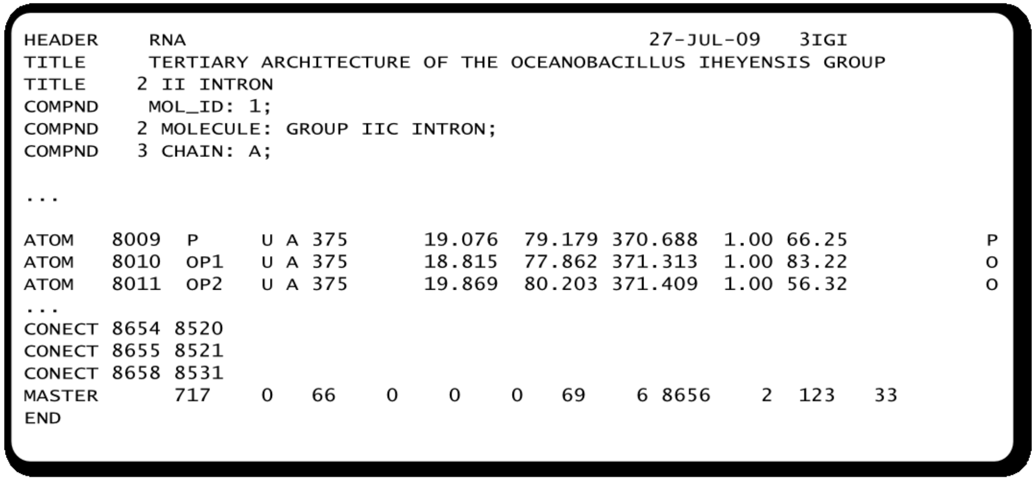


```
Filename: AM286415_b.bpseq
Organism: Yersinia enterocolitica subsp. enterocolitica 8081
Accession Numbers: AM286415
Citation and related information available at http://www.rna.ccbb.utexas.edu
1 U 0
...
117 U 0
118 U 236
119 G 235
120 C 234
121 C 233
122 U 232
123 G 231
124 G 230
...
230 C 124
231 C 123
232 A 122
233 G 121
234 G 120
235 C 119
236 A 118
...
```

```
80      dG = -33.48 [Initially -35.60]
1       U       0       2       80      1
2       G       1       3       79      2
3       G       2       4       78      3
4       G       3       5       77      4
5       A       4       6       76      5
6       U       5       7       0       6
7       G       6       8       75      7
...
75      U       74      76      7       75
76      U       75      77      5       76
77      C       76      78      4       77
78      C       77      79      3       78
79      U       78      80      2       79
80      A       79      0       1       80
```

CT (MFold)

RNA file formats: Secondary Structures



```
HEADER    RNA                                     27-JUL-09   3IGI
TITLE     TERTIARY ARCHITECTURE OF THE OCEANOBACILLUS IHEYENSIS GROUP
TITLE    2 II INTRON
COMPND    MOL_ID: 1;
COMPND   2 MOLECULE: GROUP IIC INTRON;
COMPND   3 CHAIN: A;

...

ATOM   8009  P     U A 375      19.076  79.179 370.688  1.00 66.25           P
ATOM   8010  OP1   U A 375      18.815  77.862 371.313  1.00 83.22           O
ATOM   8011  OP2   U A 375      19.869  80.203 371.409  1.00 56.32           O
...
CONECT 8654 8520
CONECT 8655 8521
CONECT 8658 8531
MASTER      717    0   66    0    0    0   69    6 8656    2  123   33
END
```

PDB

RNA file formats: Secondary Structures

```
<?xml version="1.0"?>
<!DOCTYPE rnaml SYSTEM "rnaml.dtd">
<rnaml version="1.0">
   <molecule id=“xxx">
      <sequence> ... </sequence>
      <structure> ... </structure>
    </molecule>
  <interactions> ... </interactions>
</rnaml>
```

RNAML

RNA file formats: Secondary Structures

```
<?xml version="1.0"?>
<!DOCTYPE rnaml SYSTEM "rnaml.dtd">
<rnaml version="1.0">
   <molecule id=“xxx">
      <sequence>
         <numbering-system id="1" used-in-file="false">
            <numbering-range>
               <start>1</start><end>387</end>
            </numbering-range>
         </numbering-system>
         <numbering-table length="387">
            2    3    4    5    6    7    8...
         </numbering-table>
         <seq-data>
            UGUGCCCGGC AUGGGUGCAG UCUAUAGGGU...
         </seq-data>
         ...
      </sequence>
      <structure> ... </structure>
    </molecule>
  <interactions> ... </interactions>
</rnaml>
```

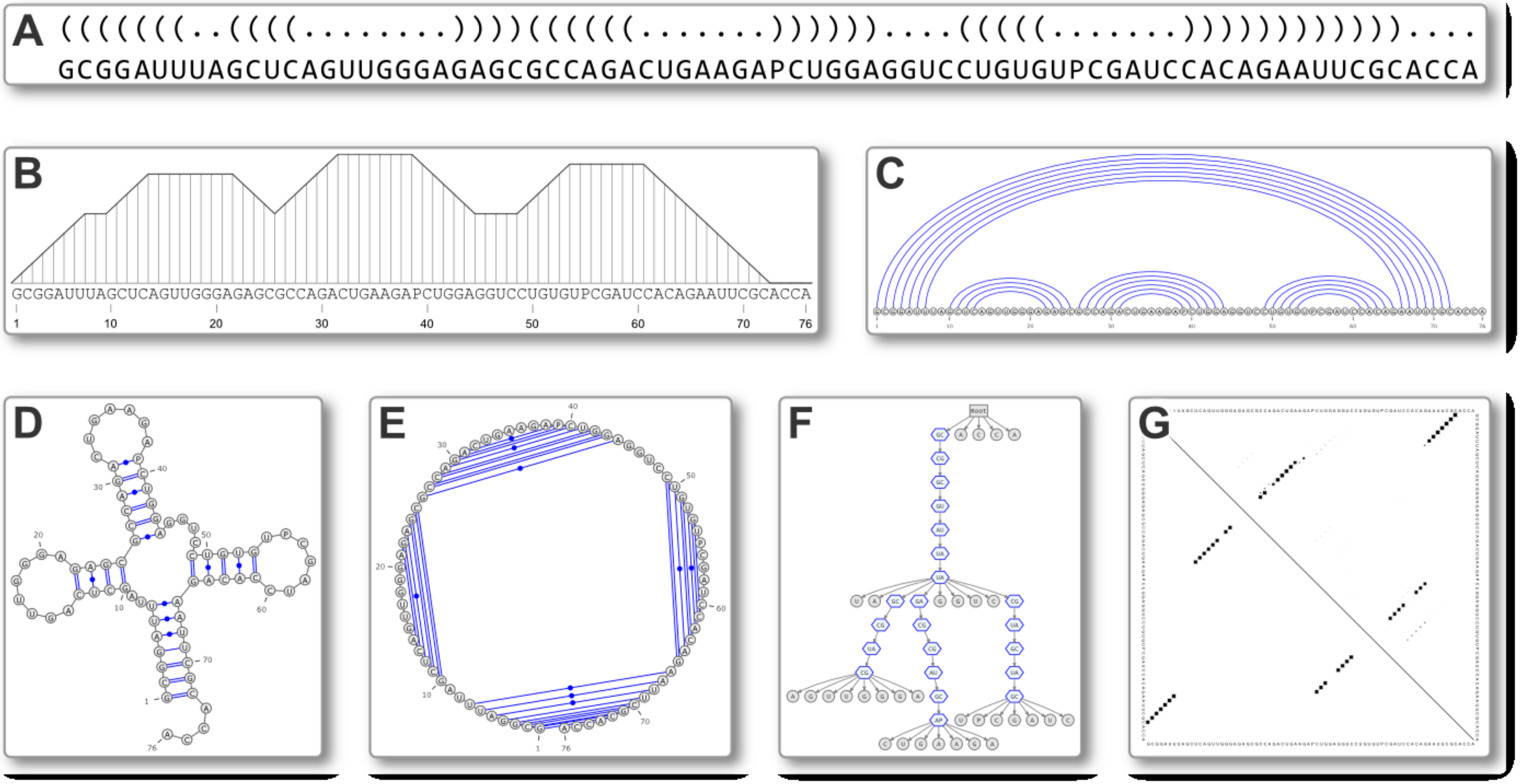
RNAML

RNA file formats: Secondary Structures

```
<?xml version="1.0"?>
<!DOCTYPE rnaml SYSTEM "rnaml.dtd">
<rnaml version="1.0">
   <molecule id=“xxx">
      <sequence> ... </sequence>
      <structure>
         <model id=“yyy">
            <base> ... </base> ...
            <str-annotation>
               ...
               <base-pair>
                  <base-id-5p><base-id><position>2</position></base-id></base-id-5p>
                  <base-id-3p><base-id><position>260</position></base-id></base-id-3p>
                  <edge-5p>+</edge-5p>
                  <edge-3p>+</edge-3p>
                  <bond-orientation>c</bond-orientation>
               </base-pair>
               <base-pair comment="?">
                  <base-id-5p><base-id><position>4</position></base-id></base-id-5p>
                  <base-id-3p><base-id><position>259</position></base-id></base-id-3p>
                  <edge-5p>S</edge-5p>
                  <edge-3p>W</edge-3p>
                  <bond-orientation>c</bond-orientation>
               </base-pair>
               ...
            </str-annotation>
        </model>
      </structure>
    </molecule>
  <interactions> ... </interactions>
</rnaml>
```

