Multiple Sequence Alignment Construction, Visualization, and Analysis Using Partial Order Graphs

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Overview of Talk

- Intro to Partial Order Multiple Sequence Alignment Representation
- Multiple Sequence Alignment Construction Using Partial Order Graphs
- Conclusions

Q: Why Do Multiple Sequence Alignment?

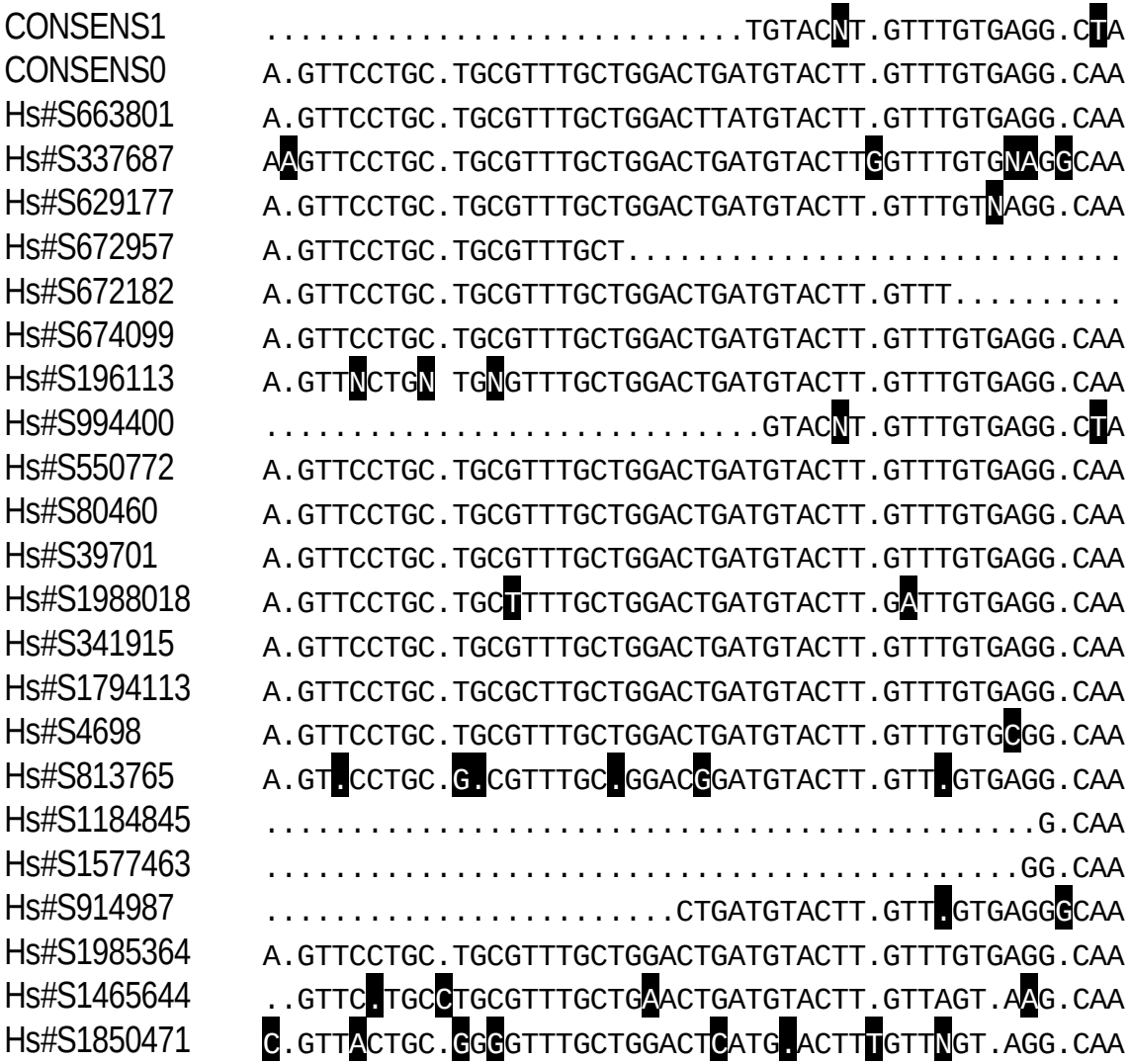
A: To model the process which constructed a set of sequences from a common source sequence.

A multiple sequence alignment allows biologists to infer:

- Protein Structure
- Protein Function
- Protein Domains
- Protein Active Sites
- Splice Sites
- Regulatory Motifs
- Single Nucleotide Polymorphisms
- mRNA Isoforms

For example, protein sequences that are >30% identical often have the same structure and function.

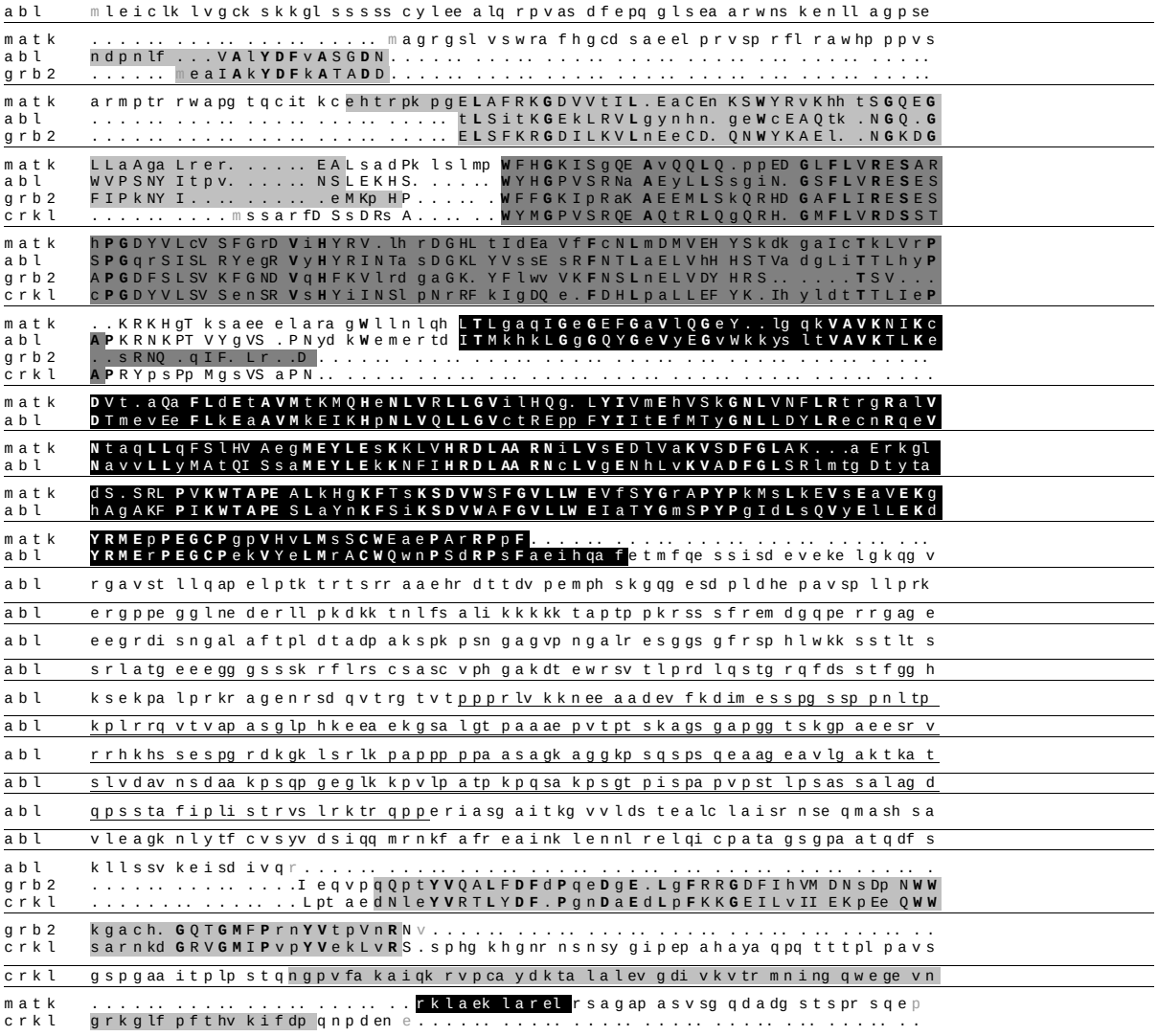
Row Column Multiple Sequence Alignment RC-MSA



