RNA Secondary Structure Prediction

02-710 Computational Genomics

Seyoung Kim

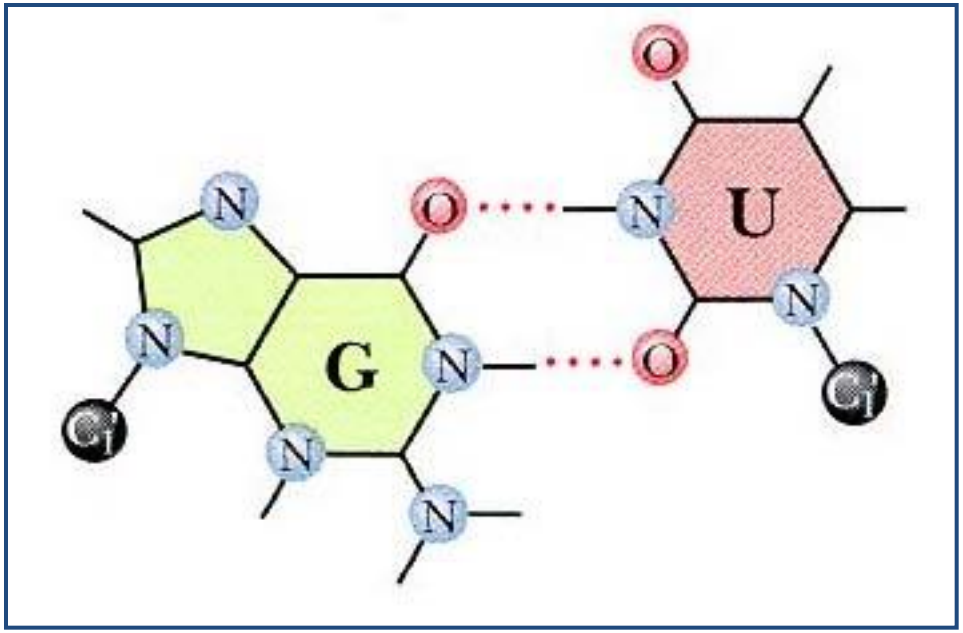
Outline

- RNA folding
- Dynamic programming for RNA secondary structure prediction
- Covariance model for RNA structure prediction

RNA Basics

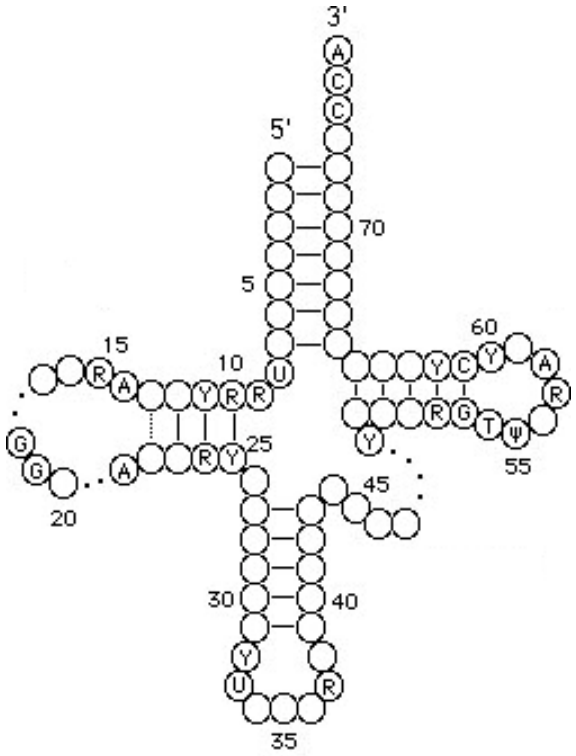
- RNA bases A,C,G,U
- Canonical Base Pairs
  - A-U
  - G-C
  - G-U

    “wobble” pairing
  - Bases can only pair with **one** other base.



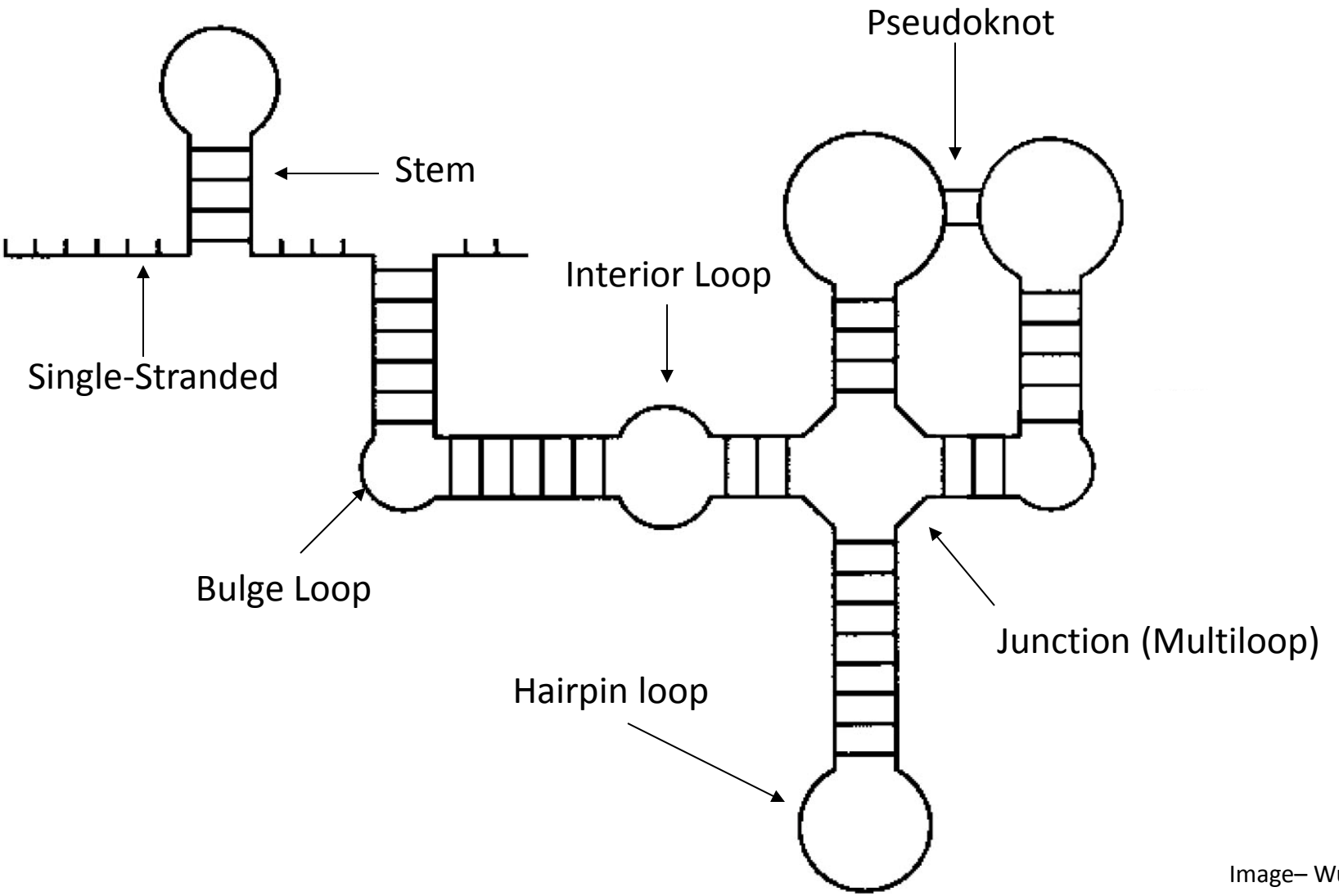
RNA Basics

- transfer RNA (tRNA)
- messenger RNA (mRNA)
- ribosomal RNA (rRNA)
- small interfering RNA (siRNA)
- micro RNA (miRNA)
- small nucleolar RNA (snoRNA)



http://www.genetics.wustl.edu/eddy/tRNAscan-SE/

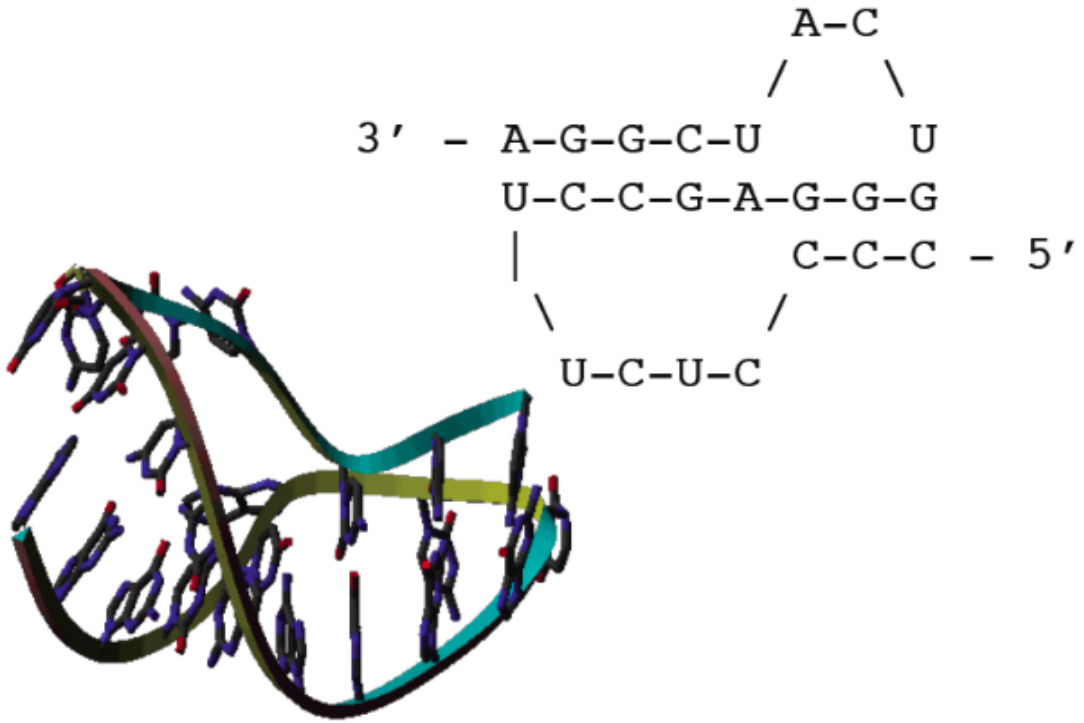
RNA Secondary Structure



Image– Wuchty

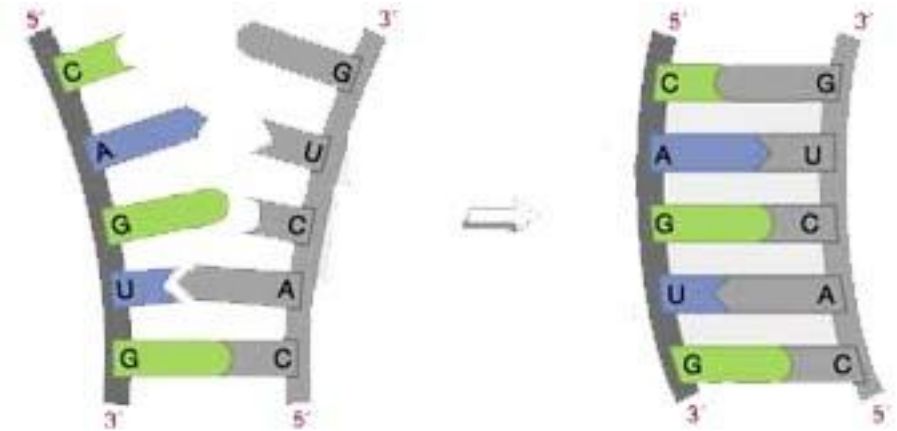
Pseudoknots

- Pseudoknots: a nucleic acid secondary structure containing at least two stem-loop structures which half of one stem is intercalated between the two halves of another stem.



