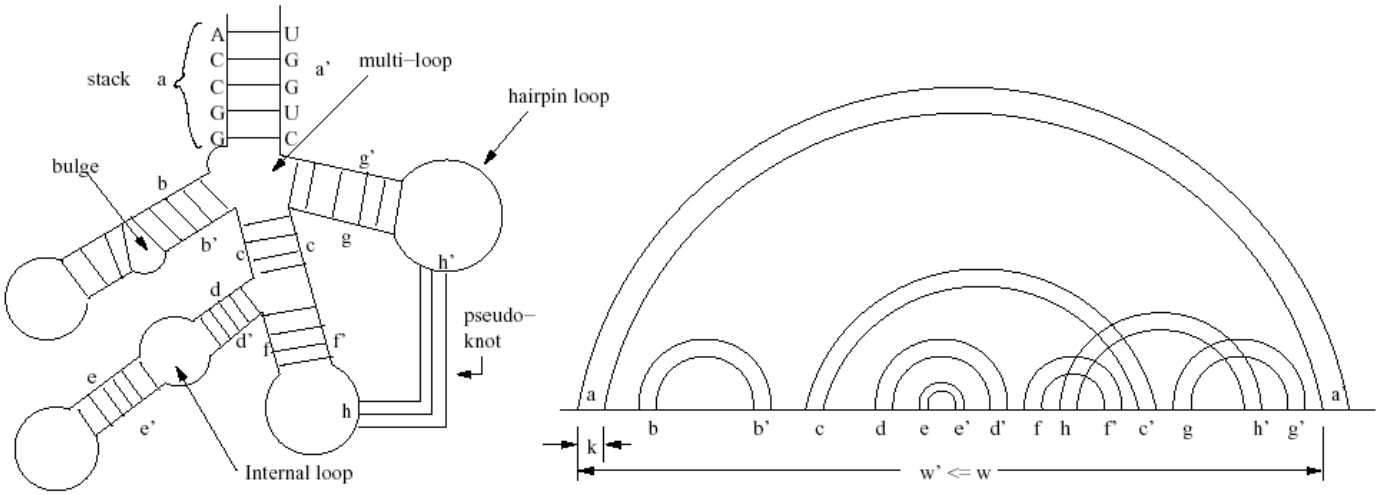
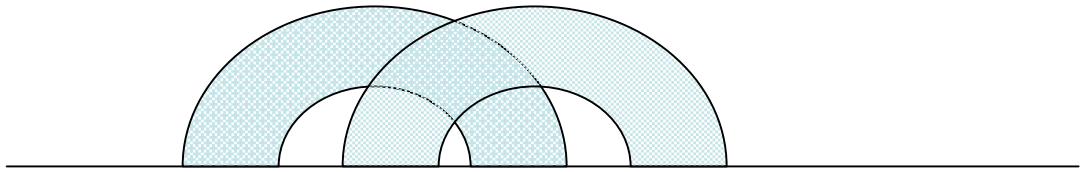
ncRNA Structure including simple pseudo-knots

RNA structure (including pseusoknots)



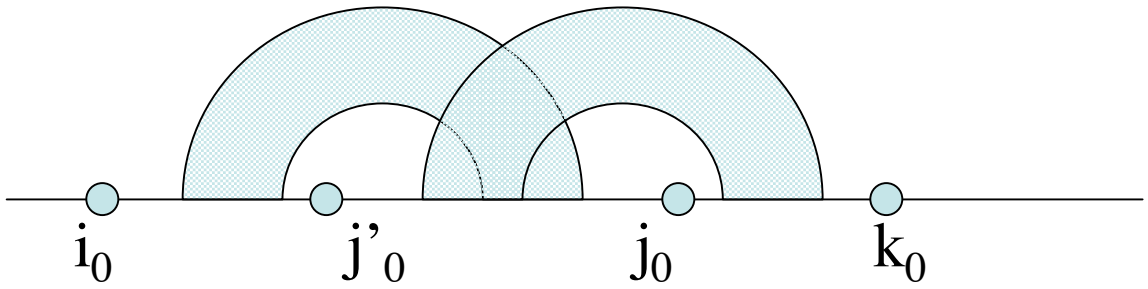
- Basics:
  - Structure is described by a set of base-pairings M.
  - Normally, the base-pairs do not interleave.