RC-MSA Representation Does Not Reveal Large Scale Features

While it is easy to interpret single residue changes in this format,

Large scale changes are not easy to interpret.



The Scale of Features of Interest Should Inform MSA Representation

- Features from single residue changes can be easily seen in RC-MSA Representation:
  - **Regulatory Motifs**
  - **Single Nucleotide Polymorphisms**
  - **Promoter Binding Sites**

- Features from large scale changes cannot:
  - **Protein Domain Differences**
  - **Alternative Splicing**
  - **Genome Duplications**

Degeneracy of RC-MSA Representation

Alignment A is biologically equivalent to alignment A'.

```
A:   .....ACATGTCGAT.....AGGTG
     TGCAC.....TCGATACATAAGGTG

A':  ACATG.....TCGAT.....AGGTG
     .....TGCACTCGATACATAAGGTG
```

However, they look different solely due to representation degeneracy.

We'd like a representation that is not degenerate.

What do we really want to know about an MSA?

1. The order of letters within a sequence. 5’ to 3’ or N-terminal to C-terminal.
2. Which letters are aligned between sequences.

One sequence can impose its order on another sequence only through alignment.

What do we really want to do with an MSA?

- We want to use it as an object in multiple sequence alignment method.
- We want to analyze it for biologically interesting features.

Partial Order Multiple Sequence Alignment PO-MSA

Conventional Format *(RC-MSA)*

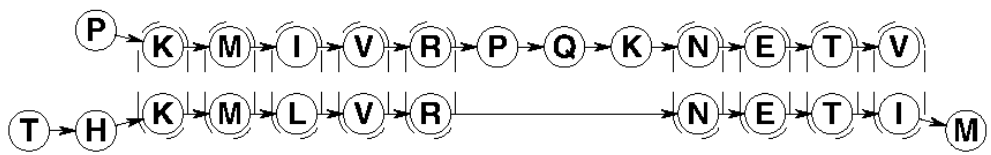
A

```
. . P K M I V R P Q K N E T V .
T H . K M L V R . . . N E T I M
```

Draw each sequence as a directed graph: node for each letter, connect by directed edges