Sequence Alignment as a method to determine structure

- Bases pair in order to form backbones and determine the secondary structure
- Aligning bases based on their ability to pair with each other gives an algorithmic approach to determining the optimal structure



Base Pair Maximization – Dynamic Programming Algorithm

Simple Example: Maximizing Base Pairing

$$S(i,j) = \max \begin{cases} S(i+1,j-1)+1 & [\text{if } i,j \text{ base pair}] \\ S(i+1,j) \\ S(i,j-1) \\ \max_{i<k<j} S(i,k) + S(k+1,j) \end{cases}$$

S(i,j) is the folding of the subsequence of the RNA strand from index i to index j which results in the highest number of base pairs

Images – Sean Eddy

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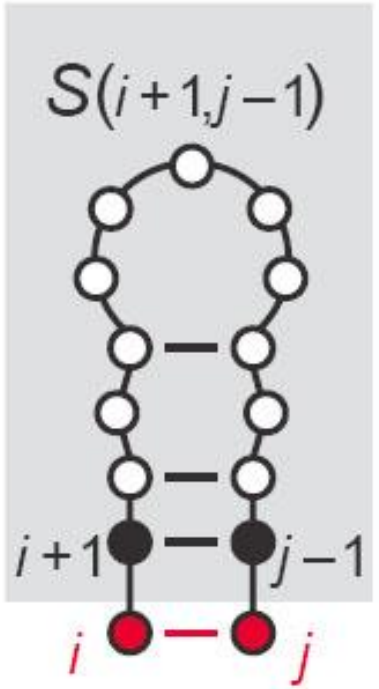
Base pair at i and j



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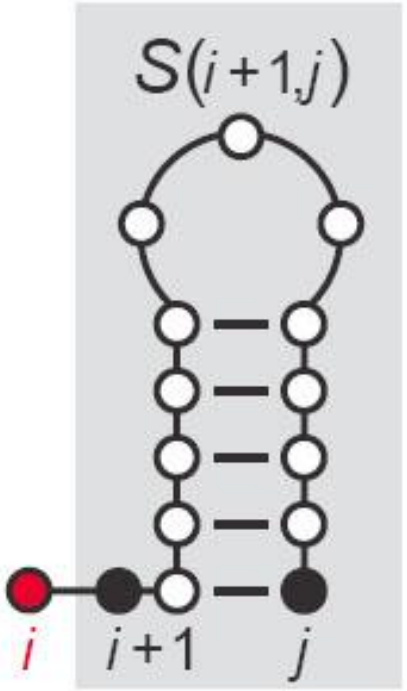
Unmatched at i



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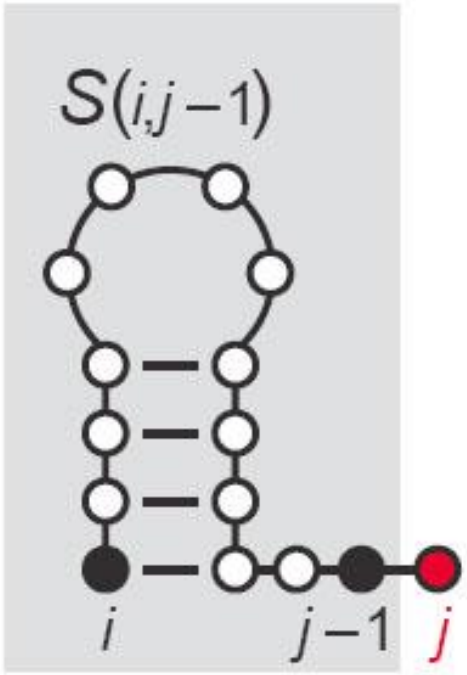

Umatched at j

Images – Sean Eddy

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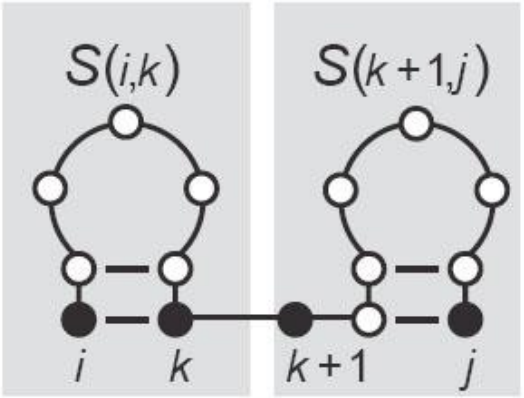
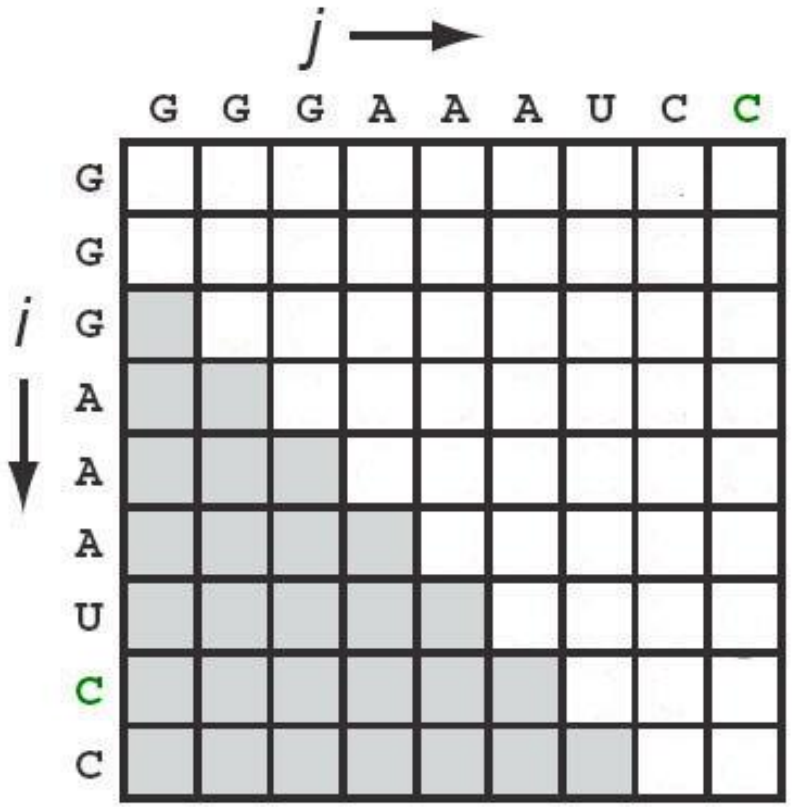


Bifurcation

Images – Sean Eddy

Base Pair Maximization – Dynamic Programming Algorithm

- Alignment Method
  - Align RNA strand to itself
  - Score increases for feasible base pairs
- Each score independent of overall structure
- Bifurcation adds extra dimension



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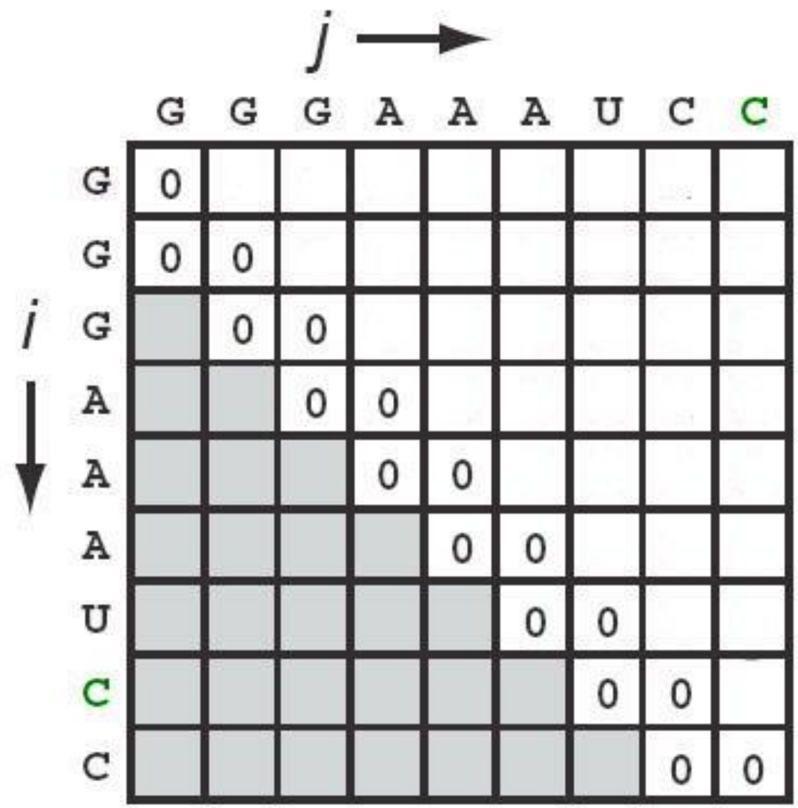

Initialize first two diagonal arrays to 0

Images – Sean Eddy

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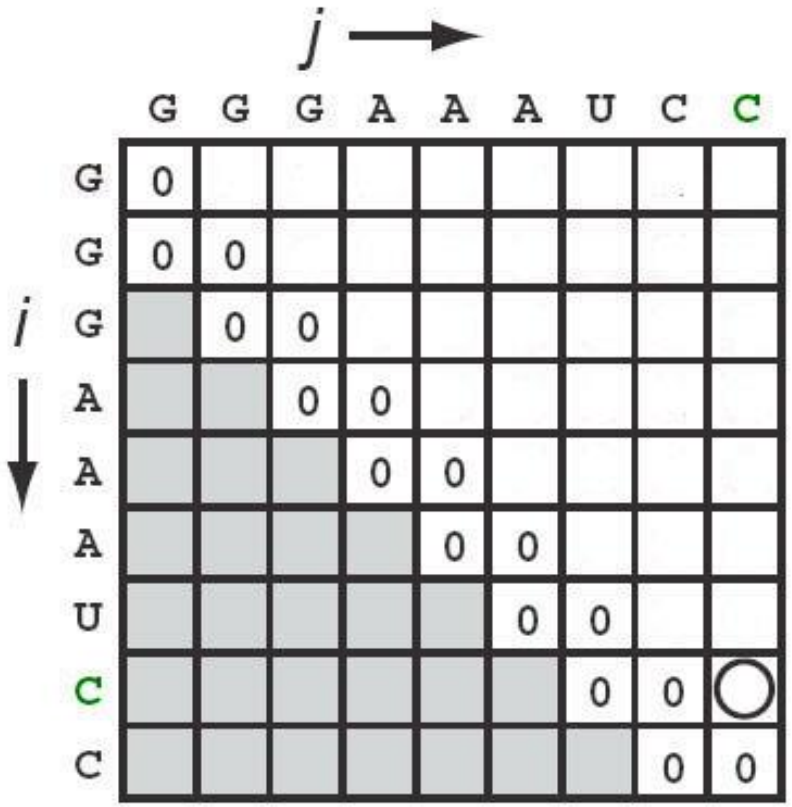

