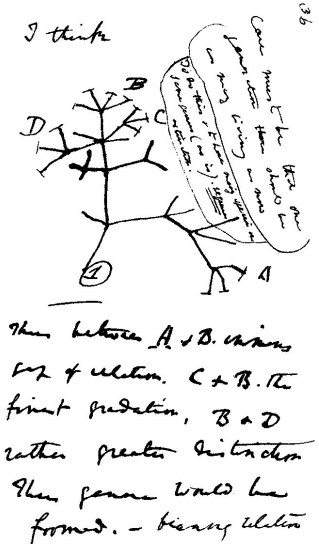
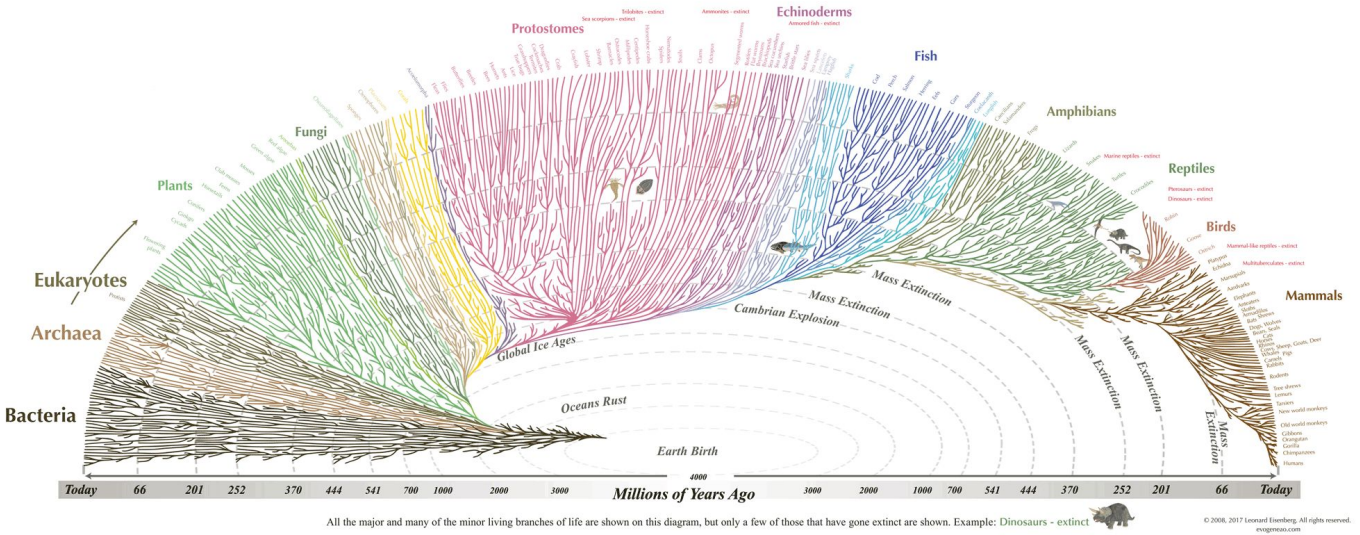
Week 4: Sequence alignment

- Global alignment
  - Dynamic programming
  - Needleman-Wunsch algorithm
- Local alignment
  - Smith-Waterman algorithm
  - BLAST

Sequence evolution





deletion mutation insertion

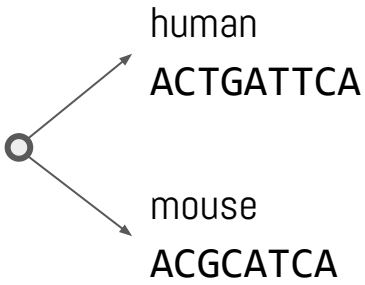
ACATGGTCA → AC*TGGTCA → ACTG**A**TCA → ACTGAT**T**CA

Evolutionary time

human
ACTGATTCA

mouse
ACGCATCA

Sequence alignment



Sequences can be aligned by allowing for **gaps** and **mismatches**.

```
ACTGATTCA
ACGCA-TCA
```

```
ACTGATTCA
AC-GCATCA
```

```
ACTG-ATTCA
AC-GCAT-CA
```

Which alignment is correct?

A scoring scheme:

- Match: 2
- Mismatch: -3
- Gap: -2

We will come back to this!

2+2-3-3+2-2+2+2+2 = 4

2+2-2+2-3-3+2+2+2 = 4

2+2-2+2-2+2+2-2+2+2 = 8

Alignment is gap placement.