RNAML

Secondary Structure representations



http://varna.lri.fr

First contact

- Run the web start version of VARNA at:

  http://varna.lri.fr/downloads.html

- Locate and save on disk a bunch of secondary structures from the RNA Strand Database (CT or BPseq):

  http://www.rnasoft.ca/strand/

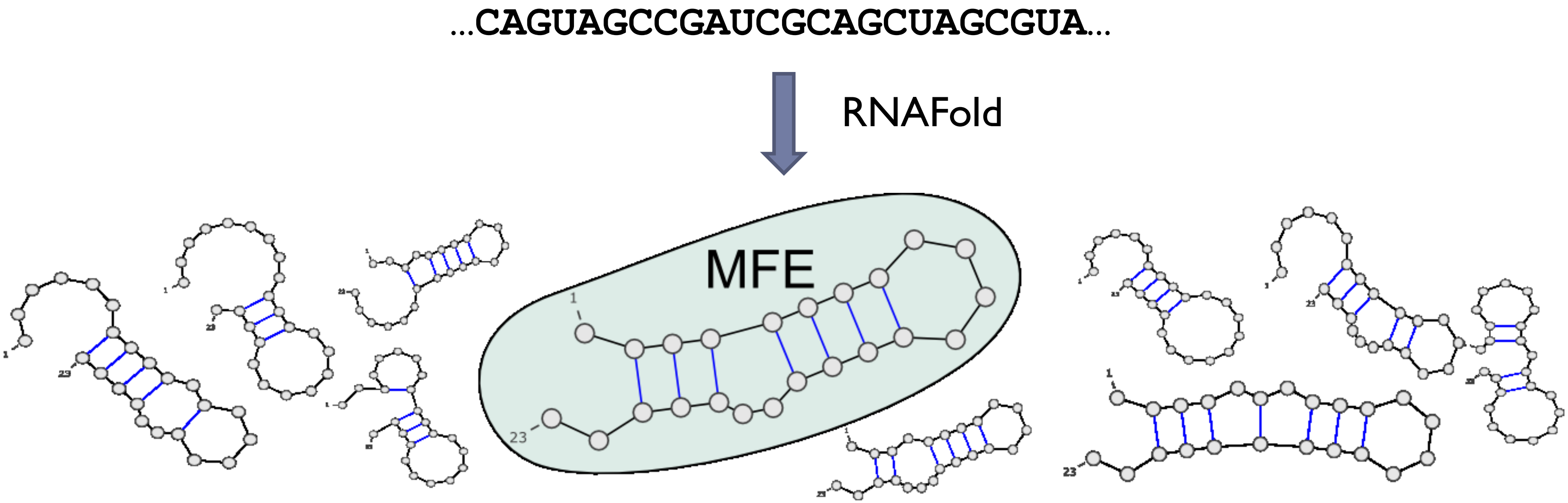
- Load these files and using the region highlight feature of VARNA, highlight a region of interest.

  Menu ▶ Edit ▶ Annotation ▶ New ▶ Region

Basic prediction

Minimal free-energy folding

Minimal Free-Energy (MFE) Folding



…CAGUAGCCGAUCGCAGCUAGCGUA…

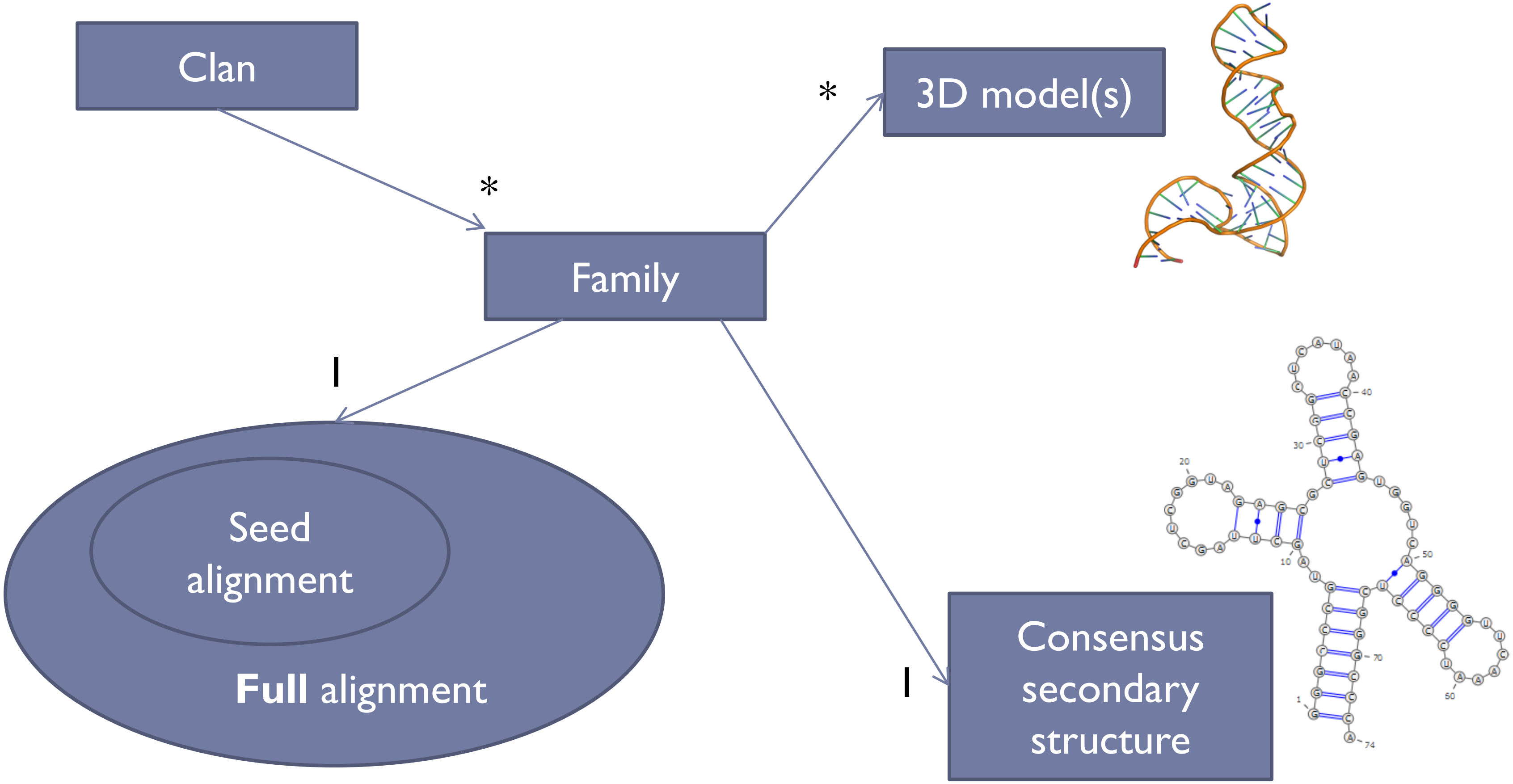
RNAFold

MFE

- Turner model associates energy to each compatible secondary structure.
- Vienna RNA package implements a $O(n^3)$ algorithm for computing the most stable folding…
- … but also offers nice visualization features.

RFAM: RNA functional families

http://rfam.sanger.ac.uk/



Clan

*

Family

*

3D model(s)

I

Seed alignment

Full alignment

I

Consensus secondary structure

Minimal Free-Energy folding of RNA

- Get the RFAM alignment for the the D1-D4 domain of the Group II intron (RFAM ID: RF02001 – Seed – Stockholm format)

  `http://rfam.sanger.ac.uk/`

- Load the *A. Capsulatum* (`Acidobacterium_capsu.1`) sequence in VARNA.
- Run RNAFold on this sequence using the Vienna RNA web tools suite:

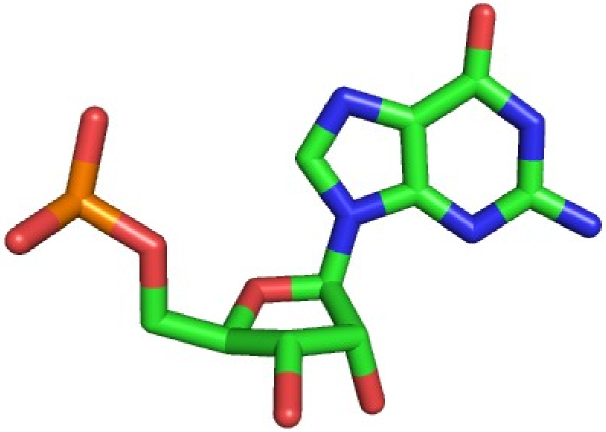
  `http://rna.tbi.univie.ac.at/`

- Retrieve the result (Vienna format) and compare it with the consensus structure.
- Rerun RNAFold using more recent energy parameters
  (*Show advanced options* → *Turner 2004 energy model*)
- Compare the predictions in both models.

Advanced structural features

Tertiary motifs and pseudoknots

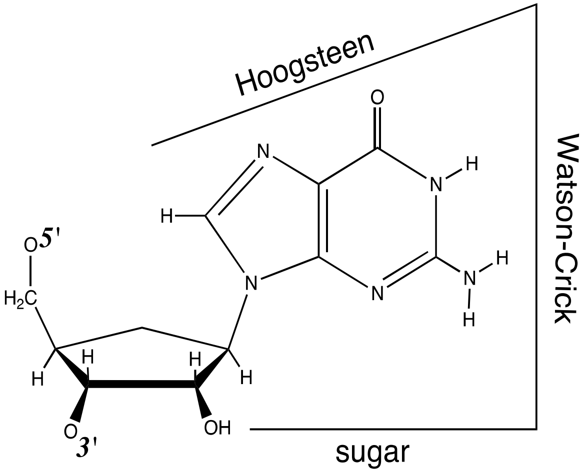
Non canonical interactions



RNA nucleotides bind through edge/edge interactions.

