BSC 4934: Q'BIC Capstone Workshop

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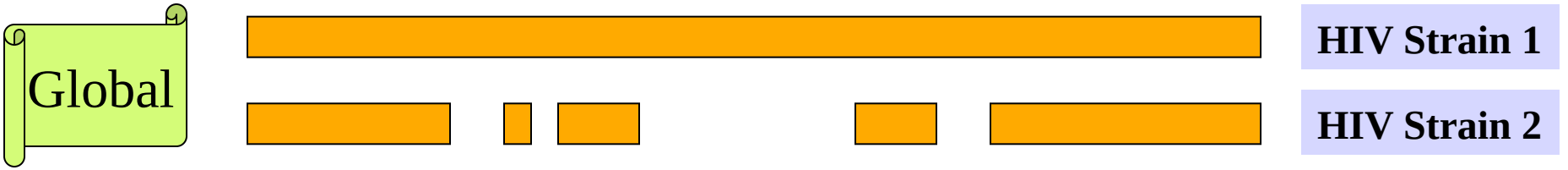
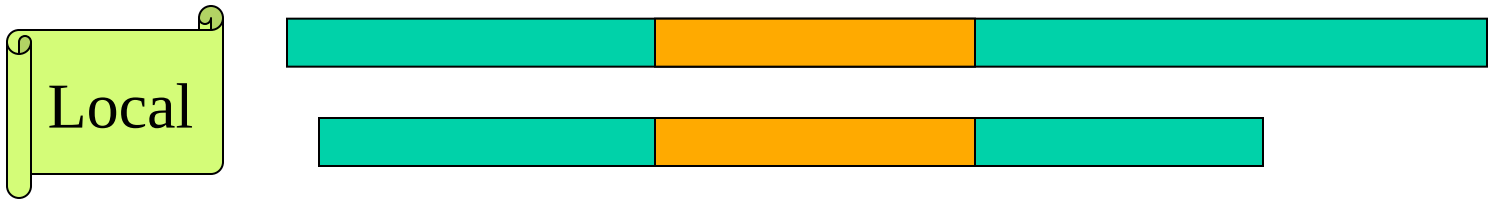
http://www.cis.fiu.edu/~giri/teach/BSC4934_Su09.html

24 June through 7 July, 2009

Types of Sequence Alignments - 1

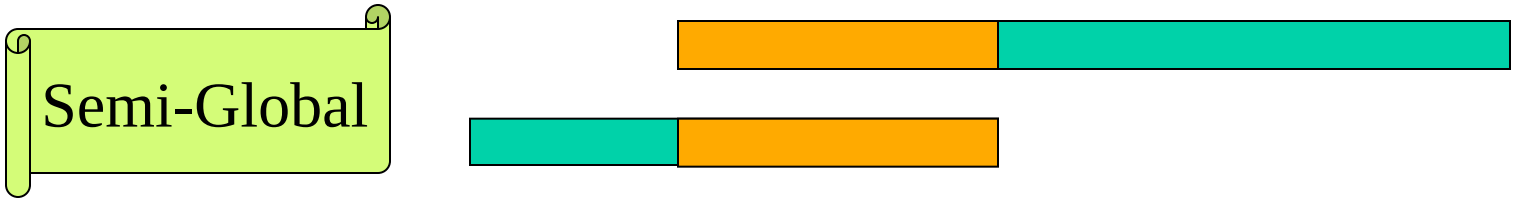


- Global Alignment: similarity over entire length

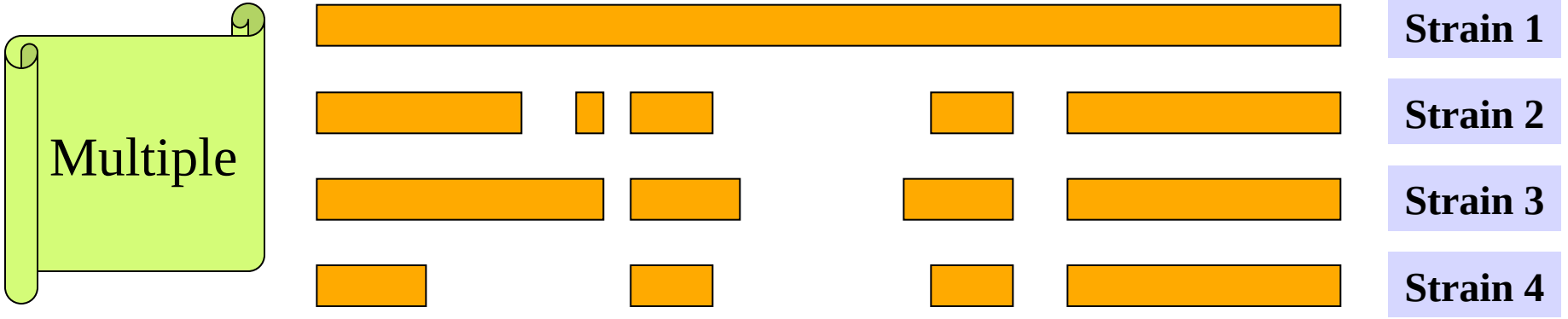


- Local Alignment: no overall similarity, but some segment(s) is/are similar

Types of Sequence Alignments - 2



- Semi-global Alignment: end segments may not be similar



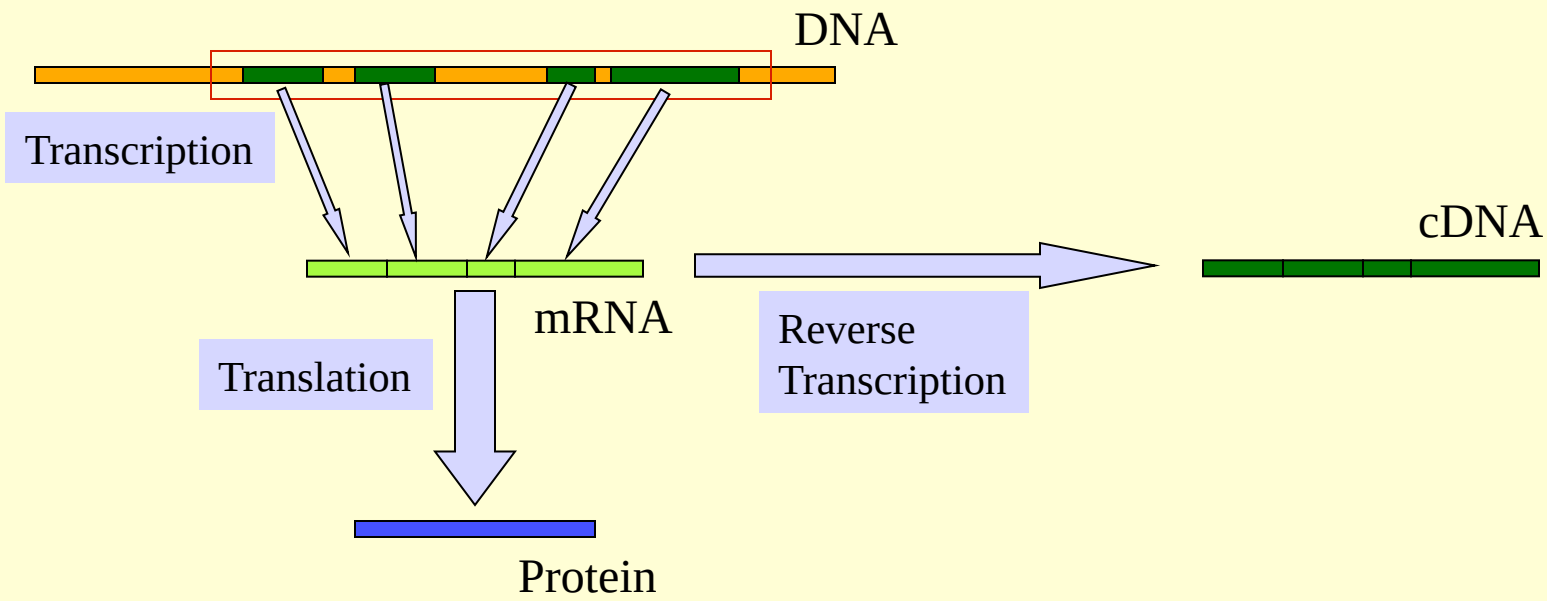
- Multiple Alignment: similarity between sets of sequences