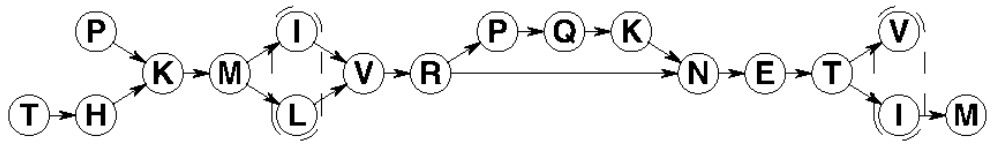
B

C



Fuse aligned, identical letters *(PO-MSA)*

D



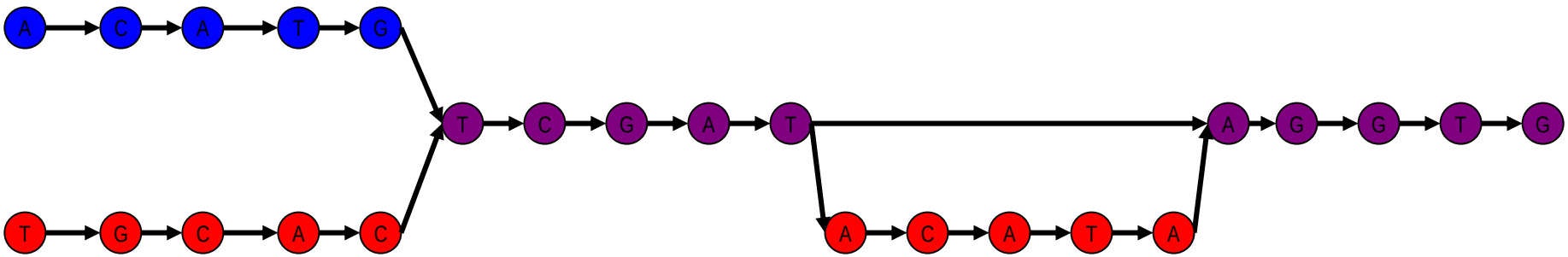
Returning to the previous example…

In the PO-MSA format, both *A* and *A'*

A:
```
.....ACATGTCGAT.....AGGTG
TGCAC.....TCGATACATAAGGTG
```

A':
```
ACATG.....TCGAT.....AGGTG
.....TGCACTCGATACATAAGGTG
```

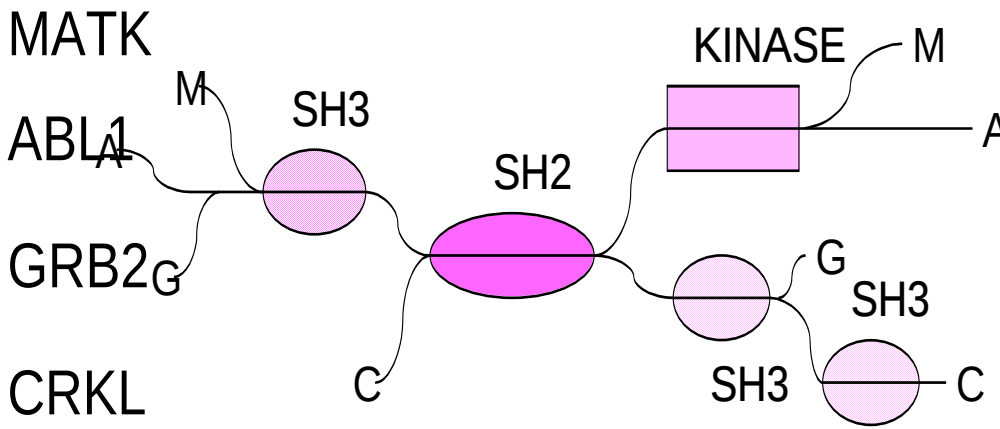
Can be represented as



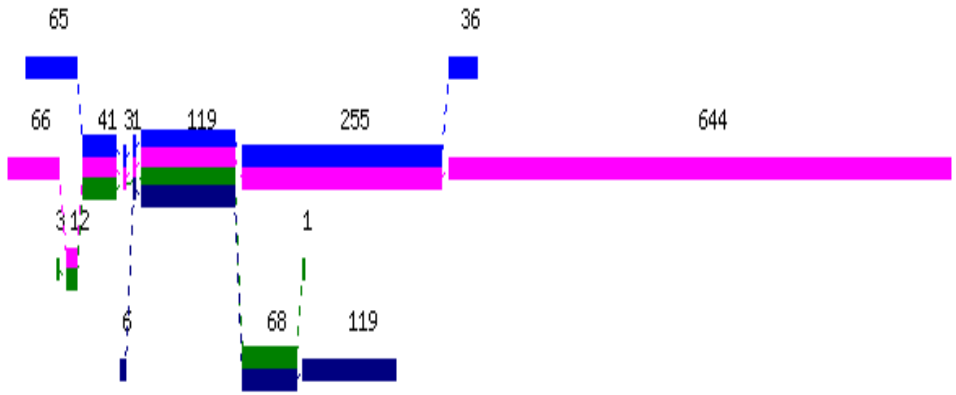
Real Example: Human SH2 Domain Containing Proteins

```
abl    mleiclklvgckskkglsssssscyleealqrpvasdfepqglseaarwnskenllagpse

matk   ......................magrgslvswrafhgcdsaeelprvsprflrawhpppvs
abl    ndpnlf...VAlYDFvASGDN.....................................
grb2   ......meaIAkYDFkATADD.....................................

matk   armptrrwapgtqcitkcehtrpkpgELAFRKGDVVtIL.EaCEnKSWYRvKhhtSGQEG
abl    ..........................tLSitKGEkLRVLgynhn.geWcEAQtk.NGQ.G
grb2   ..........................ELSFKRGDILKVLnEeCD.QNWYKAEl..NGKDG

matk   LLaAgaLrer......EALsadPklslmpWFHGKISgQEAvQQLQ.ppEDGLFLVRESAR
abl    WVPSNYItpv......NSLEKHS......WYHGPVSRNaAEyLLSsgiN.GSFLVRESES
grb2   FIPkNYI..........eMKpHP......WFFGKIpRaKAEEMLSkQRHDGAFLIRESES
crkl   ..........mssarfDSsDRsA......WYMGPVSRQEAQtRLQgQRH.GMFLVRDSST

matk   hPGDYVLcVSFGrDViHYRV.lhrDGHLtIdEaVfFcNLmDMVEHYSkdkgaIcTkLVrP
abl    SPGqrSISLRYegRVyHYRINTasDGKLYVssEsRFNTLaELVhHHSTVadgLiTTLhyP
grb2   APGDFSLSVKFGNDVqHFKVlrdgaGK.YFlwvVKFNSLnELVDYHRS......TSV...
crkl   cPGDYVLSVSenSRVsHYiINSlpNrRFkIgDQe.FDHLpaLLEFYK.IhyldtTTLIeP

matk   ..KRKHgTksaeeelaragWllnlqhLTLgaqIGeGEFGaVlQGeY..lgqkVAVKNIKc
abl    APKRNKPTVYgVS.PNydkWemertdITMkhkLGgGQYGeVyEGvWkkysltVAVKTLKe
grb2   ..sRNQ.qIF.Lr..D.....................................................
crkl   APRYpsPpMgsVSaPN.....................................................

matk   DVt.aQaFLdEtAVMtKMQHeNLVRLLGVilHQg.LYIVmEhVSkGNLVNFLRtrgRalV
abl    DTmevEeFLkEaAVMkEIKHpNLVQLLGVctREppFYIItEfMTyGNLLDYLRecnRqeV

matk   NtaqLLqFSlHVAegMEYLEsKKLVHRDLAARNiLVsEDlVaKVSDFGLAK...aErkgl
abl    NavvLLyMAtQISsaMEYLEkKNFIHRDLAARNcLVgENhLvKVADFGLSRlmtgDtyta

matk   dS.SRLPVKWTAPEALkHgKFTsKSDVWSFGVLLWEVfSYGrAPYPkMsLkEVsEaVEKg
abl    hAgAKFPIKWTAPESLaYnKFSiKSDVWAFGVLLWEIaTYGmSPYPgIdLsQVyELLEKd

matk   YRMEpPEGCPgpVHvLMsSCWEaePArRPpF.....................................
abl    YRMErPEGCPekVYeLMrACWQwnPSdRPsFaeihqafetmfqessisdevekelgkqgv

abl    rgavstllqapelptktrtsrraaehrdttdvpemphskgqgesdpldhepavspllprk

abl    ergppegglnederllpkdkktnlfsalikkkkktaptppkrsssfremdgqperrgage

abl    eegrdisngalaftpldtadpakspkpsngagvpngalresggsgfrsphlwkksstlts

abl    srlatgeeegggssskrflrscsascvphgakdtewrsvtlprdlqstgrqfdsstfggh

abl    ksekpalprkragenrsdqvtrgtvtppprlvkkneeaadevfkdimesspgssppnltp

abl    kplrrqvtvapasglphkeeaekgsalgtpaaaepvtptskagsgapggtskgpaeesrv

abl    rrhkhssespgrdkgklsrlkpappppaasagkaggkpsqspsqeaageavlgaktkat

abl    slvdavnsdaakpsqpgeglkkpvlpatpkpqsakpsgtpispapvpstlpsassalagd

abl    qpsstafiplistrvslrktrqpperiasgaitkgvvldstealclaisrnseqmashsa

abl    vleagknlytfcvsyvdsiqqmrnkfafreainklennlrelqicpatagsgpaatqdfs

abl    kllssvkeisdivqr.....................................................
grb2   ..............Ieqvpgqpt YVQALFDFdPqeDgE.LgFRRGDFIhVMDNsDpNWW
crkl   ..............LptaedNleYVRTLYDF.PgnDaEdLpFKKGEILvIIEKpEeQWW

grb2   kgach.GQTGMFPrnYVtpVnRNv.....................................
crkl   sarnkdGRVGMIPvpYVekLvRS.sphgkhgnrnsnsygipepahayaqpqtttplpavs

crkl   gspgaaitplpstqngpvfakaiqkrvpcaydktalalevgdivkvtrmninggqwegevn

matk   ......................rklaeklarelrsagapasvsgqdadgstsprsqep
crkl   grkglfpfthvkifdpqnpdene.................................
```

RC-MSA in Text Format



Hand Rendered PO-MSA Showing Domain Structure



POAVIZ Rendered PO-MSA Reflects Domain Structure

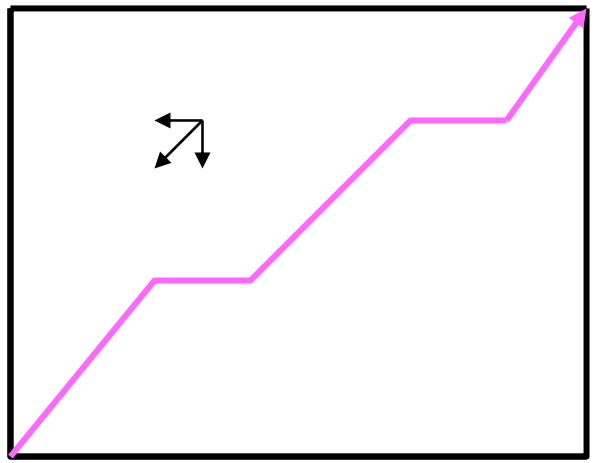
What do we really want to do with an MSA?