How many possible alignments?

Dynamic programming

Solve a given complex problem by:

1. Breaking it into **subproblems** and
2. Storing the results of subproblems to avoid computing the same results again.

Two key properties of a problem that suggest that the given problem can be solved using DP.

1. Overlapping Subproblems
    - Given problem can be recursively broken down into subproblems that can be related to each other. That is, total no. of subproblems is polynomial.
2. Optimal Substructure
    - The optimal solution can be produced by combining optimal solutions of subproblems.

Richard Bellman

Optimal decision processes, involved time series & planning - thus 'dynamic' & 'programming'.

"It's impossible to use the word dynamic in a pejorative sense"; DP was "something not even a Congressman could object to."

Dynamic programming

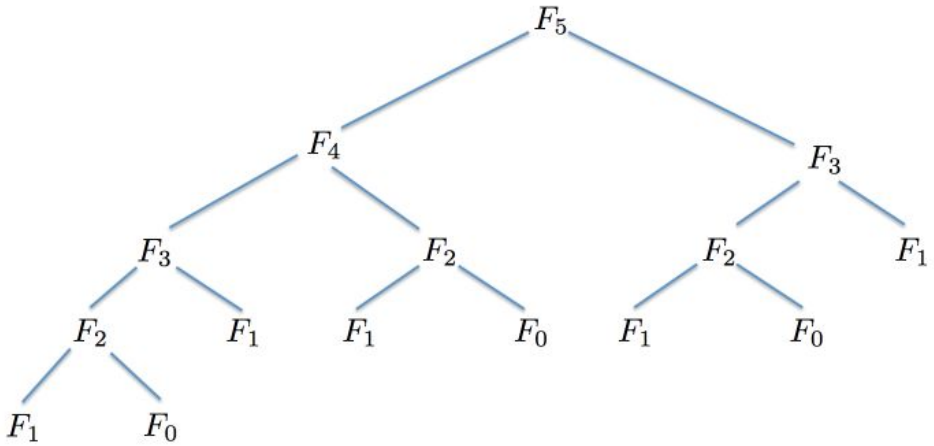
Hemachandra/Fibonacci numbers: 0, 1, 1, 2, 3, 5, 8, 13, 21, 34, 55, 89, 144, ...

$$F_0 := 0;\ F_1 := 1;$$

$$F_n = F_{n-1} + F_{n-2},\ \text{for all } n \geq 2.$$

A trivial algorithm for computing F_n:

```
naive_fib(n):
     if n ≤ 1: return n
     else: return naive_fib(n − 1) +
                  naive_fib(n − 2)
```



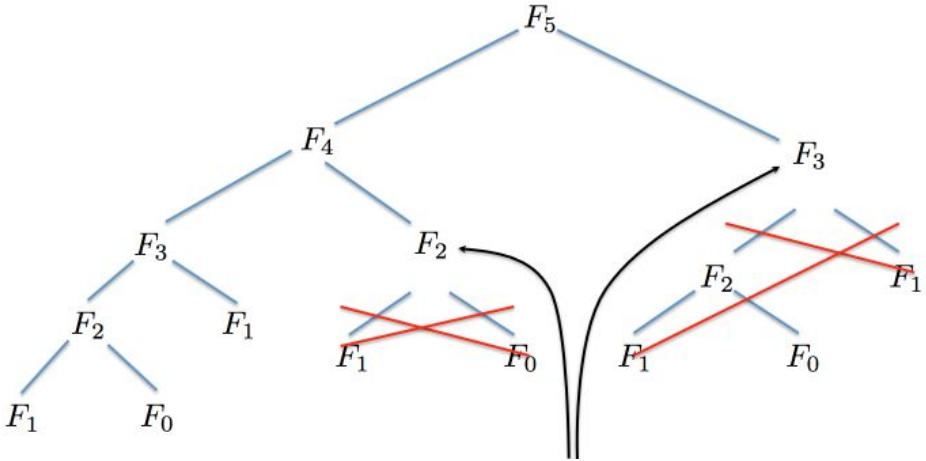
Dynamic programming

Hemachandra/Fibonacci numbers: $F_0 := 0;\ F_1 := 1;\ F_n = F_{n-1} + F_{n-2}$, for all $n \geq 2$.

Never recompute a subproblem $F(k)$, $k \leq n$, if it has been computed before.
Memoization: Remembering previously computed values.