Non canonical are weaker, but cluster into modules that are structurally constrained, evolutionarily conserved, and functionally essential.

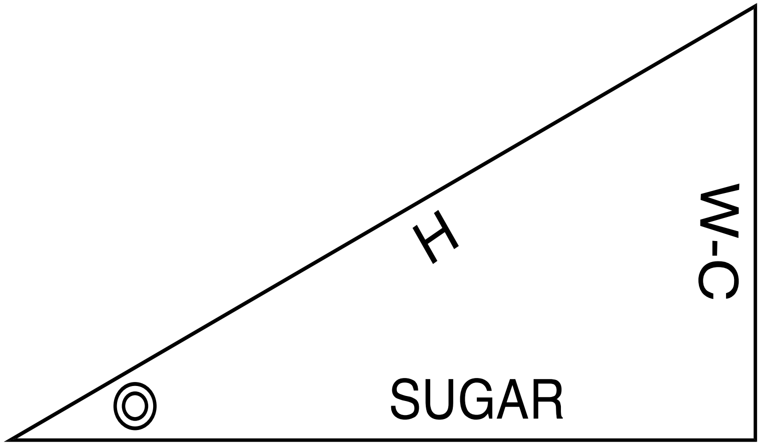
Non canonical interactions



RNA nucleotides bind through edge/edge interactions.

Non canonical are weaker, but cluster into modules that are structurally constrained, evolutionarily conserved, and functionally essential.

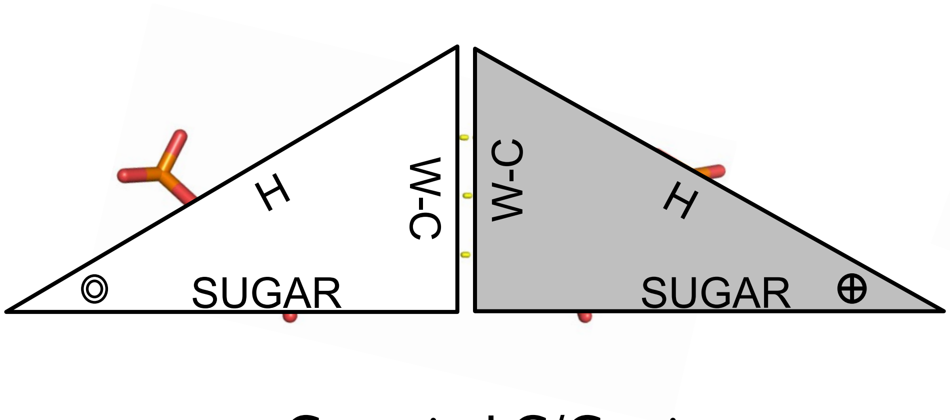
Non canonical interactions



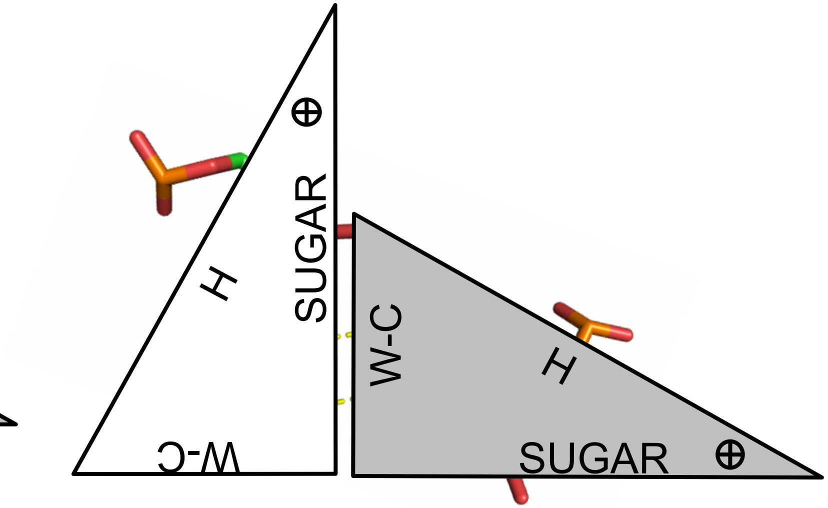
RNA nucleotides bind through edge/edge interactions.

Non canonical are weaker, but cluster into modules that are structurally constrained, evolutionarily conserved, and functionally essential.

Non canonical interactions



Canonical G/C pair
(WC/WC cis)

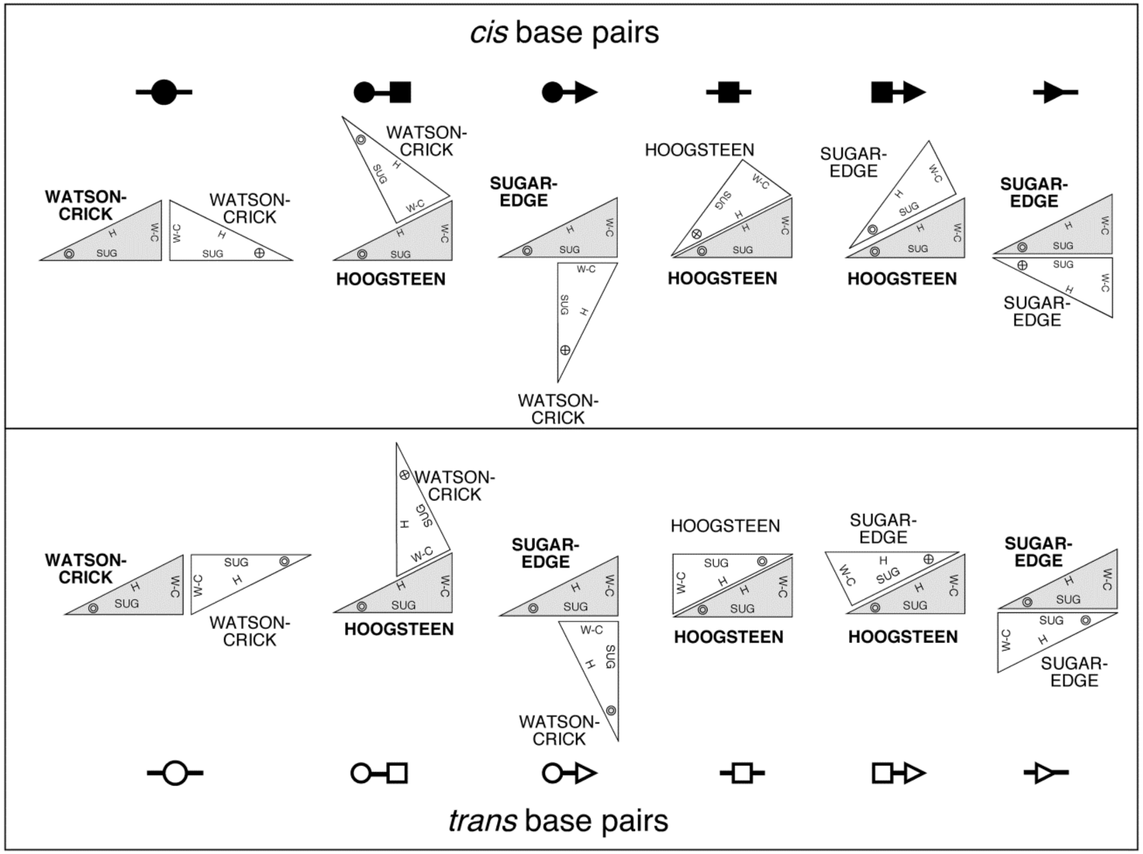


Non Canonical G/C pair
(Sugar/WC trans)

RNA nucleotides bind through edge/edge interactions.

Non canonical are weaker, but cluster into modules that are structurally constrained, evolutionarily conserved, and functionally essential.