Incorporating pseudoknots in structure prediction



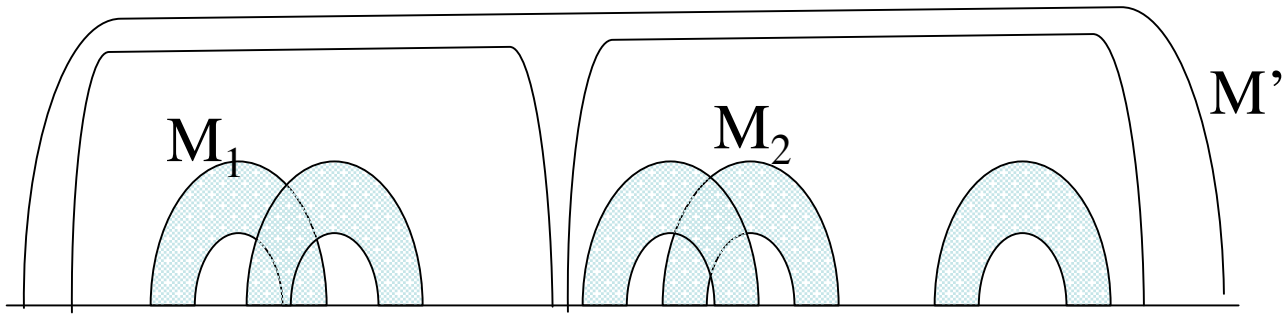
- Pseudoknots are only loosely defined.
- If any interleaving is allowed, then simply select a structure in which a max number of nucleotides can be paired.
- Under some restrictive notions, the structure problem becomes NP-hard.

Simple pseudoknots



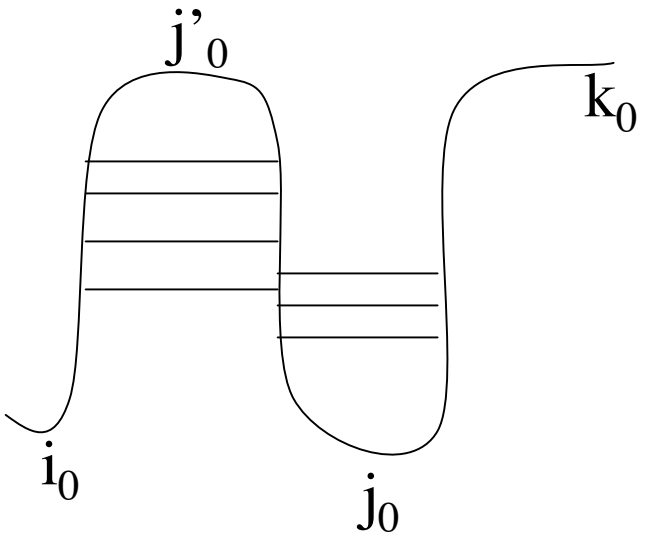
- A region $[i_0,k_0]$ forms a simple pseudoknot if there exist positions j'_0, j_0 s.t.
  - Each $(i,j) \in M$ satisfies either
    - $i_0 \le i < j'_0 \le j < j_0$ or $j'_0 \le i < j_0 \le j \le k_0$
    - Within one of the two groups, there is no interleaving.
    - $i<i'<j'_0$ or $j'_0 \le i<i'$ implies $j>j'$.

Simple pseudoknotted structure



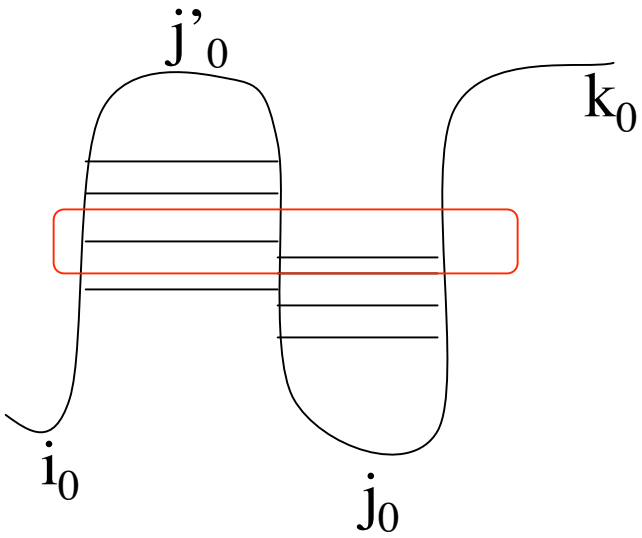
- Collection of simple pseudoknots (M_i) and other basepairs M'.
- None of the simple pseudoknot regions overlap.
- M' is a secondary structure without pseudoknots for the sequence obtained by excising all pseudoknotted regions.

Main idea



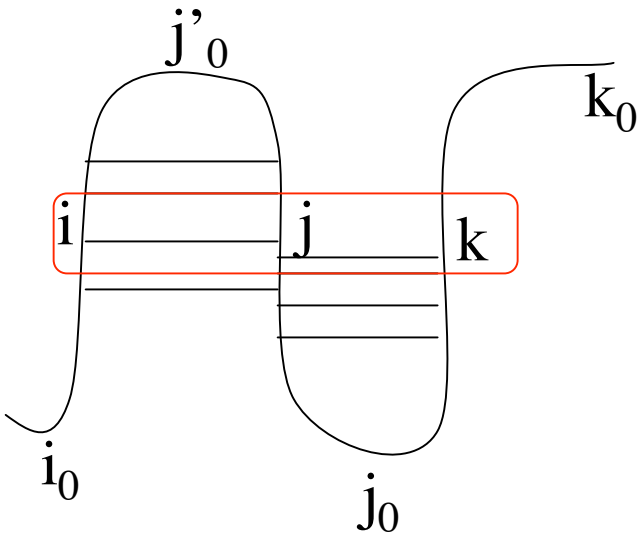
- Rotate the sequence so that it forms two loops.
- Each allowed base-pair is a horizontal line in exactly one of the two loops. The horizontal lines in the two loops are non-crossing.
- All base-pairs can be ordered.