Fill in squares sweeping diagonally

Images – Sean Eddy

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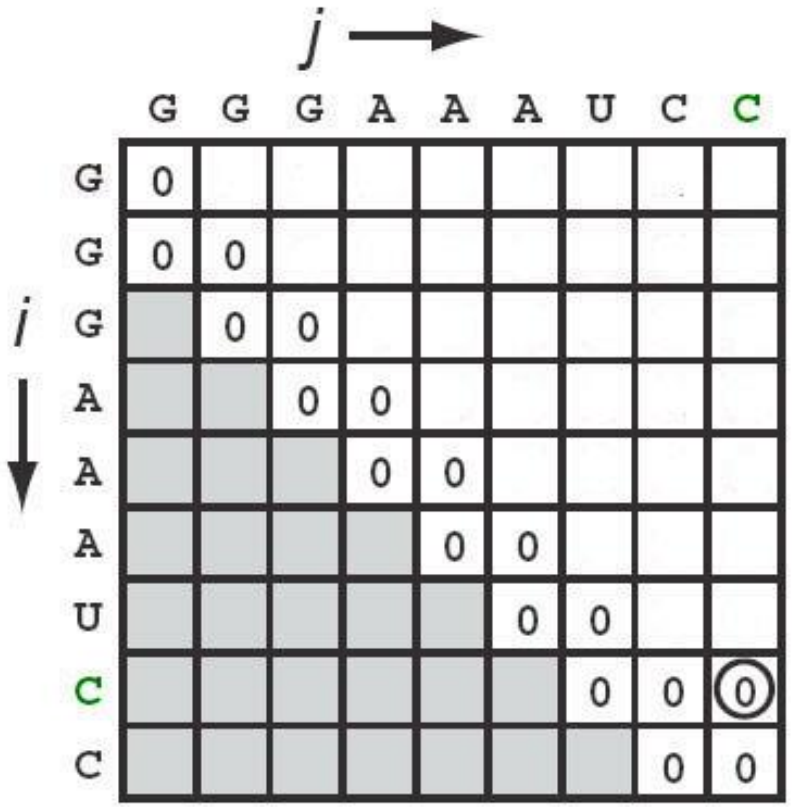
Bases cannot pair, similar to unmatched alignment



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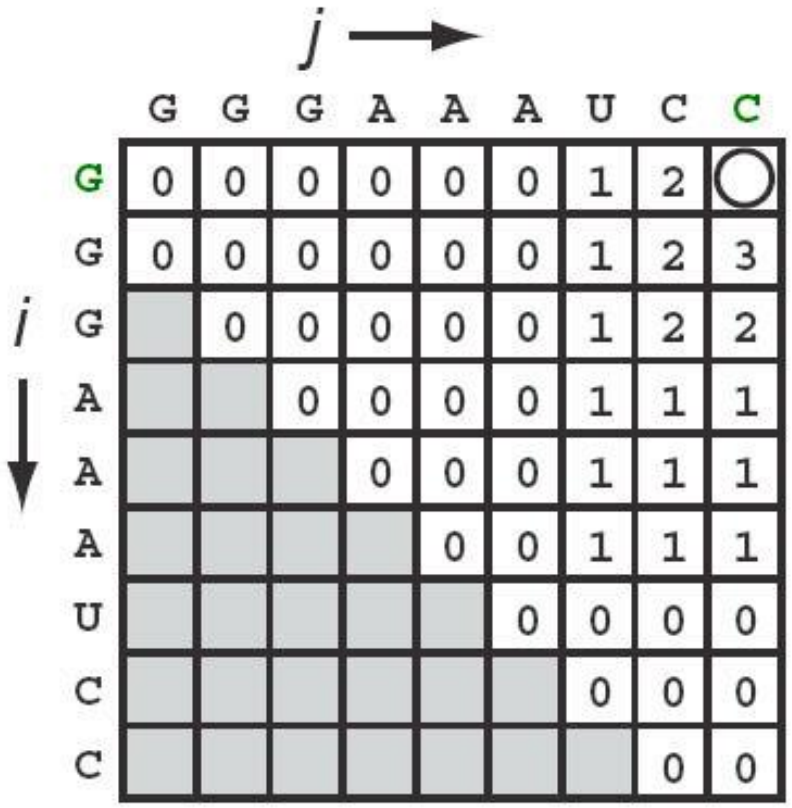

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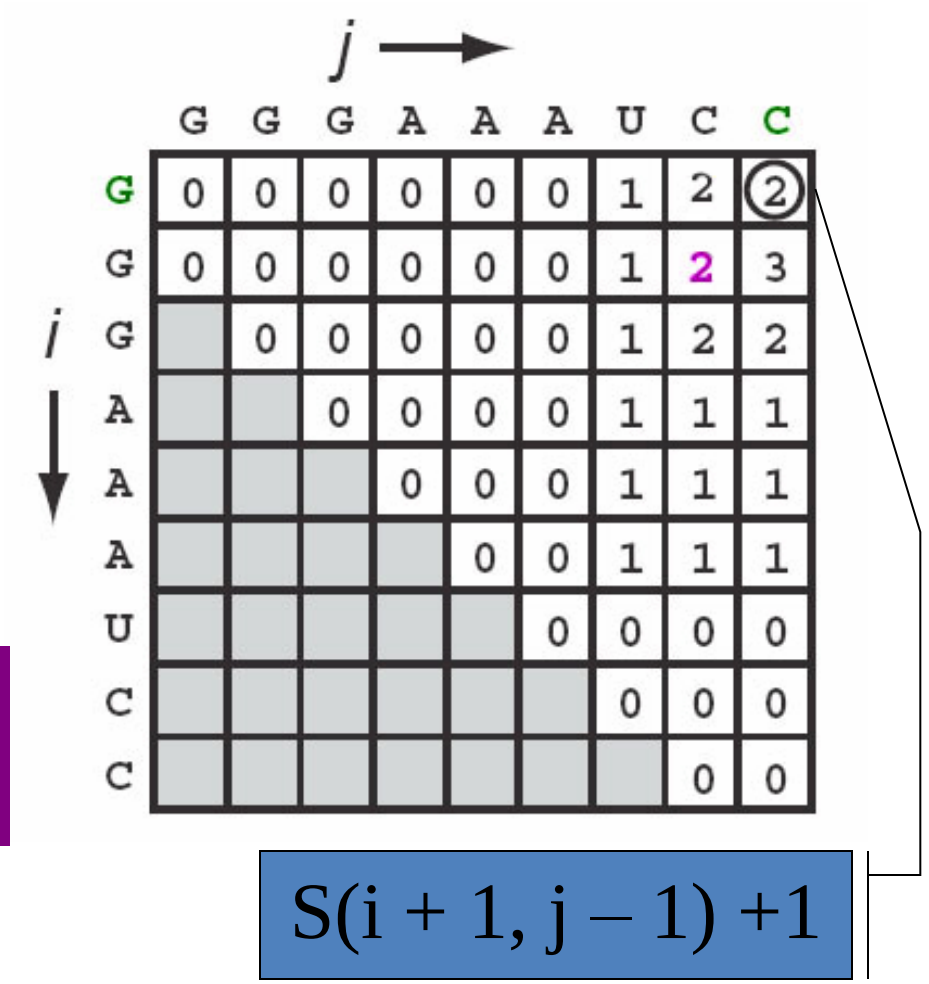

Dynamic Programming – possible paths

Images – Sean Eddy

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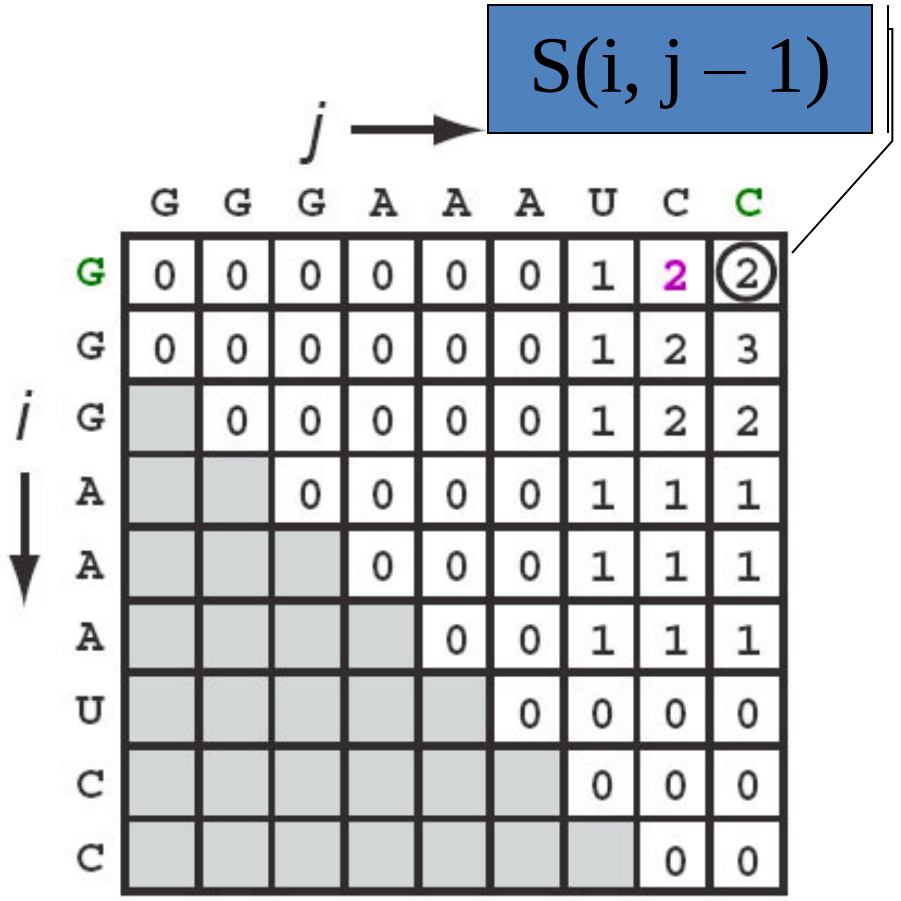