Improved algorithm for computing F_n:

```
memo = { }
fib(n):
     if n in memo: return memo[n]
     else if n = 0: return 0
     else if n = 1: return 1
     else: f = fib(n − 1) + fib(n − 2)
     memo[n] = f
     return f
```



These values are already computed and stored in memo when runtime processes these nodes of the recursion.

Dynamic programming

1. Overlapping Subproblems
2. Optimal Substructure

DP ≈ recursion + memoization (reuse)

- Remember (memoize) previously solved "subproblems"; e.g., in Fibonacci, we memoized the solutions to the subproblems $F_0, F_1, \cdots, F_{n-1}$, while unraveling the recursion.
- If we encounter a subproblem that has already been solved, reuse solution.
- Runtime ≈ (no. of subproblems) * (time per subproblem)

Needleman-Wunsch algorithm

1. Scoring function: substitution matrix & gap penalty
2. Matrix initialization & filling
3. Traceback

Align **GCAT** with **GAT**

Step 1

A scoring scheme:

- Match: 1
- Mismatch: -2
- Gap: -1

	—	G	C	A	T
—					
G					
A					
T					

Needleman-Wunsch algorithm

1. Scoring function: substitution matrix & gap penalty
2. Matrix initialization & filling
3. Traceback

Align **GCAT** with **GAT**

Step 2

$$M(0, j) = j*p$$

$$M(i, 0) = i*p$$

$$M(i, j) = \mathrm{MAX}\begin{cases} M(i-1, j) + p, & \text{top} \\ M(i, j-1) + p, & \text{left} \\ M(i-1, j-1) + S(A_i, B_j) & \text{diagonal} \end{cases}$$

	—	G	C	A	T
—					
G					
A					
T					

Needleman-Wunsch algorithm

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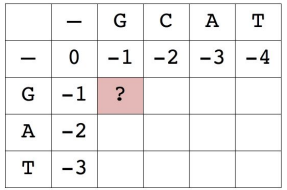
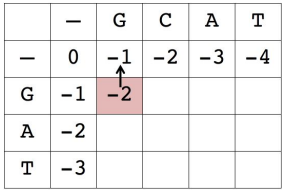
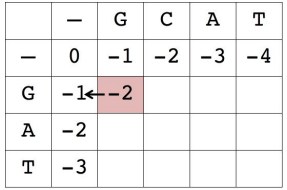
	—	G	C	A	T
—	0	−1	−2	−3	−4
G	−1				
A	−2				
T	−3				

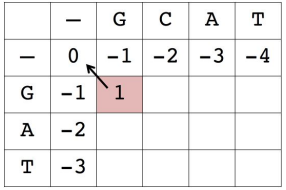
Needleman-Wunsch algorithm

1. Scoring function: substitution matrix & gap penalty
2. Matrix initialization & filling
3. Traceback









Step 2

$$M(0, j) = j*p$$

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$$M(i, j) = \mathrm{MAX}\begin{cases} M(i-1, j) + p, & \text{top} \\ M(i, j-1) + p, & \text{left} \\ M(i-1, j-1) + S(A_i, B_j) & \text{diagonal} \end{cases}$$

	—	G	C	A	T
—	0	-1	-2	-3	-4
G	-1	1			
A	-2				
T	-3				

Needleman-Wunsch algorithm

1. Scoring function: substitution matrix & gap penalty
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Align **GCAT** with **GAT**

Step 2

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	–	G	C	A	T
–	0	-1	-2	-3	-4
G	-1	1	0	-1	-2
A	-2	0	0	1	0
T	-3	-1	-2	0	2