Main idea



- The base-pairs have a total order that a d.p. can exploit.
- $(i,j) < (i',j')$ if one of the following holds
  - $i' < j' < i < j$
  - $i < i' < j < j'$
  - $i < i' < j' < j < j_0$
  - $j'_0 < i' < i < j < j'$

D.P.

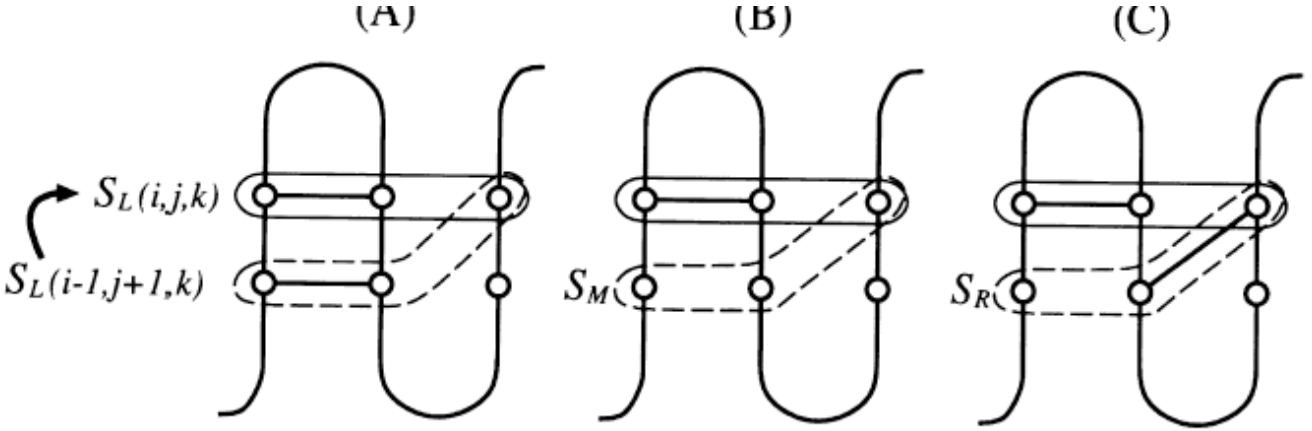


- We need to construct an increasing path of base-pairs.
- Consider a 'frontier' triple (i,j,k).
- We have the following cases:
  - (i,j) form a base-pair
  - (j,k) form a base-pair
  - Neither (i,j) nor (j,k) form a base-pair

Recurrences

- $S_L(i,j,k)$ is the optimum score of a frontier (i,j,k) assuming that
  - $i<j'_0<j<j_0<k$
  - (i,j) form a base-pair
- $S_R(i,j,k)$ is the optimum score of a frontier (i,j,k) assuming that $i<j'_0<j<j_0<k$, and
  - (j,k) form a base-pair
- $S_M(i,j,k)$ is the optimum score of a frontier (i,j,k) assuming that $i<j'_0<j<j_0<k$, and
  - Neither (i,j) nor (j,k) form a base-pair

Computing S_L



$$S_L(i,j,k) = v(a_i,a_j) + \max\begin{cases} S_L(i-1,j+1,k) \\ S_M(i-1,j+1,k) \\ S_R(i-1,j+1,k) \end{cases}$$

Putting it all together

$$S_R(i,j,k) = v(a_j, a_k) + \max \left\{ \begin{array}{l} S_L(i,j+1,k-1), \\ S_M(i,j+1,k-1), \\ S_R(i,j+1,k-1) \end{array} \right\},$$

$$S_M(i,j,k) = \max \left\{ \begin{array}{lll} S_M(i-1,j,k), & S_M(i,j+1,k), & S_M(i,j,k-1), \\ S_L(i-1,j,k), & S_L(i,j+1,k), & \\ S_R(i,j+1,k), & S_R(i,j,k-1) & \end{array} \right\},$$

$$S_{\text{pseudo}}(i_0, k_0) = \max_{i_0 \leqslant i<j<k \leqslant k_0} \{S_L(i,j,k),\ S_M(i,j,k),\ S_R(i,j,k)\}.$$

Computing optimal pseudo-knotted structures

- Let S(i,j) be the opt score of a simple pseudoknotted structure.
  - Either (I,j) is a simple pseudoknot
    - $S(i,j) = S_{pseudo}(i,j)$
  - Or, not
    - $S(i,j) = \max\{ v(a_i,a_j) + S(i+1,j-1),$
      $\max k \{ S(i,k-1)+S(k,j)\}$
      $\}$

$$S(i,j) = \max\left\{S_{\text{pseudo}}(i,j),\ S(i+1,j-1) + v(a_i,a_j), \max_{i<k\leqslant j}\{S(i,k-1)+S(k,j)\}\right\}$$

