Sibiria

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University of Vienna

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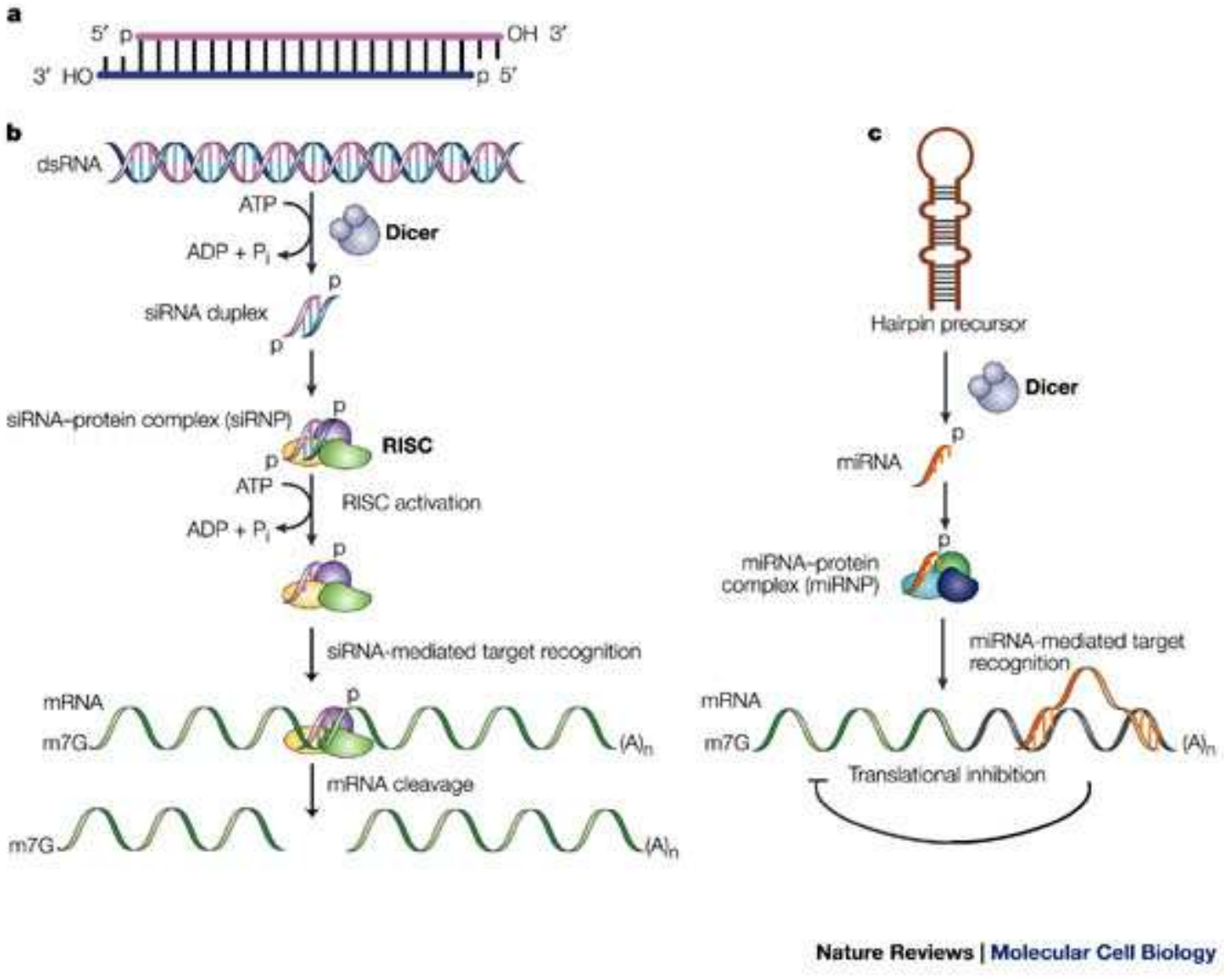
Outline

Outline

Why fold 2 RNA molecules

- Many RNA functions mediated by intermolecular RNA interactions
  - miRNA-siRNA pathway
  - RNA editing
- Design of custom-made RNA molecules
  - e.g. “openers” for binding sites

Intermolecular interactions of RNA molecules



2. Target accessibility and duplex thermodynamics

- Sfold by Ding & Lawrence
- Internal stability profile of small RNAs (5' and 3' end, average internal stability, . . .)
- probability profiling for prediction of unstructured regions in the target RNA:
  - generate a representative statistical sample of target RNA structures
  - calculate the probability that w consecutive positions, starting at position i, are within an unstructured region

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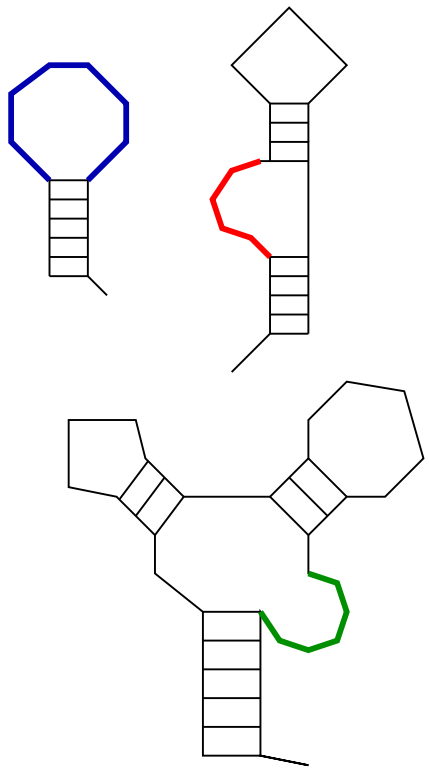
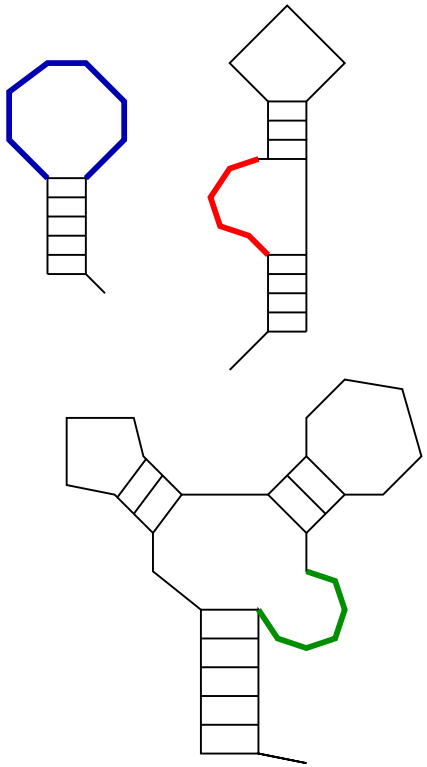
Complete ensemble of secondary structures

Probability of an unstructured region

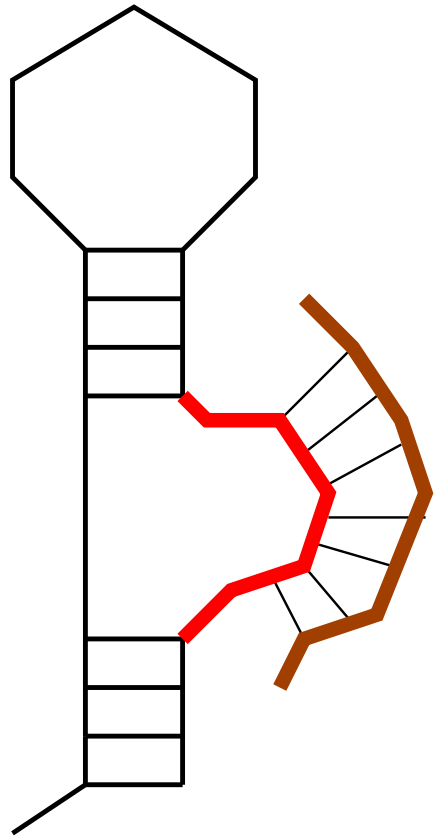


Complete ensemble of secondary structures

Probability of an unstructured region



Probability of interaction in unstructured regions



Outline

Basic Algorithms

- Mc Caskill: Equilibrium partition function for RNA secondary structure
- ViennaRNA Package

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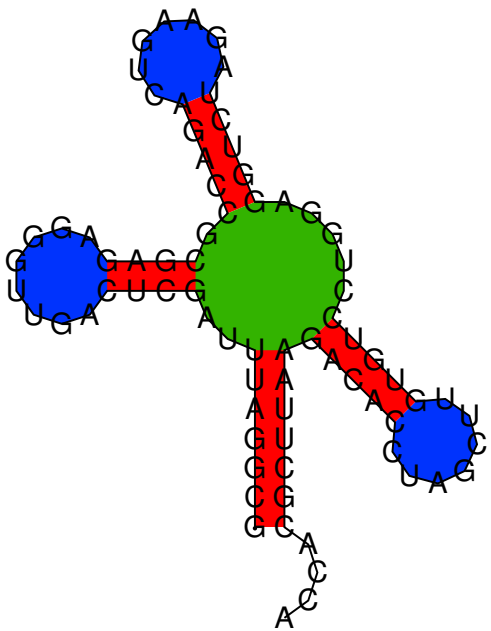
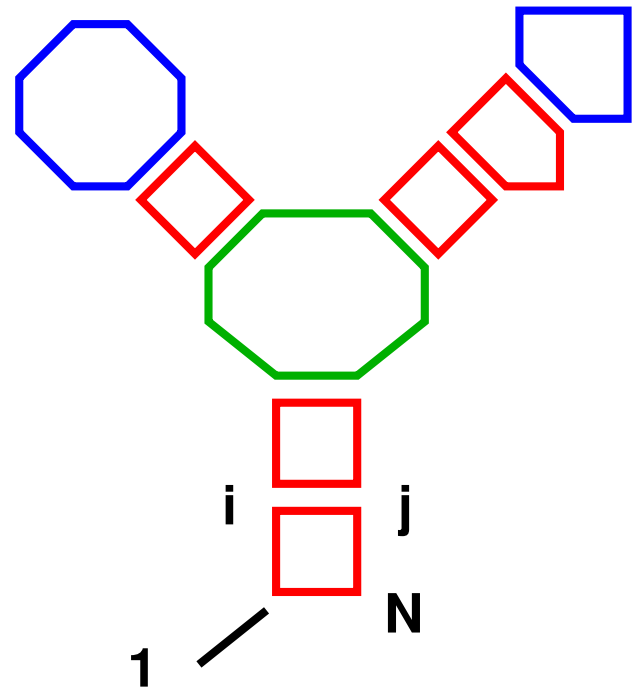
RNA Secondary Structure



- Hairpin Loops
- Interior Loops
- Multiloops
- Exterior Loops

Loop decomposition of RNA Secondary Structure

- $F(S) = \sum_{L \in S} F_L$.



Probability of a given secondary structure

- $Q = \sum_S e^{-[F(S)/kT]}$
- $P(S) = \frac{1}{Q} e^{-[F(S)/kT]}$

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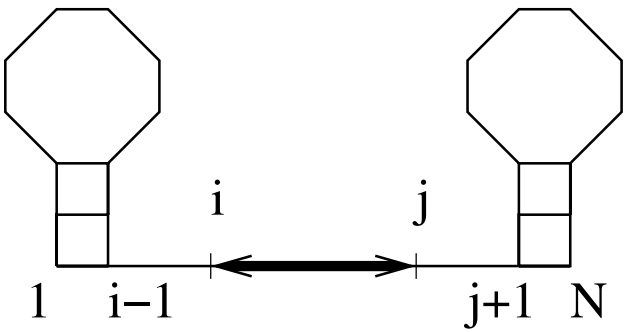
Outline

$Pr_u[i,j]$: region i,j contains no secondary structure

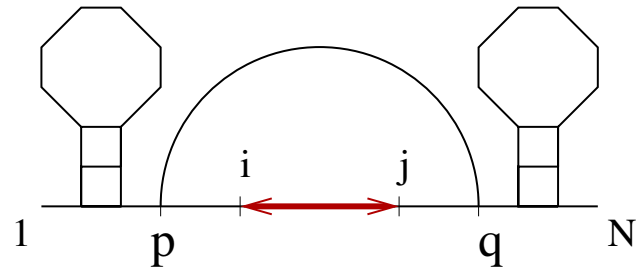
trivial case: region i,j is exterior to all loops.



$$Pr_u[i,j] = \frac{Q[1,i-1]Q[j+1,N]}{Q[1,N]}$$

$Pr_u[i,j]$: region i,j contains no secondary structure

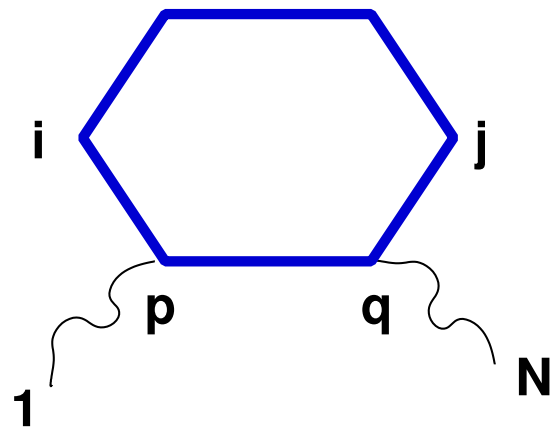
region i,j is spanned by a basepair (p,q).



$$Pr_u[i,j] \;\; += \sum_{\substack{p<i \\ j<q}} Prob[p,q] \frac{Qpq_u[i,j]}{Qb[p,q]}.$$

$Qpq_u[i,j]$: unpaired region i,j enclosed by pair (p,q):

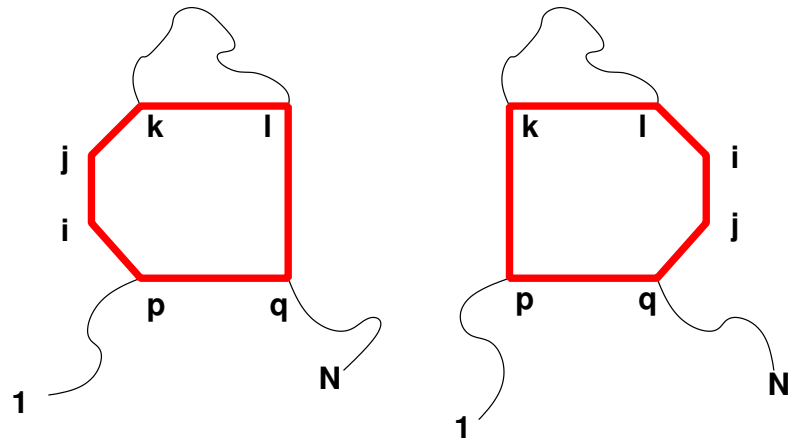
base pair (p,q) closes a Hairpin loop:



$$Qpq_u[i,j] = e^{-\beta H(p,q)} + \ldots$$

$Qpq_u[i,j]$: unpaired region i,j enclosed by pair (p,q):