Needleman-Wunsch algorithm

1. Scoring function: substitution matrix & gap penalty
2. Matrix initialization & filling
3. Traceback

Align **GCAT** with **GAT**

```
GCAT
G-AT
```

$$M(0, j) = j*p$$

$$M(i, 0) = i*p$$

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Step 3

	—	G	C	A	T
—	0	−1	−2	−3	−4
G	−1	1	0	−1	−2
A	−2	0	0	1	0
T	−3	−1	−2	0	2

Needleman-Wunsch algorithm

1. Scoring function: substitution matrix & gap penalty
2. Matrix initialization & filling
3. Traceback

Align **ATGCT** with **ATTACA**

$$M(0, j) = j*p$$

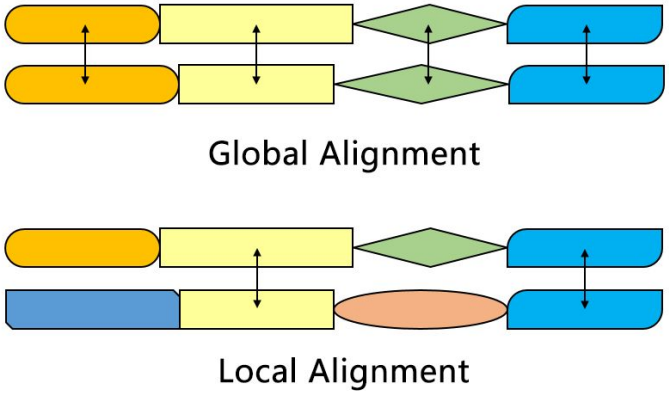
$$M(i, 0) = i*p$$

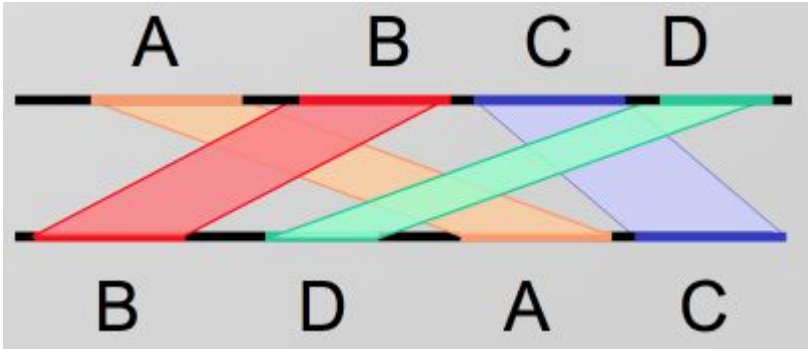
$$M(i, j) = \mathrm{MAX}\begin{cases} M(i-1, j) + p, & \text{top} \\ M(i, j-1) + p, & \text{left} \\ M(i-1, j-1) + S(A_i, B_j) & \text{diagonal} \end{cases}$$

	-	A	T	T	A	C	A
-							
A							
T							
G							
C							
T							

Global & local alignment

A local alignment of strings *s* and *t* is an alignment of a substring of *s* with a substring of *t*.





Smith-Waterman algorithm

Similar to Needleman-Wunsch, with 3 changes:

- First row/column set to 0.
- No negative scores, set to 0.
- Backtrack from cell with highest score, stop at 0.

Align **GCAT** with **GCT**

$$M(0, j) = 0$$

$$M(i, 0) = 0$$

$$M(i, j) = \mathrm{MAX}\begin{cases} 0, & \\ M(i-1, j) + p, & \text{top} \\ M(i, j-1) + p, & \text{left} \\ M(i-1, j-1) + S(A_i, B_j) & \text{diagonal} \end{cases}$$

	–	G	C	A	T
–					
G					
C					
T					

Smith-Waterman algorithm

Similar to Needleman-Wunsch, with 3 changes:

- First row/column set to 0.
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$$M(i, 0) = 0$$

$$M(i, j) = \mathrm{MAX}\begin{cases} 0, \\ M(i-1, j) + p, \\ M(i, j-1) + p, \\ M(i-1, j-1) + S(A_i, B_j) \end{cases}$$

top

left

diagonal

Align **GCAT** with **GCT**

GC
GC

	-	G	C	A	T
-	0	0	0	0	0
G	0	1	0	0	0
C	0	0	2	1	0
T	0	0	1	1	2

Substitution matrix to measure similarity in sequence alignments

Margaret Dayhoff

Applying math & computational techniques to the sequencing of proteins and nucleic acids.

- 1965: First collection of protein seqs.
- Single-letter code for amino acids.
- 1966: 'Evolutionary trees'.
- **1978: First AA similarity-scoring matrix.**
- 1980: Launched the Protein Information Resource, the first online database system that could be accessed by telephone line.

Substitution matrix: A collection of scores for aligning nucleotides or amino acids with one another.

- The scores represent the relative ease with which one nucleotide or amino acid may mutate into or substitute for another.
- Purely statistical, nothing directly to do with structure/biochemistry.