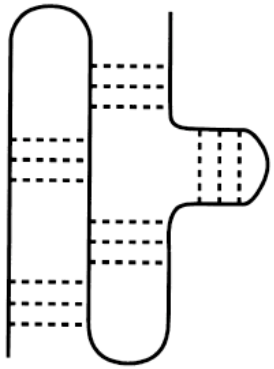
Time Complexity

- We compute $S_{pseudo}(i,j)$ for all (i,j). Each computation is $O(n^3)$. Total time?
- For each i_0, perform the following computation:

for $i = i_0$ **to** $n - 2$ **do**
  for $j = n - 1$ **downto** $i + 1$ **do**
    for $k = j + 1$ **to** n **do**

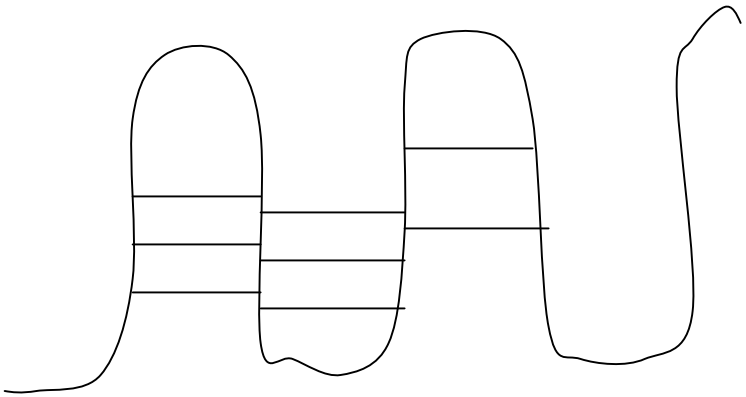
- $S_{pseudo}(i_0,k_0) = \max_{i0 \le i<j \le k0}\{S_L(i,j,k_0),\ldots\}$
- Total time $O(n^4)$

Recursive pseudoknots



- Each loop of the RNA structure is a recursive pseudoknotted RNA structure.
- Optimal recursive pseudoknotted RNA structure problem can be solved in $O(n^5)$ time.

Open Questions

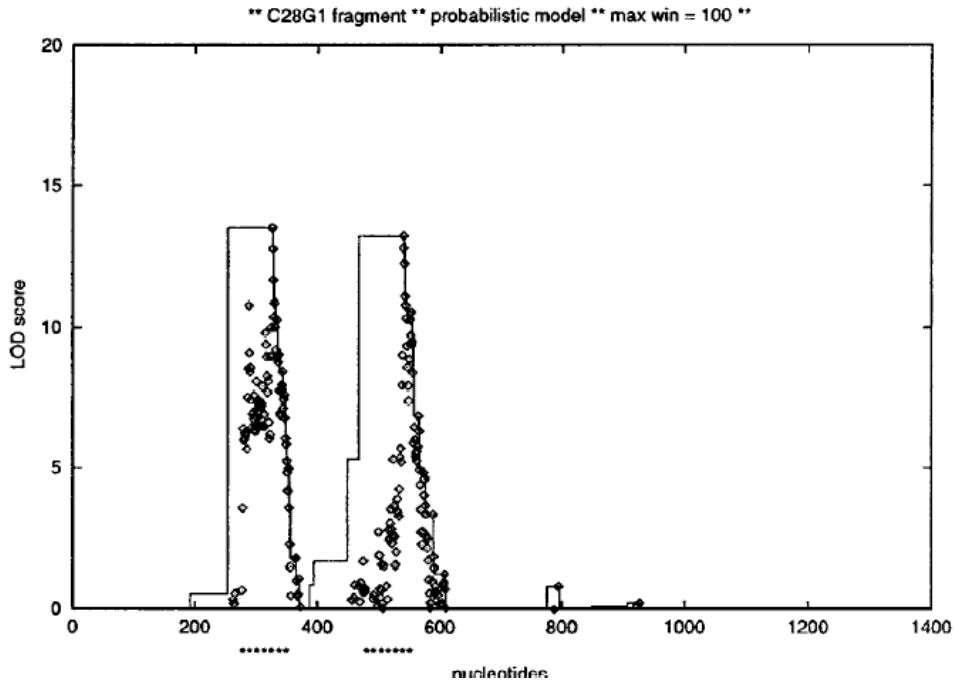


- There should be a direct generalization of simple pseudoknots to the following structure.
- Rivas and Eddy do consider such a generalization, but a systematic treatment is missing.
- Q: Given a pseudo-knotted structure, is it an Akutsu simple pseudoknotted structure?
  - Linear time algorithm was devised recently for this problem.