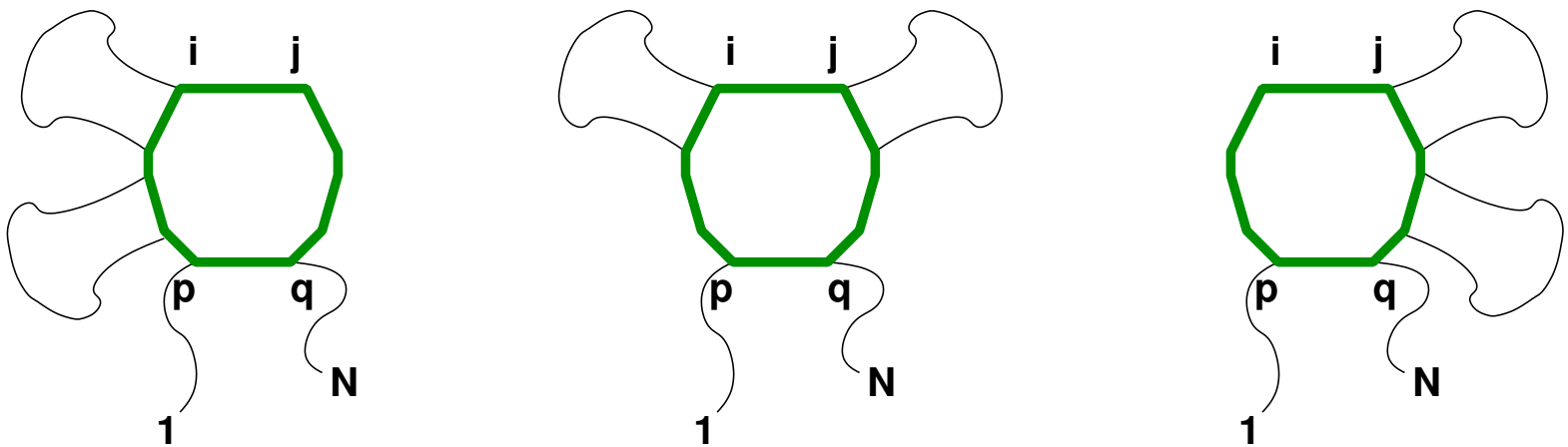
base pair (p,q) closes an Interior loop:



$$Qpq_u[i,j] += \sum_{\substack{p<i\leq j<k \\ l<i\leq j<q}} e^{-\beta I(p,q,k,l)} Qb[k,l] + \ldots$$

$Qpq_u[i,j]$: unpaired region i,j enclosed by pair (p,q):

base pair (p,q) closes a Multiloop:



$$Qpq_u[i,j] += \sum_{p<i\leq j<q}$$

$$\Big(Qm2[p+1,i-1]e^{-\beta u(j-i+1)} + Qm[p+1,i-1]e^{-\beta u(j-i+1)}Qm[j+1,q-1] + e^{-\beta u(j-i+1)}Qm2[j+1,q-1]\Big).$$

$Pr_u[i,j]$: region i,j contains no secondary structure

$$Pr_u[i,j] = \frac{Q[1,i-1]Q[j+1,N]}{Q[1,N]} + \sum_{p<i<j<q} Prob[p,q]\frac{Qpq_u[i,j]}{Qb[p,q]}.$$

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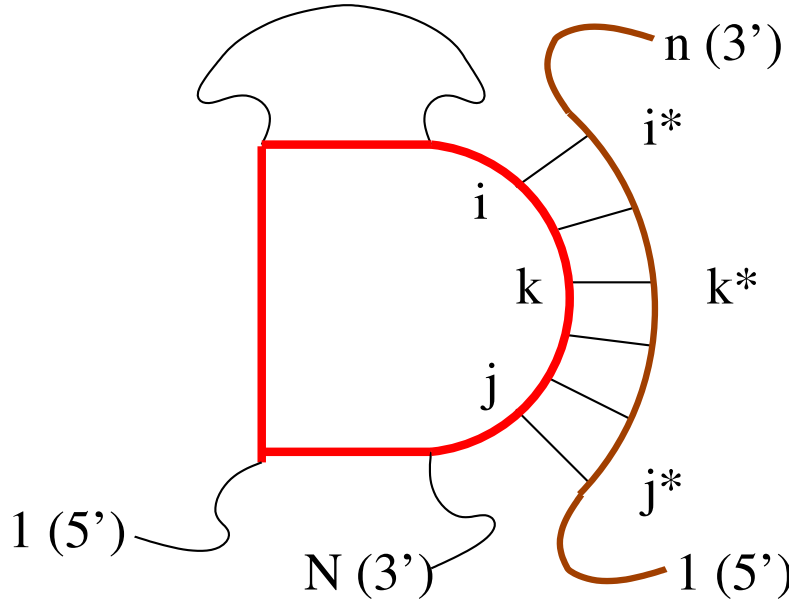
unstructured region i, j is paired to i^*, j^*:



$$Qup[i,j,i^*,j^*] = Pr_u[i,j] \sum_{\substack{i<k<j \\ i^*>k^*>j^*}} Qp[i,k,i^*,k^*] e^{-\beta I(k,k^*;j,j^*)}.$$

unstructured region i, j is paired to i^*, j^*:

$$Pup[i, j, i^*, j^*] = \frac{Qup[i, j, i^*, j^*]}{Qup}.$$

where

$$Qup = \sum_{i,\ j,\ i^*, j^*} Qup[i, j, i^*, j^*].$$

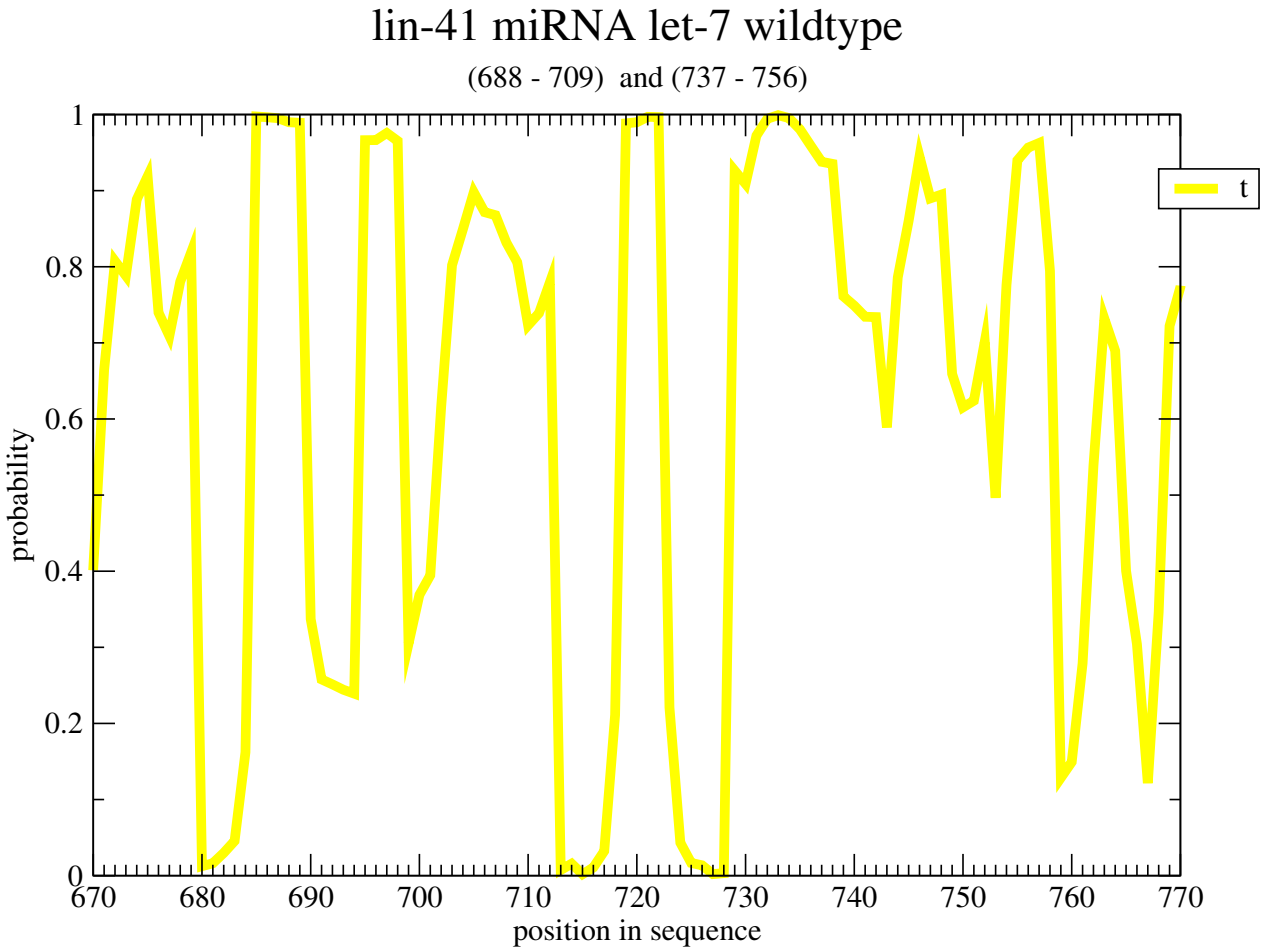
position i is contained in a target site for a small RNA

$$Pup[i,j] = \sum_{i^*,j^*} Pup[i,j,i^*,j^*].$$

and

$$Pup[k] = \sum_{i \leq k \leq j} Pup[i,j].$$

Interaction of let-7 miRNA with target gene lin-1



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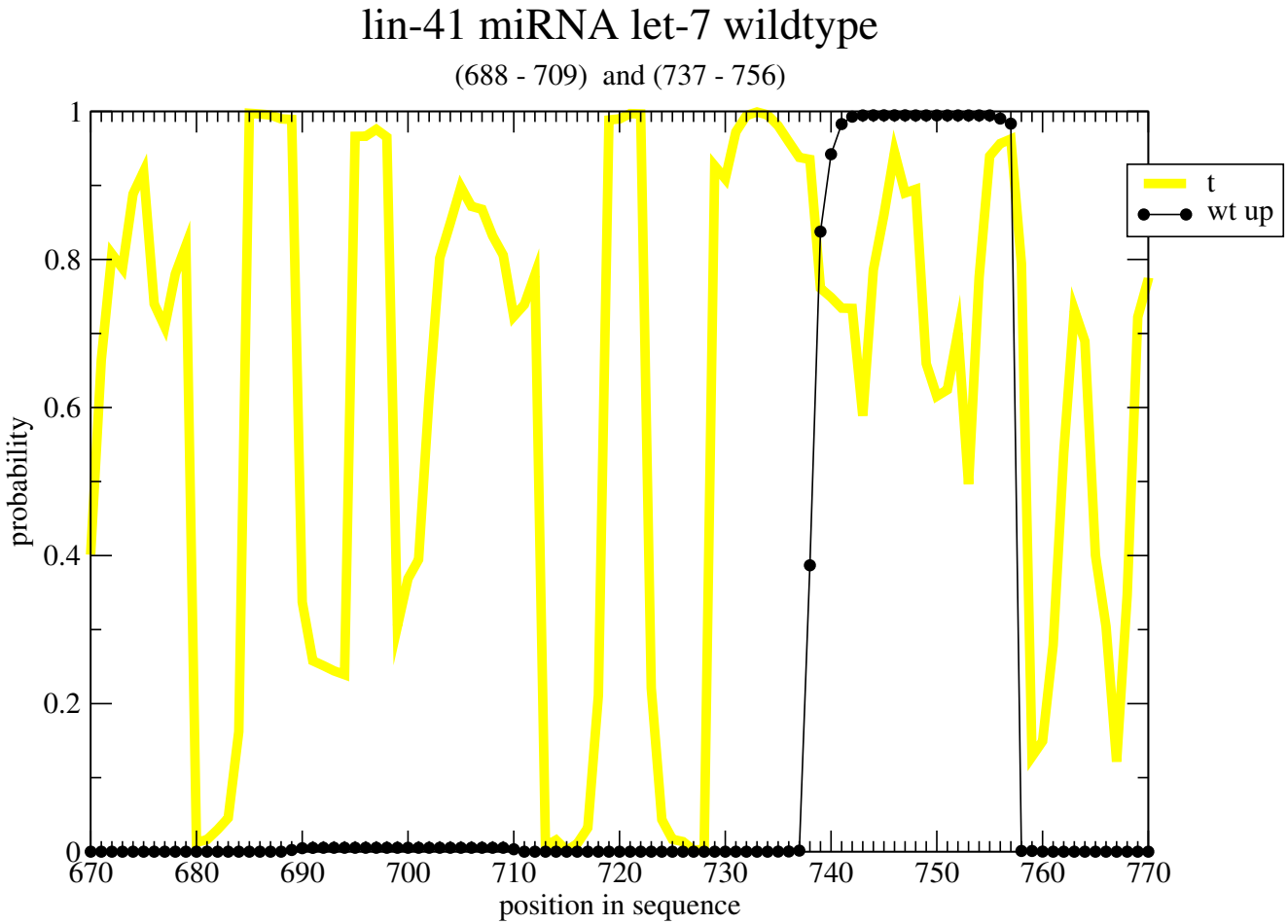