Sequence Alignment

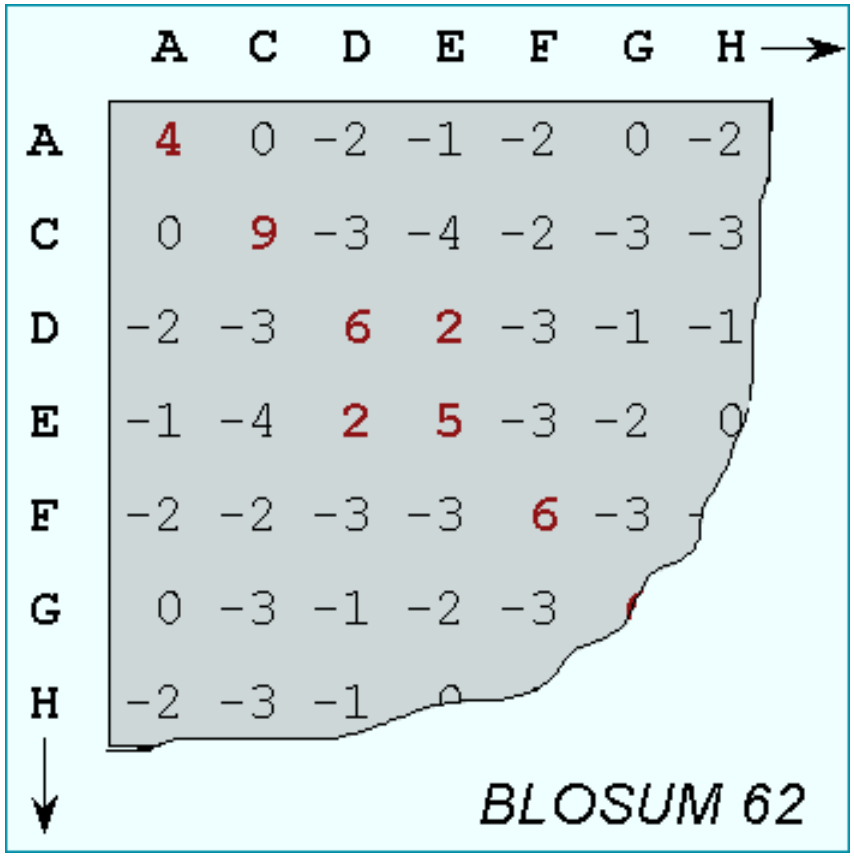
- Global:
  - Needleman-Wunsch-Sellers (1970).
- Local:
  - Smith-Waterman (1981)
  - Useful when commonality is small and global alignment is meaningless. Often unaligned portions "mask" short stretches of aligned portions. Example: comparing long stretches of anonymous DNA; aligning proteins that share only some motifs or domains.
- Dynamic Programming (DP) based.

Why gaps?

- Example: Finding the gene site for a given (eukaryotic) cDNA requires "gaps".
- What is cDNA? cDNA = Copy DNA



How to score mismatches?



BLAST & FASTA

- FASTA

  [Lipman, Pearson '85, '88]

- Basic Local Alignment Search Tool

  [Altschul, Gish, Miller, Myers, Lipman '90]

BLAST Overview

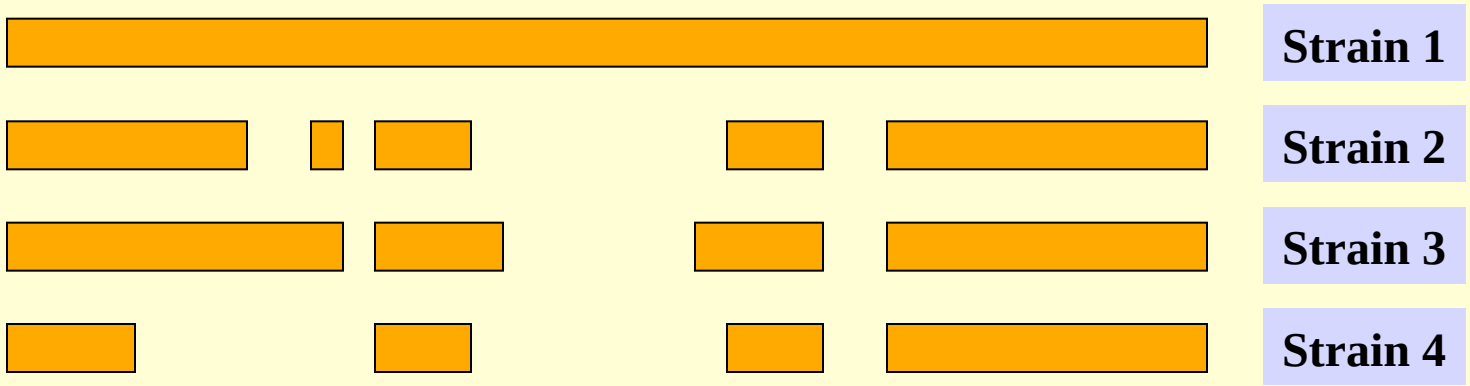
- Program(s) to search all sequence databases
- Tremendous Speed/Less Sensitive
- Statistical Significance reported
- WWWBLAST, QBLAST (send now, retrieve results later), Standalone BLAST, BLASTcl3 (Client version, TCP/IP connection to NCBI server), BLAST URLAPI (to access QBLAST, no local client)

BLAST Strategy & Improvements

- Lipman et al.: speeded up finding "runs" of "hot spots".
- Eugene Myers '94: "Sublinear algorithm for approximate keyword matching".
- Karlin, Altschul, Dembo '90, '91: "Statistical Significance of Matches"

Why Gaps?

- Example: Aligning HIV sequences.