	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
Ala	4																			
Arg	−1	5																		
Asn	−2	0	6																	
Asp	−2	−2	1	6																
Cys	0	−3	−3	−3	9															
Gln	−1	1	0	0	−3	5														
Glu	−1	0	0	2	−4	2	5													
Gly	0	−2	0	−1	−3	−2	−2	6												
His	−2	0	1	−1	−3	0	0	−2	8											
Ile	−1	−3	−3	−3	−1	−3	−3	−4	−3	4										
Leu	−1	−2	−3	−4	−1	−2	−3	−4	−3	2	4									
Lys	−1	2	0	−1	−3	1	1	−2	−1	−3	−2	5								
Met	−1	−1	−2	−3	−1	0	−2	−3	−2	1	2	−1	5							
Phe	−2	−3	−3	−3	−2	−3	−3	−3	−1	0	0	−3	0	6						
Pro	−1	−2	−2	−1	−3	−1	−1	−2	−2	−3	−3	−1	−2	−4	7					
Ser	1	−1	1	0	−1	0	0	0	−1	−2	−2	0	−1	−2	−1	4				
Thr	0	−1	0	−1	−1	−1	−1	−2	−2	−1	−1	−1	−1	−2	−1	1	5			
Trp	−3	−3	−4	−4	−2	−2	−3	−2	−2	−3	−2	−3	−1	1	−4	−3	−2	11		
Tyr	−2	−2	−2	−3	−2	−1	−2	−3	2	−1	−1	−2	−1	3	−3	−2	−2	2	7	
Val	0	−3	−3	−3	−1	−2	−2	−3	−3	3	1	−2	1	−1	−2	−2	0	−3	−1	4

Wikipedia; Eddy (2004)

Substitution matrix to measure similarity in sequence alignments

Substitution matrix: Each scores is a log-odds score equal to the logarithm of the ratio of the likelihoods of two hypotheses: the residues can substitute for one another or not.

$$s(a,b) = \frac{1}{\lambda} \log \frac{p_{ab}}{f_a f_b}$$

- p_{ab}: likelihood of these two residues being correlated because they're homologous.
  - p_{ab} are the target frequencies: the probability that we expect to observe residues *a* and *b* aligned in homologous sequence alignments.
- $f_a f_b$: likelihood of these two residues being uncorrelated and unrelated, occurring independently.
  - f_a and f_b are background frequencies: the probabilities that we expect to observe amino acids *a* and *b* on average in any protein sequence.
- λ: a scaling factor, usually set to something that lets helps round off all the terms in the score matrix to sensible integers.

Eddy (2004)

Substitution matrix to measure similarity in sequence alignments

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$$s(a,b) = \frac{1}{\lambda} \log \frac{p_{ab}}{f_a f_b}$$

- p_{ab}: likelihood of these two residues being correlated because they're homologous.
- $f_a f_b$: likelihood of these two residues being uncorrelated and unrelated, occurring independently.
- λ: a scaling factor

Assuming that each aligned residue pair is statistically independent of the others (biologically dubious, but mathematically convenient):

- The score of an alignment ("**alignment score**") = sum of individual log-odds scores for each aligned residue pair.

Eddy (2004)

Substitution matrix to measure similarity in sequence alignments

	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
Ala	4																			
Arg	−1	5																		
Asn	−2	0	6																	
Asp	−2	−2	1	6																
Cys	0	−3	−3	−3	9															
Gln	−1	1	0	0	−3	5														
Glu	−1	0	0	2	−4	2	5													
Gly	0	−2	0	−1	−3	−2	−2	6												
His	−2	0	1	−1	−3	0	0	−2	8											
Ile	−1	−3	−3	−3	−1	−3	−3	−4	−3	4										
Leu	−1	−2	−3	−4	−1	−2	−3	−4	−3	2	4									
Lys	−1	2	0	−1	−3	1	1	−2	−1	−3	−2	5								
Met	−1	−1	−2	−3	−1	0	−2	−3	−2	1	2	−1	5							
Phe	−2	−3	−3	−3	−2	−3	−3	−3	−1	0	0	−3	0	6						
Pro	−1	−2	−2	−1	−3	−1	−1	−2	−2	−3	−3	−1	−2	−4	7					
Ser	1	−1	1	0	−1	0	0	0	−1	−2	−2	0	−1	−2	−1	4				
Thr	0	−1	0	−1	−1	−1	−1	−2	−2	−1	−1	−1	−1	−2	−1	1	5			
Trp	−3	−3	−4	−4	−2	−2	−3	−2	−2	−3	−2	−3	−1	1	−4	−3	−2	11		
Tyr	−2	−2	−2	−3	−2	−1	−2	−3	2	−1	−1	−2	−1	3	−3	−2	−2	2	7	
Val	0	−3	−3	−3	−1	−2	−2	−3	−3	3	1	−2	1	−1	−2	−2	0	−3	−1	4

BLOSUM (BLOcks SUbstitution Matrix) for protein sequence alignment.