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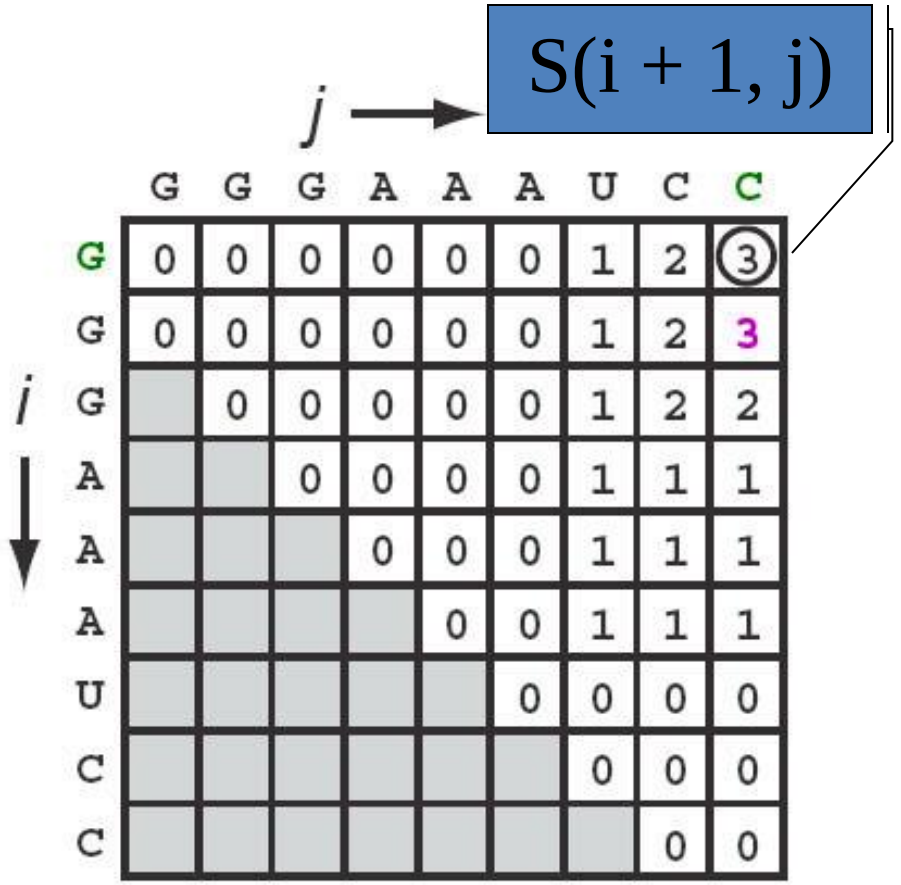

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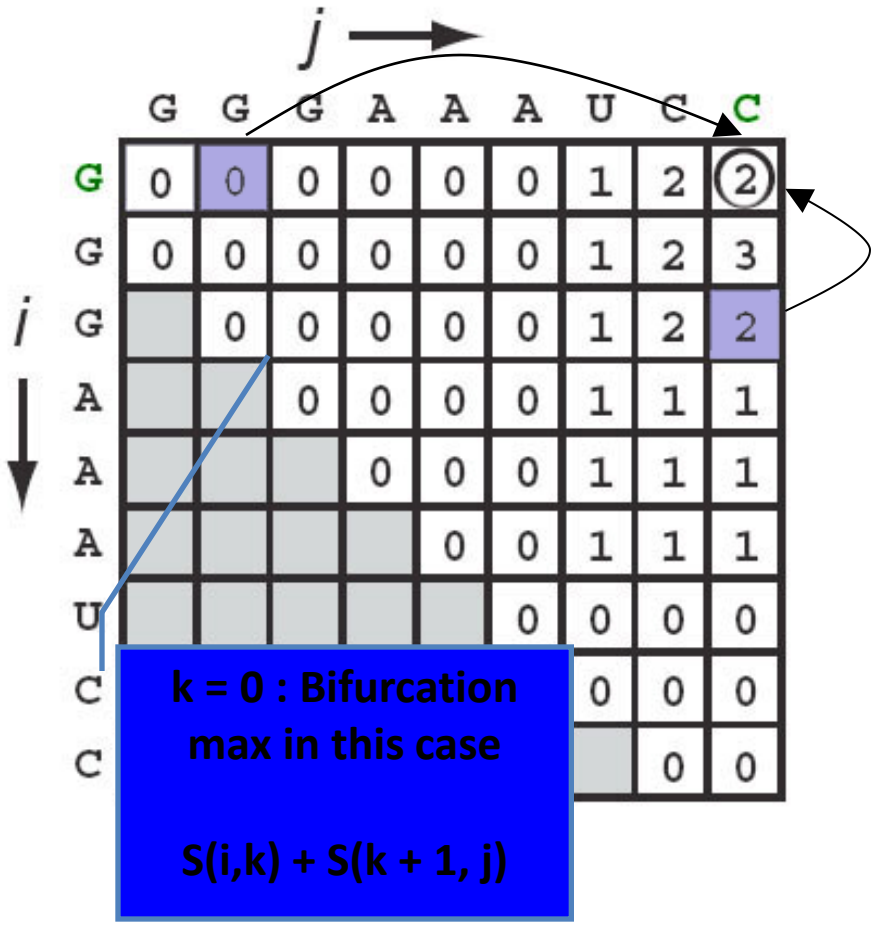

Bifurcation – add values for all k

Images – Sean Eddy

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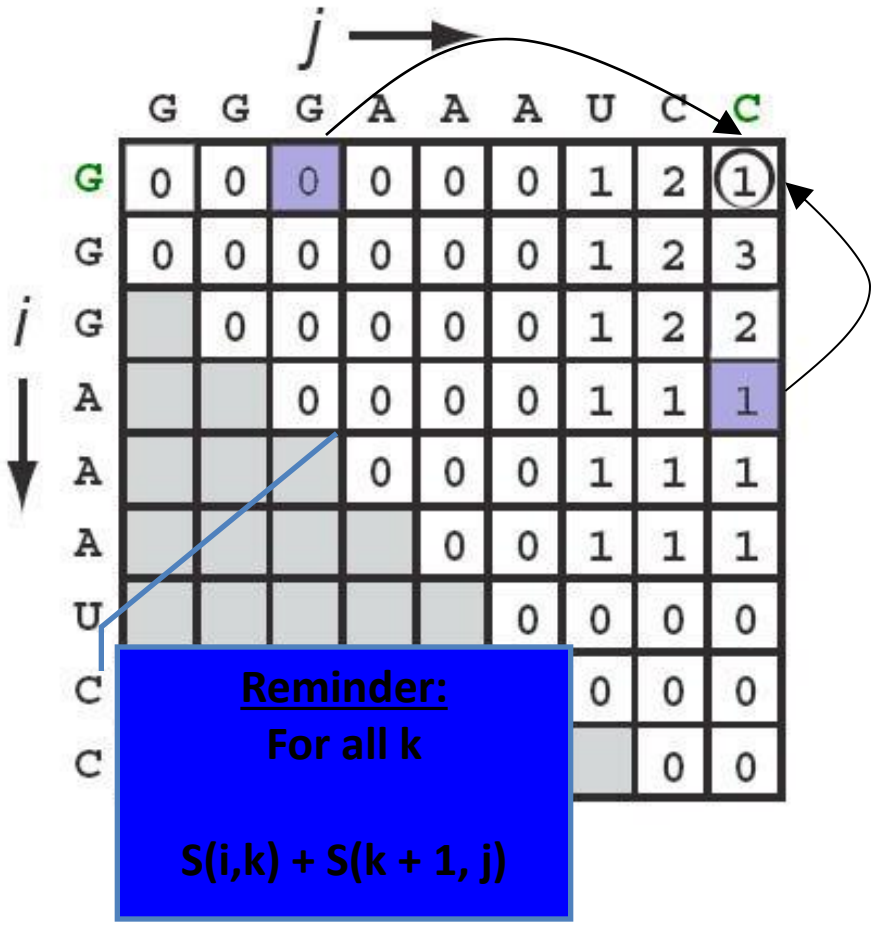

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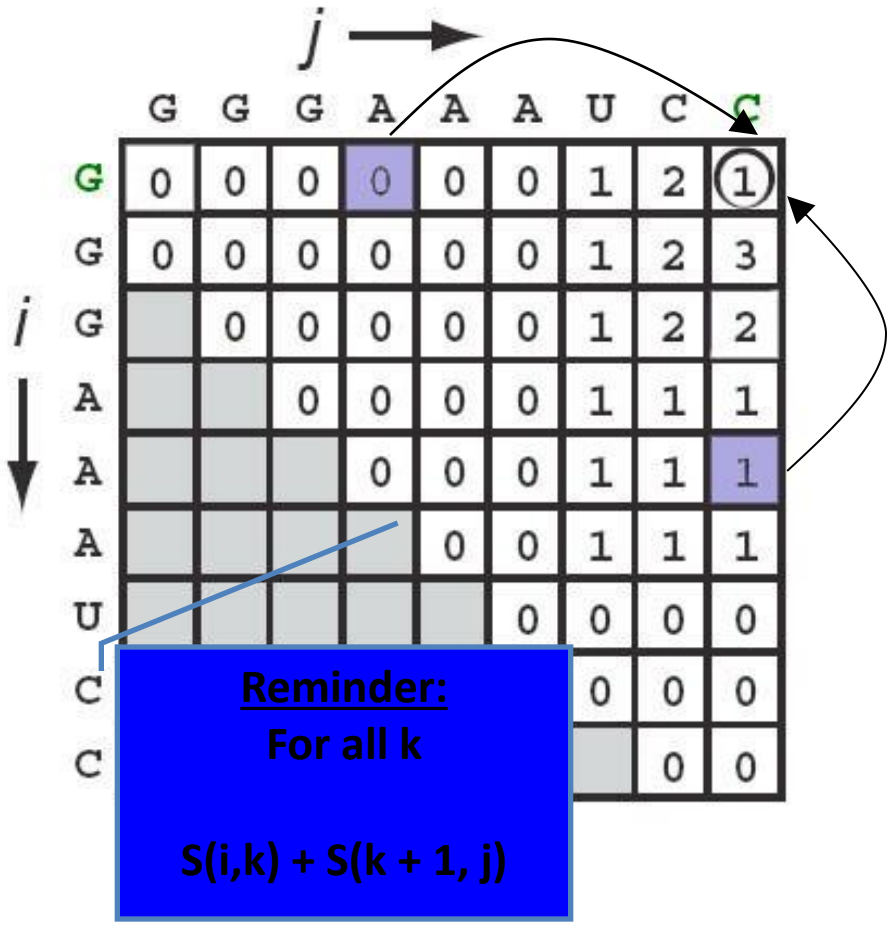