BLAST Variants

- **Nucleotide BLAST**
  - **Standard blastn**
  - **MEGABLAST** (Compare large sets, Near-exact searches)
  - **Short Sequences** (higher E-value threshold, smaller word size, no low-complexity filtering)
- **Protein BLAST**
  - **Standard blastp**
  - **PSI-BLAST** (Position Specific Iterated BLAST)
  - **PHI-BLAST** (Pattern Hit Initiated BLAST; reg expr. Or Motif search)
  - **Short Sequences** (higher E-value threshold, smaller word size, no low-complexity filtering, PAM-30)
- **Translating BLAST**
  - **Blastx**: Search nucleotide sequence in protein database (6 reading frames)
  - **Tblastn**: Search protein sequence in nucleotide dB
  - **Tblastx**: Search nucleotide seq (6 frames) in nucleotide DB (6 frames)

BLAST Cont'd

- **RPS BLAST**
  - Compare protein sequence against Conserved Domain DB; Helps in predicting rough structure and function
- **Pairwise BLAST**
  - blastp (2 Proteins), blastn (2 nucleotides), tblastn (protein-nucleotide w/ 6 frames), blastx (nucleotide-protein), tblastx (nucleotide w/6 frames-nucleotide w/ 6 frames)
- **Specialized BLAST**
  - Human & Other finished/unfinished genomes
  - *P. falciparum*: Search ESTs, STSs, GSSs, HTGs
  - VecScreen: screen for contamination while sequencing
  - IgBLAST: Immunoglobin sequence database

BLAST Credits

- Stephen Altschul
- Jonathan Epstein
- David Lipman
- Tom Madden
- Scott McGinnis
- Jim Ostell
- Alex Schaffer
- Sergei Shavirin
- Heidi Sofia
- Jinghui Zhang

Databases used by BLAST

- **Protein**
  - nr (everything), swissprot, pdb, alu, individual genomes
- **Nucleotide**
  - nr, dbest, dbsts, htgs (unfinished genomic sequences), gss, pdb, vector, mito, alu, epd
- **Misc**

BLAST Parameters and Output

- Type of sequence, nucleotide/protein
- Word size, w
- Gap penalties, p_1 and p_2
- Neighborhood Threshold Score, T
- Score Threshold, S
- E-value Cutoff, E
- Number of hits to display, H
- Database to search, D
- Scoring Matrix, M
- Score s and E-value e
  - E-value e is the expected number of sequences that would have an alignment score greater than the current score s.

Scoring Matrix to Use

- PAM 40 — Short alignments with high similarity (70-90%)
- PAM 160 — Members of a protein family (50-60%)
- PAM 250 — Longer alignments (divergent sequences) (~30%)

- BLOSUM90 — Short alignments with high similarity (70-90%)
- BLOSUM80 — Members of a protein family (50-60%)
- BLOSUM62 — Finding all potential hits (30-40%)
- BLOSUM30 — Longer alignments (divergent sequences) (<30%)

BLAST

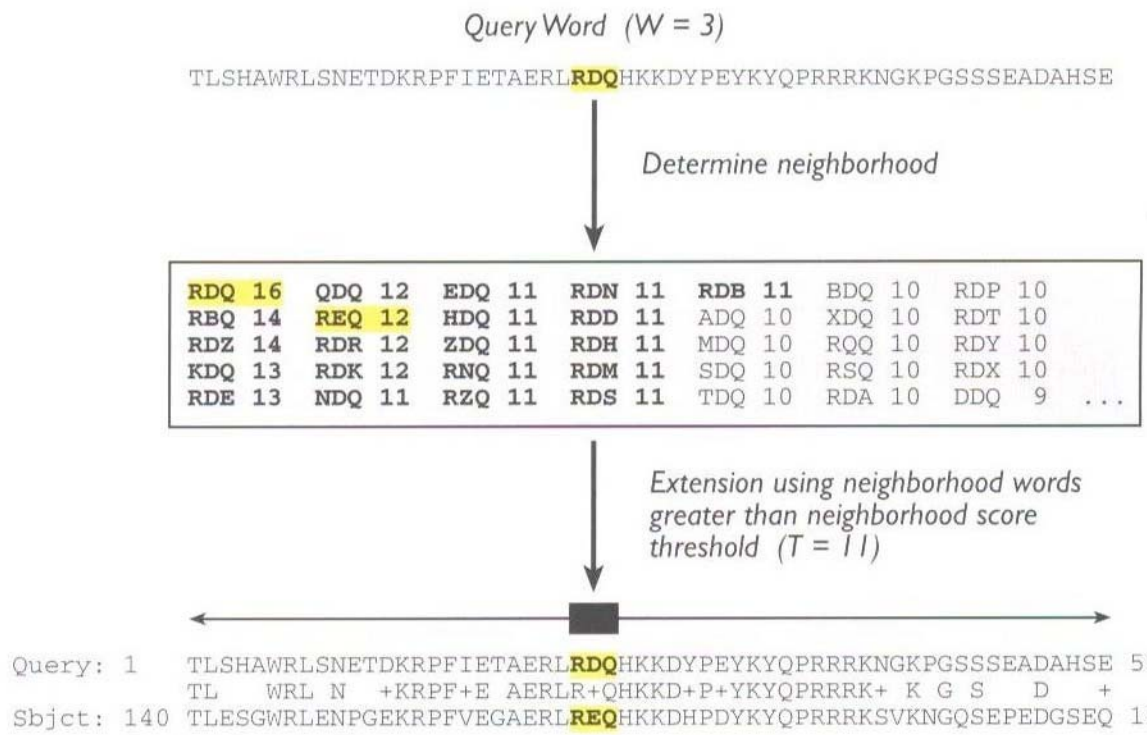


FIGURE 11.7 The initiation of a **BLAST** search. The search begins with query words of a given length (here, three amino acids) being compared against a scoring matrix to determine additional three-letter words "in the neighborhood" of the original query word. Any occurrences of these neighborhood words in sequences within the target database then are investigated. See text for details.

Rules of Thumb

- Results of searches using different scoring systems may be compared directly using normalized scores.
- If S is the (raw) score for a local alignment, the **normalized** score S' (in bits) is given by

  S’ = (λS - lnK)/ln2

  The parameter λ scales for the scoring system, while K scales for the search space size.
- **Statistically significant normalized score**,

$$S' > \log\left(\frac{N}{E}\right)$$

  where E-value = E, and N = size of search space.
- Read **http://www.ncbi.nlm.nih.gov/Education/BLASTinfo/glossary2.html** for information about the various terms being used here.

Rules of Thumb

- Most sequences with significant similarity over their entire lengths are homologous.
- Matches that are > 50% identical in a 20-40 aa region occur frequently by chance.
- Distantly related homologs may lack significant similarity. Homologous sequences may have few absolutely conserved residues.
- A homologous to B & B to C ⇒ A homologous to C.
- Low complexity regions, transmembrane regions and coiled-coil regions frequently display significant similarity without homology.
- Greater evolutionary distance implies that length of a local alignment required to achieve a statistically significant score also increases.

Rules of Thumb

- Results of searches using different scoring systems may be compared directly using normalized scores.
- If S is the (raw) score for a local alignment, the **normalized** score S' (in bits) is given by