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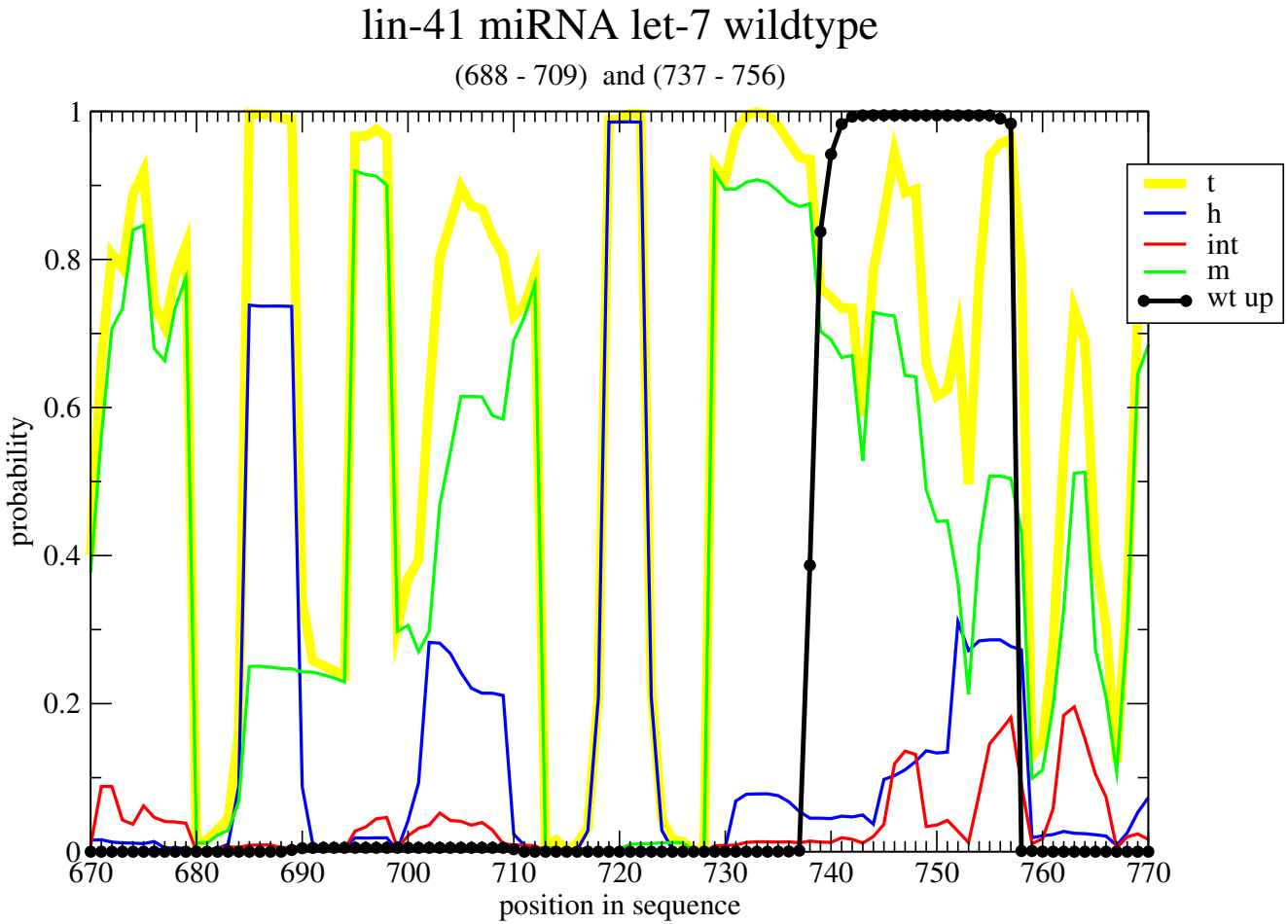

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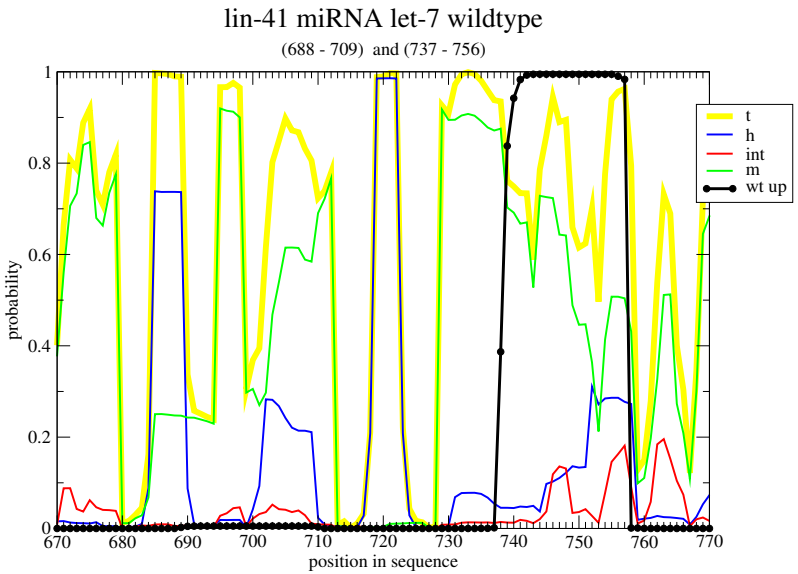
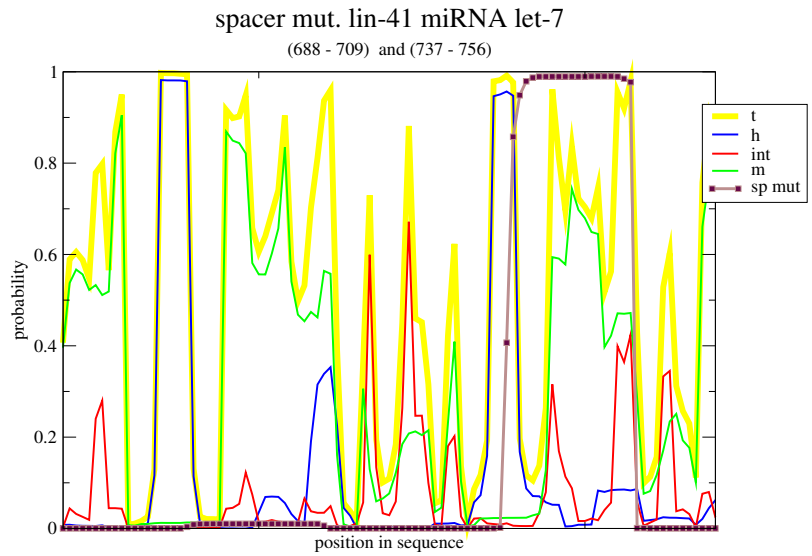
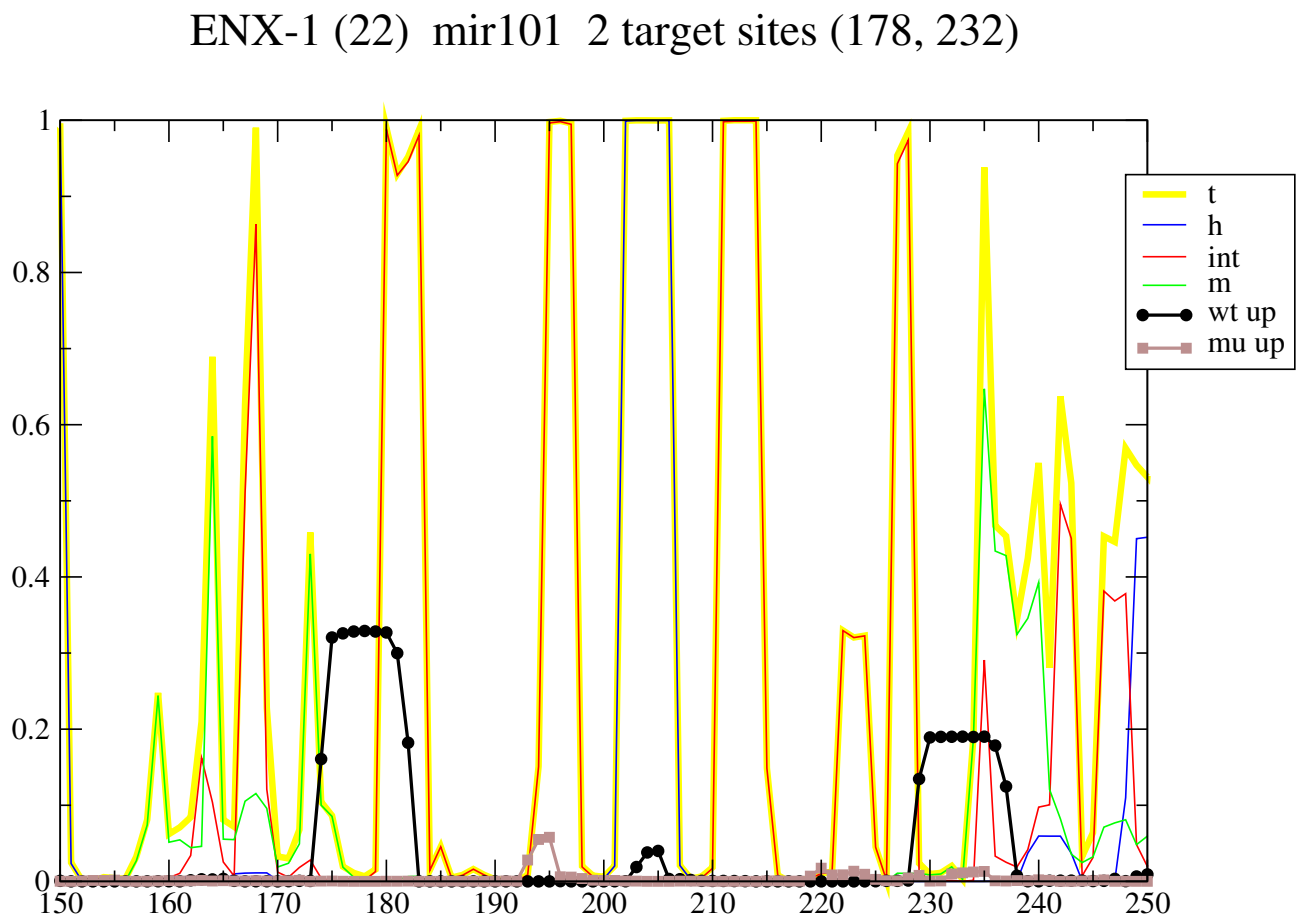