We want to analyze it for biologically interesting features.

We want to use it as an object for building multiple sequence alignments.

Multiple Sequence Alignment Construction Using PO-MSAs

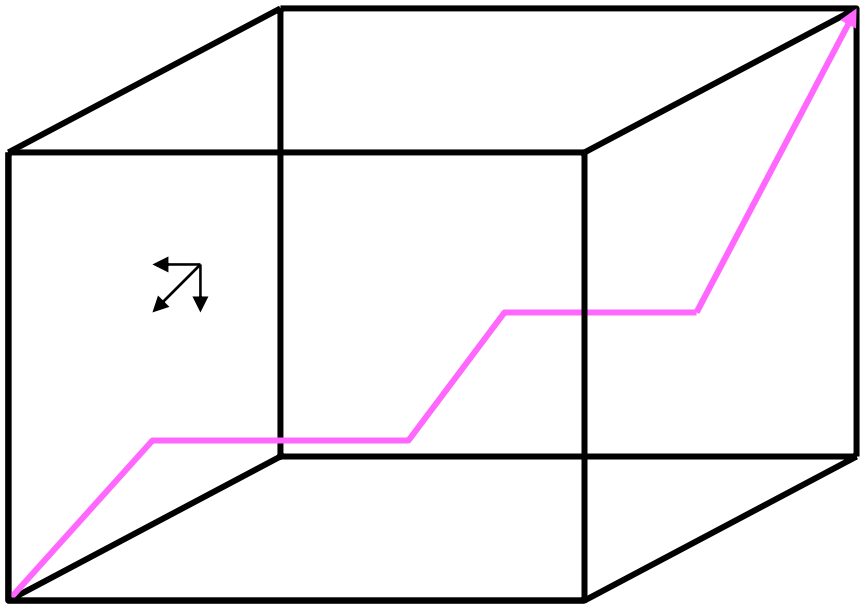
Pair-wise Sequence Alignment Using Dynamic Programming



Finding a PSA = Finding a path through a 2-Dim matrix.

It's $O(L^2)$, where L is the sequence length.

Multiple Sequence Alignment Using Dynamic Programming

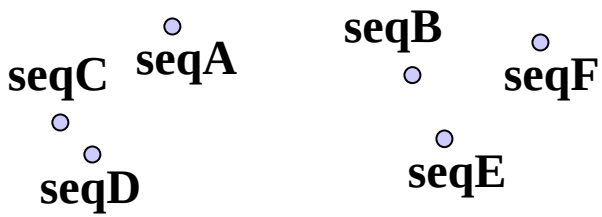


Finding an MSA = Finding a path through an N-Dim matrix. It's $O(L^N)$, where N is the number of sequences and L is the sequence length.

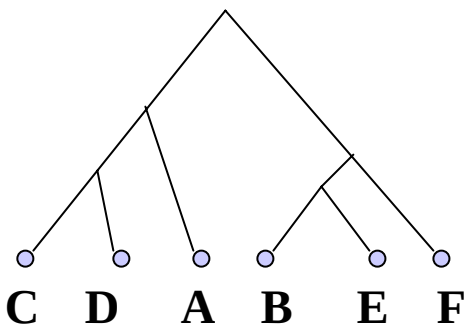
Note: More than 5 sequences takes a prohibitive amount of time.
Heuristic methods, such as those used by CLUSTAL W, are used instead.

Progressive Alignment (CLUSTAL W) Approach

1. Compute pairwise distances of all N sequences.

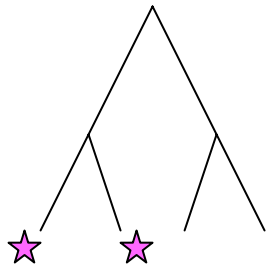


2. Build Guide Tree



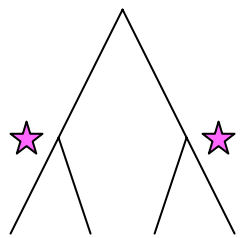
3. Align N sequences using guide tree.

a. Use standard PSA to align leaf sequence.



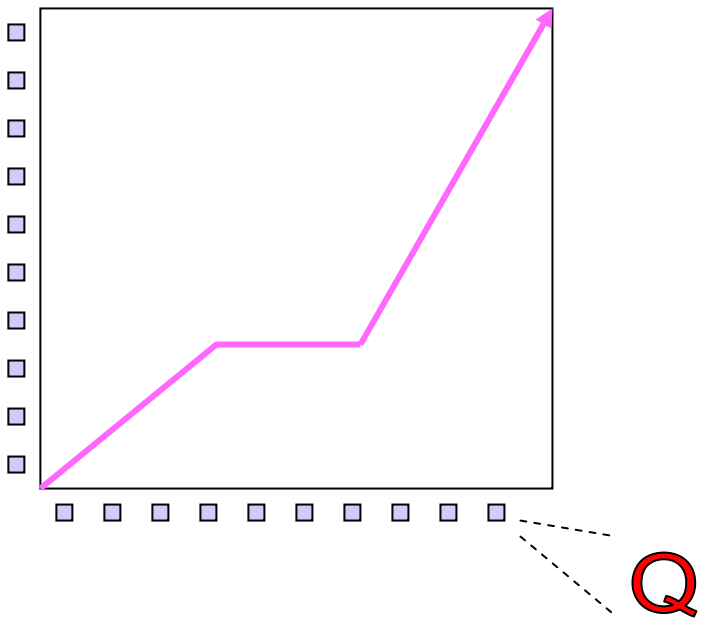
b. Profile multiple sequence alignments at branch nodes.

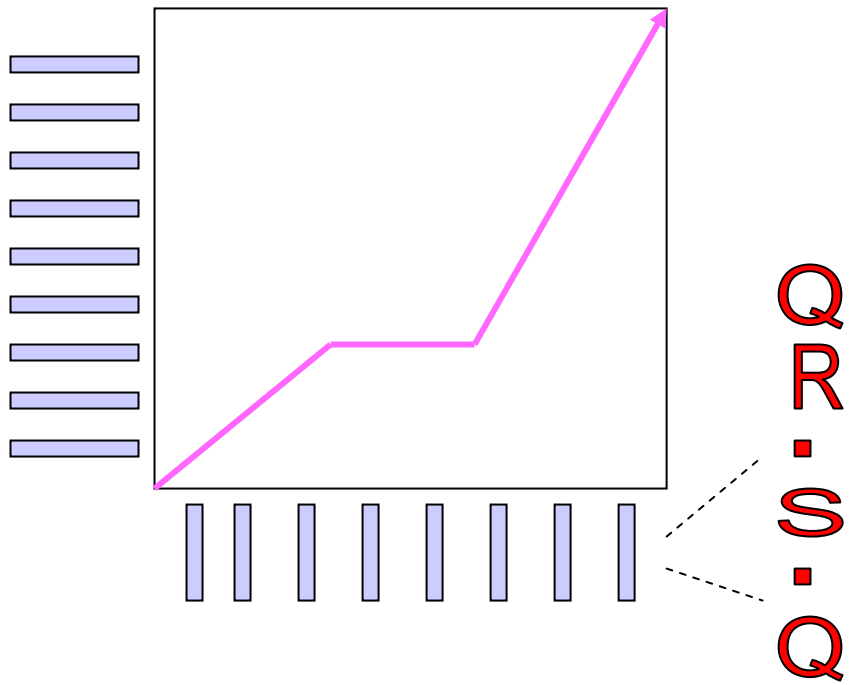
c. Use standard PSA on profiles.



d. Recurse.