$$S' = \frac{\lambda - \ln(K)}{\ln(2)}$$

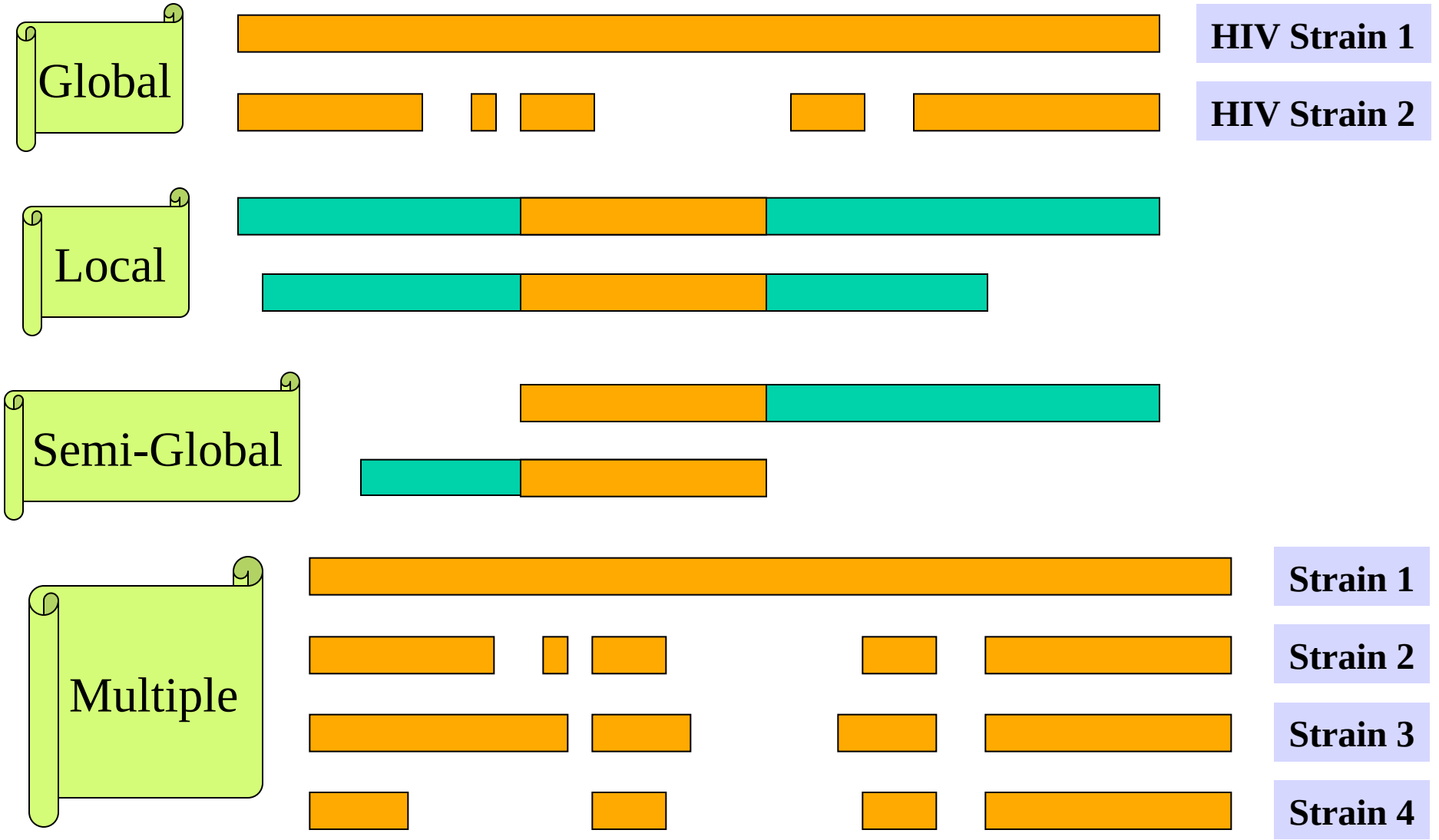
The parameters depend on the scoring system.

- **Statistically significant normalized score**,

$$S' > \log\left(\frac{N}{E}\right)$$

where E-value = E, and N = size of search space.

Types of Sequence Alignments



Global Alignment: An example

V: G A A T T C A G T T A
W: G G A T C G A

		G	A	A	T	T	C	A	G	T	T	A
	0	0	0	0	0	0	0	0	0	0	0	0
G	0											
G	0											
A	0											
T	0											
C	0											
G	0											
A	0											

Given

δ[I, J] = Score of Matching
the I^{th} character of sequence V &
the J^{th} character of sequence W

Compute

S[I, J] = Score of Matching
First I characters of sequence V &
First J characters of sequence W

