# CAP 5510: Introduction to Bioinformatics

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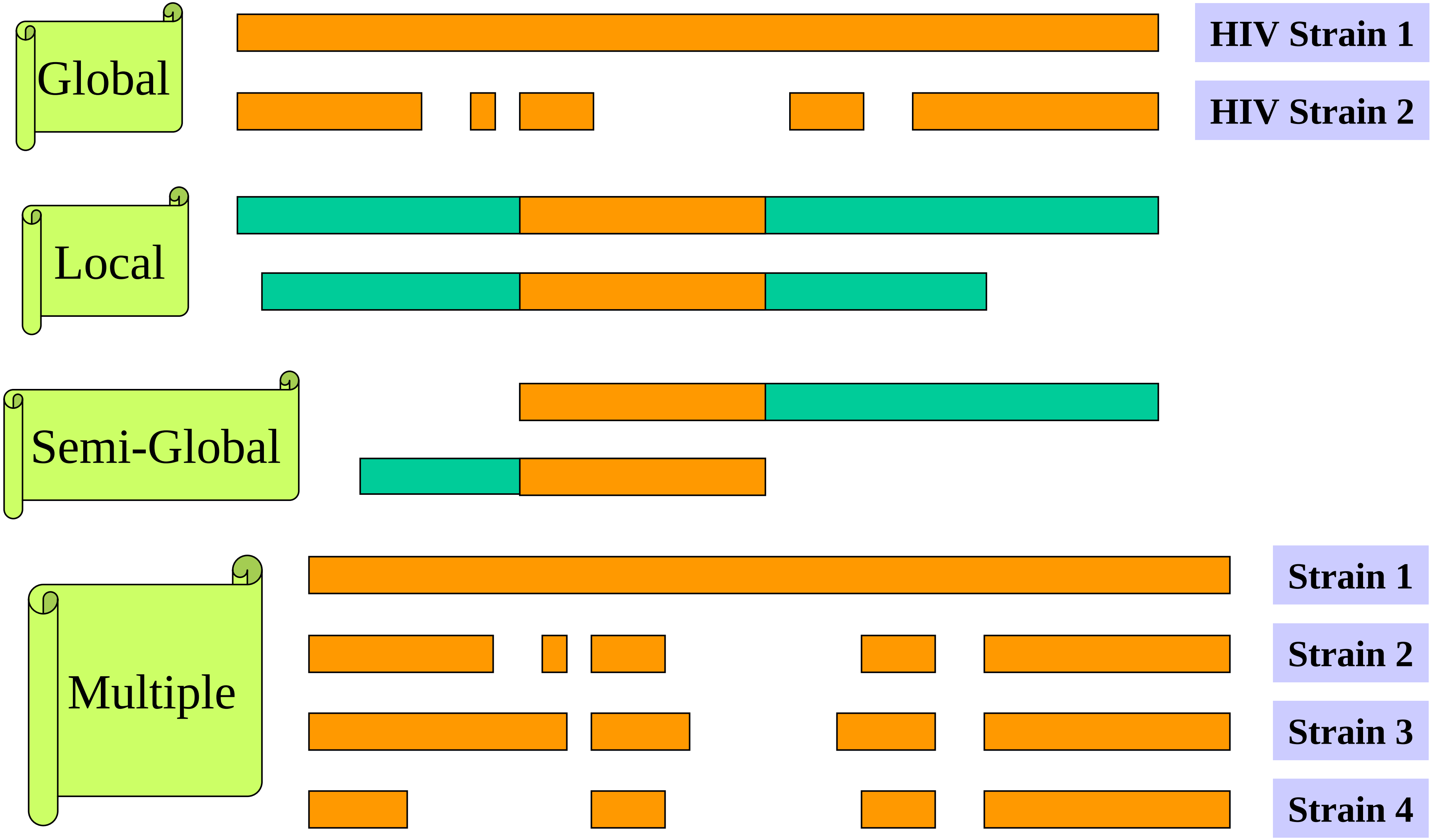
# Types of Sequence Alignments



Global

HIV Strain 1

HIV Strain 2

Local

Semi-Global

Multiple

Strain 1

Strain 2

Strain 3

Strain 4

# 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)

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

Global Alignment

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- $S[I, J] = \text{MAXIMUM} \{ 0,\ S[I-1, J-1] + \delta(V[I], W[J]),\ S[I-1, J] + \delta(V[I], —),\ S[I, J-1] + \delta(— , W[J]) \}$

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 |

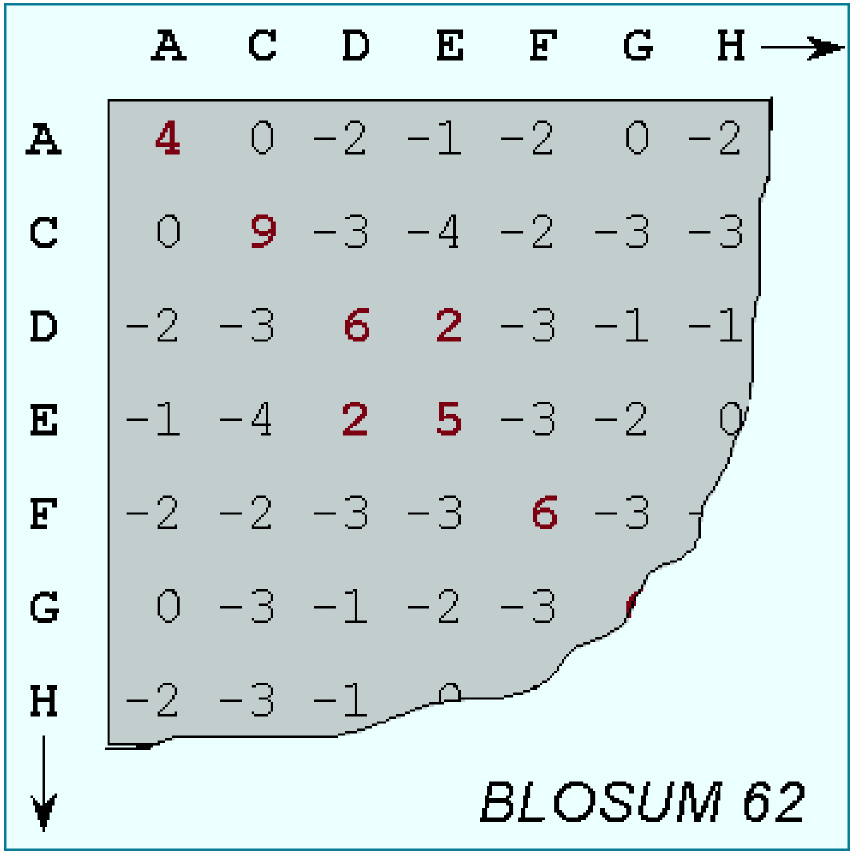
Match +1
Mismatch –1
Gap (-1, -1)

```
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 = -∞?
  - Longest Common Substring Problem
- What if the average entry is positive?
  - Global Alignment

# How to score mismatches?



|   | A | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|
| A | 4 | 0 | -2 | -1 | -2 | 0 | -2 |
| C | 0 | 9 | -3 | -4 | -2 | -3 | -3 |
| D | -2 | -3 | 6 | 2 | -3 | -1 | -1 |
| E | -1 | -4 | 2 | 5 | -3 | -2 | 0 |
| F | -2 | -2 | -3 | -3 | 6 | -3 | - |
| G | 0 | -3 | -1 | -2 | -3 | 6 |  |
| H | -2 | -3 | -1 | 0 |  |  |  |

BLOSUM 62

# 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

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

# 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”

# 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**

# 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.