Leontis/Westhof nomenclature: A *visual grammar* for tertiary motifs



cis	*trans*	
●	○	Watson-Crick edge
■	□	Hoogsteen edge
◀	◁	Sugar edge

— AU *cis* Watson-Crick base pair

= GC *cis* Watson-Crick base pair

-○- GU *cis* Watson-Crick base pair

G or Ⓖ *syn* oriented

Ⓑ bifurcated base pair

Ⓦ water-mediated base pair

---- single H-bond interaction

├─┤ indicates base stacking

➔ indicates change in strand orientation

Leontis/Westhof, NAR 2002

\+ Tools to infer base-pairs from experimentally-derived 3D models

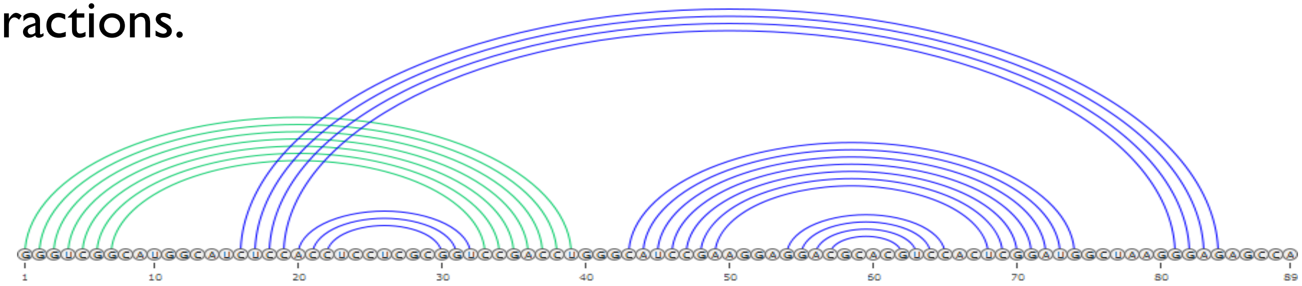
RNAView, MC-Annotate…

Automated annotation of 3D RNA models

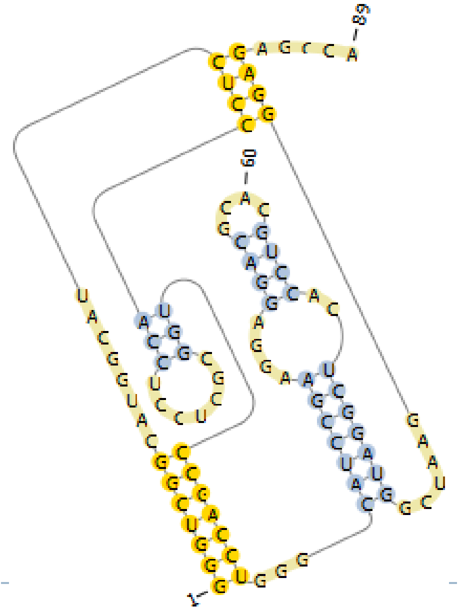
- Get from the NDB and compile (see Readme) the RNAView software*
  `http://ndbserver.rutgers.edu/services/download/`
- Retrieve the 3IGI model from the RSCB PDB as a PDB file.
- Annotate it using RNAview (-p option) to create a RNAML file
- Visualize the output RNAML file within VARNA
- Run RNAFold (default options) on the sequence and compare the prediction with the one inferred from the 3D model.

Pseudoknots

- Pseudoknots are complex topological models indicated by crossing interactions.



- Pseudoknots are largely ignored by computational prediction tools:
  - Lack of accepted energy model
  - Algorithmically challenging
- Yet heuristics can be sometimes efficient.
- Visualizing of secondary structure with pseudoknots is supported by:
  - PseudoViewer
  - VARNA



Predicting and visualizing Pseudoknots

- Get seq./struct. data for a pseudoknot tmRNA the PseudoBase (ID: PKB210)

  `http://pseudobaseplusplus.utep.edu/`

- Visualize the structure using VARNA and the Pseudoviewer:

  `http://pseudoviewer.inha.ac.kr/`

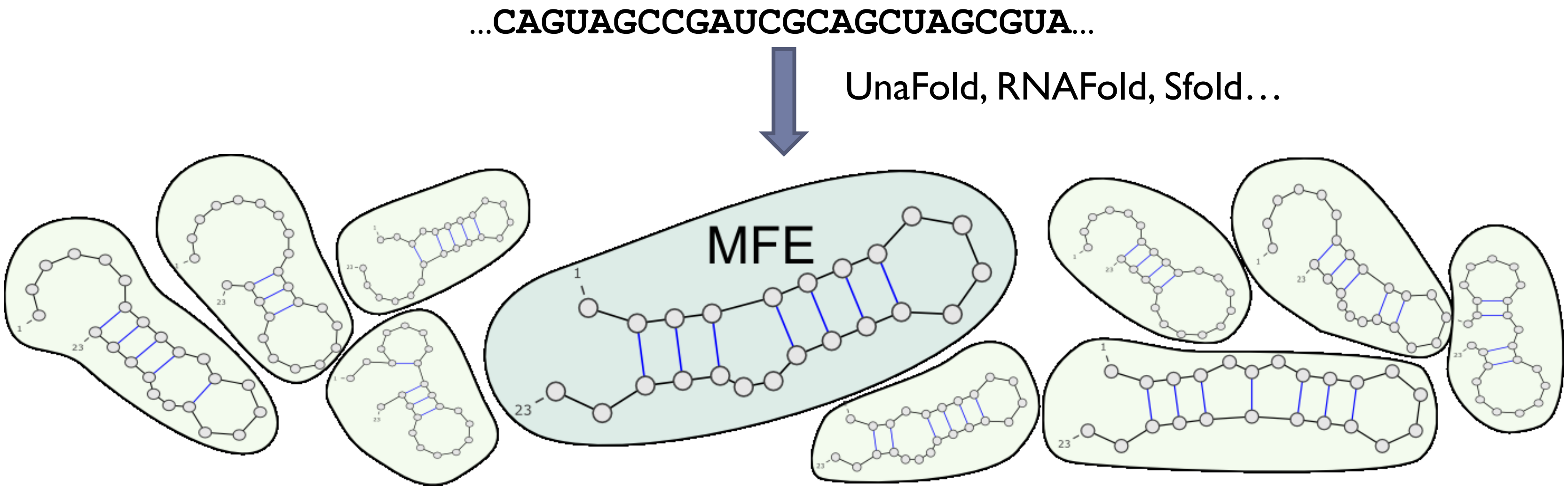
- Fold this sequence using RNAFold and compare the result to the native structure

- Fold this sequence using Pknots-RG (*Program type: Enforcing PK*):

  `http://bibiserv.techfak.uni-bielefeld.de/pknotsrg/`

Ensemble approaches in RNA folding

- RNA *in silico* paradigm shift:
  - From single structure, minimal free-energy folding…
  - … to ensemble approaches.



...**CAGUAGCCGAUCGCAGCUAGCGUA**...

UnaFold, RNAFold, Sfold…

Ensemble diversity? Structure likelihood? Evolutionary robustness?

Example:

[redacted]

Comparative data

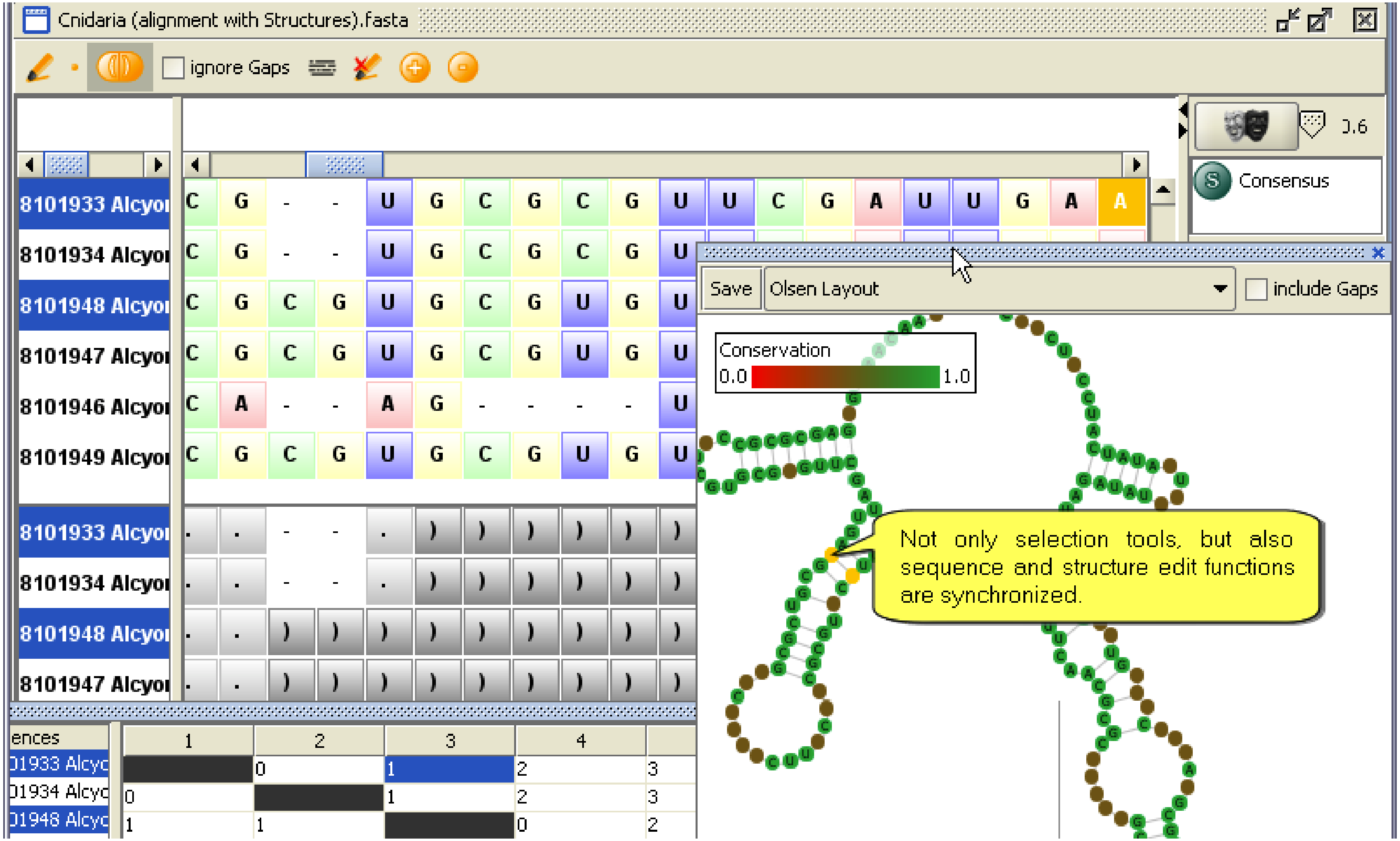
RNA Alignment curation

- Different tools for different tasks
  - ‘top down’ Structure guided modelling
    - S2S/Assemble
    - Interactive 3D modelling – edit structure based on fold predictions and manual manipulation
    - Alignments arise from RNA structure comparisons
  - ‘Bottom up’
    - Use evolutionary information (conservation patterns) to infer structural homology
    - Alignment methods like locaRNA or R-COFFEE maximise similarity in base pair contacts
    - Still need to curate/correlate with respect to other evidence for homology
  - Why curate when no structure is available
    - INFERNAL – tool to search genomes for matches to RFAM alignments
      - ☐ Functional modules, etc.