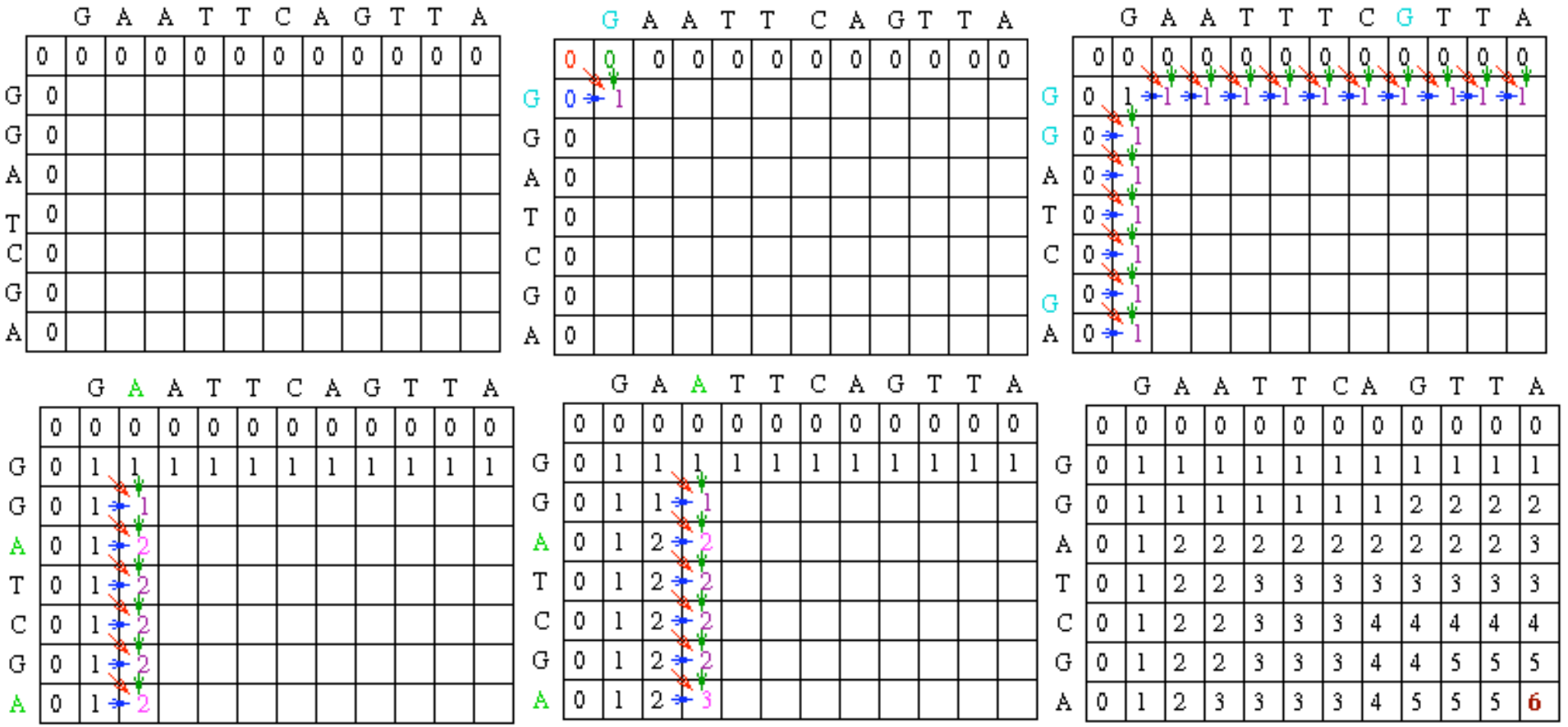
Recurrence Relation

S[I, J] = MAXIMUM {
S[I-1, J-1] + δ(V[I], W[J]),
S[I-1, J] + δ(V[I], —),
S[I, J-1] + δ(— , W[J]) }

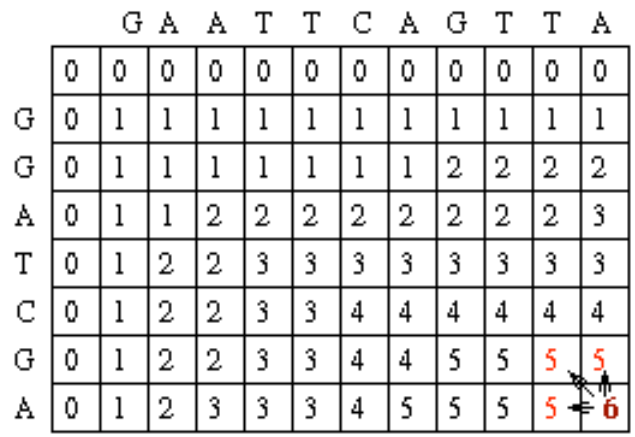
Global Alignment: An example

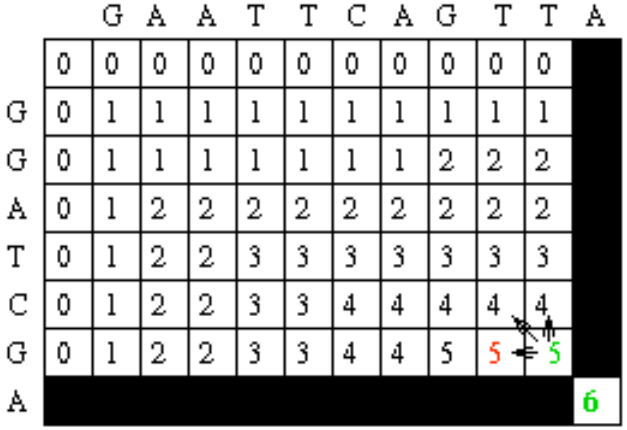
$$S[I, J] = \text{MAXIMUM}\{ S[I-1, J-1] + \delta(V[I], W[J]),\ S[I-1, J] + \delta(V[I], —),\ S[I, J-1] + \delta(— , W[J]) \}$$

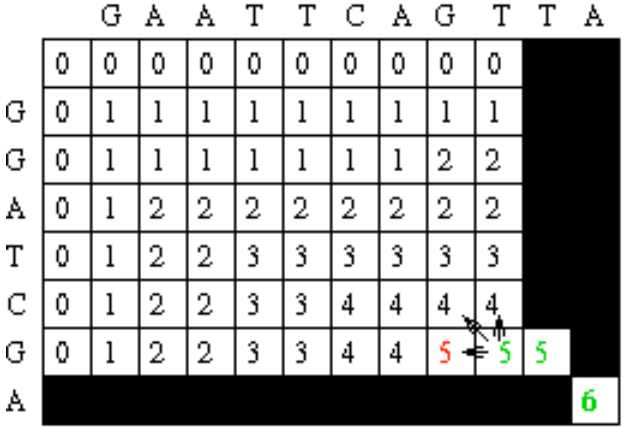
```
V: G A A T T C A G T T A
W: G G A T C G A
```

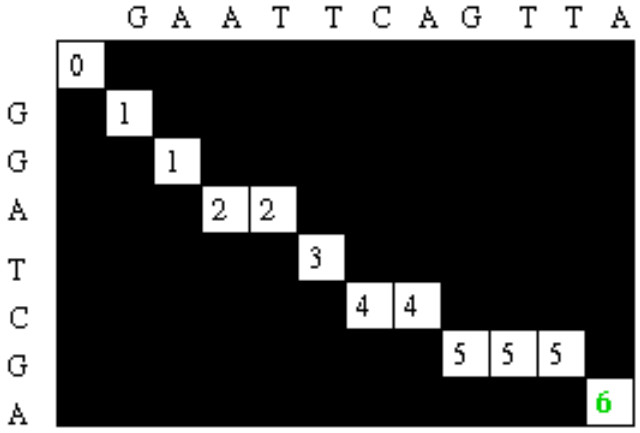


Traceback



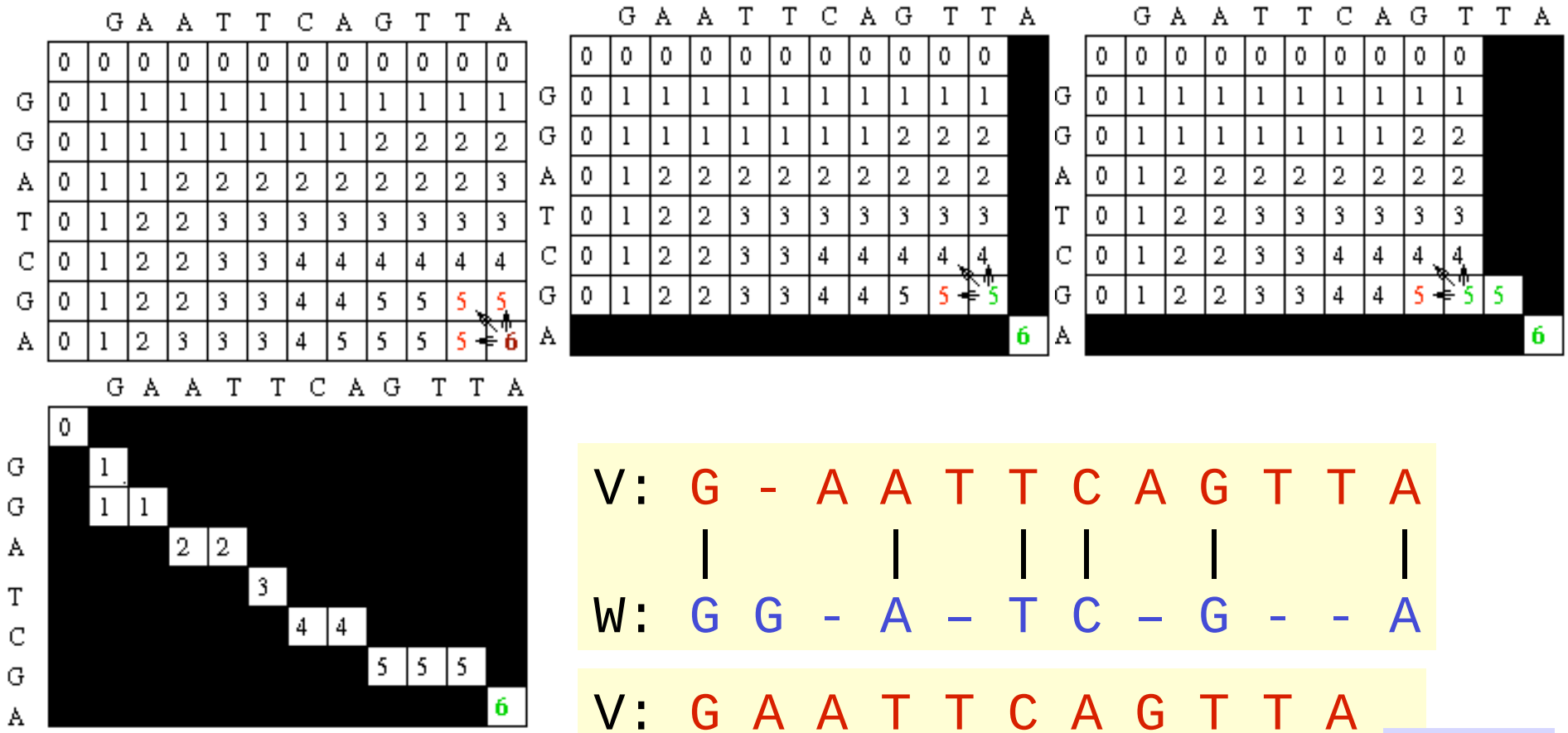







```
V: G A A T T C A G T T A
   |   |   | |   |     |
W: G G A - T C - G - - A
```