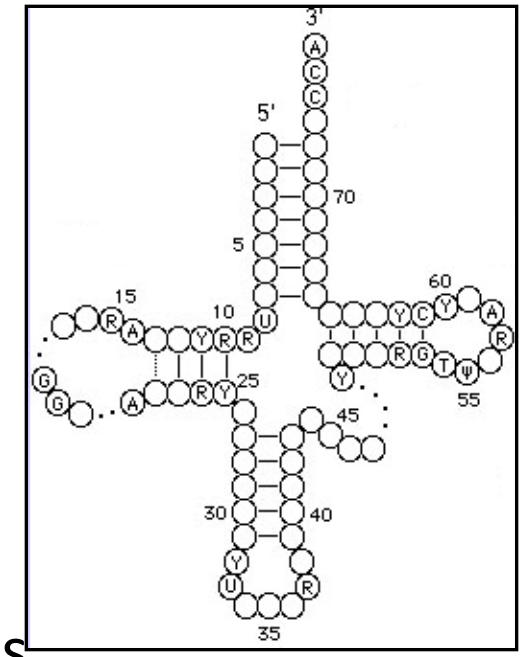
Bifurcation – add values for all k

Images – Sean Eddy

Base Pair Maximization - Drawbacks

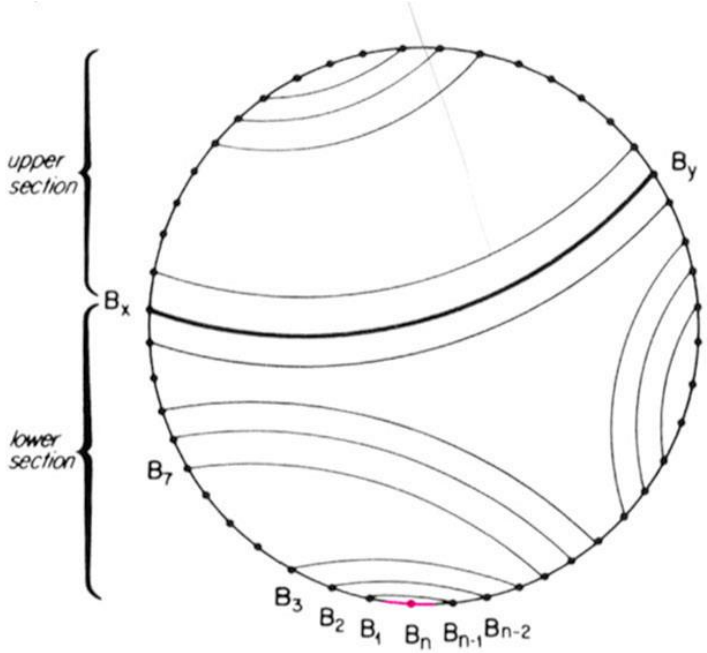
- Base pair maximization will not necessarily lead to the most stable structure
  - May create structure with many interior loops or hairpins which are energetically unfavorable
- Comparable to aligning sequences with scattered matches – not biologically reasonable

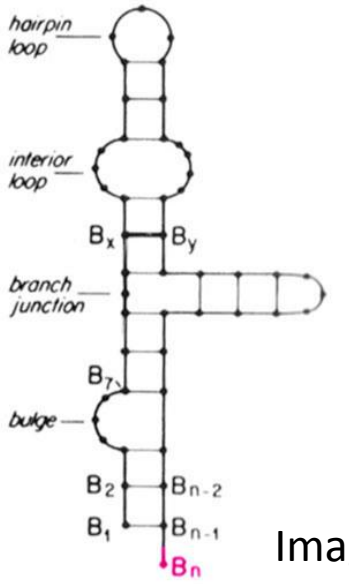
Energy Minimization



- Thermodynamic Stability
  - Estimated using experimental techniques
  - Theory : Most Stable is the Most likely
- No Pseudknots due to algorithm limitations
- Uses Dynamic Programming alignment technique
- Attempts to maximize the score taking into account thermodynamics
- MFOLD and ViennaRNA

Energy Minimization Results

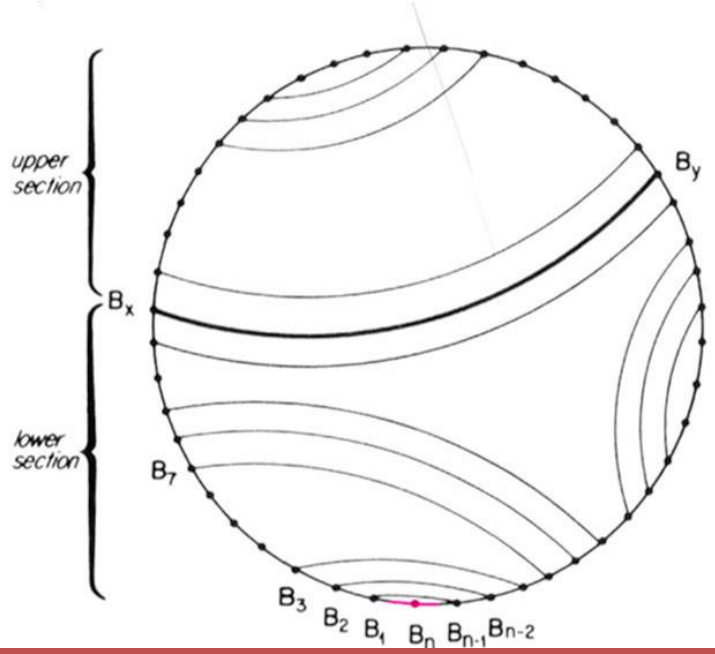


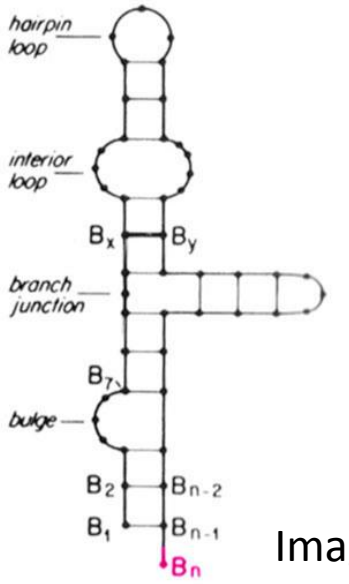


Images – David Mount

- Linear RNA strand folded back on itself to create secondary structure
- Circularized representation uses this requirement
  - Arcs represent base pairing

Energy Minimization Results



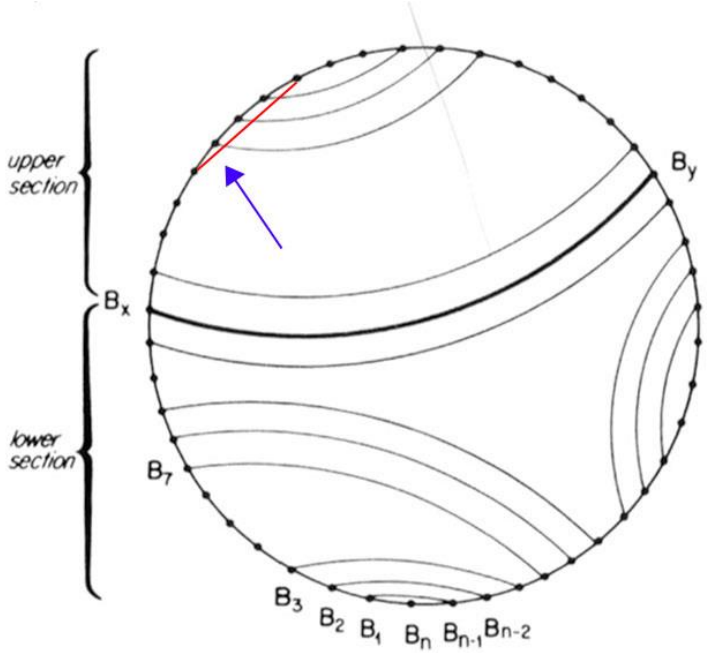


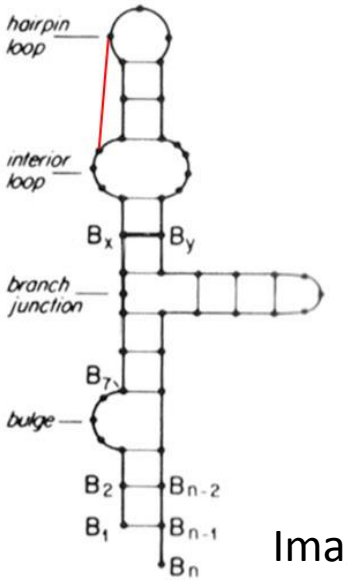
Images – David Mount

- All loops must have at least 3 bases in them
  - Equivalent to having 3 base pairs between all arcs

Exception: Location where the beginning and end of RNA come together in circularized representation

Trouble with Pseudoknots





Images – David Mount

- Pseudoknots cause a breakdown in the Dynamic Programming Algorithm.
- In order to form a pseudoknot, checks must be made to ensure base is not already paired – this breaks down the recurrence relations

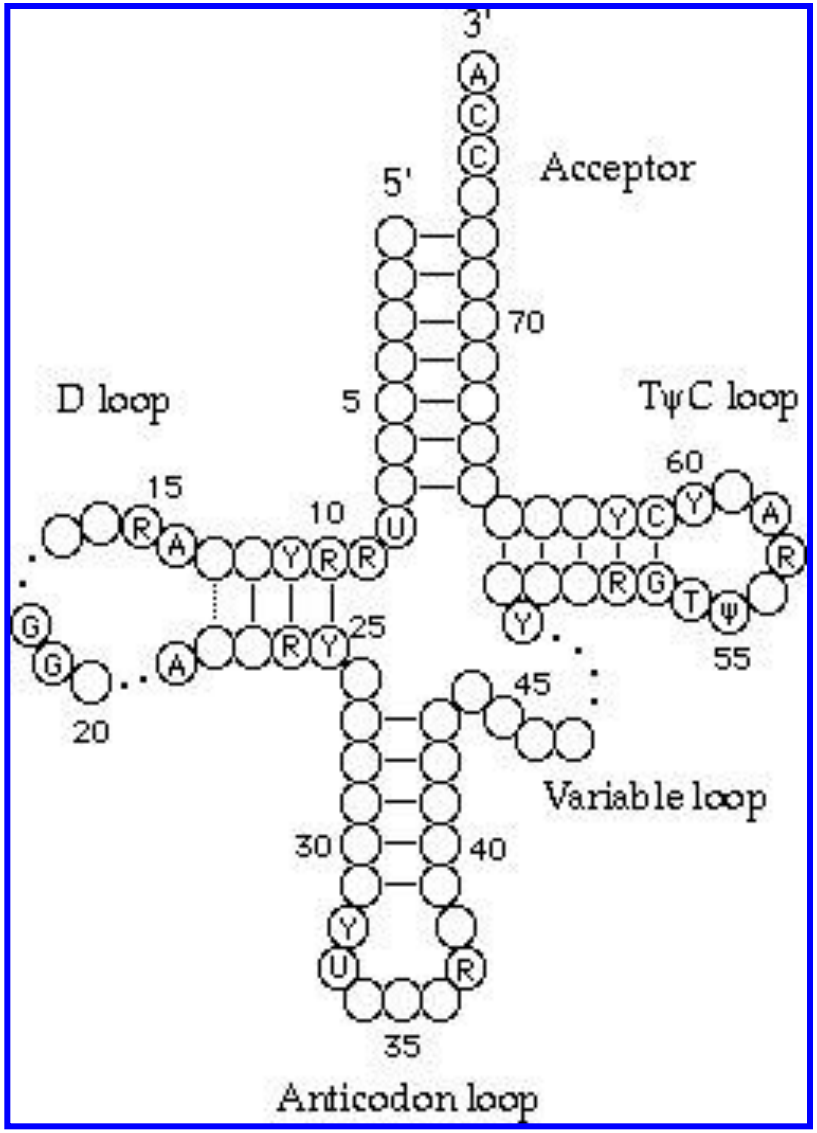
Energy Minimization Drawbacks

- Compute only one optimal structure
- Usual drawbacks of purely mathematical approaches
  - Similar difficulties in other algorithms
    - Protein structure
    - Exon finding