A selection of tools ..

- RALEE (based on Emacs)
  - Favourite for hardcore RNA modellers – (,), space and delete to edit
- 4SALE
  - Visual editor also accesses RNA alignment and folding services
- BoulderAle: http://boulderale.sourceforge.net/
  - Web based RNA alignment annotator/editor (up to 1000 nucleotides)
  - Uses VARNA for 2D visualization & KineMAGE for 3D structure
    - Stockholm file + Vienna files + GFF
  - Model 2D structure based on isostericity
  - Curate alignments to align bases that can form similar base-base interactions
- Jalview – new kid on the block…

4SALE



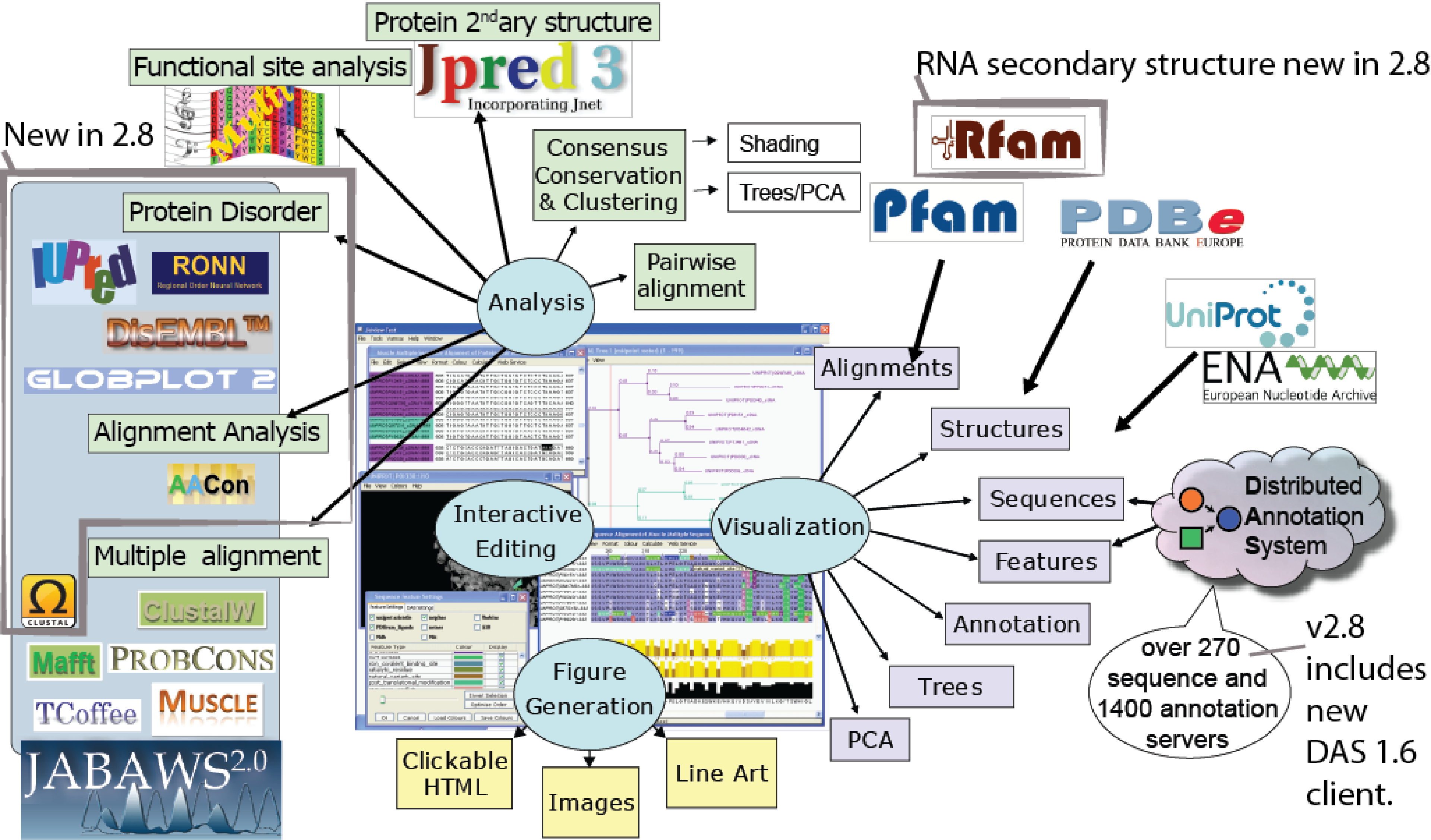
8101933 Alcyo C G - - U G C G C G U U C G A U U G A A
8101934 Alcyo C G - - U G C G C G U
8101948 Alcyo C G C G U G C G U G U
8101947 Alcyo C G C G U G C G U G U
8101946 Alcyo C A - - A G - - - - U
8101949 Alcyo C G C G U G C G U G U

8101933 Alcyo . . - - .))))))
8101934 Alcyo . . - - .))))))
8101948 Alcyo . .)))))))))
8101947 Alcyo . .)))))))))

ences	1	2	3	4	
01933 Alcyc		0	1	2	3
01934 Alcyc	0		1	2	3
01948 Alcyc	1	1		0	2

Not only selection tools, but also sequence and structure edit functions are synchronized.

Upcoming Jalview features



Protein 2ndary structure

Functional site analysis

New in 2.8

Protein Disorder

Alignment Analysis

Multiple alignment

Consensus Conservation & Clustering → Shading, Trees/PCA

Pairwise alignment

Analysis

Interactive Editing

Figure Generation

Clickable HTML

Images

Line Art

Visualization

Alignments

Structures

Sequences

Features

Annotation

Trees

PCA

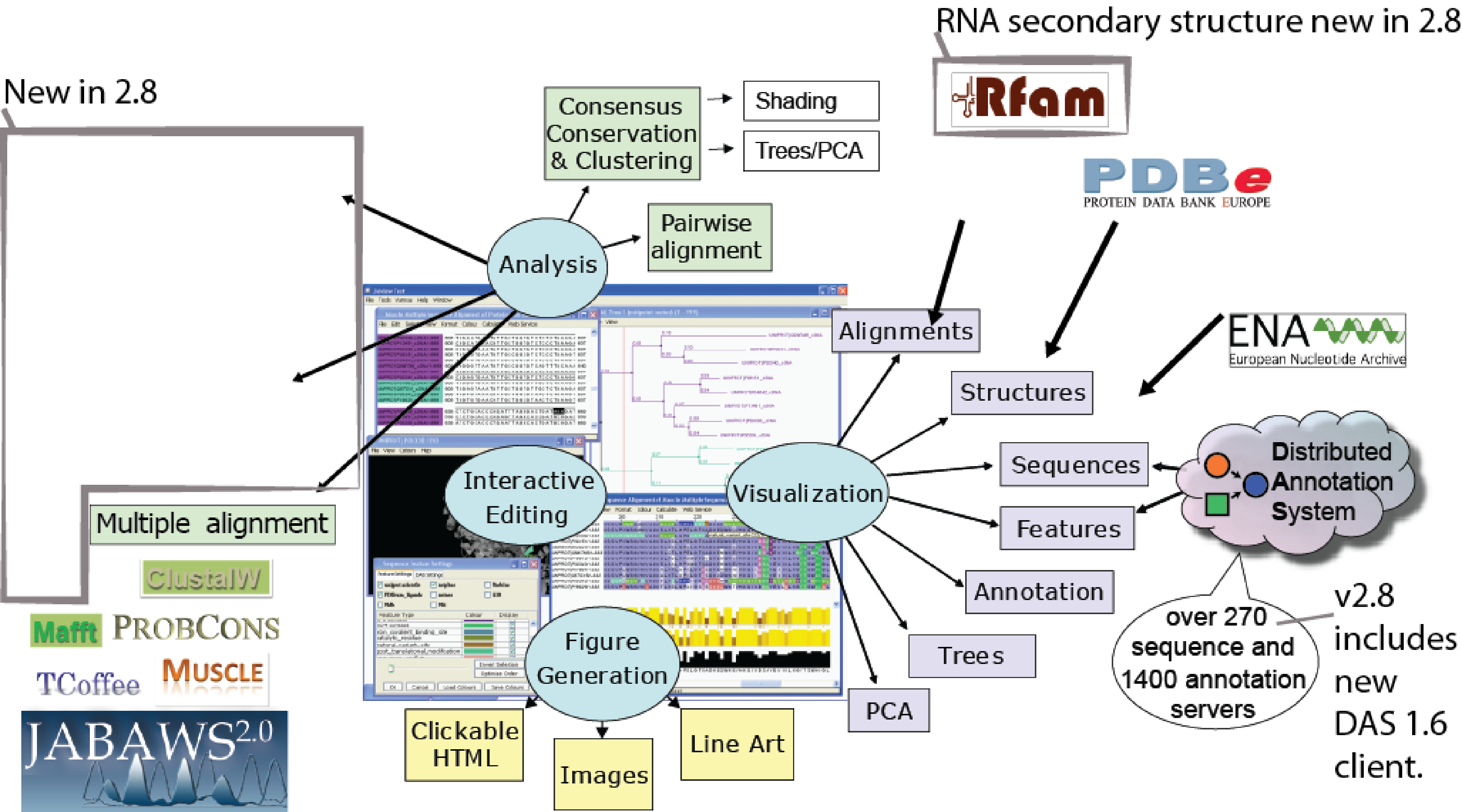
RNA secondary structure new in 2.8

Distributed Annotation System

over 270 sequence and 1400 annotation servers

v2.8 includes new DAS 1.6 client.