Alternative Traceback




```
V: G - A A T T C A G T T A
   |   |   | |   |     |
W: G G - A – T C – G - - A
```

```
V: G A A T T C A G T T A
   |   |   | |   |     |
W: G G A – T C – G - - A
```

Previous

Improved Traceback

		G	A	A	T	T	C	A	G	T	T	A
	0	0	0	0	0	0	0	0	0	0	0	0
G	0	×1	←1	← 1	← 1	← 1	← 1	← 1	×1	← 1	← 1	← 1
G	0	×1	↑1	↑1	↑1	↑1	↑1	↑1	×2	← 2	← 2	← 2
A	0	↑1	↑1	×2	← 2	← 2	← 2	×2	↑2	↑2	↑2	×3
T	0	↑1	← 2	↑2	×3	×3	← 3	← 3	← 3	×3	×3	↑3
C	0	↑1	↑2	↑2	↑3	↑3	×4	← 4	← 4	← 4	← 4	← 4
G	0	↑1	↑2	↑2	↑3	↑3	↑4	↑4	×5	← 5	← 5	← 5
A	0	↑1	↑2	×3	↑3	↑3	↑4	×5	↑5	↑5	↑5	×6

Improved Traceback

		G	A	A	T	T	C	A	G	T	T	A
	0	0	0	0	0	0	0	0	0	0	0	0
G	0	×1	←1	← 1	← 1	← 1	← 1	← 1	×1	← 1	← 1	← 1
G	0	×1	↑1	↑1	↑1	↑1	↑1	↑1	×2	← 2	← 2	← 2
A	0	↑1	↑1	×2	← 2	← 2	← 2	×2	↑2	↑2	↑2	×3
T	0	↑1	← 2	↑2	×3	×3	← 3	← 3	← 3	×3	×3	↑3
C	0	↑1	↑2	↑2	↑3	↑3	×4	← 4	← 4	← 4	← 4	← 4
G	0	↑1	↑2	↑2	↑3	↑3	↑4	↑4	×5	← 5	← 5	← 5
A	0	↑1	↑2	×3	↑3	↑3	↑4	×5	↑5	↑5	↑5	×6

Improved Traceback

		G	A	A	T	T	C	A	G	T	T	A
	0	0	0	0	0	0	0	0	0	0	0	0
G	0	×1	←1	← 1	← 1	← 1	← 1	← 1	×1	← 1	← 1	← 1
G	0	×1	↑1	↑1	↑1	↑1	↑1	↑1	×2	← 2	← 2	← 2
A	0	↑1	↑1	×2	← 2	← 2	← 2	×2	↑2	↑2	↑2	×3
T	0	↑1	← 2	↑2	×3	×3	← 3	← 3	← 3	×3	×3	↑3
C	0	↑1	↑2	↑2	↑3	↑3	×4	← 4	← 4	← 4	← 4	← 4
G	0	↑1	↑2	↑2	↑3	↑3	↑4	↑4	×5	← 5	← 5	← 5
A	0	↑1	↑2	×3	↑3	↑3	↑4	×5	↑5	↑5	↑5	×6

```
V: G A - A T T C A G T T A
   |   |   |   |   |     |
W: G - G A - T C - G - - A
```

Subproblems

- Optimally align V[1..I] and W[1..J] for every possible values of I and J.
- Having optimally aligned
  - V[1..I-1] and W[1..J-1]
  - V[1..I] and W[1..J-1]
  - V[1..I-1] and W[1, J]

  it is possible to optimally align V[1..I] and W[1..J]

- $O(mn)$,
  where m = length of V,
  and n = length of W.

Generalizations of Similarity Function

- Mismatch Penalty = α
- Spaces (Insertions/Deletions, InDels) = β
- Affine Gap Penalties:
  (Gap open, Gap extension) = (γ,δ)
- Weighted Mismatch = $\Phi(a,b)$
- Weighted Matches = $\Omega(a)$

Alternative Scoring Schemes

		G	A	A	T	T	C	A	G	T	T	A
	0	-2	-3	-4	-5	-6	-7	-8	-9	-10	-11	-12
G	-2	× 1	← -1	← -2	← -3	← -4	← -5	← -6	← -7	← -8	← -9	← -10
G	-3	↑-1	× -1	← -3	← -4	← -5	← -6	← -7	× -5	← -7	← -8	← -9
A	-4	↑-2	× 0	× 0	← -2	← -3	← -4	← -5	← -6	← -7	← -8	× -7
T	-5	↑-3	↑ -2	↑-2	× 1	← -1	← -2	← -3	← -4	← -5	← -6	← -7
C	-6	↑-4	↑ -3	↑-3	↑-1	× -1	× 0	← -2	← -3	← -4	← -5	← -6
G	-7	↑-5	↑-4	↑-4	↑-2	↑-3	↑-2	× -2	× -1	← -3	← -4	← -5
A	-8	↑-6	↑-5	↑-5	↑-3	↑-4	↑-3	× -1	↑-3	× -3	× -5	× -3

Match +1
Mismatch –2
Gap (-2, -1)

```
V: G A A T T C A G T T A
   |   | |   |   |     |
W: G G A T - C – G - - A
```

Local Sequence Alignment

- Example: comparing long stretches of anonymous DNA; aligning proteins that share only some motifs or domains.
- Smith-Waterman Algorithm

Recurrence Relations (Global vs Local Alignments)

- S[I, J] = MAXIMUM {
  S[I-1, J-1] + δ(V[I], W[J]),
  S[I-1, J] + δ(V[I], —),
  S[I, J-1] + δ(— , W[J]) }

Global Alignment

- S[I, J] = MAXIMUM { 0,
  S[I-1, J-1] + δ(V[I], W[J]),
  S[I-1, J] + δ(V[I], —),
  S[I, J-1] + δ(— , W[J]) }

Local Alignment

Local Alignment: Example

		G	A	A	T	T	C	A	G	T	T	A
	0	0	0	0	0	0	0	0	0	0	0	0
G	0	× 1	0	0	0	0	0	0	0	0	0	0
G	0	× 1	← 0	0	0	0	0	0	× 1	0	0	0
A	0	0	× 2	× 1	0	0	0	× 1	0	0	0	× 1
T	0	0	↑ 0	× 1	× 2	← 1	0	0	0	× 1	× 1	0
C	0	0	0	0	↑ 0	× 0	× 2	0	0	0	0	0
G	0	0	0	0	0	0	0	0	× 1	0	0	0
A	0	0	× 1	× 1	0	0	0	× 1	0	0	0	× 1