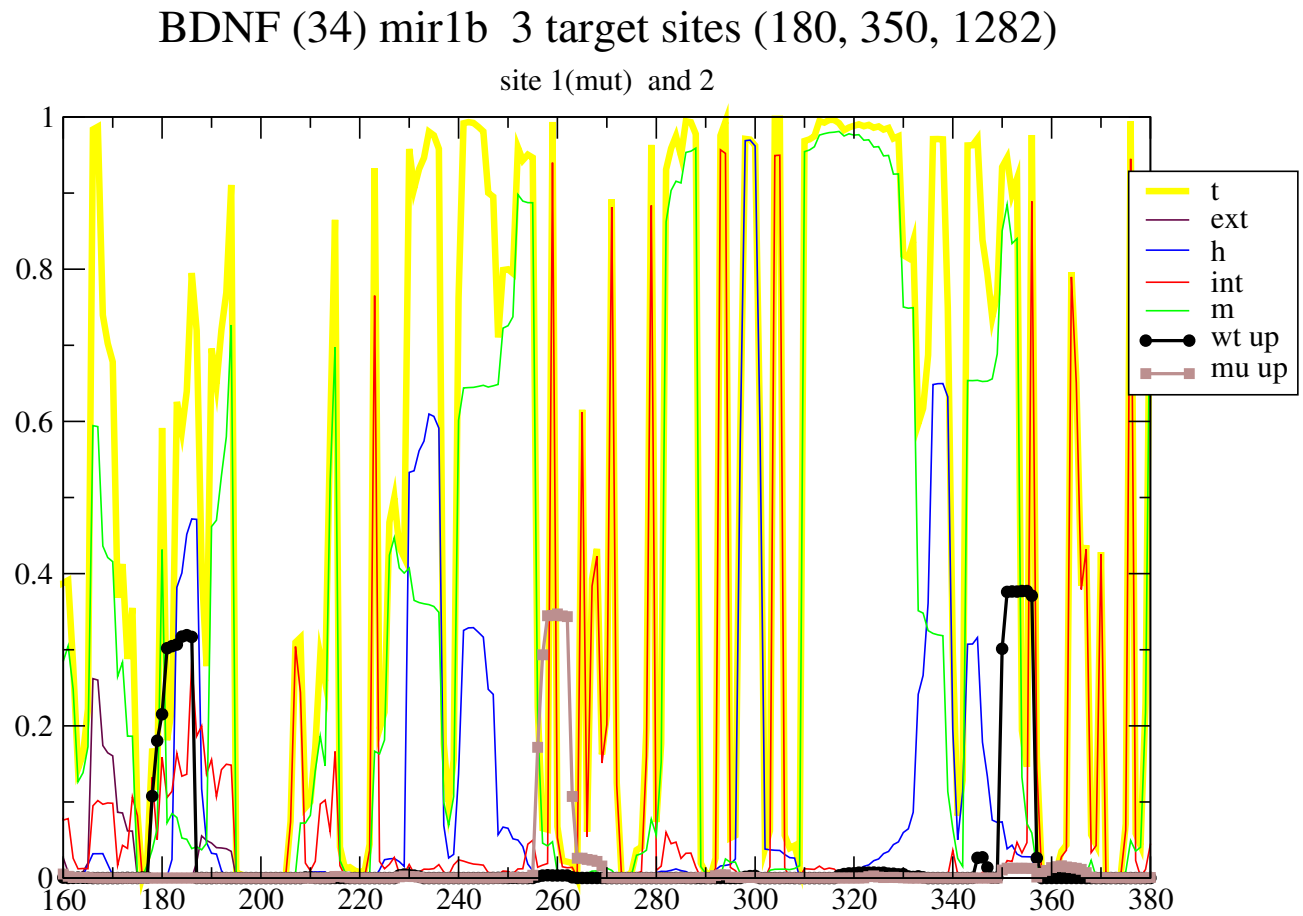


Interaction of human enx-1 with miRNA mir101



Interaction of human bdnf with miRNA mir1b

176-185 p=0.99, 348-358, p<0.01



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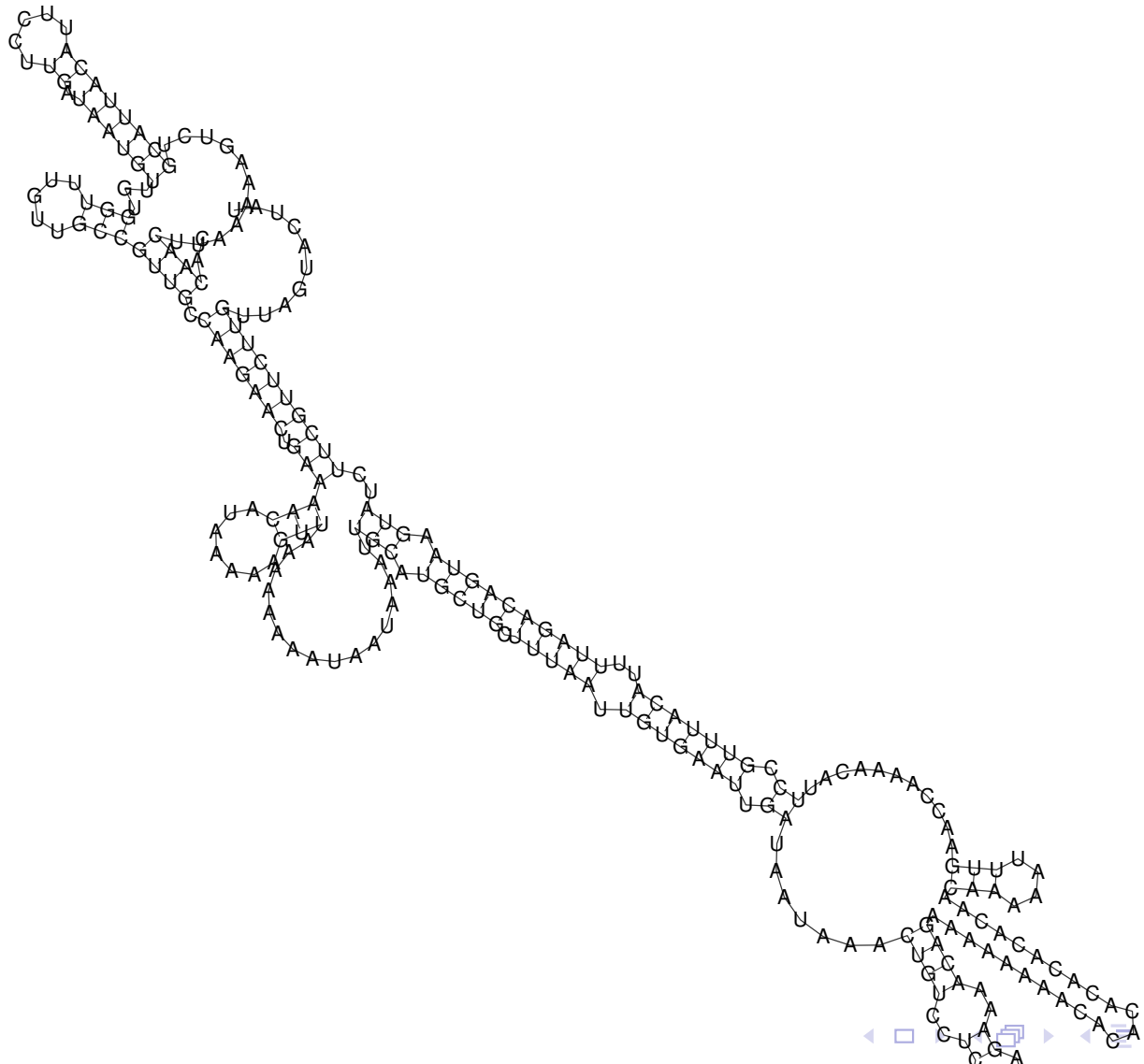
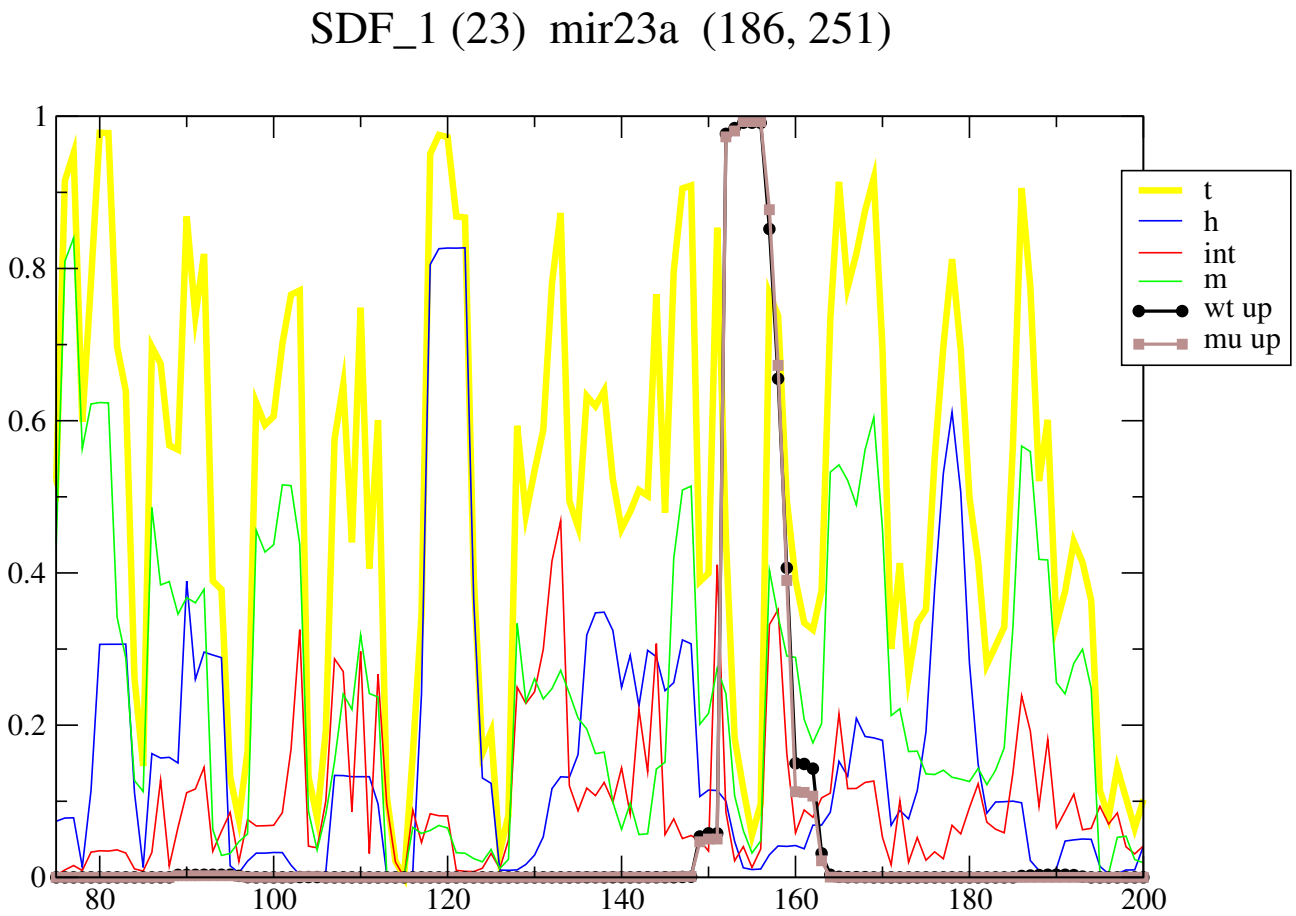
seed sequence: ACATTCC

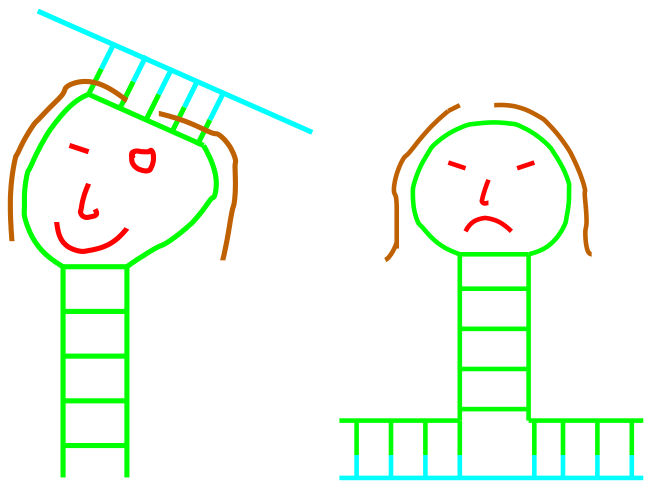


Interaction of human sdf-1 with miRNA mir23a

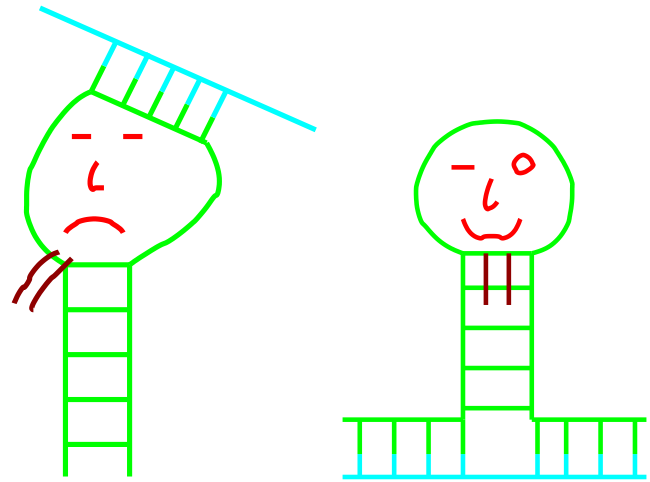


2 Parts of one Solution

RNAup



RNAcofold



Outline

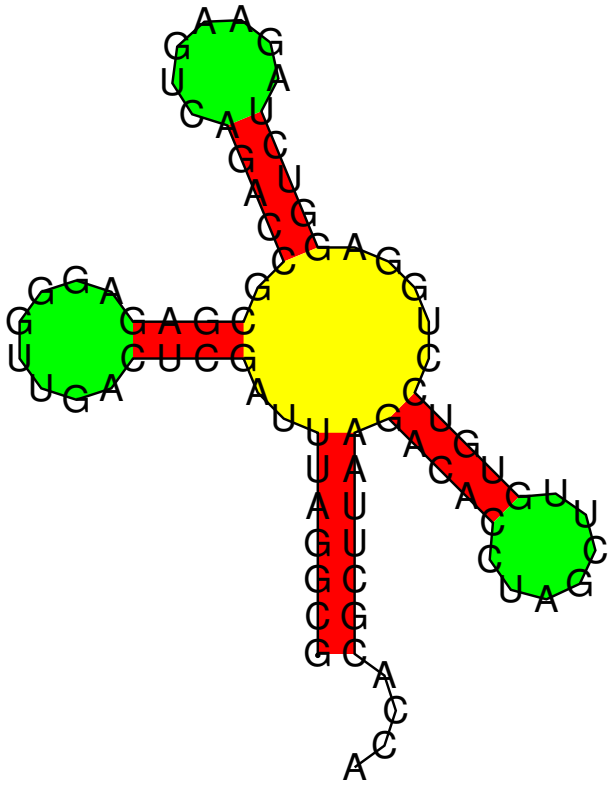
RNAcofold

- Variant of RNAfold
- Concatenate the 2 molecules
- Define cut between molecules

RNA Secondary Structure

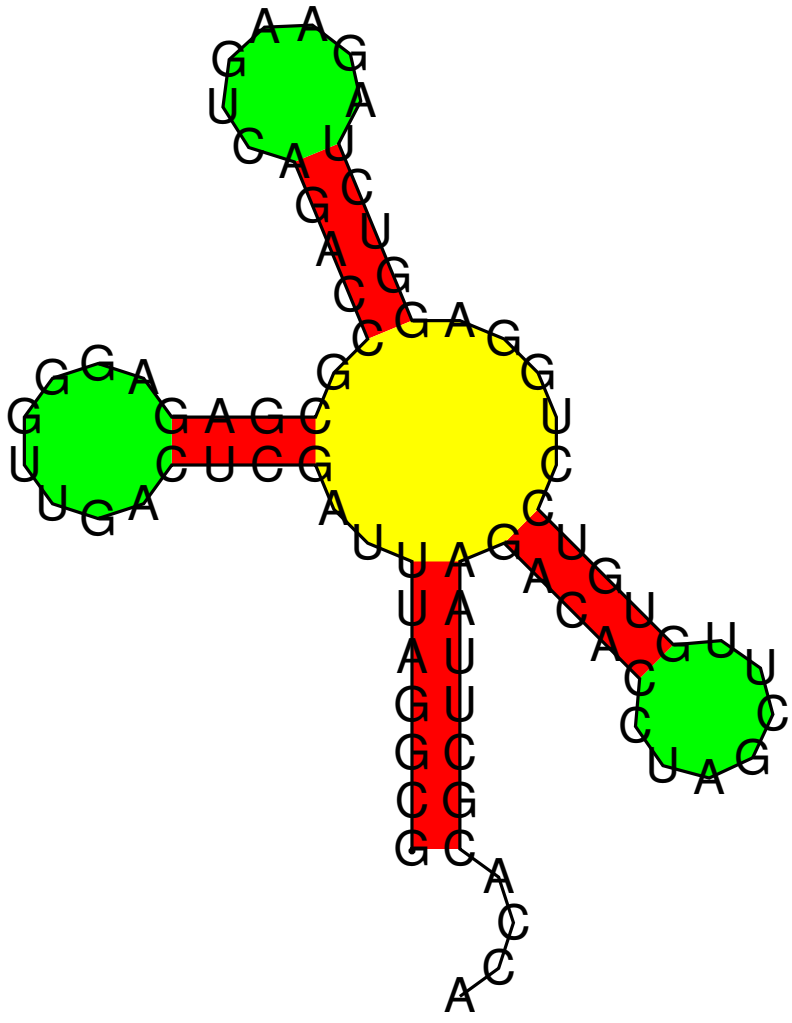
Free Energy

Free Energy is sum of Loop contributions:

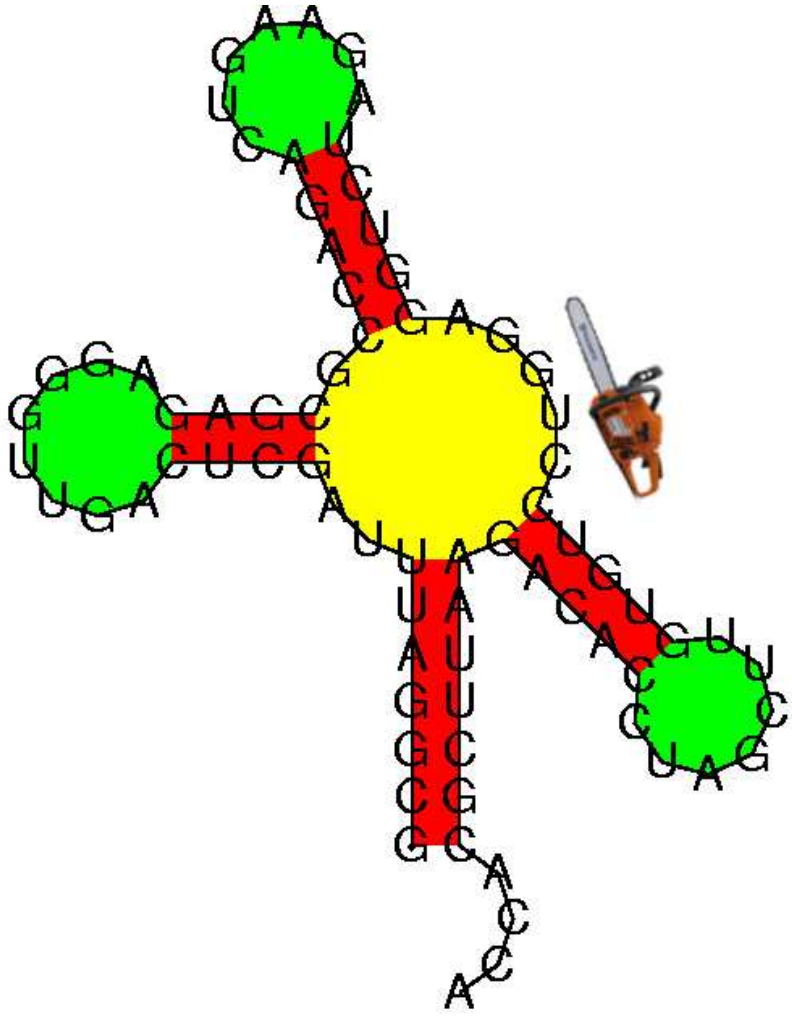


- Interior Loops
- Hairpin Loops
- Multi loops
- **Exterior Loops**

Going exterior



Going exterior



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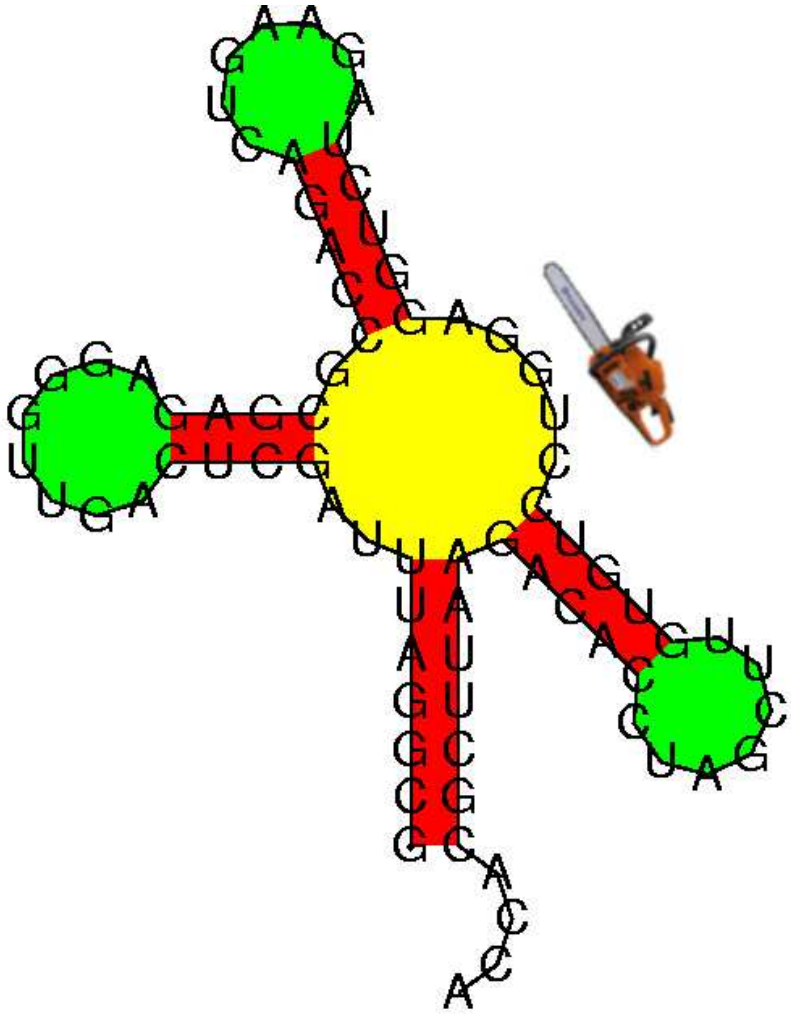

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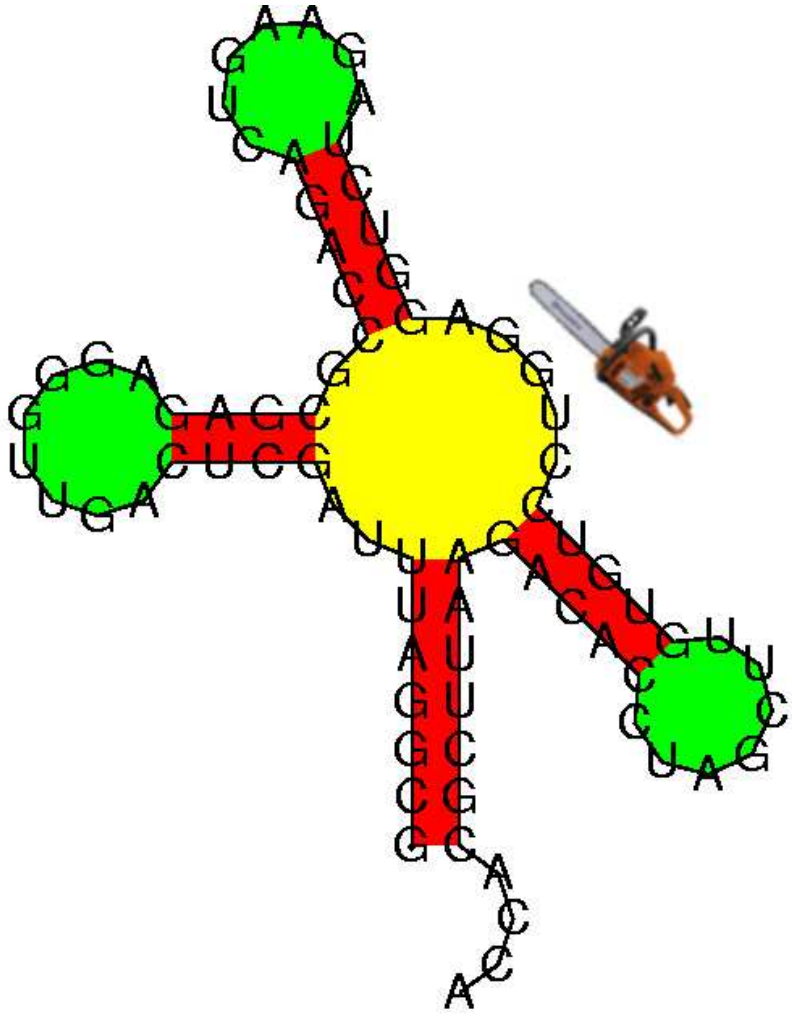

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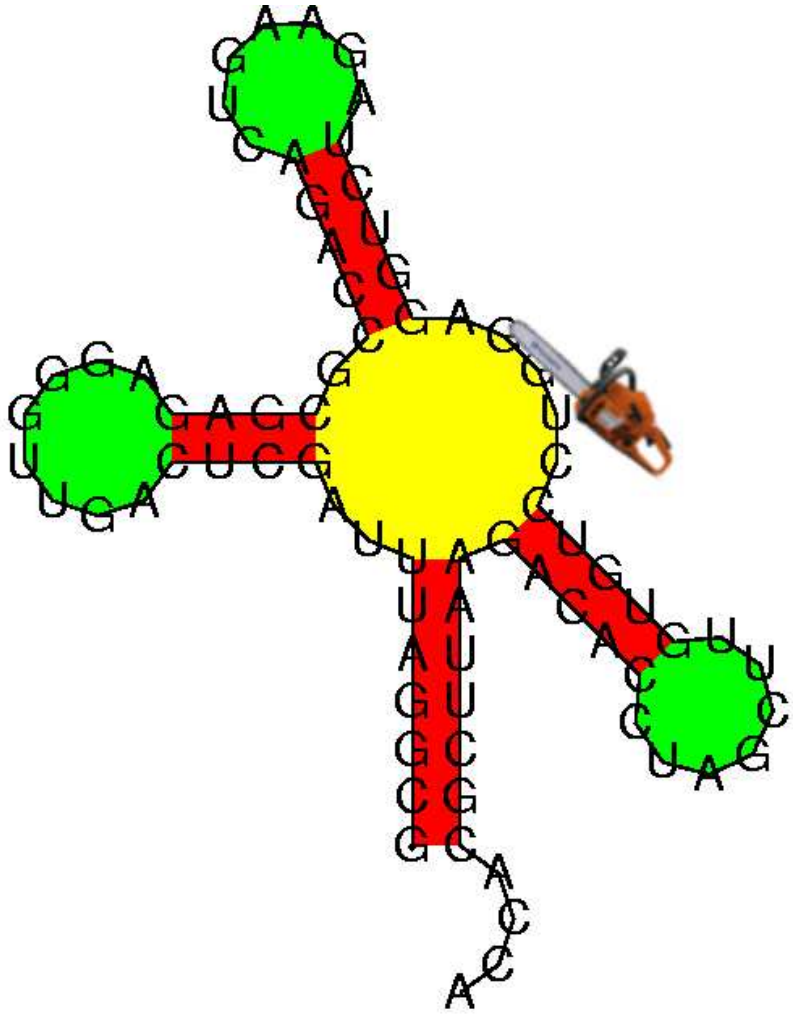

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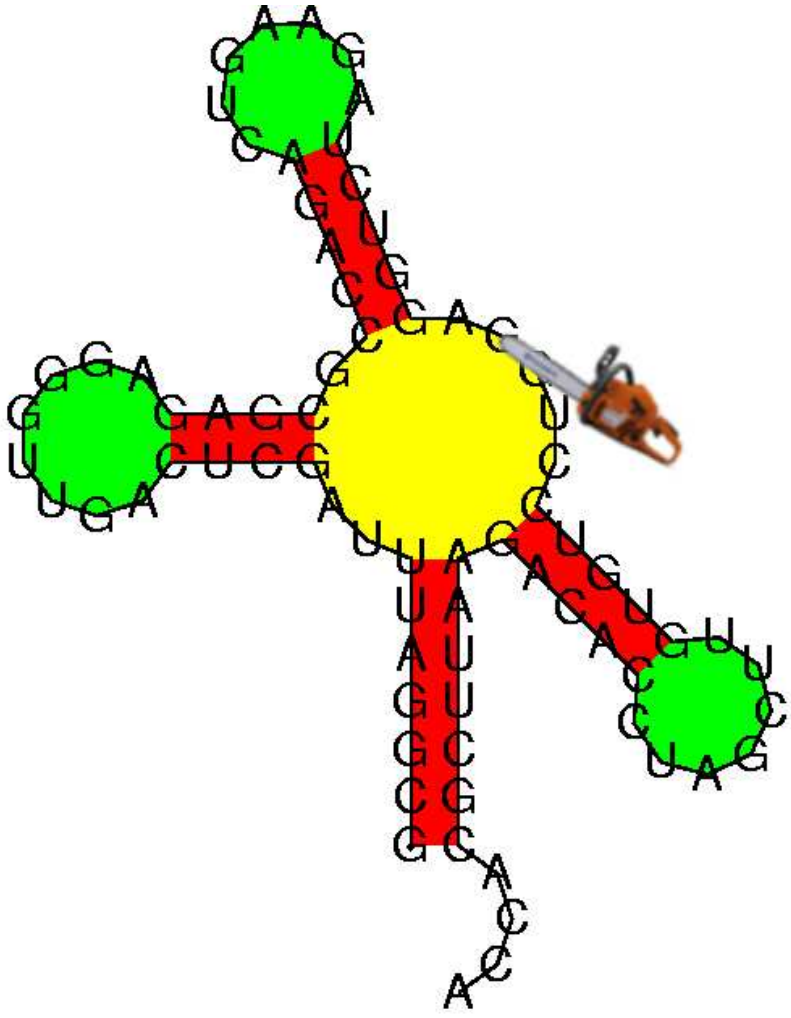

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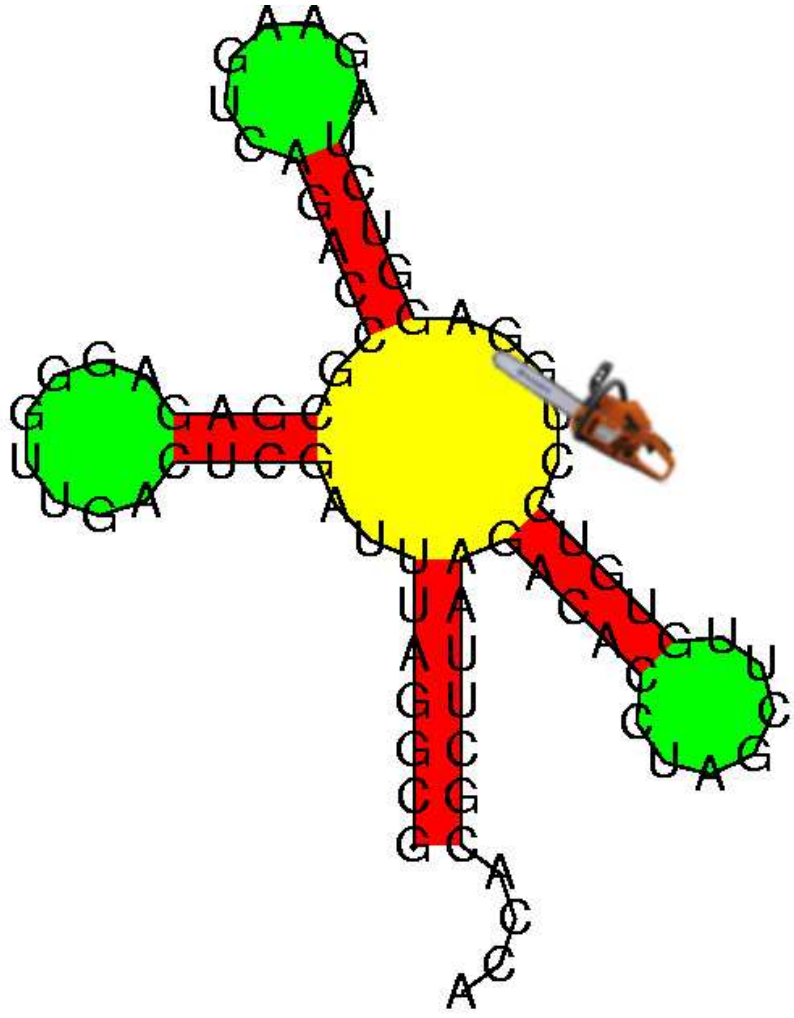