- Scan the BLOCKS database for very conserved regions of protein families (w/o gaps in the alignment).
- Count the relative frequencies of AA and their substitution probabilities.
- Calculate a log-odds score for each of the 210 possible substitution pairs of the 20 standard amino acids.

Henikoff & Henikoff (1992)

Substitution matrix to measure similarity in sequence alignments

	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
Ala	4																			
Arg	−1	5																		
Asn	−2	0	6																	
Asp	−2	−2	1	6																
Cys	0	−3	−3	−3	9															
Gln	−1	1	0	0	−3	5														
Glu	−1	0	0	2	−4	2	5													
Gly	0	−2	0	−1	−3	−2	−2	6												
His	−2	0	1	−1	−3	0	0	−2	8											
Ile	−1	−3	−3	−3	−1	−3	−3	−4	−3	4										
Leu	−1	−2	−3	−4	−1	−2	−3	−4	−3	2	4									
Lys	−1	2	0	−1	−3	1	1	−2	−1	−3	−2	5								
Met	−1	−1	−2	−3	−1	0	−2	−3	−2	1	2	−1	5							
Phe	−2	−3	−3	−3	−2	−3	−3	−3	−1	0	0	−3	0	6						
Pro	−1	−2	−2	−1	−3	−1	−1	−2	−2	−3	−3	−1	−2	−4	7					
Ser	1	−1	1	0	−1	0	0	0	−1	−2	−2	0	−1	−2	−1	4				
Thr	0	−1	0	−1	−1	−1	−1	−2	−2	−1	−1	−1	−1	−2	−1	1	5			
Trp	−3	−3	−4	−4	−2	−2	−3	−2	−2	−3	−2	−3	−1	1	−4	−3	−2	11		
Tyr	−2	−2	−2	−3	−2	−1	−2	−3	2	−1	−1	−2	−1	3	−3	−2	−2	2	7	
Val	0	−3	−3	−3	−1	−2	−2	−3	−3	3	1	−2	1	−1	−2	−2	0	−3	−1	4

BLOSUM (BLOcks SUbstitution Matrix) for protein sequence alignment.

- The rarer the amino acid is, the more surprising it would be to see two of them align together by chance.
- L/L more common than W/W:
  - $p_{LL} = 0.0371$, $p_{WW} = 0.0065$
- W is a much rarer amino acid:
  - $f_L = 0.099$, $f_W = 0.013$.

Check with $\lambda = 0.347$.

Eddy (2004)

Substitution matrix to measure similarity in sequence alignments

	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
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Gly	0	−2	0	−1	−3	−2	−2	6												
His	−2	0	1	−1	−3	0	0	−2	8											
Ile	−1	−3	−3	−3	−1	−3	−3	−4	−3	4										
Leu	−1	−2	−3	−4	−1	−2	−3	−4	−3	2	4									
Lys	−1	2	0	−1	−3	1	1	−2	−1	−3	−2	5								
Met	−1	−1	−2	−3	−1	0	−2	−3	−2	1	2	−1	5							
Phe	−2	−3	−3	−3	−2	−3	−3	−3	−1	0	0	−3	0	6						
Pro	−1	−2	−2	−1	−3	−1	−1	−2	−2	−3	−3	−1	−2	−4	7					
Ser	1	−1	1	0	−1	0	0	0	−1	−2	−2	0	−1	−2	−1	4				
Thr	0	−1	0	−1	−1	−1	−1	−2	−2	−1	−1	−1	−1	−2	−1	1	5			
Trp	−3	−3	−4	−4	−2	−2	−3	−2	−2	−3	−2	−3	−1	1	−4	−3	−2	11		
Tyr	−2	−2	−2	−3	−2	−1	−2	−3	2	−1	−1	−2	−1	3	−3	−2	−2	2	7	
Val	0	−3	−3	−3	−1	−2	−2	−3	−3	3	1	−2	1	−1	−2	−2	0	−3	−1	4

BLOSUM (BLOcks SUbstitution Matrix) for protein sequence alignment.

- A/L pairs are slightly more frequent in homologous alignments than K/E pairs:
  - $p_{AL} = 0.0044$, $p_{KE} = 0.0041$.
- But, A and L are more common amino acids:
  - $p_A = 0.074$, $p_L = 0.099$, $p_K = 0.058$, $p_E = 0.054$.