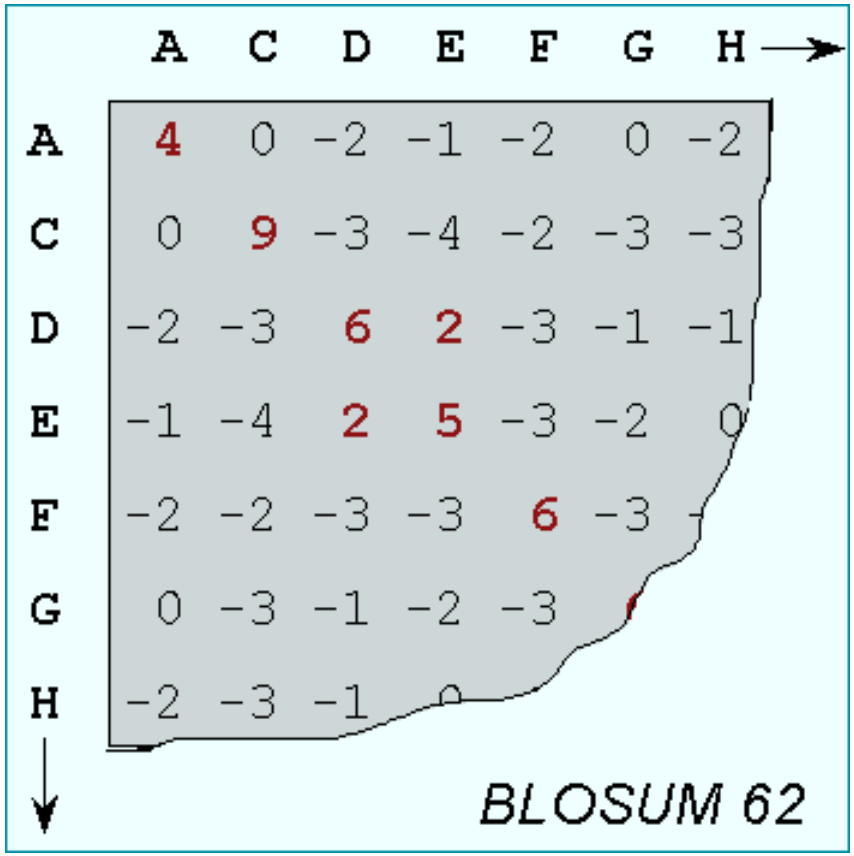
Match +1
Mismatch –1
Gap (-1, -1)

```
V: - G A A T T C A G T T A
     |   | |   |
W: G G - A T - C – G - - A
```

Properties of Smith-Waterman Algorithm

- How to find all regions of "high similarity"?
  - Find all entries above a threshold score and traceback.
- What if: Matches = 1 & Mismatches/spaces = 0?
  - Longest Common Subsequence Problem
- What if: Matches = 1 & Mismatches/spaces = $-\infty$?
  - Longest Common Substring Problem
- What if the average entry is positive?
  - Global Alignment

How to score mismatches?



BLOSUM n Substitution Matrices

- For each amino acid pair a, b
  - For each BLOCK
    - Align all proteins in the BLOCK
    - Eliminate proteins that are more than n% identical
    - Count F(a), F(b), F(a,b)
    - Compute Log-odds Ratio

$$\log\left(\frac{F(a,b)}{F(a)F(b)}\right)$$

Multiple Alignments

- Global
  - ClustalW, ClustalX
  - MSA
  - T-Coffee
- Local
  - BLOCKS
  - eMOTIF
  - GIBBS
  - HMMER
  - MACAW
  - MEME
- Other
  - Profile Analysis from msa (UCSD)
  - SAM HMM (from msa)

Multiple Alignments: CLUSTALW

* identical

: conserved substitutions

. semi-conserved substitutions

```
gi|2213819          CDN-ELKSEAIIEHLCASEFALR------------MKIKEVKKENGDKK 223
gi|12656123         ----ELKSEAIIEHLCASEFALR------------MKIKEVKKENGD-   31
gi|7512442          CKNKNDDDNDIMETLCKNDFALK------------IKVKEITYINRDTK 211
gi|1344282          QDECKFDYVEVYETSSSGAFSLLGRFCGAEPPPHLVSSHHELAVLFRTDH 400
                  : .   : *  . . *:*               . :*:
```

Red: AVFPMLW (Small & hydrophobic)

Blue: DE (Acidic)

Magenta: RHK (Basic)

Green: STYHCNGQ (Hydroxyl, Amine, Basic)

Gray: Others

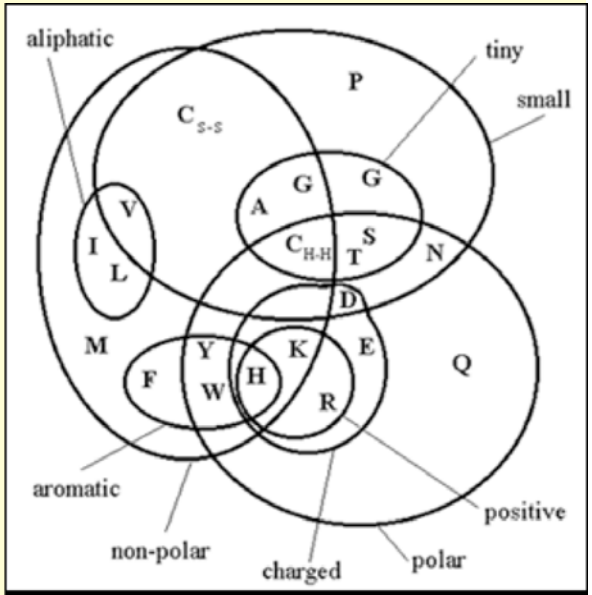


Figure 1. A Venn diagram showing the relationship of the 20 naturally occurring amino acids to a selection of physio-chemical properties thought to be important in the determination of protein structure.

Multiple Alignments

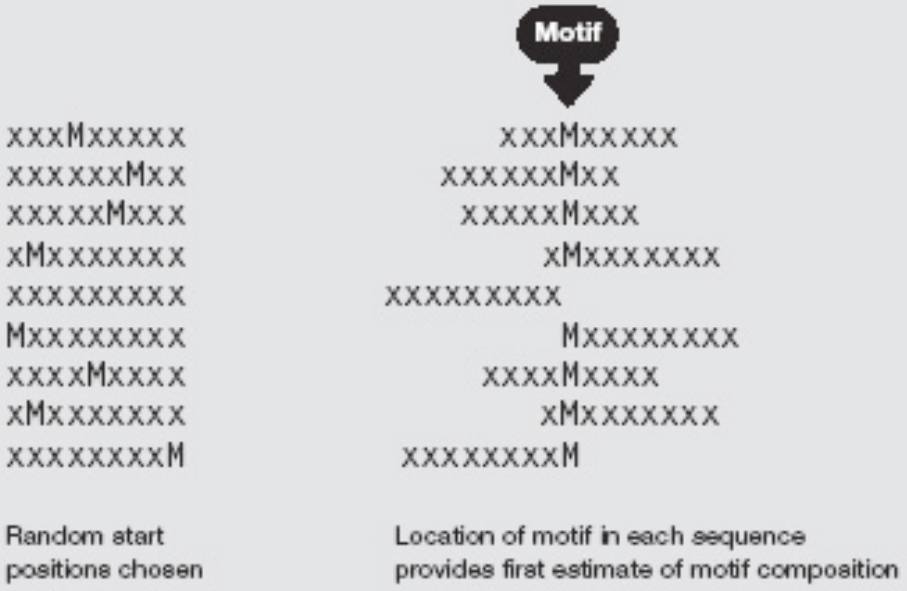
- **Family alignment for the ITAM domain (Immunoreceptor tyrosine-based activation motif)**

```
CD3D_MOUSE/1-2   EQLYQPLRDR EDTQ-YSRLG GN
Q90768/1-21      DQLYQPLGER NDGQ-YSQLA TA
CD3G_SHEEP/1-2   DQLYQPLKER EDDQ-YSHLR KK
P79951/1-21      NDLYQPLGQR SEDT-YSHLN SR
FCEG_CAVPO/1-2   DGIYTGLSTR NQET-YETLK HE
CD3Z_HUMAN/3-0   DGLYQGLSTA TKDT-YDALH MQ
C79A_BOVIN/1-2   ENLYEGLNLD DCSM-YEDIS RG
C79B_MOUSE/1-2   DHTYEGLNID QTAT-YEDIV TL
CD3H_MOUSE/1-2   NQLYNELNLG RREE-YDVLE KK
CD3Z_SHEEP/1-2   NPVYNELNVG RREE-YAVLD RR
CD3E_HUMAN/1-2   NPDYEPIRKG QRDL-YSGLN QR
CD3H_MOUSE/2-0   EGVYNALQKD KMAEAYSEIG TK
Consensus/60%    -.lYpsLspc pcsp.YspLs pp
```