Pair-wise Sequence Alignment of Leaf Nodes V. Branch Nodes





- PSA of sequences at leaf nodes: **Requires a scoring function which can score a match between residues.**
- PSA of profiles at branch nodes: **Requires a scoring function which can score a match between profiles of columns of residues and gaps.**

Problem with Aligning Profiles: Gap Artifacts!

Alignment *A* is biologically equivalent to alignment *A'*.

A:
```
.....ACATGTCGAT.....AGGTG
TGCAC.....TCGATACATAAGGTG
```

A':
```
ACATG.....TCGAT.....AGGTG
.....TGCACTCGATACATAAGGTG
```

If we try to align another sequence which is identical to the second sequence in the alignment…

S: `TGCACTCGATACATAAGGTG`

We find that *Score(S,A)* not equal to *Score(S,A')*, but it should be.

In doing pair-wise sequence alignment on RC-MSA profiles:

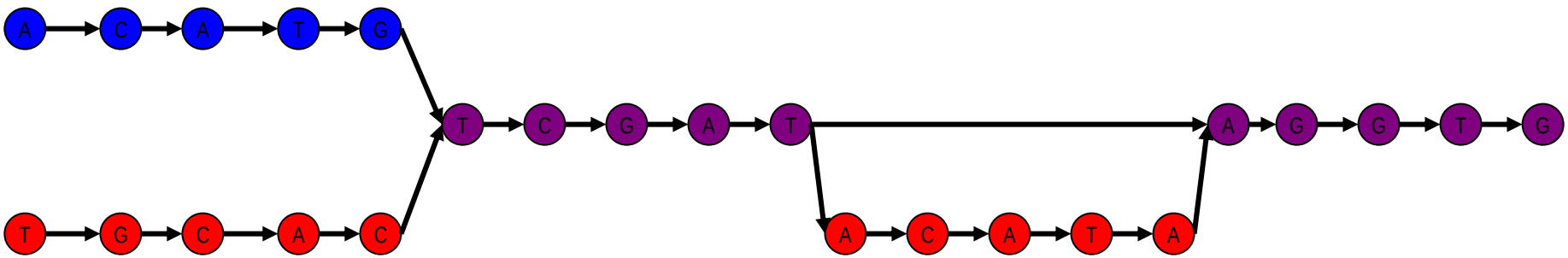
- Each column is treated in isolation.
- But interpreting what's a true gap requires looking outside of column.
- We can try to solve this problem by adjusting the scoring process.
- This results in a non-local scoring function, which violates dynamic programming.

We can instead replace the profile RC-MSA representation with the PO-MSA representation.

In the PO-MSA representation, both *A* and *A'*

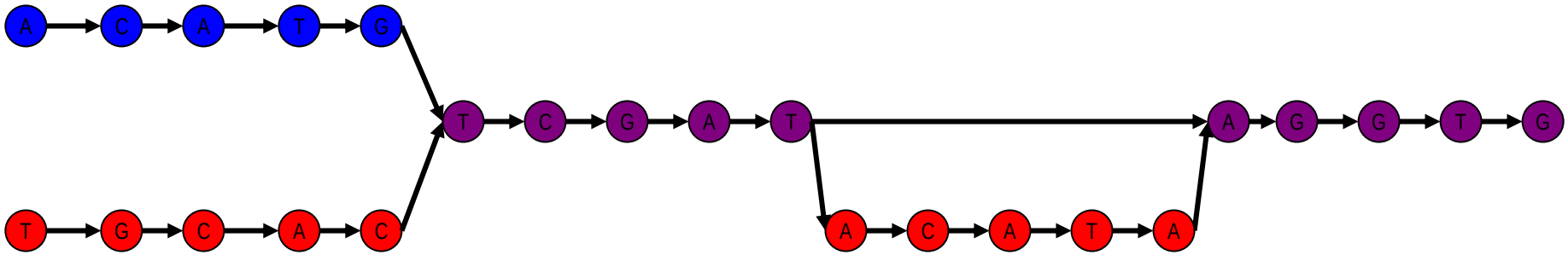
```
      .....ACATGTCGAT.....AGGTG
A:    TGCAC.....TCGATACATAAGGTG

      ACATG.....TCGAT.....AGGTG
A':   .....TGCACTCGATACATAAGGTG
```

Can be represented as

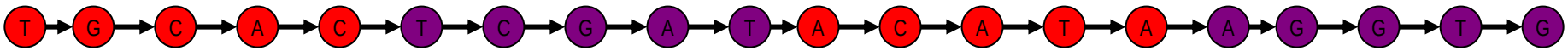


We can align *S* to *A* using Sequence to PO-MSA alignment algorithm.

A:

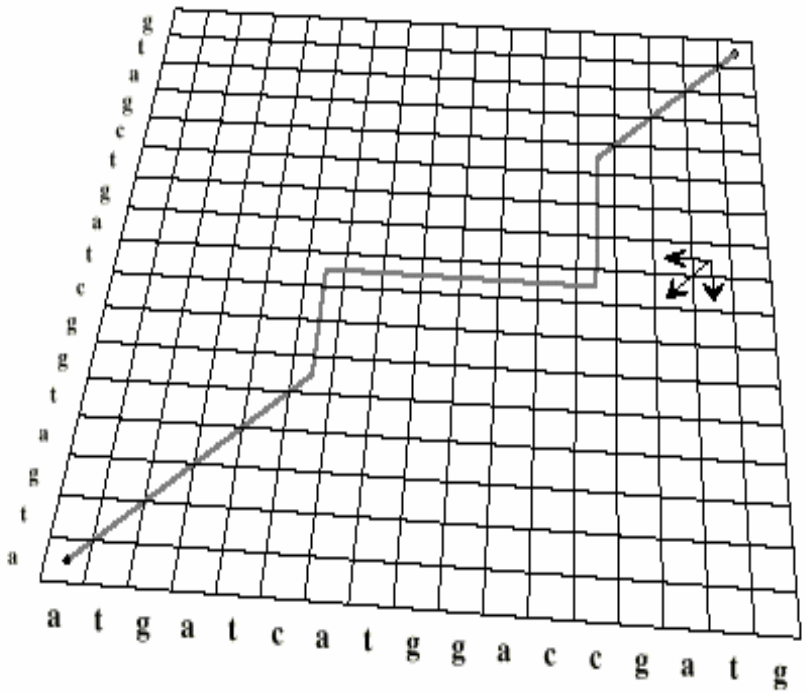


S:

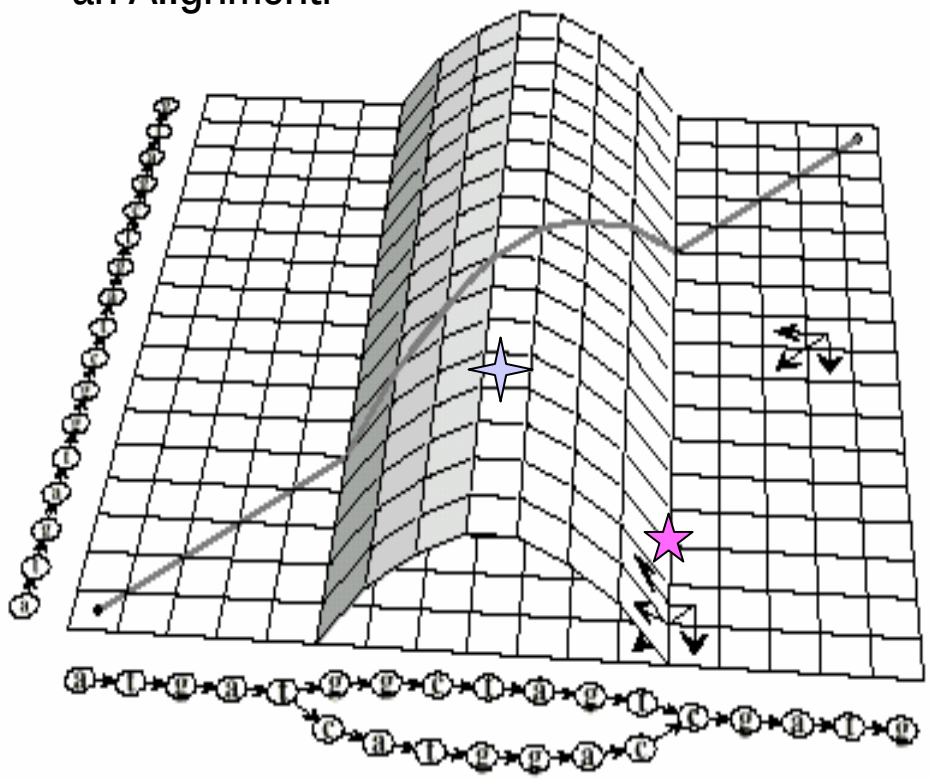


Sequence to PO-MSA Alignment Algorithm

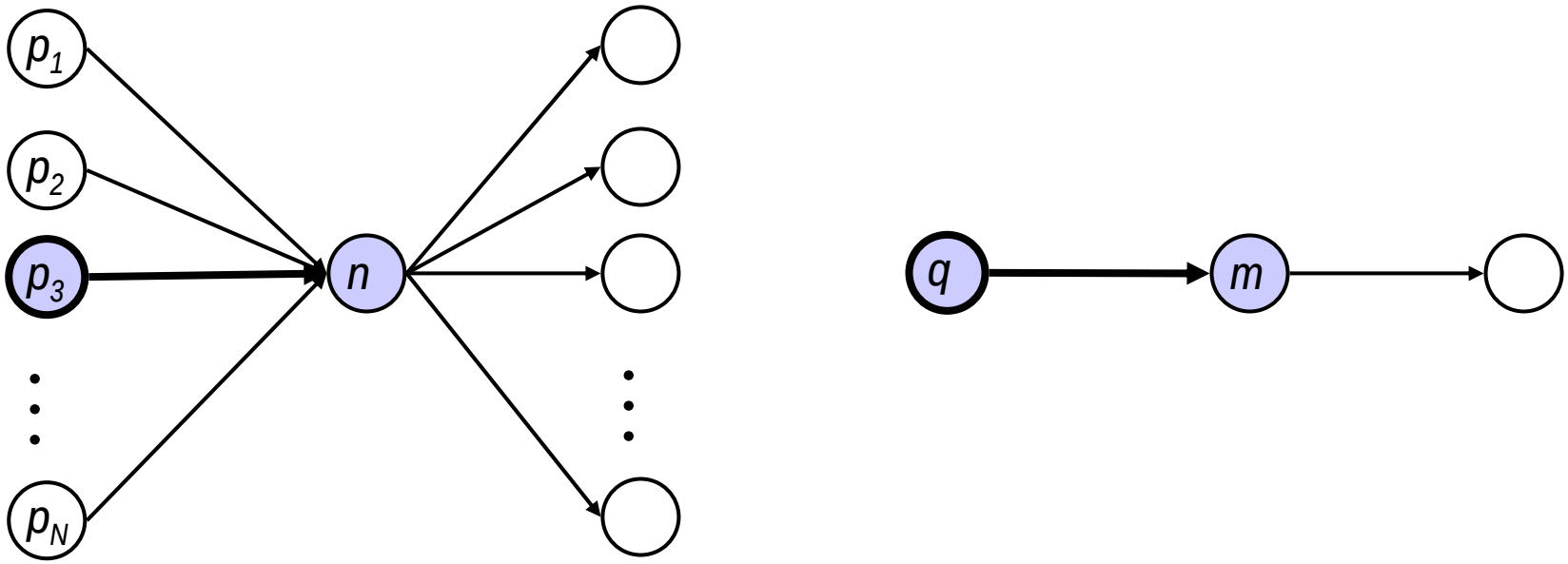
Conventional Alignment of Two Sequences



Partial Order Alignment of a Sequence to an Alignment.



Sequence to PO-MSA Alignment Algorithm Requires a Simple Extension of Sequence to Sequence Alignment Algorithm



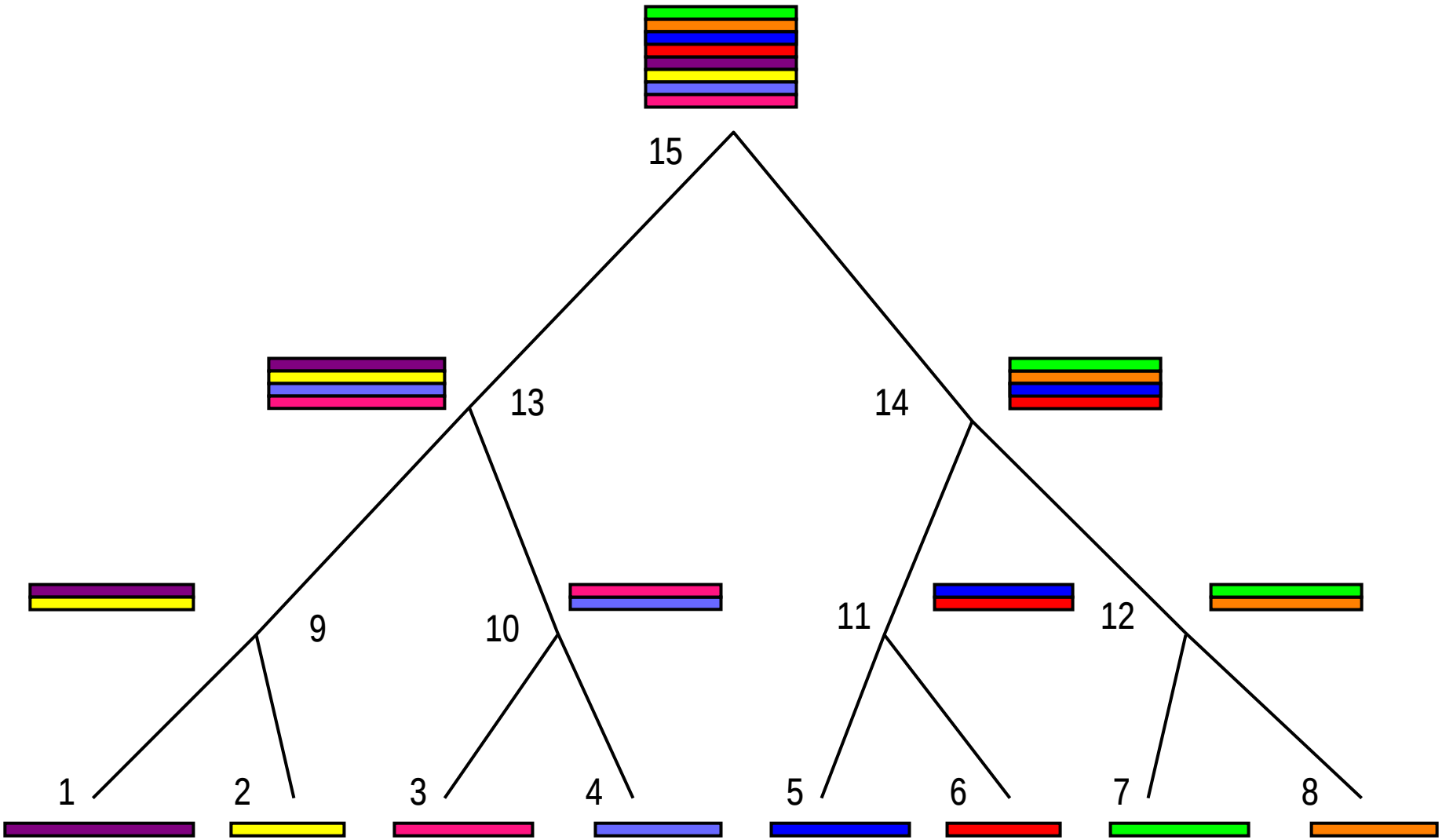
Simply extend dynamic programming move set to include partial order moves: at each position (n,m) in the matrix, choose best move by:

$$S(n,m) = \max \begin{cases} S(p,m-1) + s(n,m) \\ S(p,m) + \Delta(m) \\ S(n,m-1) + \Delta(n) \end{cases}$$

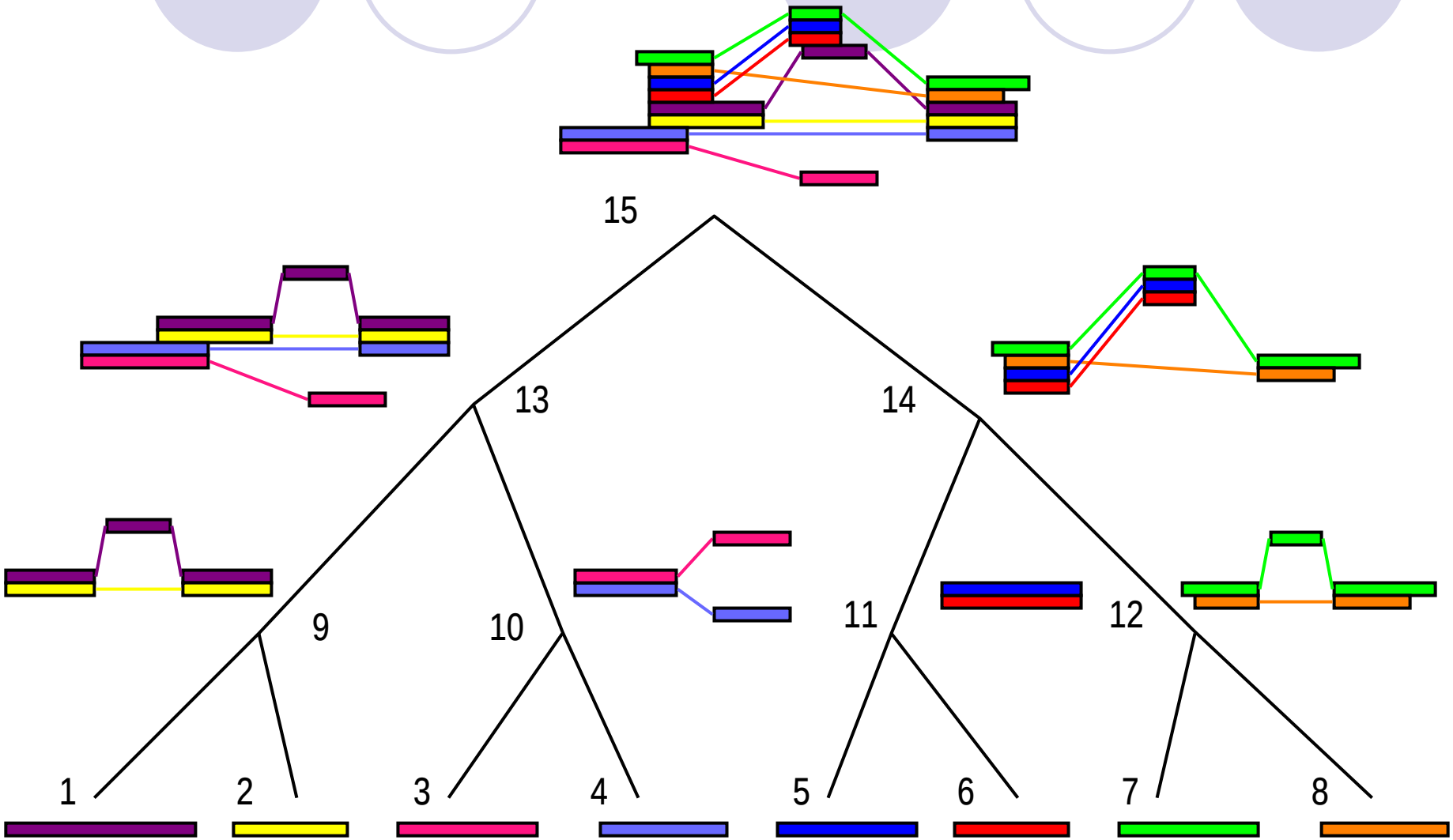
Considering all predecessor nodes that have a directed edge from p $\rightarrow$ n.

Note: MATCH and INSERT moves may have more than one incoming edge p.