Alternative Algorithms - Covariaton

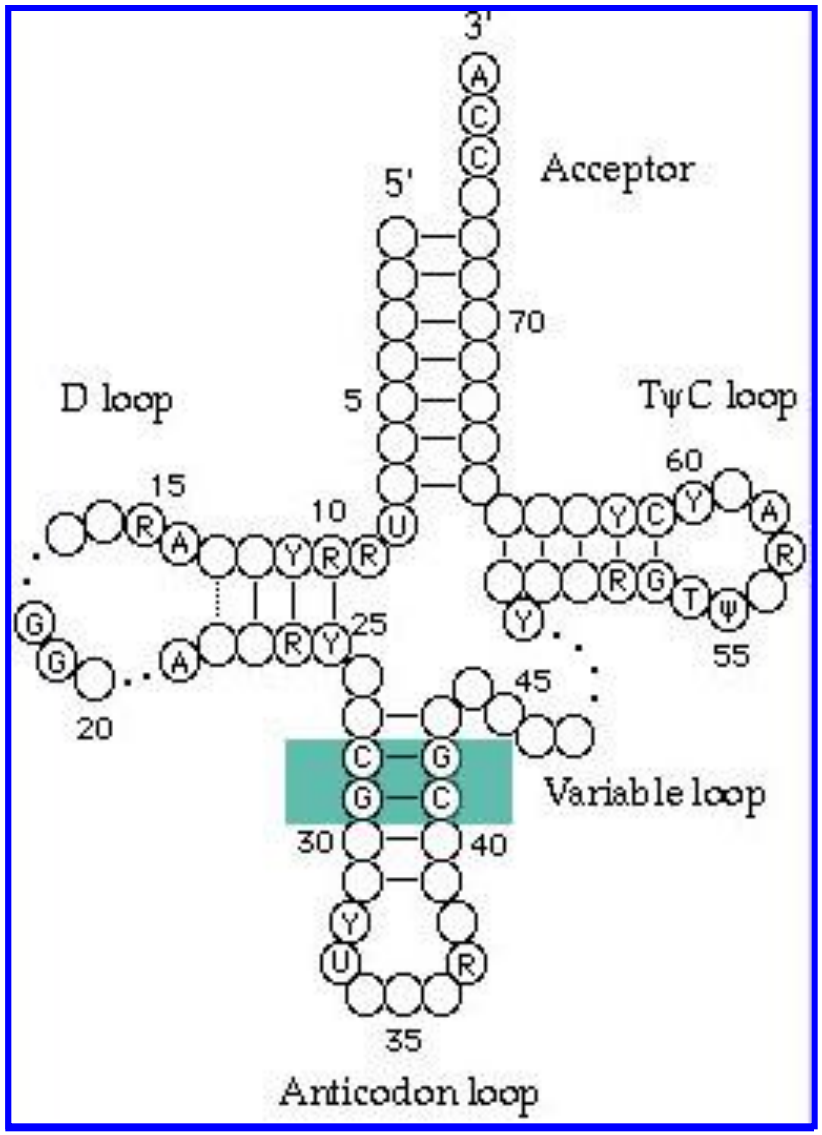
- Incorporates Similarity-based method
  - Evolution maintains sequences that are important
  - Change in sequence coincides to maintain structure through base pairs (Covariance)
    - Cross-species structure conservation example – tRNA
- Manual and automated approaches have been used to identify covarying base pairs
- Models for structure based on results
  - Ordered Tree Model
  - Stochastic Context Free Grammar

Alternative Algorithms - Covariaton



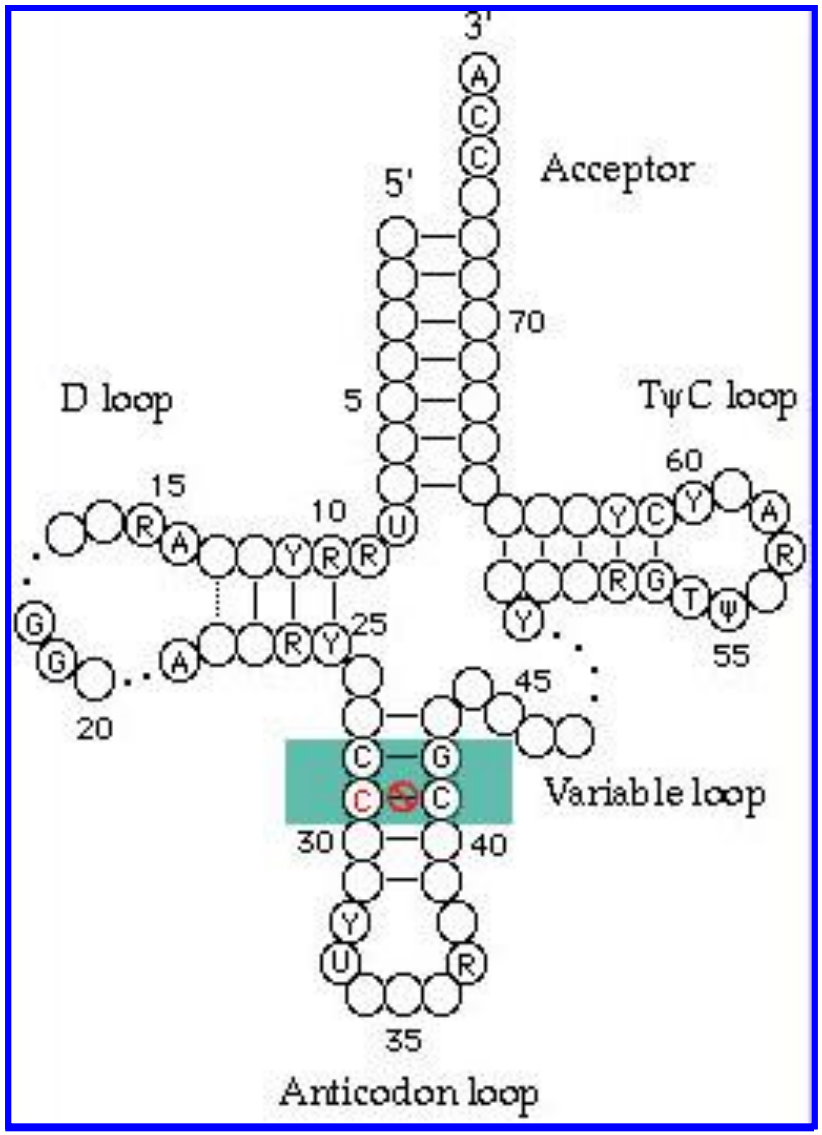
Expect areas of base pairing in tRNA to be covarying between various species

Alternative Algorithms - Covariaton



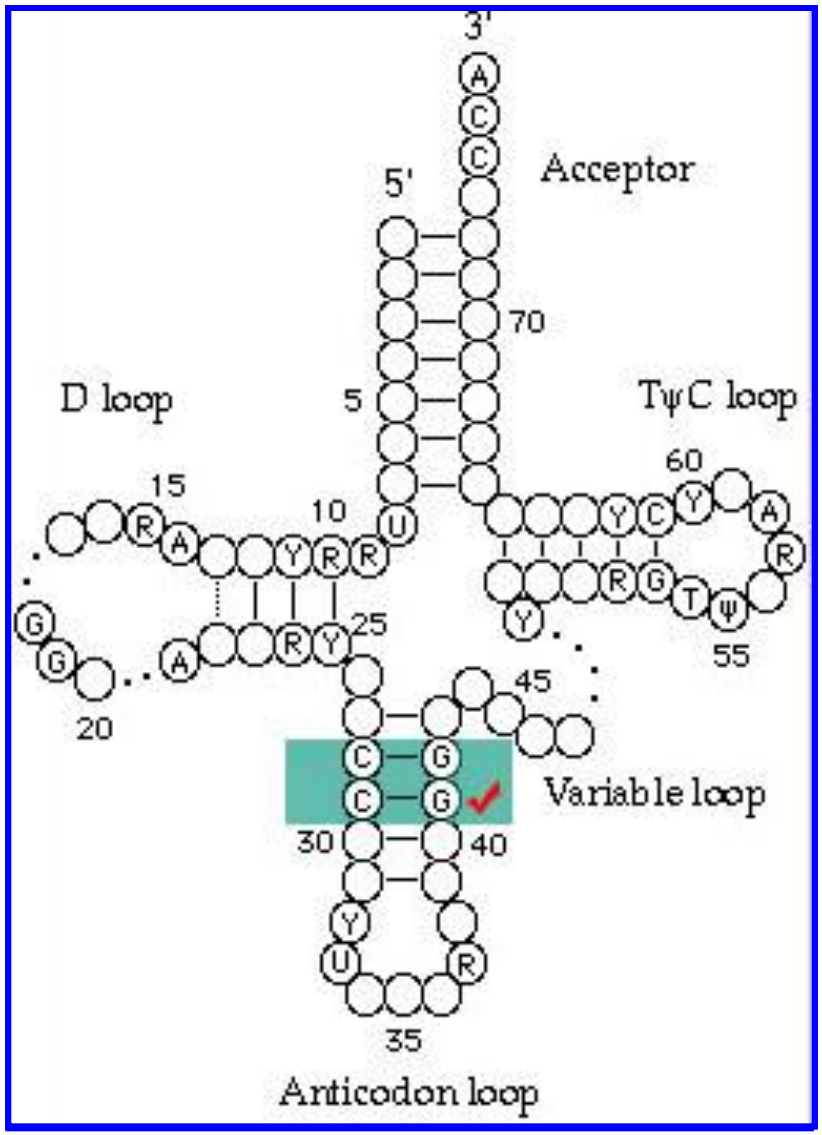
Base pairing creates same stable tRNA structure in organisms

Alternative Algorithms - Covariaton



Mutation in one base yields pairing impossible and breaks down structure

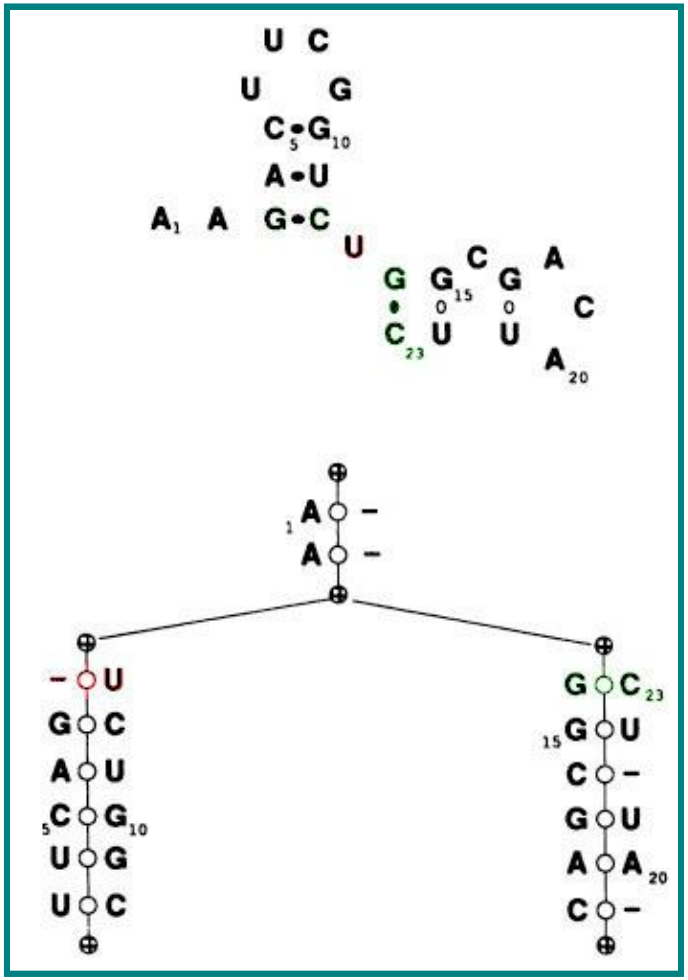
Alternative Algorithms - Covariaton



Covariation ensures ability to base pair is maintained and RNA structure is conserved