Structure as a proxy for ncRNA

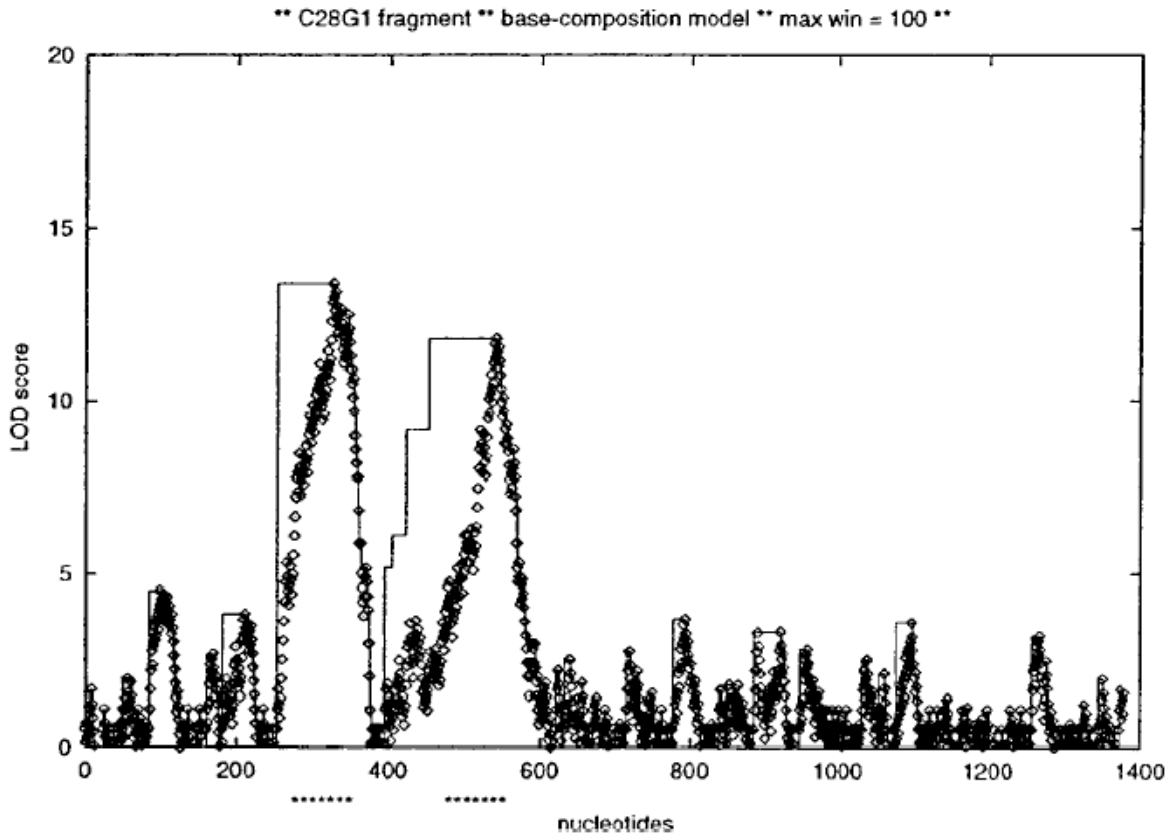
- Any genomic region with an energetically favorable fold is a candidate ncRNA?
- Rivas and Eddy show otherwise.
- They use
  - LOD score L = Pr(s|RNA)/Pr(s|Null)
  - Z-score Z = G-□/□

LOD scores for putative ncRNA



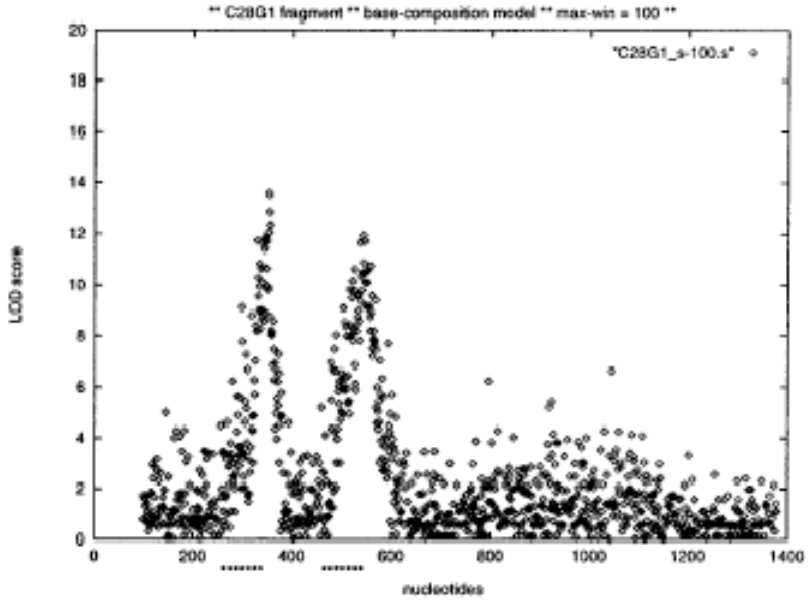
- A region of C. elegans is containing two tRNAs is chosen. The position of tRNA is indicated by stars.
- The true positions and the LOD-score correspond.
- Stronger results in M. janaschii (AT%=70)
- Weak results in E.coli (GC%=50)