Jalview’s features relevant to RNA



RNA secondary structure new in 2.8

Rfam

PDBe PROTEIN DATA BANK EUROPE

ENA European Nucleotide Archive

New in 2.8

Consensus Conservation & Clustering

Shading

Trees/PCA

Pairwise alignment

Analysis

Multiple alignment

ClustalW

Mafft

ProbCons

TCoffee

Muscle

JABAWS 2.0

Interactive Editing

Visualization

Figure Generation

Clickable HTML

Images

Line Art

Alignments

Structures

Sequences

Features

Annotation

Trees

PCA

Distributed Annotation System

over 270 sequence and 1400 annotation servers

v2.8 includes new DAS 1.6 client.

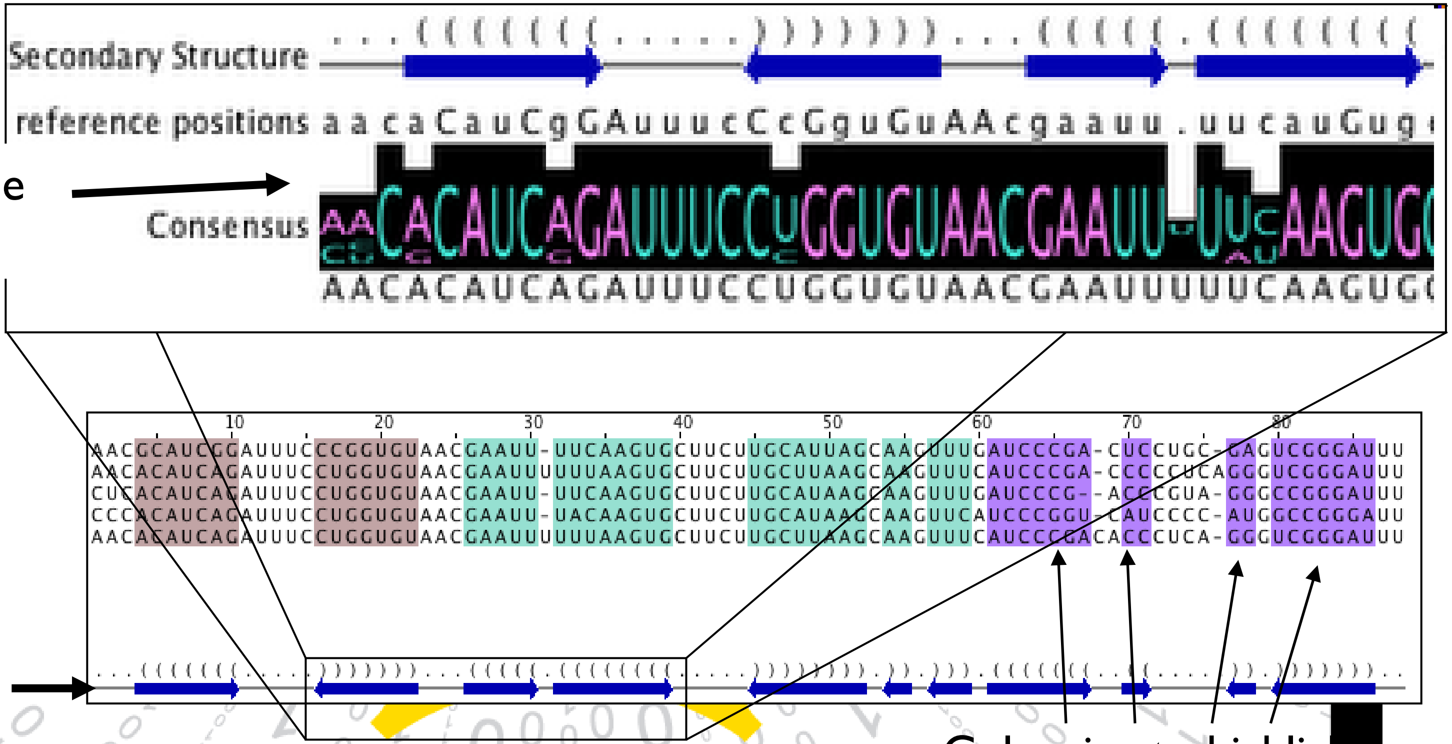
Lauren Lui, UC Santa Cruz.
http://jalview-rnasupport.blogspot.com/

Purine/pyrimidine colourscheme

Rfam

alignment fetcher

WUSS annotation
rser
(om RALEE)



```
Secondary Structure  ...((((((((......))))))))...(((((.((((((((
reference positions  aacaCauCgGAuuucCcGguGuAAcgaauu.uucauGug
Consensus            AACACAUCAGAUUUCCUGGUGUAACGAAUU-UUCAAGUG
                     AACACAUCAGAUUUCCUGGUGUAACGAAUUUUUUCAAGUG
```

```
   10        20        30        40        50        60        70        80
AACGCAUCGGAUUUCCCGGUGUAACGAAUU-UUCAAGUGCUUCUUGCAUUAGCAAGUUUGAUCCCGA-CUCCUGC-GAGUCGGGAUUU
AACACAUCAGAUUUCCUGGUGUAACGAAUUUUUUAAGUGCUUCUUGCUUAAGCAAGUUUCAUCCCGA-CCCCCUCAGGGUCGGGAUUU
CUCACAUCAGAUUUCCUGGUGUAACGAAUU-UUCAAGUGCUUCUUGCAUAAGCAAGUUUGAUCCCG--ACCCGUA-GGGCCGGGAUUU
CCCACAUCAGAUUUCCUGGUGUAACGAAUU-UACAAGUGCUUCUUGCAUAAGCAAGUUCAUCCCGGU-CAUCCCC-AUGGCCGGGAUU
AACACAUCAGAUUUCCUGGUGUAACGAAUUUUUUAAGUGCUUCUUGCUUAAGCAAGUUUCAUCCCGACACCCUCA-GGGUCGGGAUUU

...((((((((....))))))))...(((((.((((((((.....))))))))).)).))).((((((((..((.....)).))))))))..
```

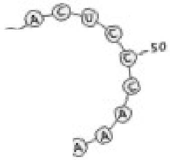
Colouring to highlig
helical structure

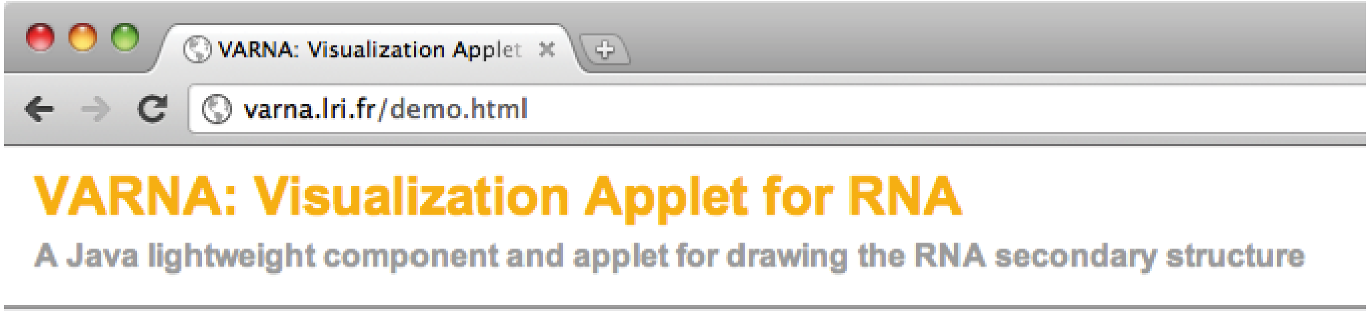
SUMMER OF CODE // 2010

code.google.com/soc

Google

Jan Engelhardt (Uni. Leipzig)





varna.lri.fr/demo.html

VARNA: Visualization Applet for RNA

A Java lightweight component and applet for drawing the RNA secondary structure

Google

SUMMER OF CODE 2011

RNA alignment tutorial with Locarna and Jalview

1. Start Development version of Jalview

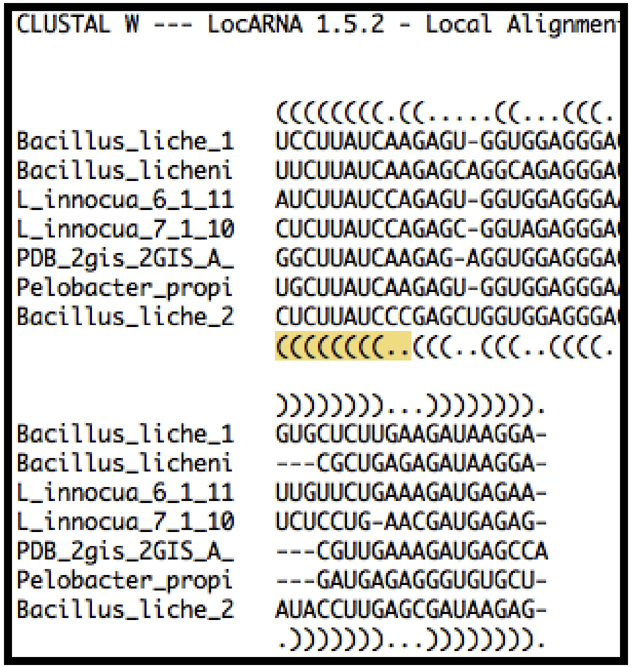
   http://www.compbio.dundee.ac.uk/users/ws-dev1/jalview/develop/webstart/jalview_1G.jnlp