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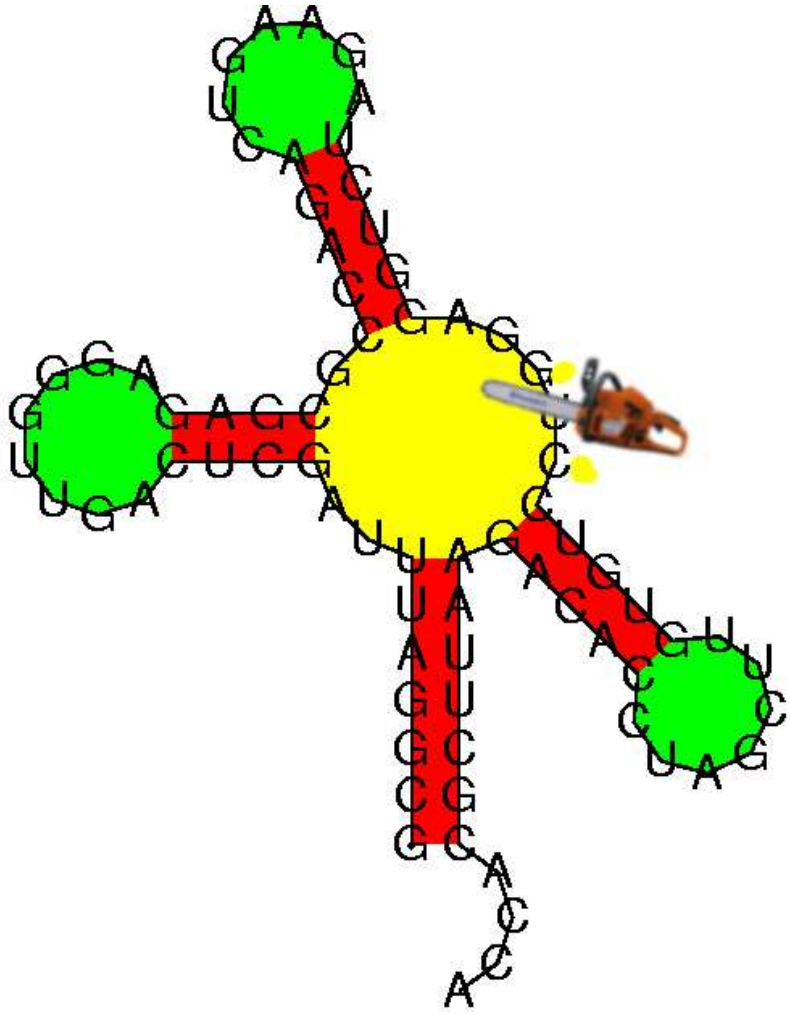

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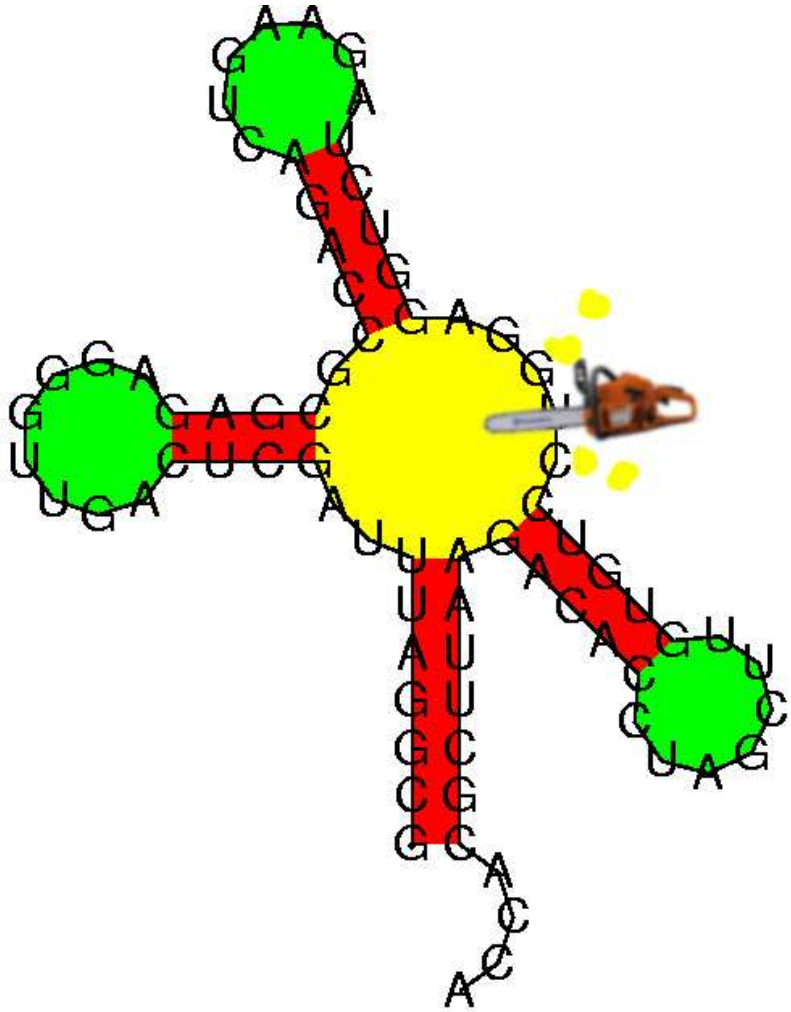

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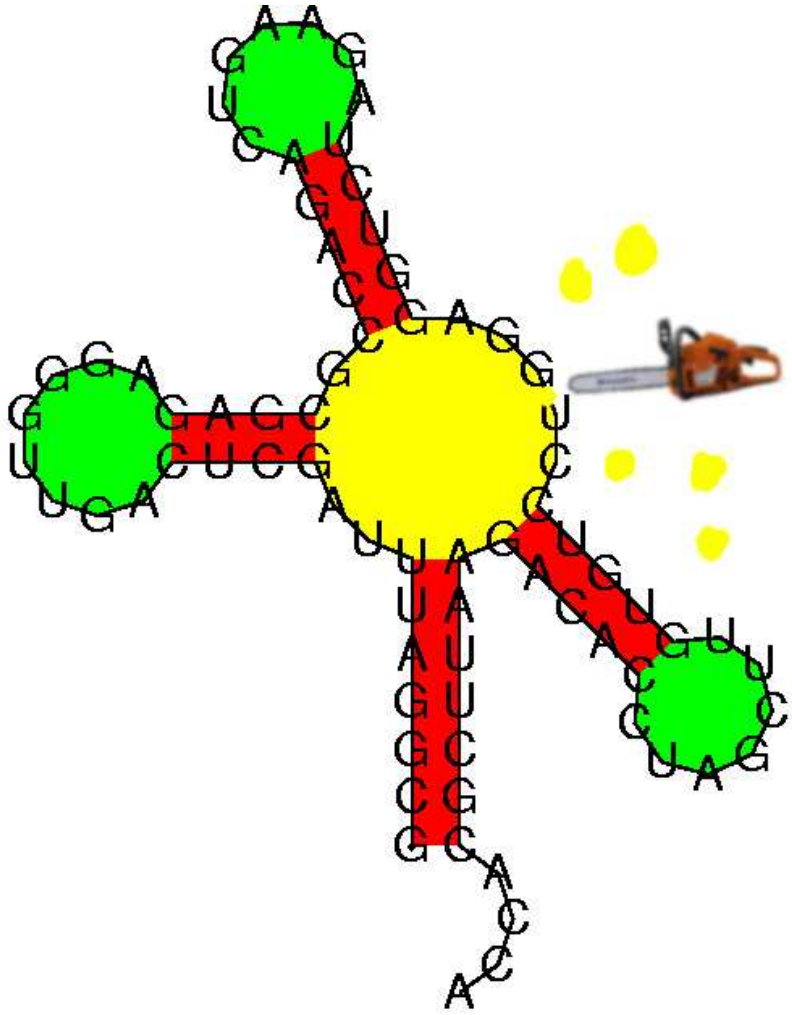

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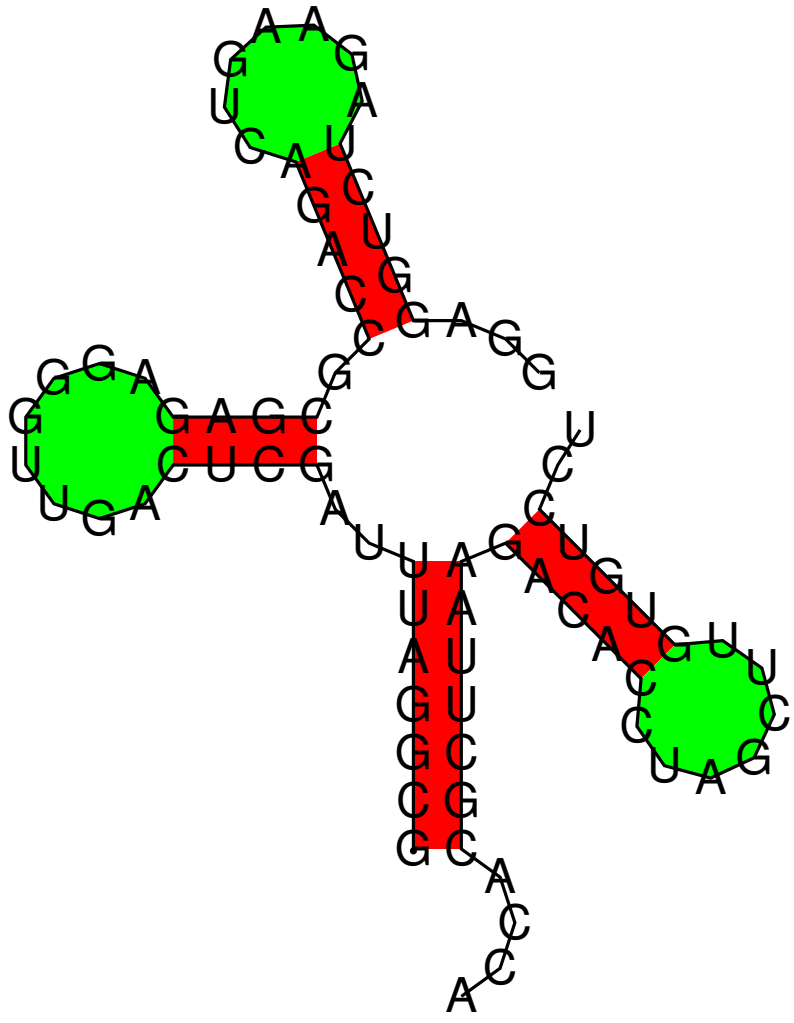

Partition Function

recursion

Partition function of segment i, j

$$Q_{ij} = Q_{i+1,j} + \sum_{i<k\leq j} Q^B_{i,k} Q_{k+1,j}$$

$$Q^B_{i,j} = \mathcal{H}(i,j) + \sum_{i<k<l<j} Q^B_{kl} \mathcal{I}(i,j;k,l) + Q^M_{i+1,j-1} a$$

$$Q^M_{i,j} = Q^M_{i+1,j} c + \sum_{i<k\leq j} Q^B_{i,k} b Q^{M1}_{k+1,j}$$

$$Q^{M1}_{i,j} = Q^{M1}_{i+1,j} + \sum_{i<k<j} Q^B_{i,k} b Q^{M1}_{k+1,j} + \sum_{i<k\leq j} Q^B_{i,k} b(j-k) c$$

Partition Function

recursion

Parts to be checked when cofolding

$$Q_{ij} = Q_{i+1,j} + \sum_{i<k\leq j} Q^B_{i,k} Q_{k+1,j}$$

$$Q^B_{i,j} = \mathcal{H}(i,j) \vee Q_{i+1,\|} Q_{\|,j-1} + \sum_{i<k<l<j} Q^B_{kl} \mathcal{I}(i,j;k,l) + Q^M_{i+1,j-1} a$$

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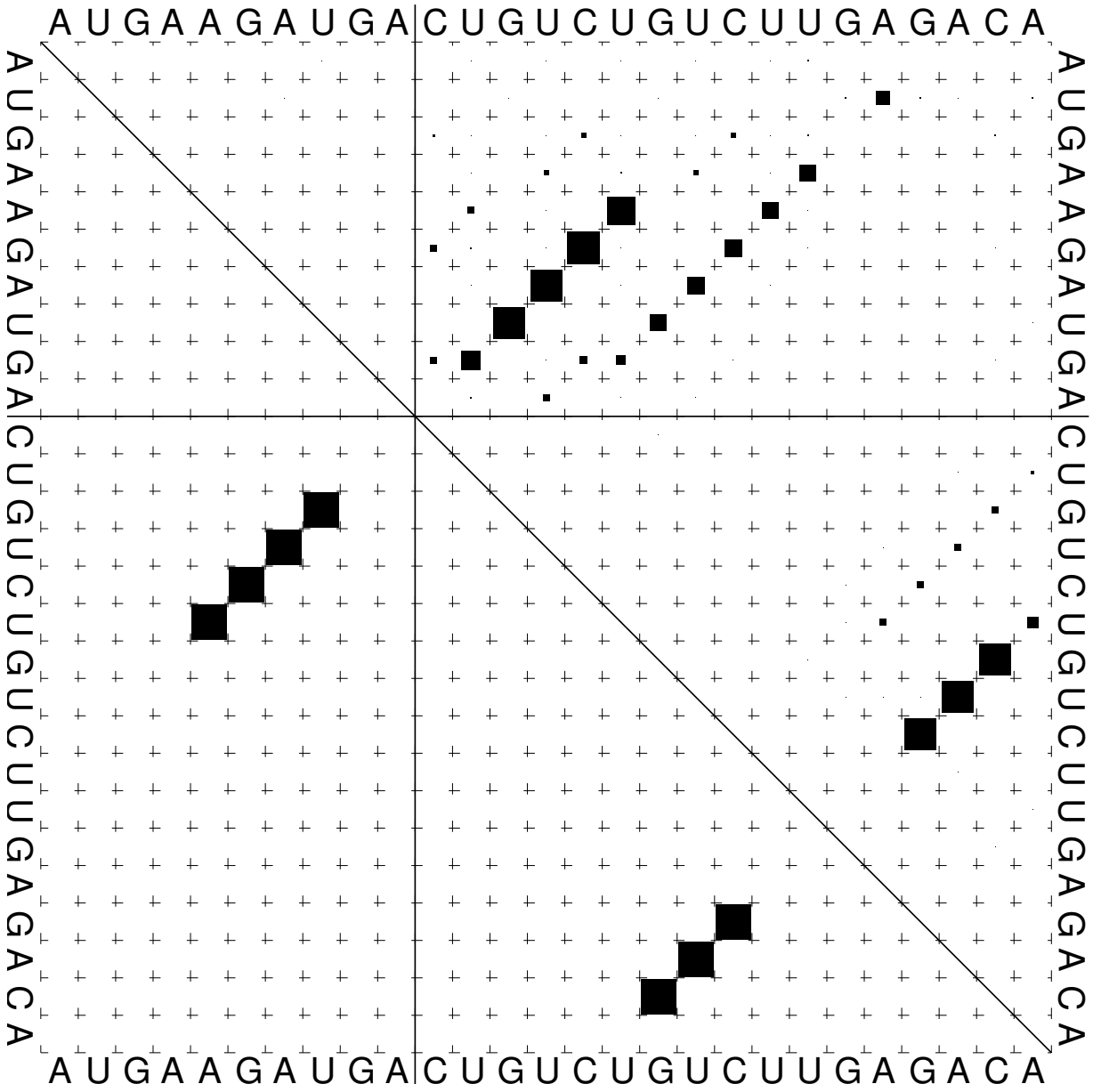
Output: Dot Plot