Correlation with GC content



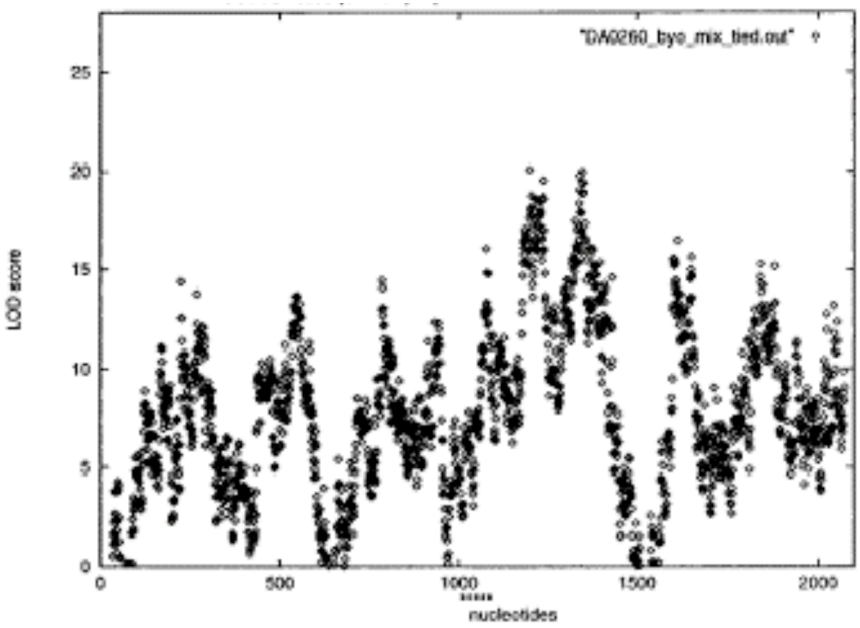
- GC content detector has results similar to structure prediction!

Significance of detected regions



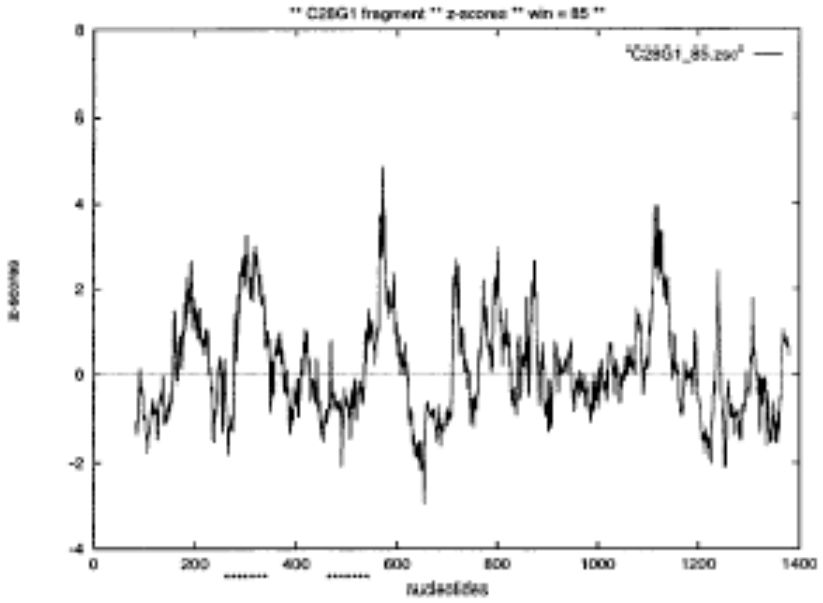
- Test for significance was using Z-scores on shuffled sequences.
- Earlier tests shuffled sequences using a large window.
- Shuffling high scoring windows led to lower Z-scores.

Testing chimeric sequence

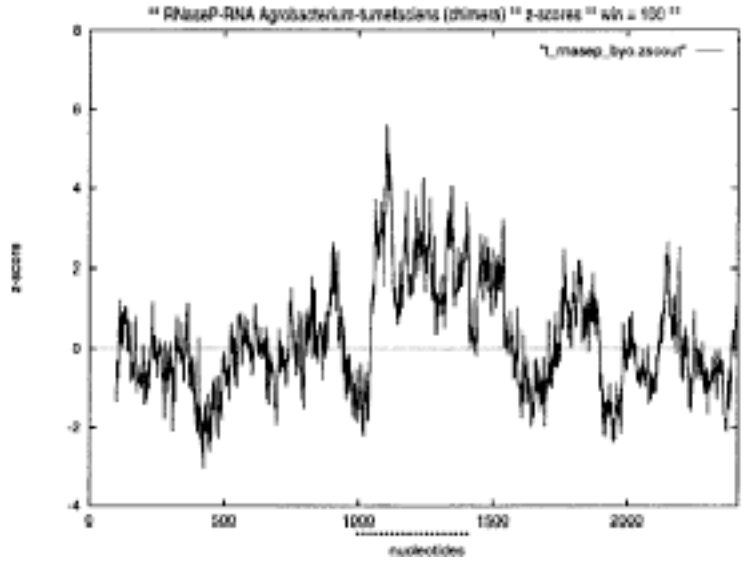


- Test on chimeric sequence. A real tRNA was embedded in 2000bp random sequence with similar GC-composition.