Check with $\lambda = 0.347$.

Eddy (2004)

Substitution matrix to measure similarity in sequence alignments

	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
Ala	4																			
Arg	−1	5																		
Asn	−2	0	6																	
Asp	−2	−2	1	6																
Cys	0	−3	−3	−3	9															
Gln	−1	1	0	0	−3	5														
Glu	−1	0	0	2	−4	2	5													
Gly	0	−2	0	−1	−3	−2	−2	6												
His	−2	0	1	−1	−3	0	0	−2	8											
Ile	−1	−3	−3	−3	−1	−3	−3	−4	−3	4										
Leu	−1	−2	−3	−4	−1	−2	−3	−4	−3	2	4									
Lys	−1	2	0	−1	−3	1	1	−2	−1	−3	−2	5								
Met	−1	−1	−2	−3	−1	0	−2	−3	−2	1	2	−1	5							
Phe	−2	−3	−3	−3	−2	−3	−3	−3	−1	0	0	−3	0	6						
Pro	−1	−2	−2	−1	−3	−1	−1	−2	−2	−3	−3	−1	−2	−4	7					
Ser	1	−1	1	0	−1	0	0	0	−1	−2	−2	0	−1	−2	−1	4				
Thr	0	−1	0	−1	−1	−1	−1	−2	−2	−1	−1	−1	−1	−2	−1	1	5			
Trp	−3	−3	−4	−4	−2	−2	−3	−2	−2	−3	−2	−3	−1	1	−4	−3	−2	11		
Tyr	−2	−2	−2	−3	−2	−1	−2	−3	2	−1	−1	−2	−1	3	−3	−2	−2	2	7	
Val	0	−3	−3	−3	−1	−2	−2	−3	−3	3	1	−2	1	−1	−2	−2	0	−3	−1	4

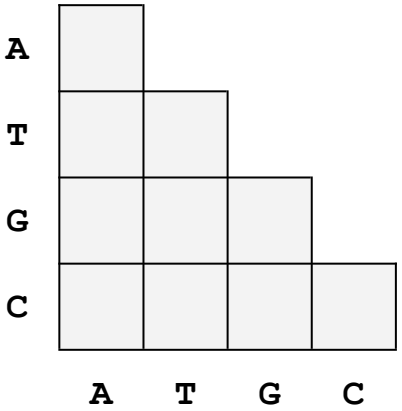
BLOSUMr: the matrix built from blocks with less than **r**% of similarity.

- E.g., BLOSUM62: built using sequences with less than 62% similarity (sequences with ≥ 62% identity were clustered)
  - BLOSUM62 among the best for detecting most weak protein similarities.
  - Default matrix for protein BLAST.

Eddy (2004)

Substitution matrix to measure similarity in sequence alignments

Substitution matrix for DNA



Making-up an arbitrary matrix by fixing the p_{ab} values → directly describes what homologous alignments are expected to look like.

- The resulting score matrix is optimal for detecting alignments that match these target frequencies.

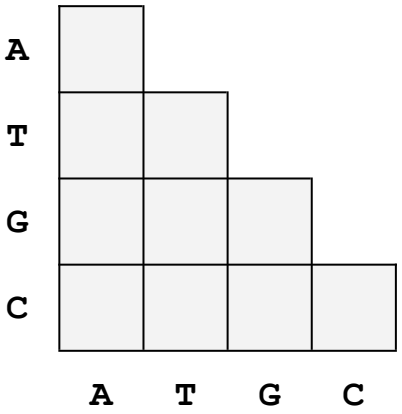
Say, the matrix should be optimized for finding 88% identity alignments.

- Assume that all mismatches are equiprobable, and composition of both alignments and background sequences is uniform at 25% for each nucleotide (f_a, f_b = 0.25 for all a,b). Then,
  - Four identities: p_{aa} = 0.22
  - 12 types of mismatch: p_{ab} = 0.01.
- If we set λ = 1, this gives +1.26 for a match and −1.83 for a mismatch.
- Setting λ = 0.25 and round off: we have a new scoring system of +4/−7.

Eddy (2004)

Substitution matrix to measure similarity in sequence alignments

Substitution matrix for DNA



Given a scoring matrix, we can back calculate target frequencies if two conditions are met:

$$s(a,b) = \frac{1}{\lambda} \log \frac{p_{ab}}{f_a f_b}$$

1. It must have at least one positive score, and
2. The expected score for random sequence alignments must be negative.

True for most score matrices:

- These properties are necessary to make local sequence alignment algorithms like BLAST and Smith-Waterman work.
- Both conditions are met by definition for matrices derived as log-odds scores, except for the useless case of $p_{ab} = f_a f_b$ for all a,b.