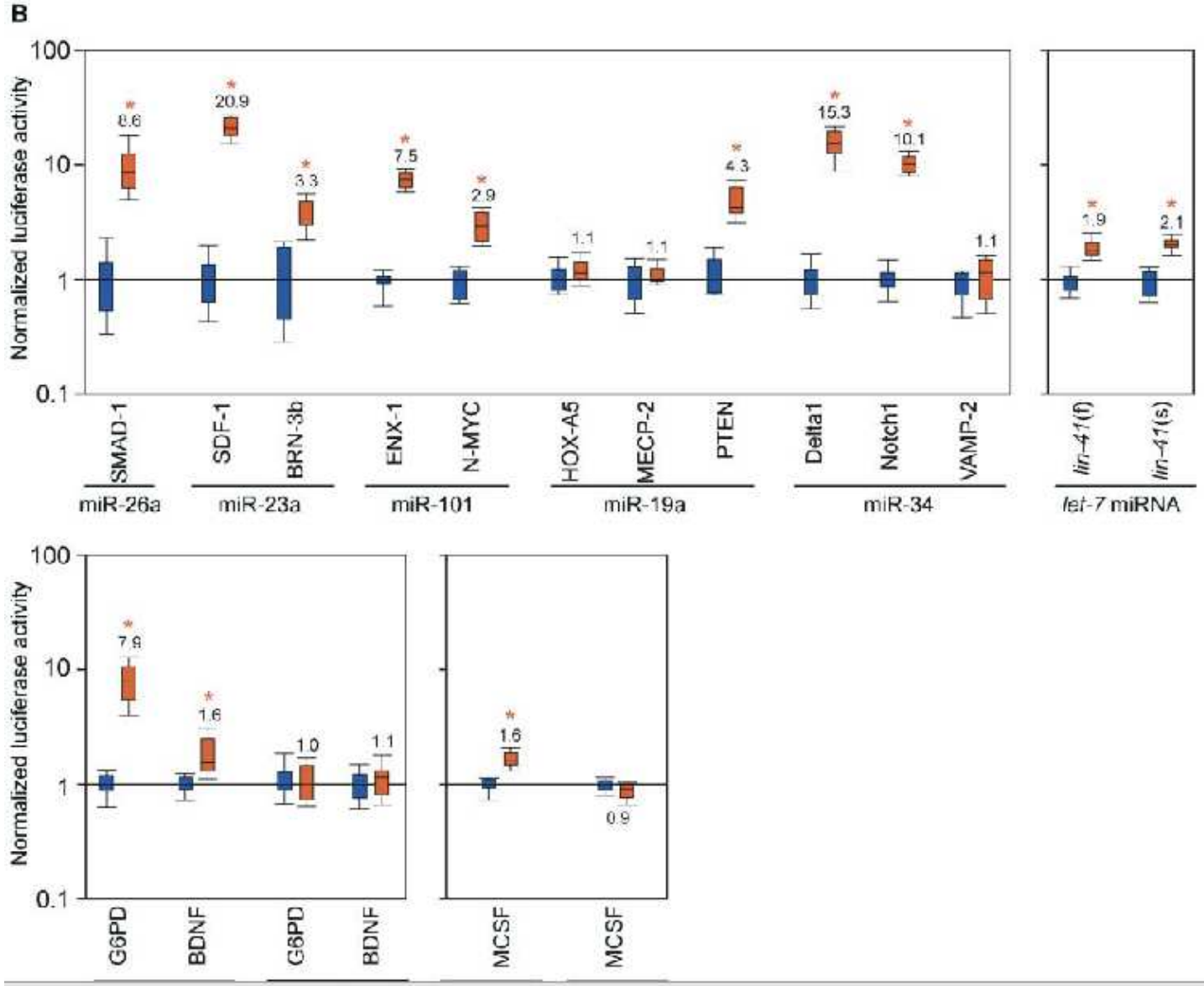


Outline

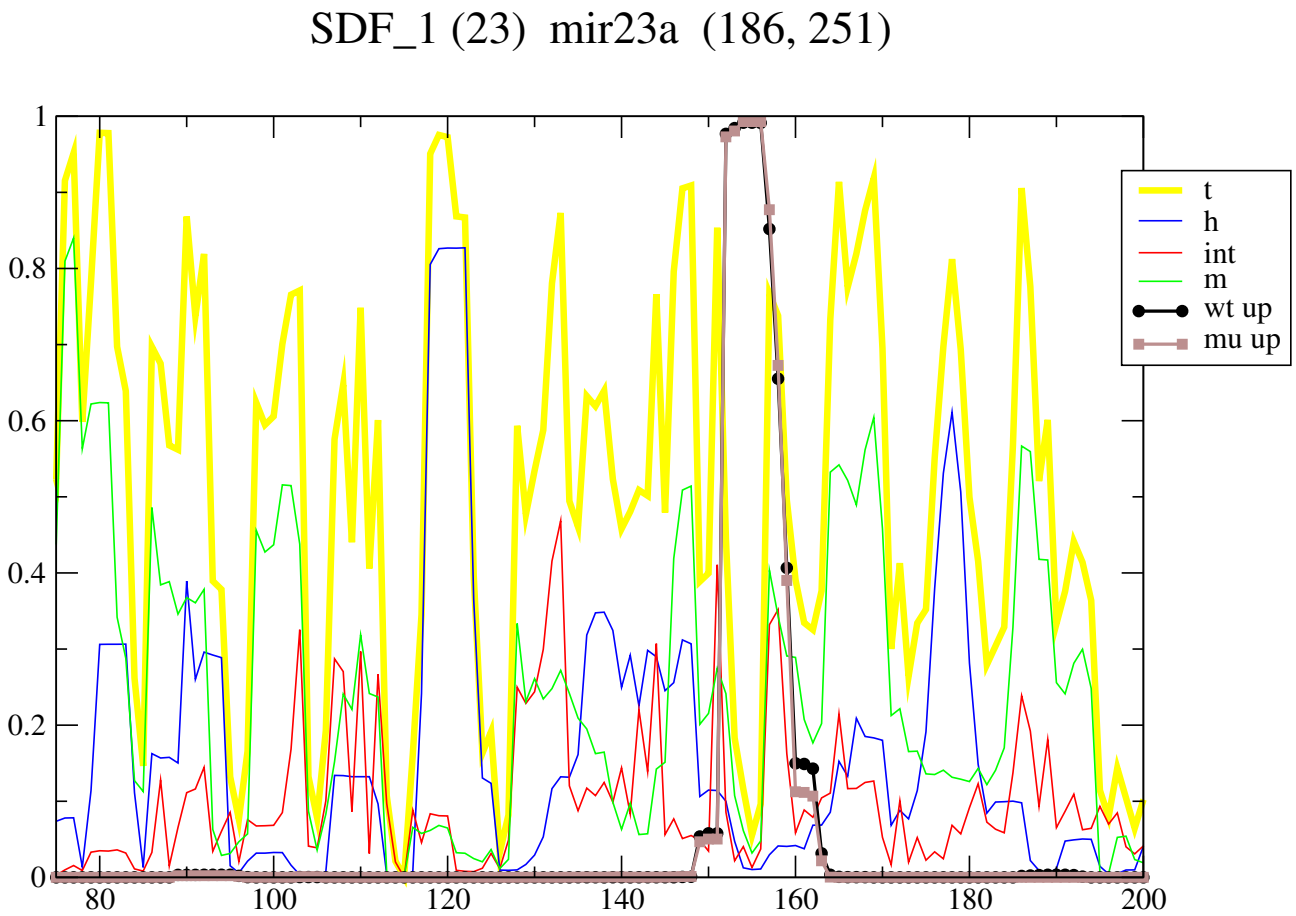
Setting

Wenn du nicht überzeugen kannst, stifte Verwirrung

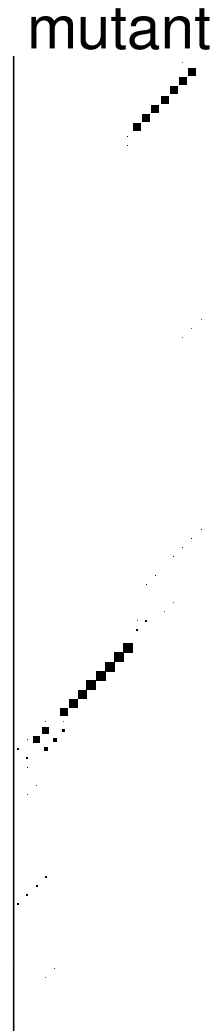
Lewis et.al, Prediction of Mammalian MicroRNA Targets



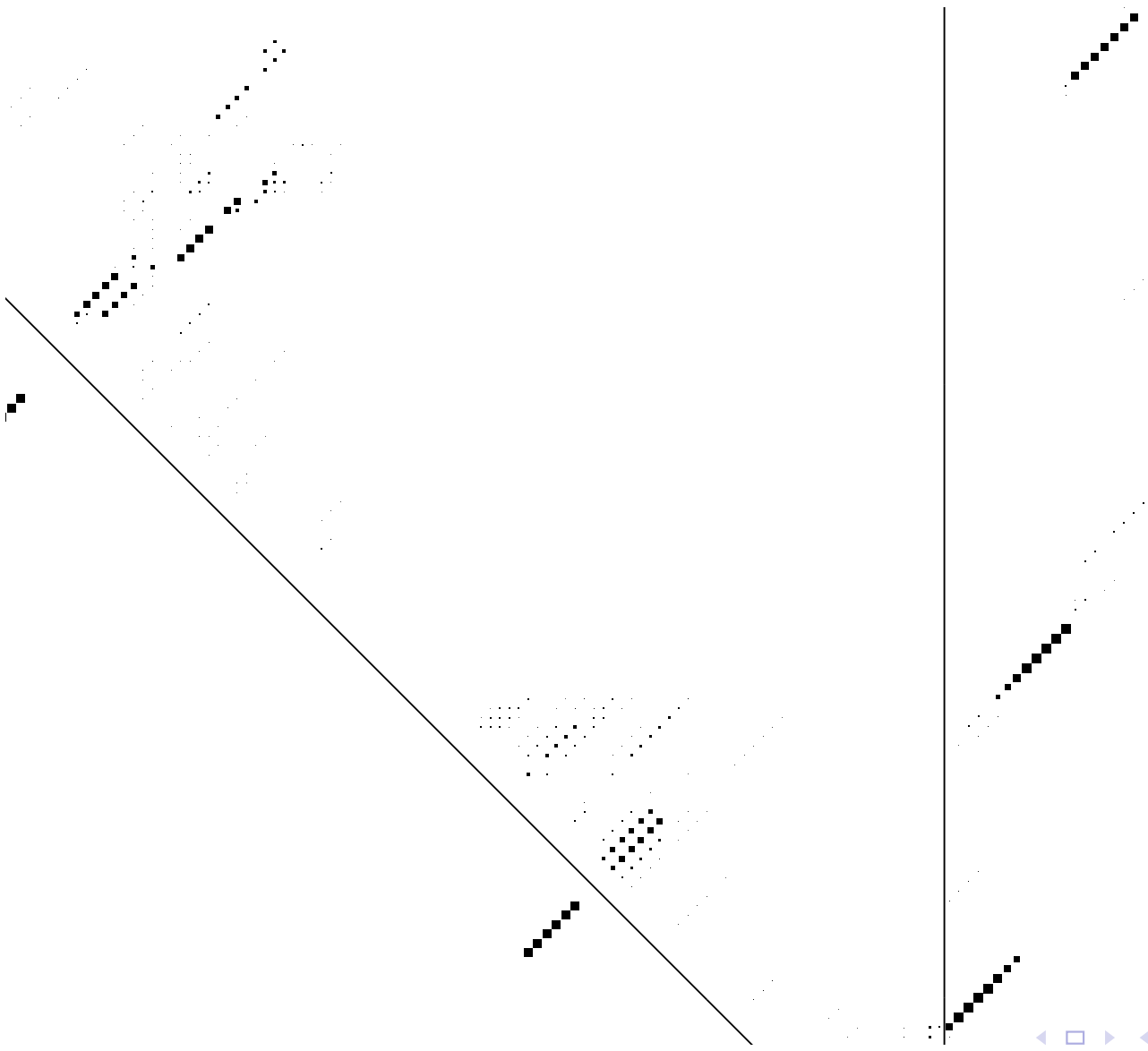
Interaction of human sdf-1 with miRNA mir23a