2. Import RF00162 from RFAM seed alignment
3. Select first 6 sequences in alignment, copy and paste to new alignment (shift + cmd/CTRL+V)
4. Select 'Edit->remove all gaps'
5. Add PDB sequence 2gis
6. Open locarna server page at http://rna.informatik.uni-freiburg.de:8080/LocARNA.jsp
7. Select/copy all 7 (ctrl+a + ctrl+c) and paste into locarna input
8. Wait a few minutes…

Viewing the locarna results in Jalview

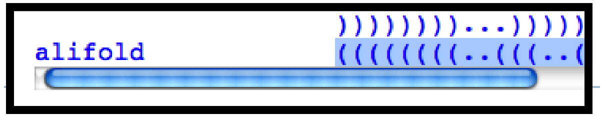
- Jalview doesn't support direct retrieval of LocaRNA results just yet

download [alignment] [matrix] [tree]

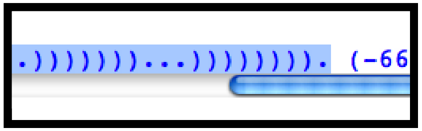


```
CLUSTAL W --- LocARNA 1.5.2 - Local Alignmen


                    ((((((((.((.....((...(((.
Bacillus_liche_1    UCCUUAUCAAGAGU-GGUGGAGGGA
Bacillus_licheni    UUCUUAUCAAGAGCAGGCAGAGGGA
L_innocua_6_1_11    AUCUUAUCCAGAGU-GGUGGAGGGA
L_innocua_7_1_10    CUCUUAUCCAGAGC-GGUAGAGGGA
PDB_2gis_2GIS_A_    GGCUUAUCAAGAG-AGGUGGAGGGA
Pelobacter_propi    UGCUUAUCAAGAGU-GGUGGAGGGA
Bacillus_liche_2    CUCUUAUCCCGAGCUGGUGGAGGGA
                    ((((((((..(((..(((..((((.


                    ))))))))...)))))))).
Bacillus_liche_1    GUGCUCUUGAAGAUAAGGA-
Bacillus_licheni    ---CGCUGAGAGAUAAGGA-
L_innocua_6_1_11    UUGUUCUGAAAGAUGAGAA-
L_innocua_7_1_10    UCUCCUG-AACGAUGAGAG-
PDB_2gis_2GIS_A_    ---CGUUGAAAGAUGAGCCA
Pelobacter_propi    ---GAUGAGAGGGUGUGCU-
Bacillus_liche_2    AUACCUUGAGCGAUAAGAG-
                    .))))))))...)))))))).
```

1. Download '[alignment]' link
2. Open in a text editor
3. Replace the lower RNA secondary structure line with the 'alifold' prediction given in the locarna output
4. Save and load into Jalview

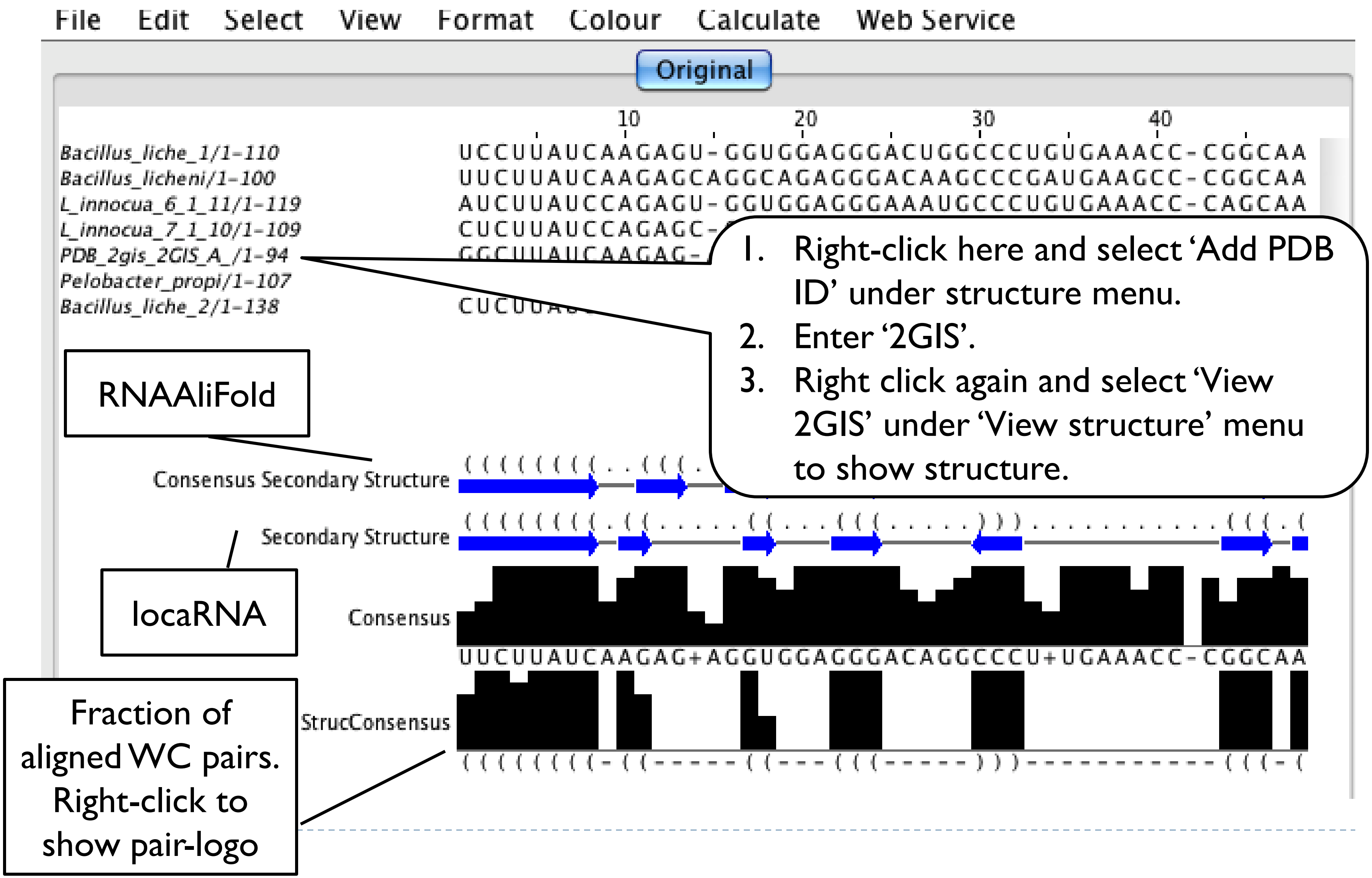


```
         ))))))))...)))))
alifold  ((((((((..(((..(
```



```
.)))))))...)))))))). (-66
```

LocaRNA and RNAliFold in Jalview



```
Bacillus_liche_1/1-110      UCCUUAUCAAGAGU-GGUGGAGGGACUGGCCCUGUGAAACC-CGGCAA
Bacillus_licheni/1-100      UUCUUAUCAAGAGCAGGCAGAGGGACAAGCCCGAUGAAGCC-CGGCAA
L_innocua_6_1_11/1-119      AUCUUAUCCAGAGU-GGUGGAGGGAAAUGCCCUGUGAAACC-CAGCAA
L_innocua_7_1_10/1-109      CUCUUAUCCAGAGC-
PDB_2gis_2GIS_A_/1-94       GGCUUAUCAAGAG-
Pelobacter_propi/1-107
Bacillus_liche_2/1-138      CUCUUAU

Consensus Secondary Structure   ((((((((..(((.
Secondary Structure             ((((((((.((.....((...(((.....)))...........(((.(
Consensus
                                UUCUUAUCAAGAG+AGGUGGAGGGACAGGCCCU+UGAAACC-CGGCAA
StrucConsensus
                                ((((((((-((-----((---(((-----)))-----------(((-(
```

1. Right-click here and select 'Add PDB ID' under structure menu.
2. Enter '2GIS'.
3. Right click again and select 'View 2GIS' under 'View structure' menu to show structure.