Recall Progressive Multiple Sequence Alignment with Profile Intermediates

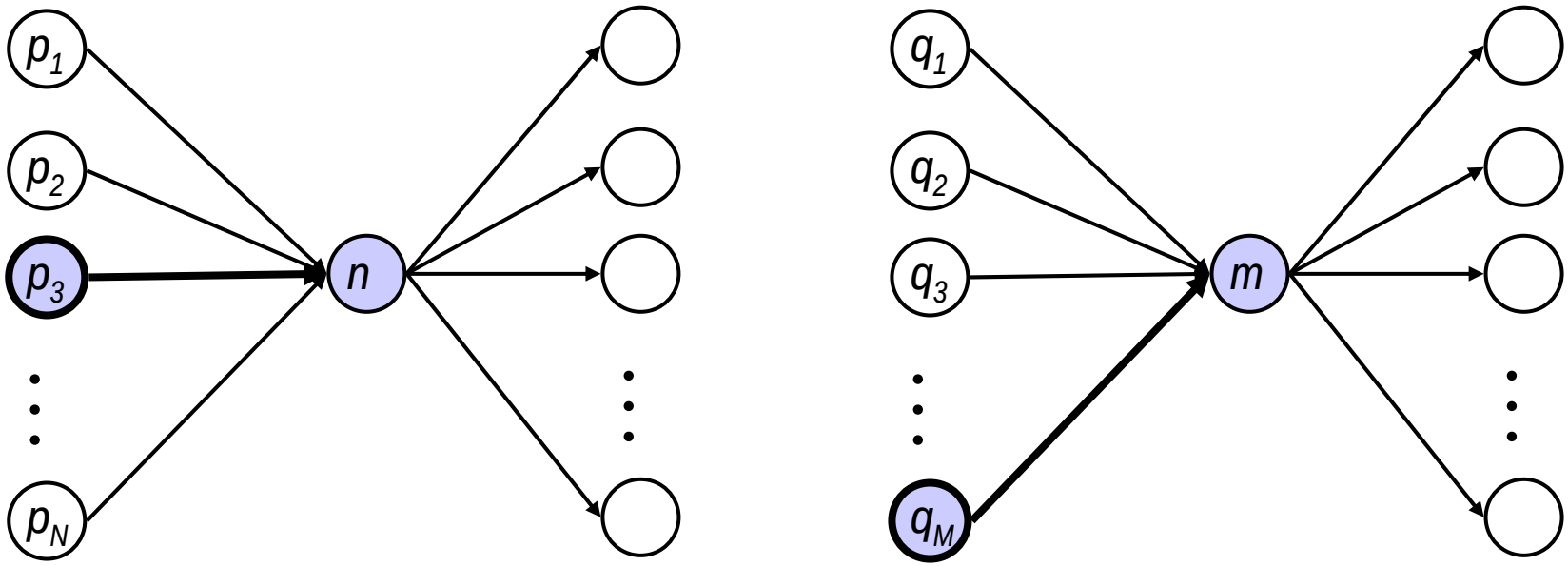


Progressive Multiple Sequence Alignment with PO-MSA Intermediates



Requires PO-MSA to PO-MSA Alignment Algorithm

PO-MSA to PO-MSA Alignment Algorithm Requires a Simple Extension of Sequence to PO-MSA Alignment Algorithm



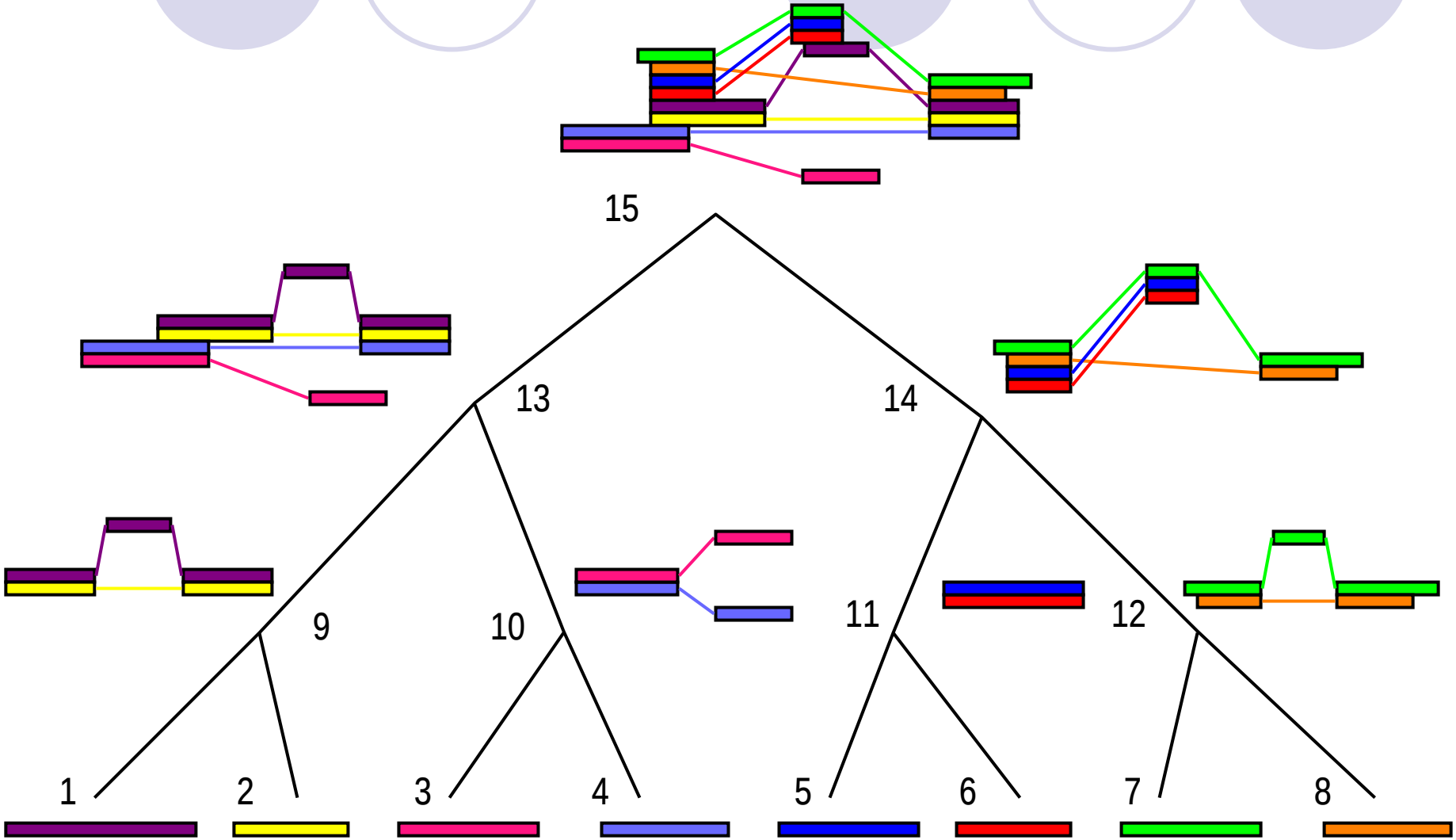
Simply extend dynamic programming move set to include partial order moves: at each position (n,m) in the matrix, choose best move by:

$$S(n,m) = \max_{\substack{p \to n \\ q \to m}} \begin{cases} S(p,q) + s(n,m) \\ S(p,m) + \Delta(n) \\ S(n,q) + \Delta(m) \end{cases}$$

Considering all predecessor nodes that have a directed edge from p $\rightarrow$ n and q $\rightarrow$ m.

Note: MATCH and INSERT moves may have more than one incoming edge p or q.

PO-MSA to PO-MSA Alignment Algorithm Finds Best Linear Match Between the PO-MSAs



Can be extended heuristically to find best match

Thesis Work

- Developed partial order alignment visualizer
- Combined partial order alignment and progressive alignment
- Applied POA to detect alternative splicing events in expressed sequence data
- Formalized relationship between PO-MSAs and HMMs

Acknowledgements

- I'd like to thank:
  - Chris Lee for all of his guidance and support.
  - Michael Quist for all of his help with this project.
  - Barmak Modrek with whom I worked on annotating alternative splicing using PO-MSAs and POA.
  - Everyone in the Lee Lab for hours of helpful discussion.
  - DOE CSGF for supporting the work.

To use or download POA or POAVIZ go to:

http://www.bioinformatics.ucla.edu/poa