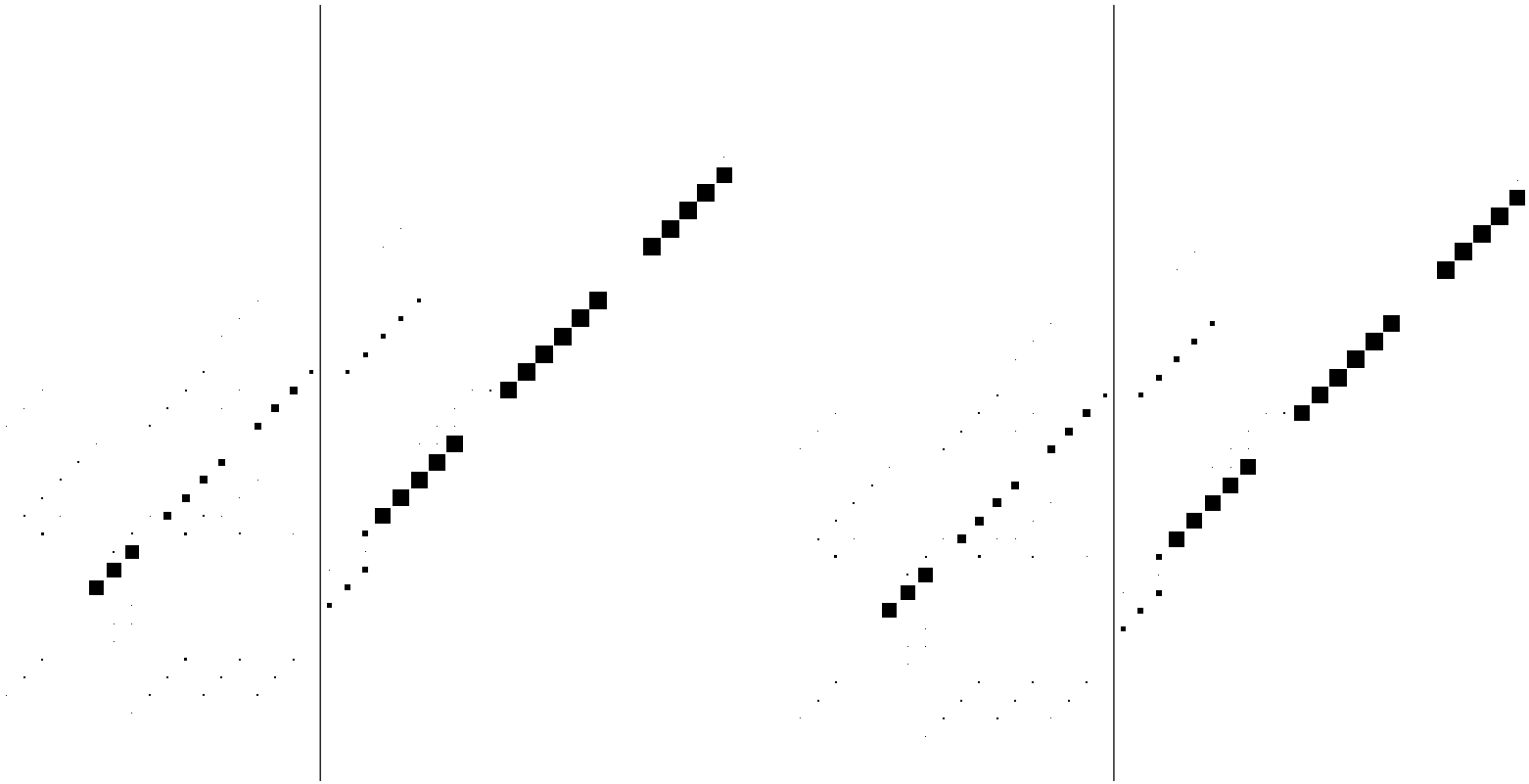
SDF1



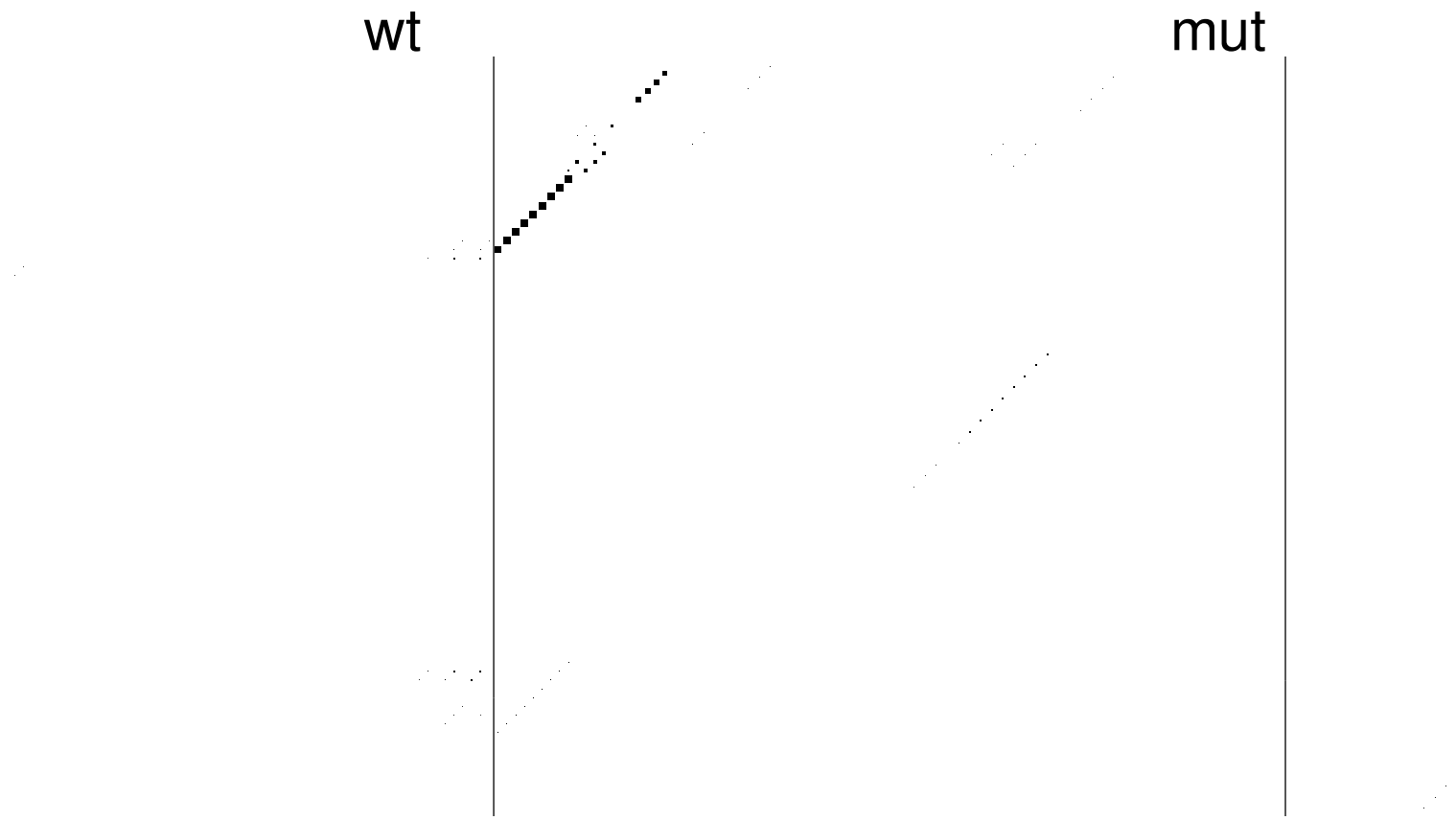
SDF1



HoxA5, position 185-230

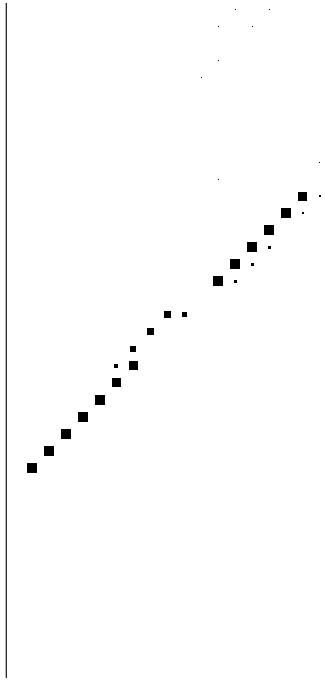


Enx1, position 150-235

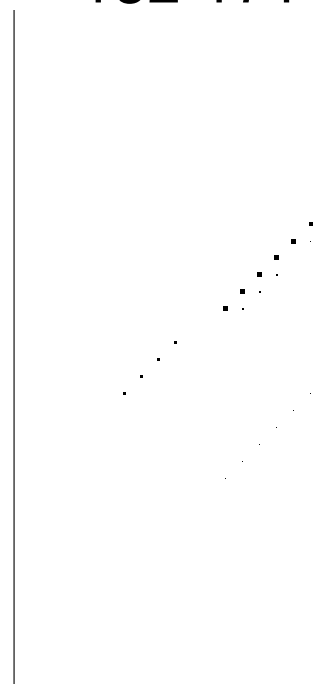


BRN3-b

wt
132-171, 30%



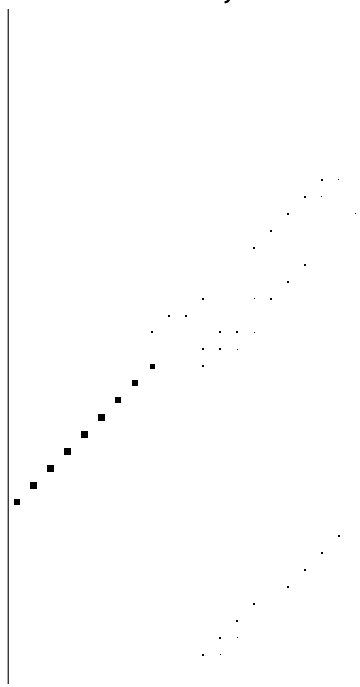
mutant
132-171



BRN3-b

wt

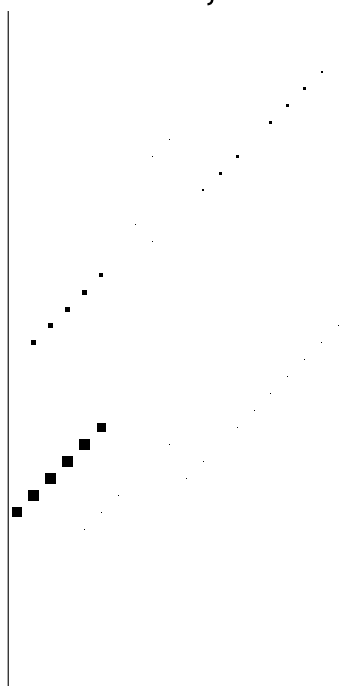
492-531, 12%



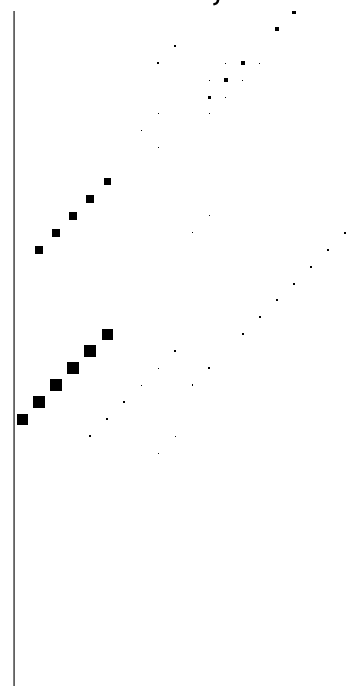
mutant

BRN3-b

wt
622-66, 40%



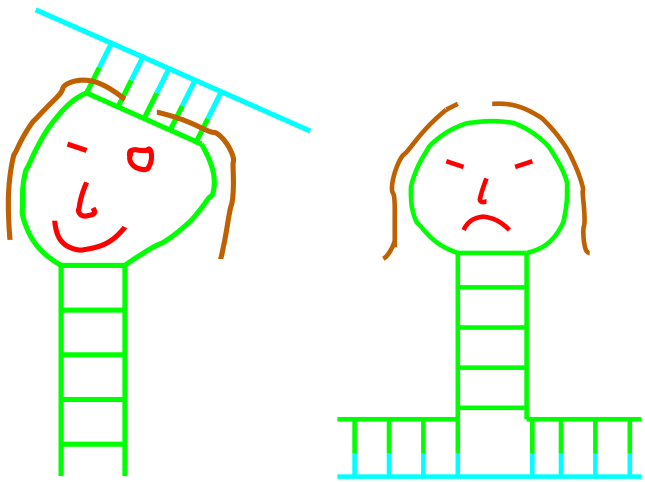
mutant
622-66, 50%



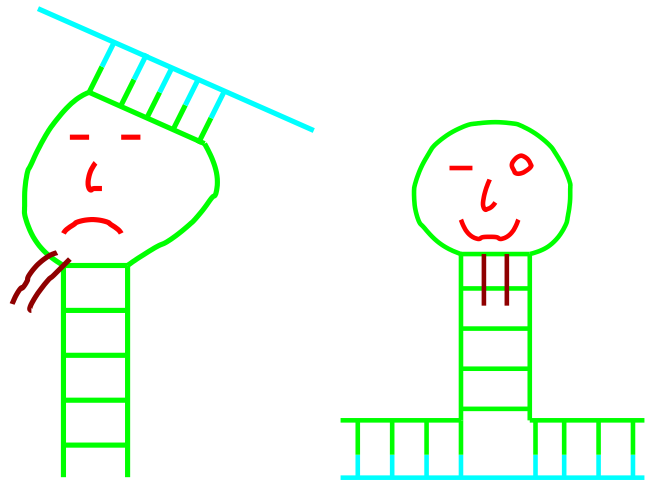
Outline

Problems

RNAup



RNAcofold



Solution?

- Great Unified Cofolding Algorithm
- Cofolding including intermolecular Pseudo-knots and pair probabilities
- Will be expensive computationally and in memory requirements

Unification of RNAcofold and RNAup

First assume no intramolecular base pairs in smaller molecule

- Start with Cofold $\mathcal{O}((n_A + n_B)^3)$ proc. $\mathcal{O}((n_A + n_B)^2)$
- Use RNAup routines, compute intermolecular partition functions ($\mathcal{O}(n^3)$)
- Use RNAup routines, compute partition functions given that pair i, j is innermost base-pair enclosing a binding site. ($\mathcal{O}(n^3)$)

Unification II

- Compute partition functions given duplex between i and j (Like Fold, ($\mathcal{O}(n^3)$))
- Compute partition functions for intra-molecular pairs given Pseudo-knot. $\mathcal{O}(n^3)$
- Run modified RNAup to get the partition functions of intermolecular pairs given Pseudo-knot. $\mathcal{O}(n^3)$

Unification III

- Will take up to six times longer than a simple Cofold
- Will take four to six times more memory.
- Still has time complexity of $\mathcal{O}(n^3)$ in processor time and $\mathcal{O}(n^2)$ in memory.

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