Binary Tree Representation of RNA Secondary Structure

- Representation of RNA structure using Binary tree
- Nodes represent
  - Base pair if two bases are shown
  - Loop if base and “gap” (dash) are shown
- Traverse root to leaves, from left to right
- Pseudoknots still not represented
- Tree does not permit varying sequences
  - Mismatches
  - Insertions & Deletions

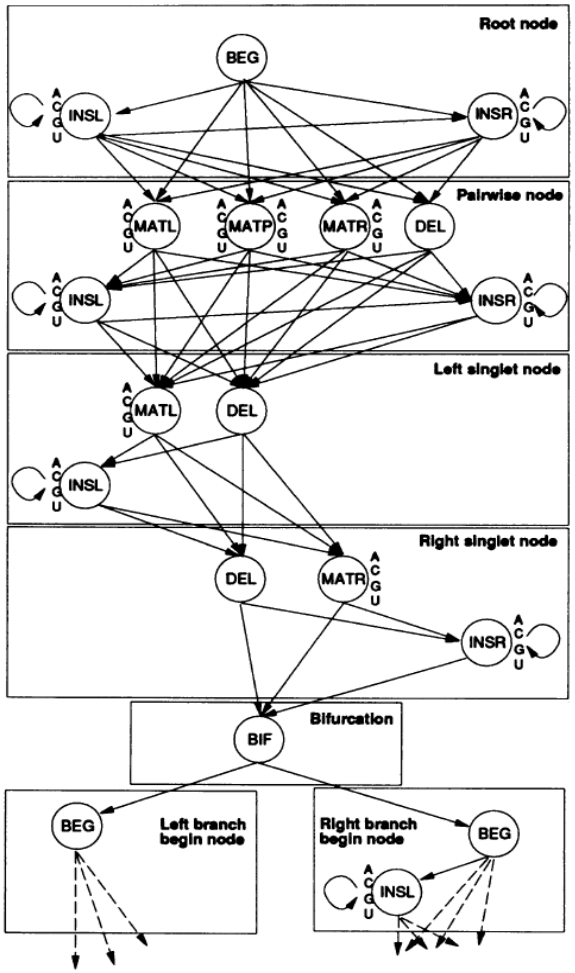


Images – Eddy et al.

Covariance Model

- HMM which permits flexible alignment to an RNA structure –
  - emission and transition probabilities
- Model trees based on finite number of states
  - Match states – sequence conforms to the model:
    - MATP – State in which bases are paired in the model and sequence
    - MATL & MATR – State in which either right or left bulges in the sequence and the model
  - Deletion – State in which there is deletion in the sequence when compared to the model
  - Insertion – State in which there is an insertion relative to model
- Transitions have probabilities
  - Varying probability – Enter insertion, remain in current state, etc
  - Bifurcation – no probability, describes path

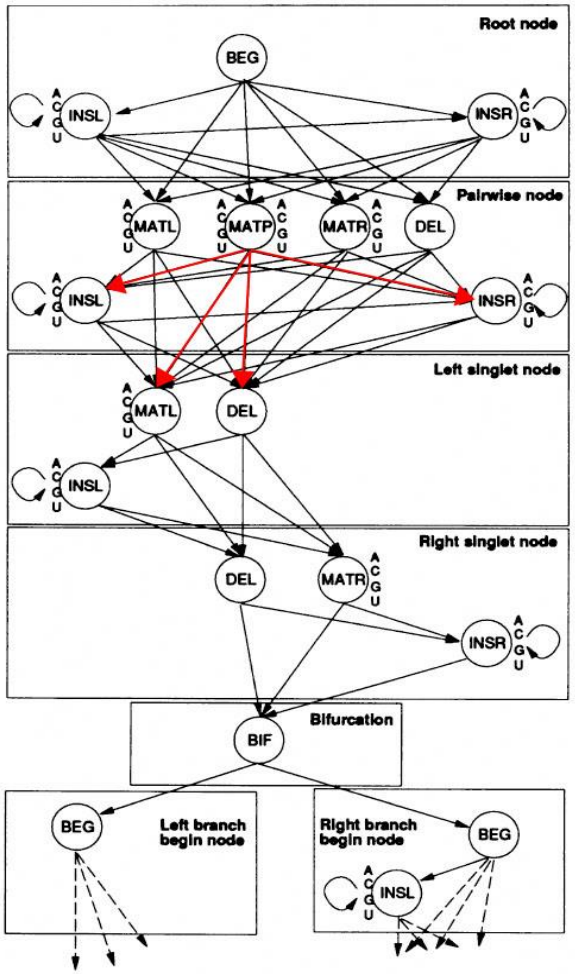
Alignment to CM Algorithm



- Calculate the probability score of aligning RNA to CM
- Three dimensional matrix – $O(n^3)$
  - Align sequence to given subtrees in CM
  - For each subsequence calculate all possible states
- Subtrees evolve from Bifurcations
  - For simplicity Left singlet is default

Images – Eddy et al.

Alignment to CM Algorithm

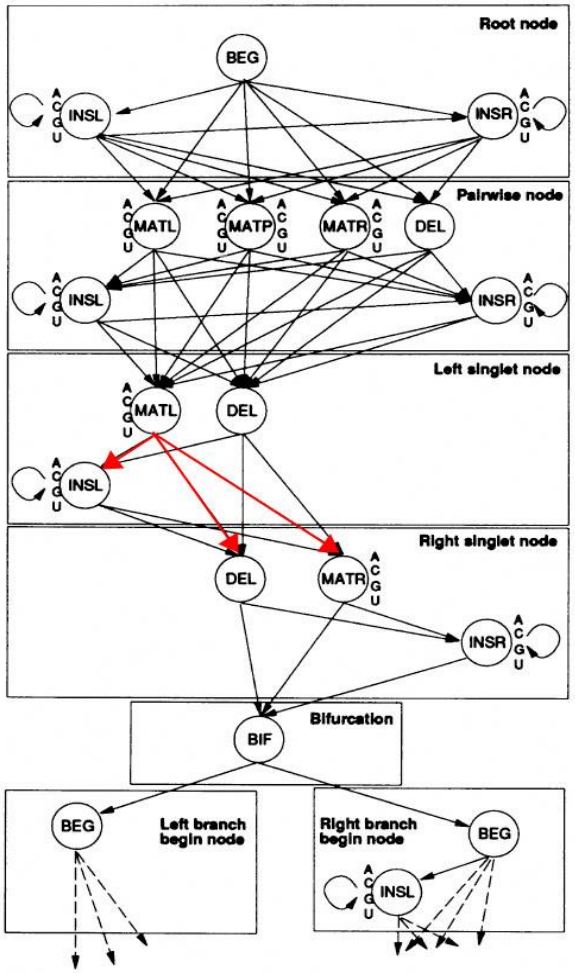


Images – Eddy et al.

- For each calculation take into account the
  - Transition (T) to next state
  - Emission probability (P) in the state as determined by training data

$$S_{i,j,y}(y = MATP) = \max_{y_{next}}[S_{i+1,j-1,y_{next}} + \log \mathcal{T}(y_{next} \mid y) + \log \mathcal{P}(x_i, x_j \mid y)]$$

Alignment to CM Algorithm



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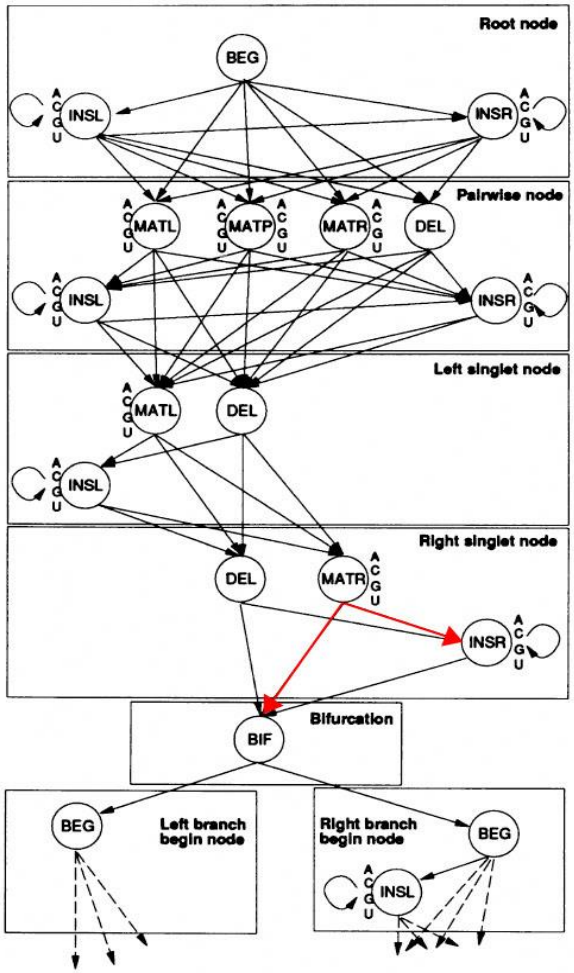

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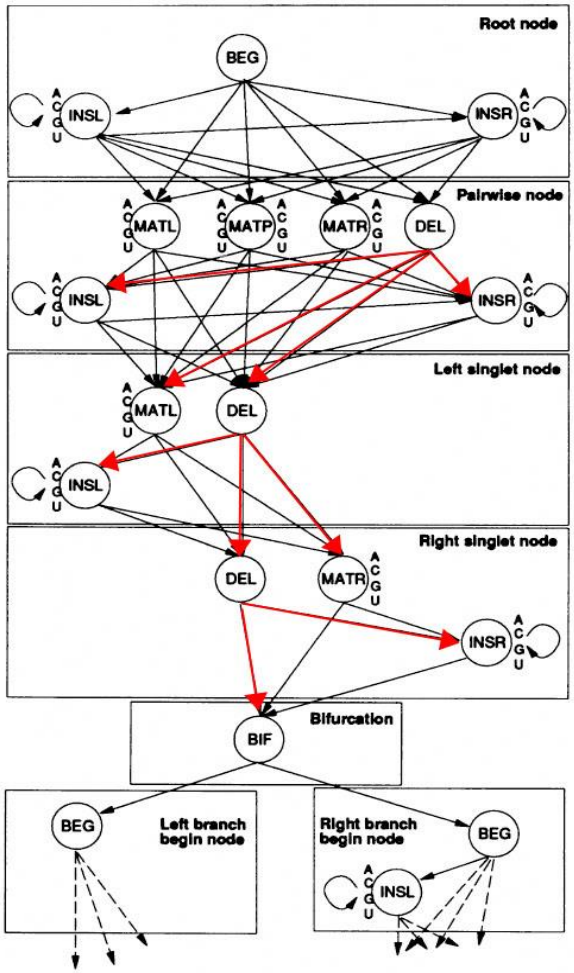