Examples:

- FASTA & WU-BLASTN: arbitrary +5/−4 scoring system;
  Optimal for detecting alignments that are 65% identical.
- NCBI BLASTN: +1/−2 scoring system; Optimal for detecting alignments that are 95% identical.

Eddy (2004)

How do we scale this up to search an entire sequence database?

Given a query sequence, and a large set of target sequences (millions),
which target sequences (if any) are related to the query?

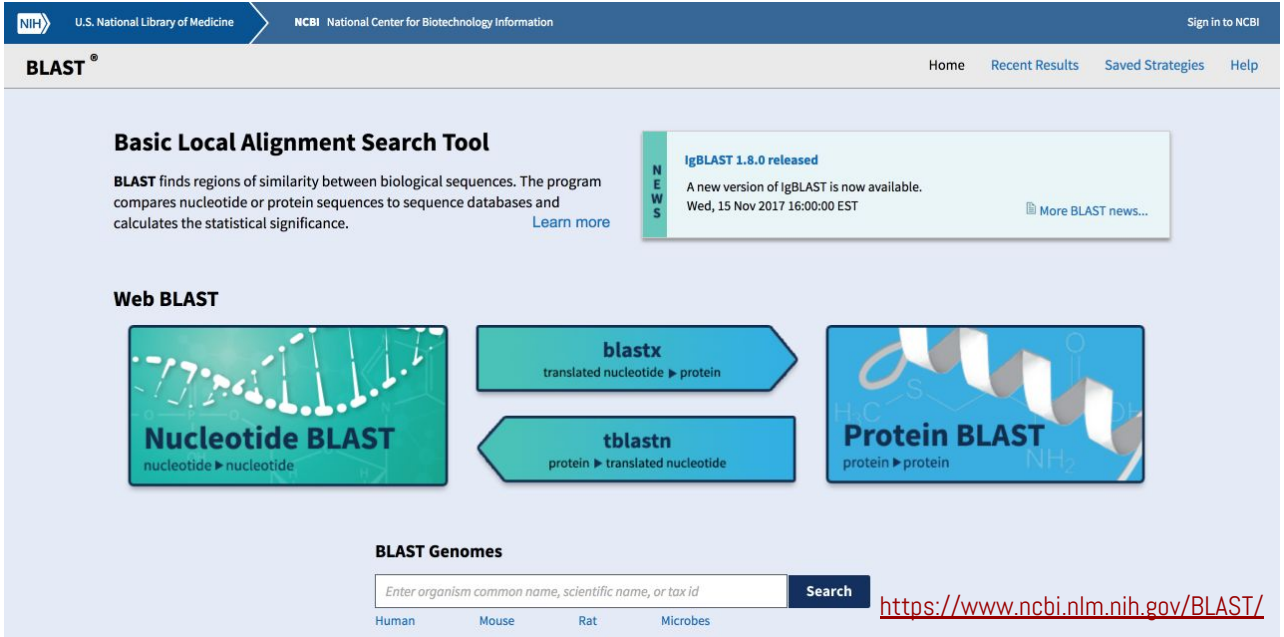
- Individual alignments need not be perfect: Once initial matches are found, they can fine-tune them later.
- Must be very fast.

Exploit the nature of the problem (most sequences will be unrelated to the query):

- If any match with % identity ≤ 90 is going to be rejected, can ignore sequences which don't have a stretch of 10 nucleotides in a row.
- Pre-screen sequences for common long stretches.
- Pre-process the database offline and index k-mers.

BLAST

TITLE	CITED BY	YEAR
Basic local alignment search tool SF Altschul, W Gish, W Miller, EW Myers, DJ Lipman Journal of molecular biology 215 (3), 403-410	136003 *	1990



https://www.ncbi.nlm.nih.gov/BLAST/

Upcoming project deadline: Project topic due on Jan 31

- Discuss with me *and* any other PI; Read recent papers.
- Briefly describe a project idea:
  - Title
  - Project advisor (if someone outside class)
  - 250-word abstract addressing the following 4 Qs:
    - What is the problem?
    - How is it addressed currently & what are the limitations?
    - What is your approach to addressing it & why is likely to be successful?
    - If successful, why does it matter (what is the impact)?
- Submit a PDF.