Increasing window size

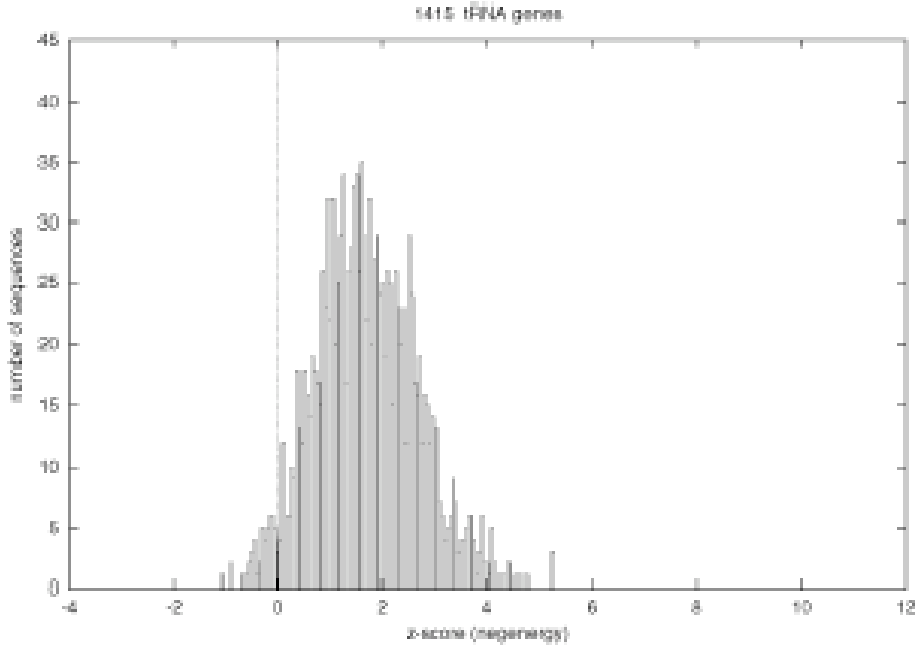


- tRNA Z-score computation with a larger window (85nt).



- For very strong signals, a Z-score > 4 may still be significant.

Z-score distribution



- Z-scores of 415 tRNA genes. 98% have Z-score lower than 4.

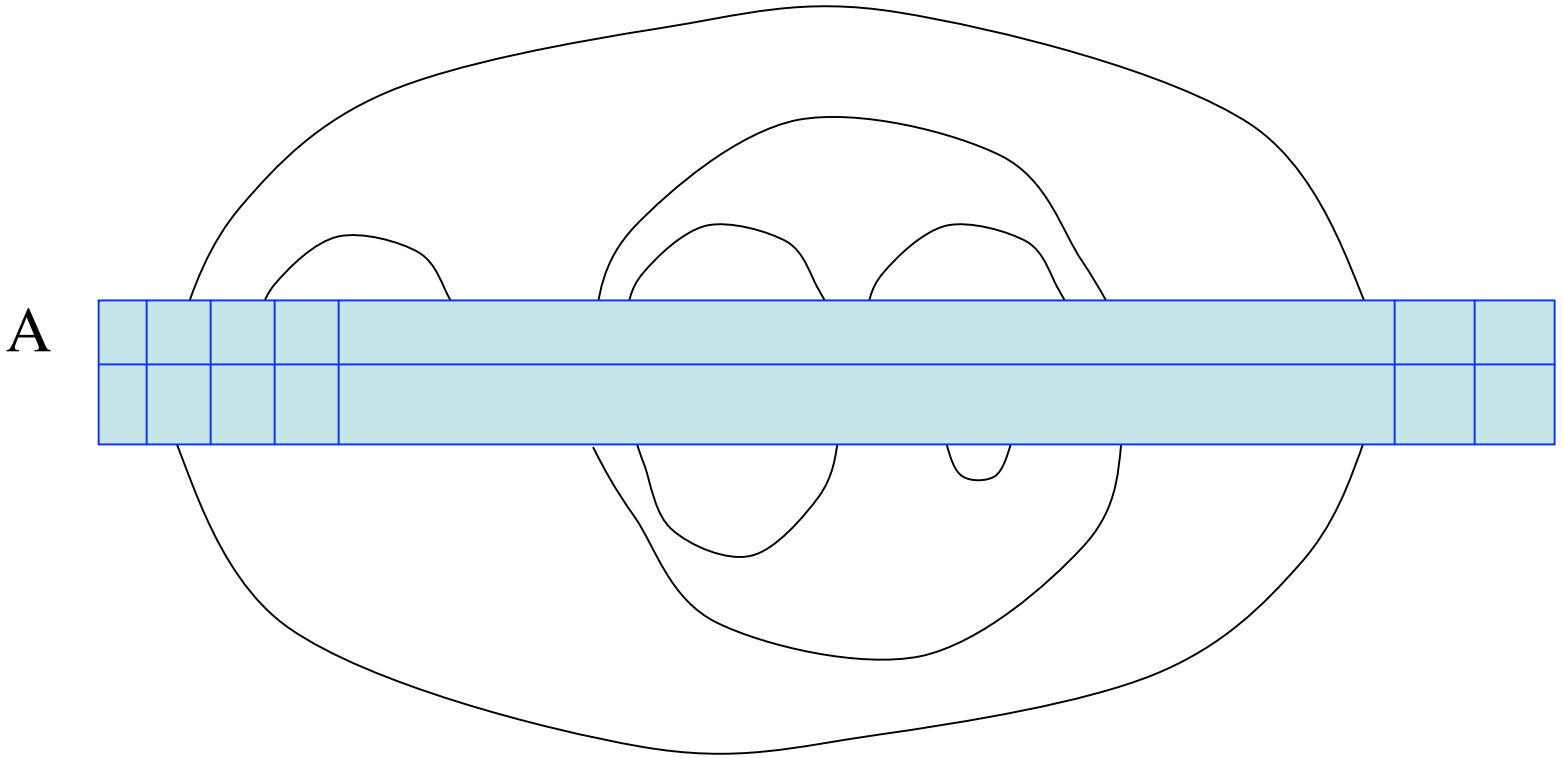
ncRNA gene detection using comparative sequence analysis