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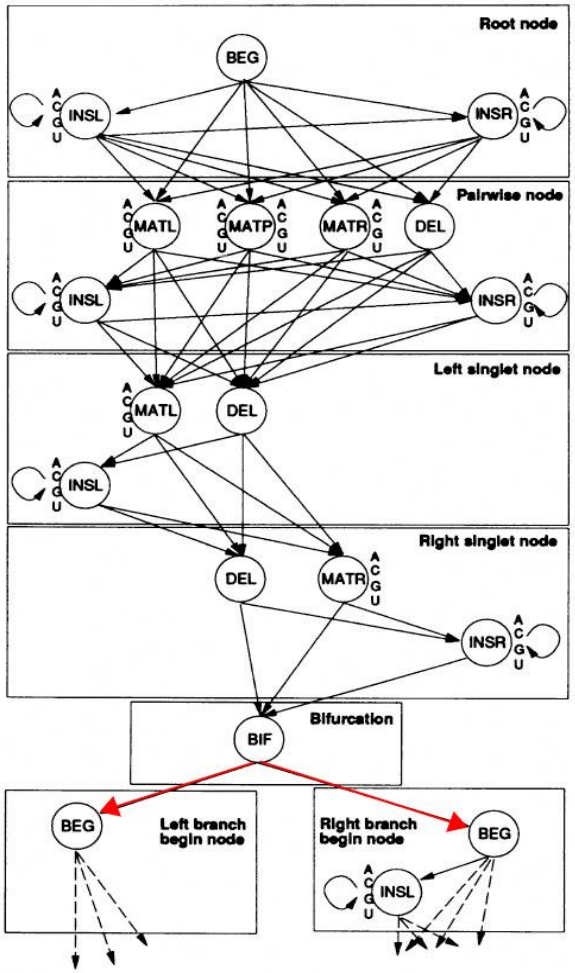
Deletion – does not have an emission probability (P) associated with it

$$S_{i,j,y}(y = DEL) = \max_{y_{next}}[S_{i,j,y_{next}} + \log \mathcal{T}(y_{next} \mid y)]$$

Alignment to CM Algorithm



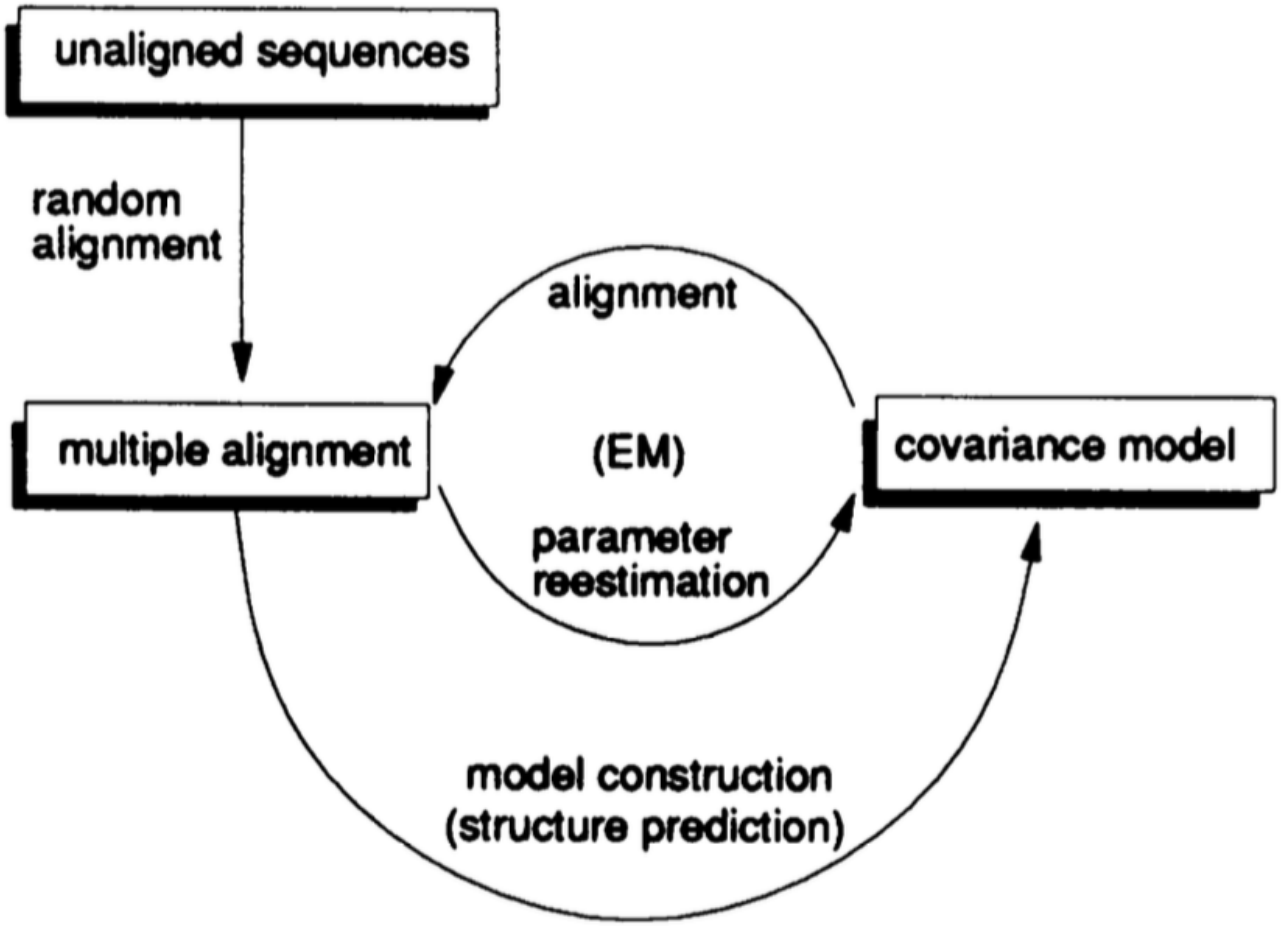
Images – Eddy et al.

- For each calculation take into account the
  - Transition (T) to next state
  - Emission probability (P) in the state as determined by training data

Bifurcation – does not have a probability associated with the state

$$S_{i,j,y}(y = BIFURC) = \max_{i-1<=mid<=j} [S_{i,mid,y_{left}} + S_{mid+1,j,y_{right}}]$$

Model Training



Covariance Model (CM) Training Algorithm

- S(i,j) = Score at indices i and j in RNA when aligned to the Covariance Model

$$S(i,j) = \max \begin{cases} S(i+1,j-1) + \mathrm{M}(i,j) \\ S(i+1,j) \\ \mathrm{S}(i,j-1) \\ \max_{i<k<j} S(i,k) + S(k+1,j) \end{cases}$$

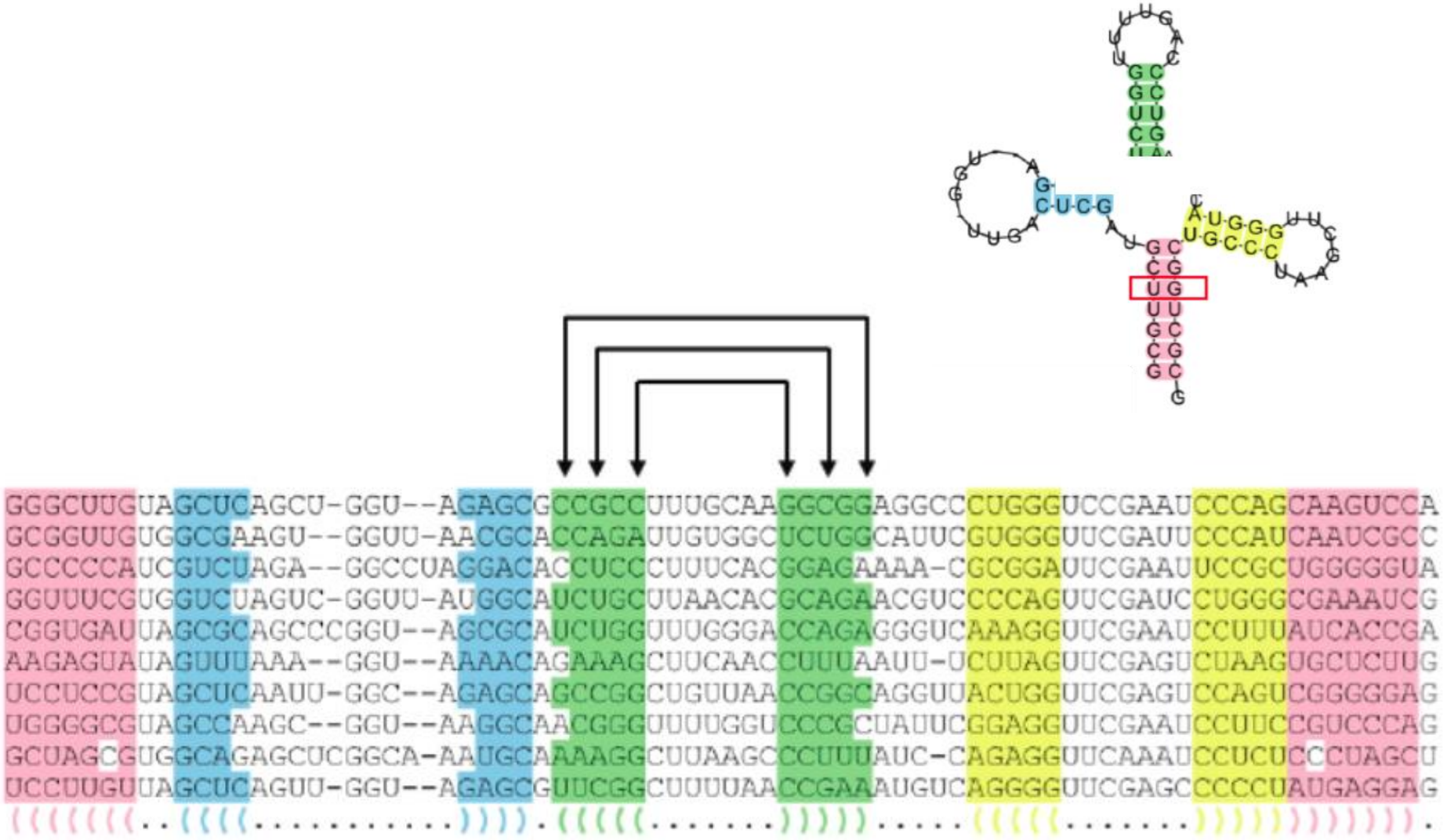
Frequency of seeing the symbols (A, C, G, T) together in locations i and j depending on symbol.

$$M_{i,j} = \sum_{x_i,x_j} f_{x_i x_j} \log_2 \frac{f_{x_i x_j}}{f_{x_i} f_{x_j}}$$

Independent frequency of seeing the symbols (A, C, G, T) in locations i or j depending on symbol.

- Frequencies obtained by aligning model to "training data" – consists of sample sequences
  - Reflect values which optimize alignment of sequences to model

Mutual information for RNA Secondary Structure Prediction



Covariance Model Drawbacks

- Needs to be well trained
- Not suitable for searches of large RNA
  - Structural complexity of large RNA cannot be modeled
  - Runtime
  - Memory requirements

References

- How Do RNA Folding Algorithms Work?. S.R. Eddy. *Nature Biotechnology*, 22:1457-1458, 2004.