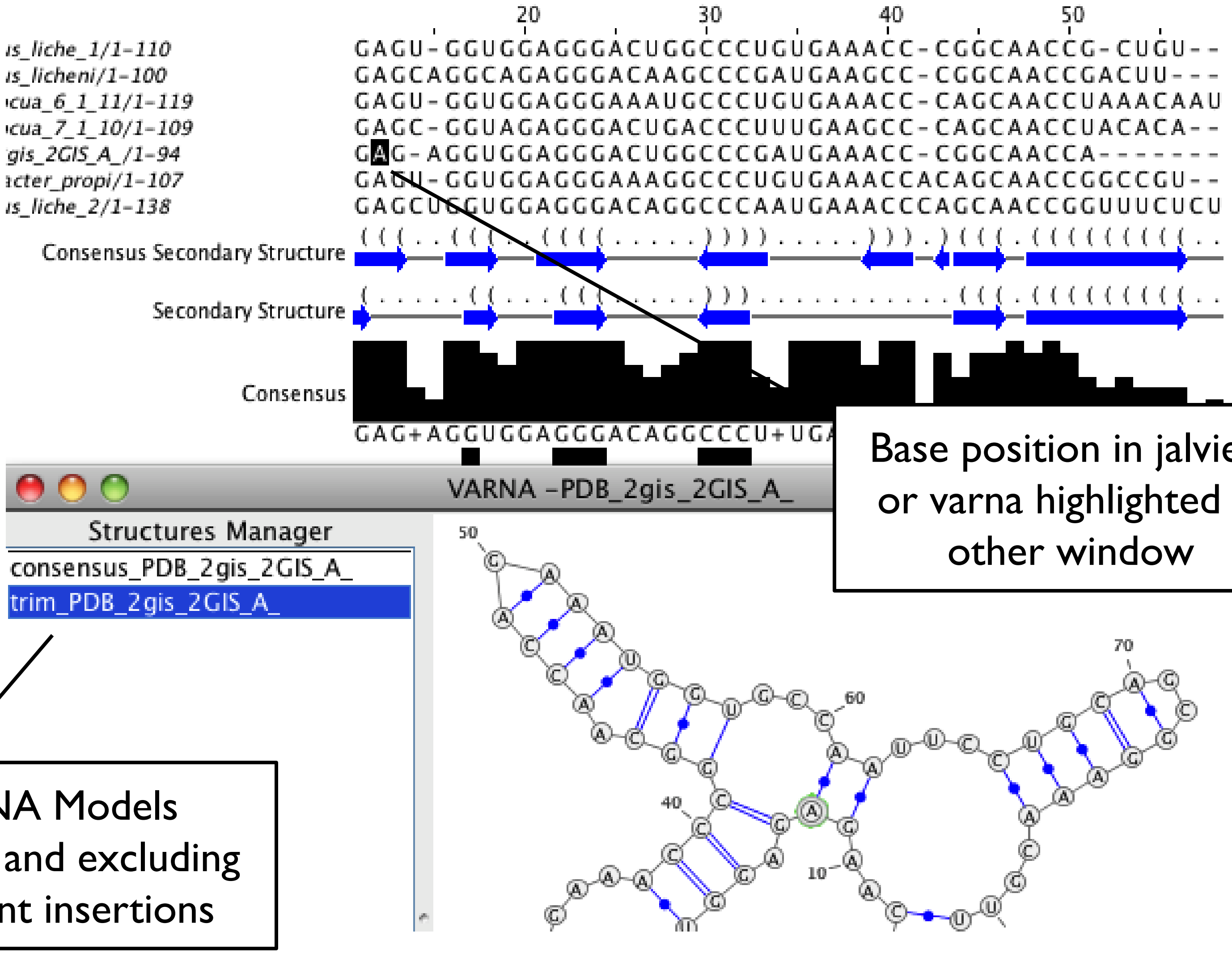
RNAAliFold

locaRNA

Fraction of aligned WC pairs. Right-click to show pair-logo

VARNA in Jalview

Linked Highlighting & Selections

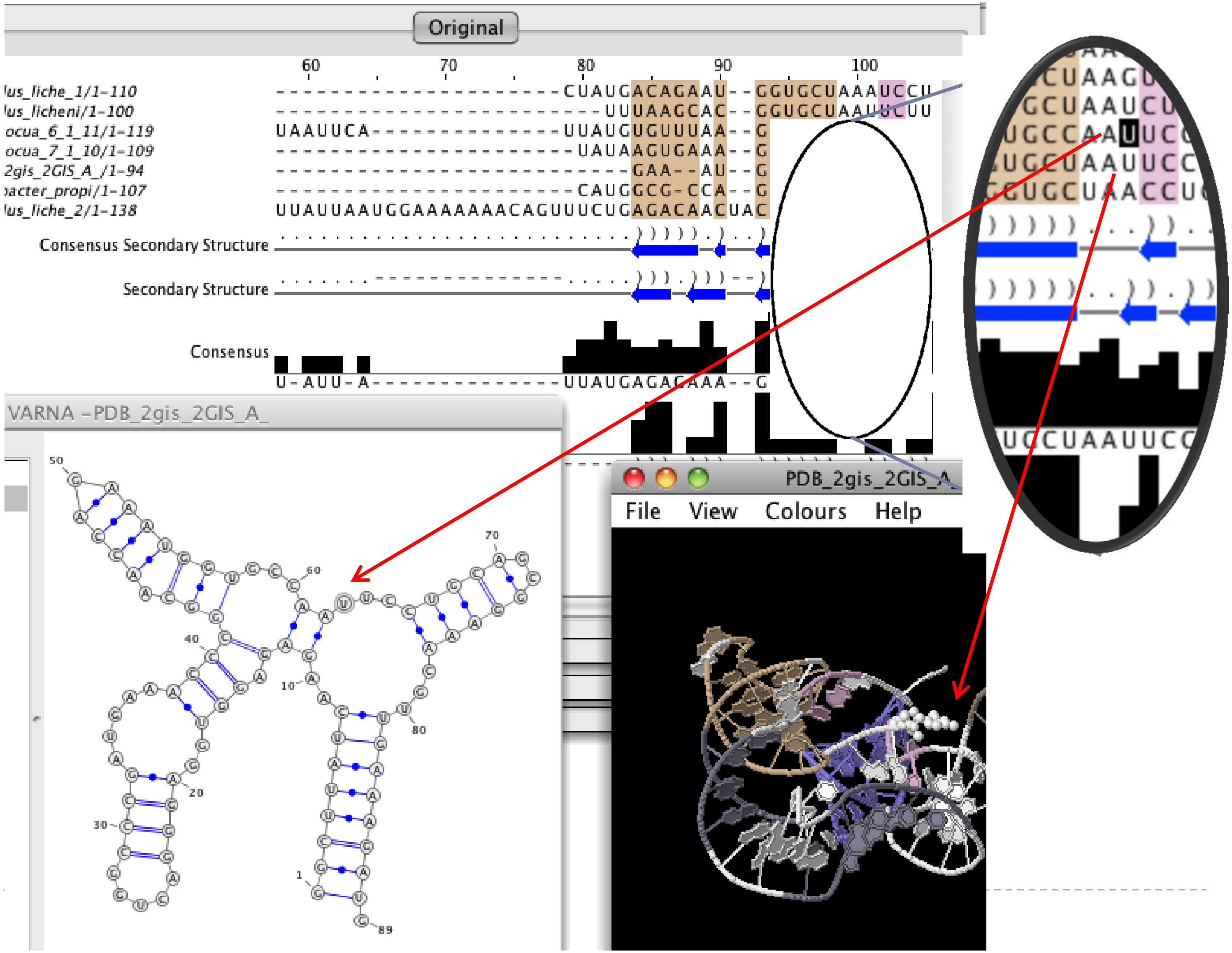


```
                     20        30        40        50
ıs_liche_1/1-110     GAGU-GGUGGAGGGACUGGCCCUGUGAAACC-CGGCAACCG-CUGU--
ıs_licheni/1-100     GAGCAGGCAGAGGGACAAGCCCGAUGAAGCC-CGGCAACCGACUU---
ıcua_6_1_11/1-119    GAGU-GGUGGAGGGAAAUGCCCUGUGAAACC-CAGCAACCUAAACAAU
ıcua_7_1_10/1-109    GAGC-GGUAGAGGGACUGACCCUUUGAAGCC-CAGCAACCUACACA--
ıgis_2GIS_A_/1-94    GAG-AGGUGGAGGGACUGGCCCGAUGAAACC-CGGCAACCA-------
ıcter_propi/1-107    GAGU-GGUGGAGGGAAAGGCCCUGUGAAACCACAGCAACCGGCCGU--
ıs_liche_2/1-138     GAGCUGGUGGAGGGACAGGCCCAAUGAAACCCAGCAACCGGUUUCUCU
```

Base position in jalview or varna highlighted in other window

VARNA Models including and excluding alignment insertions

Inspection and curation of prediction



```
                                   60        70        80        90        100
lus_liche_1/1-110                  ------------------------CUAUGACAGAAU--GGUGCUAAAUCCU
lus_licheni/1-100                  ---------------------------UUUAAGCAC--GGUGCUAAUUCUU
ocua_6_1_11/1-119                  UAAUUCA-----------------UUAUGUGUUUAA--G
ocua_7_1_10/1-109                  -------------------------UAUAAGUGAAA--G
2gis_2GIS_A_/1-94                  -----------------------------GAA--AU--G
bacter_propi/1-107                 -------------------------CAUGGCG-CCA--G
lus_liche_2/1-138                  UUAUUAAUGGAAAAAAACAGUUUCUGAGACAACUAC
Consensus Secondary Structure      ..............................)))))).)...)
Secondary Structure                .......---------------......))).)))--)
Consensus                          U-AUU-A---------------UUAUGAGACAAA--G
```

Summary/Discussion