- Idea: It is likely for a random sequence to fold into an energetically favorable structure.
- However, it is unlikely for a random sequence to fold into a structure that is similar to a query structure.

Questions

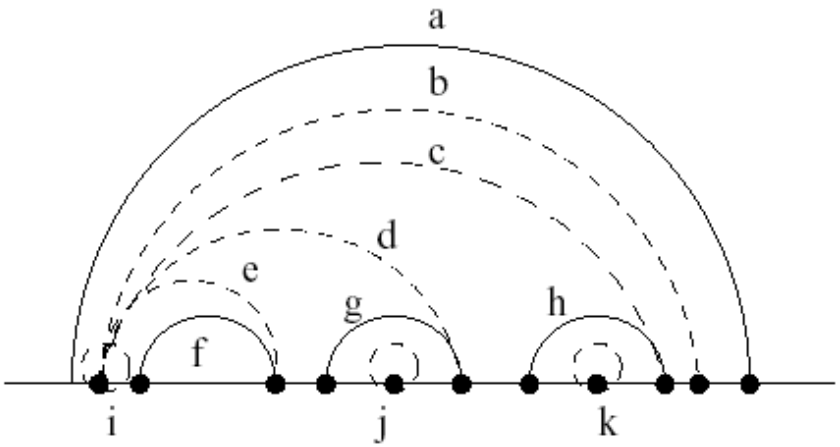
- 1. Given a query ncRNA, compute an alignment that conserves both sequence, and structure.
- 2. Given two orthologous sequences, compute an alignment that simultaneously preserves sequence and structure in both.

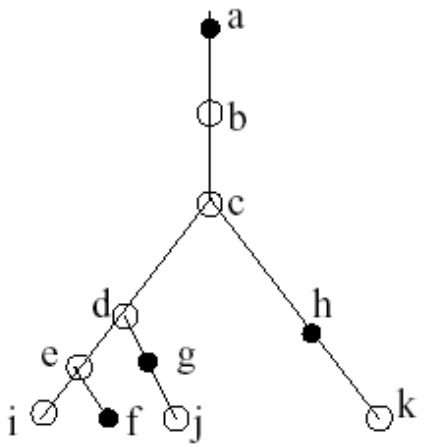
Scoring RNA alignments



$$\text{Score} = \sum_{j} \gamma(A[1,j], A[2,j]) + \sum_{(i,j),(k,l)} \gamma(i,j,k,l)$$

Binary tree representation of RNA structure





AlignRNA

procedure alignRNA
(*S is the set of base-pairs in RNA structure of s. S' is the augmented set. *)
for all intervals (i, j), $1 \leq i < j \leq n$, all nodes $v \in S'$
if $v \in S$

$$A[i,j,v] = \max \begin{cases} A[i+1, j-1, \text{child}(v)] + \delta(t[i], t[j], s[l_v], s[r_v]), \\ A[i, j-1, v] + \gamma('-', t[j]), \\ A[i+1, j, v] + \gamma('-', t[i]), \\ A[i+1, j, \text{child}[v]] + \gamma(s[l_v], t[i]) + \gamma(s[r_v], '-'), \\ A[i, j-1, \text{child}[v]] + \gamma(s[l_v], '-') + \gamma(s[r_v], t[j]), \\ A[i, j, \text{child}[v]] + \gamma(s[l_v], '-') + \gamma(s[r_v], '-'), \end{cases}$$

else if $v \in S' - S$, and v has one child

$$A[i,j,v] = \max \begin{cases} A[i, j-1, \text{child}[v]] + \gamma(s[r_v], t[j]), \\ A[i, j, \text{child}[v]] + \gamma(s[r_v], '-'), \\ A[i, j-1, v] + \gamma('-', t[j]), \\ A[i+1, j, v] + \gamma('-', t[i]), \end{cases}$$

else if $v \in S' - S$, and v has two children
$A[i,j,v] = \max_{i \leq k \leq j} \{A[i, k-1, \text{left\_child}[v]] + A[k, j, \text{right\_child}[v]]\}$
end if
end for

Filtering