Simple Modular Architecture Research Tool

Multiple Alignment

A. Estimate the amino acid frequencies in the motif columns of all but one sequence. Also obtain background.



How to Score Multiple Alignments?

- Sum of Pairs Score (SP)
  - Optimal alignment: $O(d^N)$ [Dynamic Prog]
  - Approximate Algorithm: Approx Ratio 2
    - Locate Center: $O(d^2N^2)$
    - Locate Consensus: $O(d^2N^2)$

Consensus char: char with min distance sum

Consensus string: string of consensus char

Center: input string with min distance sum

Multiple Alignment Methods

- Phylogenetic Tree Alignment (NP-Complete)
  - Given tree, task is to label leaves with strings
- Iterative Method(s)
  - Build a MST using the distance function
- Clustering Methods
  - Hierarchical Clustering
  - K-Means Clustering

Multiple Alignment Methods (Cont'd)

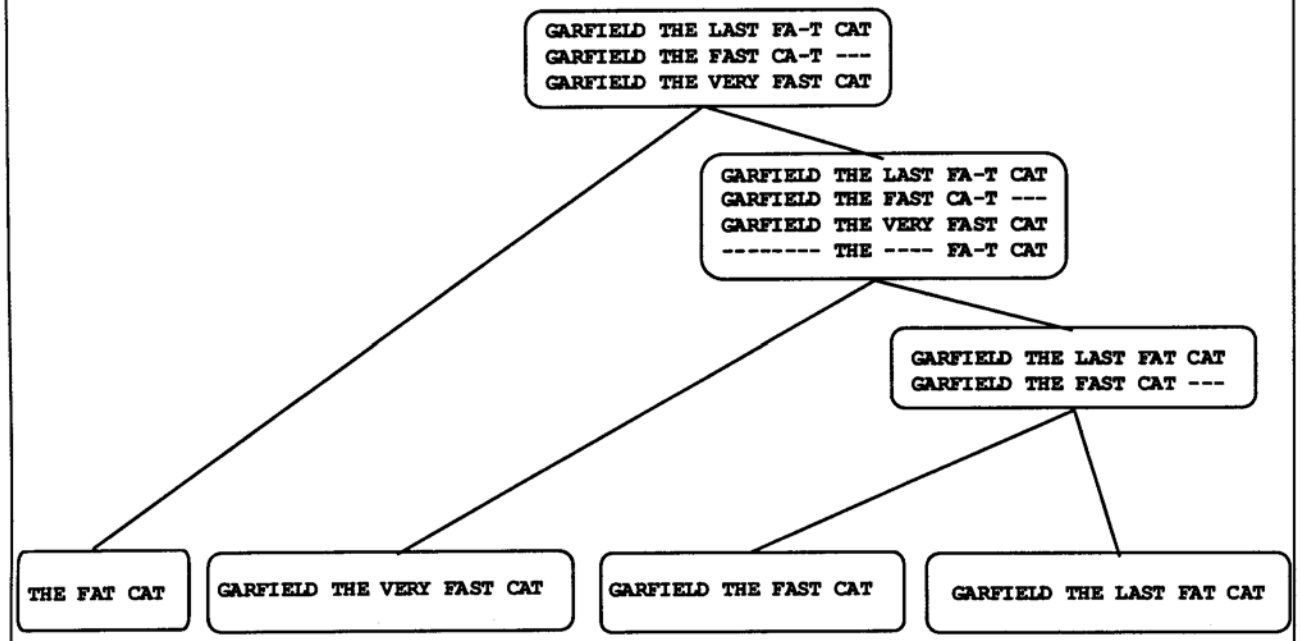
- Gibbs Sampling Method
  - Lawrence, Altschul, Boguski, Liu, Neuwald, Winton, *Science*, 1993
- Hidden Markov Model
  - Krogh, Brown, Mian, Sjolander, Haussler, *JMB*, 1994

Multiple Sequence Alignments (MSA)

- Choice of Scoring Function
  - Global vs local
  - Gap penalties
  - Substitution matrices
  - Incorporating other information
  - Statistical Significance
- Computational Issues
  - Exact/heuristic/approximate algorithms for optimal MSA
  - Progressive/Iterative/DP
  - Iterative: Stochastic/Non-stochastic/Consistency-based
- Evaluating MSAs
  - Choice of good test sets or benchmarks (BAliBASE)
  - How to decide thresholds for good/bad alignments

Progressive MSA: CLUSTALW

Figure 1. Limits of the progressive strategy.



This example shows how a progressive alignment strategy can be misled. In the initial alignment of sequences 1 and 2, ClustalW has a choice between aligning CAT with CAT and making an internal gap or making a mismatch between C and F and having a terminal gap. Since terminal gaps are much cheaper than internals, the ClustalW scoring schemes prefers the former. In the next stage, when the extra sequence is added, it turns out that properly aligning the two CATs in the previous stage would have led to a better scori ng sums-of-pairs multiple alignment.

C. Notredame, *Pharmacogenomics*, **3**(1), 2002.

Software for MSA

Table 1. Some recent and less recent available methods for MSAs.

MSA	Exact	http://www.ibc.wustl.edu/ibc/msa.html	[28]
OMA	Iterative DCA	http://bibiserv.techfak.uni-biefield.de/oma	[61]
MultAlin	Progressive	http://www.toulouse.inra.fr/multalin.html	[41]
ComAlign	Consistency-based	http://www.daimi.au.df/~ ocaprani	[75]
Praline	Iterative/progressive	jhering@nimr.mrc.ac.uk	[48]
Prrp	Iterative/Stochastic	ftp://ftp.genome.ad.jp/pub/genome/saitama-cc/	[47]
HMMER	Iterative/Stochastic/HMM	http://hmmer.wustl.edu/	[68]
GA	Iterative/Stochastic/GA	czhang@watnow.uwaterloo.ca	[52]

C. Notredame, *Pharmacogenomics*, **3**(1), 2002.

MSA: Conclusions

- Very important
  - Phylogenetic analyses
  - Identify members of a family
  - Protein structure prediction
- No perfect methods
- Popular
  - Progressive methods: CLUSTALW
  - Recent interesting ones: Prrp, SAGA, DiAlign, T-Coffee
- Review of Methods [C. Notredame, *Pharmacogenomics,* **3**(1), 2002]
  - CLUSTALW works reasonably well, in general
  - DiAlign is better for sequences with long insertions & deletions (indels)
  - T-Coffee is best available method