For written notes on this lecture, please read chapter 10 of *The Practical Bioinformatician*, ond chapter 2 and 5 of *Algorithms in Bioinformatics*.

CS2220: Introduction to Computational Biology
Unit 4: Essence of Sequence Comparison

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Plan

- **Dynamic programming**
- **Protein evolution**
- **String comparison**
- **Sequence alignment**
  - Pairwise alignment
  - Multiple alignment
- **Popular tools**
  - FASTA, BLAST, Pattern Hunter

Dynamic programming

Knapsack problem

- **Each item that can go into the knapsack has a size and a benefit**
- **The knapsack has a certain capacity**
- **What should go into the knapsack to maximize the total benefit?**

Formulation of a solution

Source: http://mat.gsia.cmu.edu/classes/dynamic/node6.html

- **Intuitively, to fill a *w*-pound knapsack, we must start by adding some item. If we add item *j*, we end up with a knapsack *k'* of size $w - w_j$ to fill …**

$$g(w) = \max_j \{ b_j + g(w - w_j) \}$$

where

– w_j and b_j be weight and benefit for item *j*

– $g(w)$ is max benefit that can be gained from a *w*-pound knapsack

Exercise #1

- **Does g(w) produce the optimal benefit? Prove it**

$$g(w) = \max_j \{ b_j + g(w - w_j) \}$$

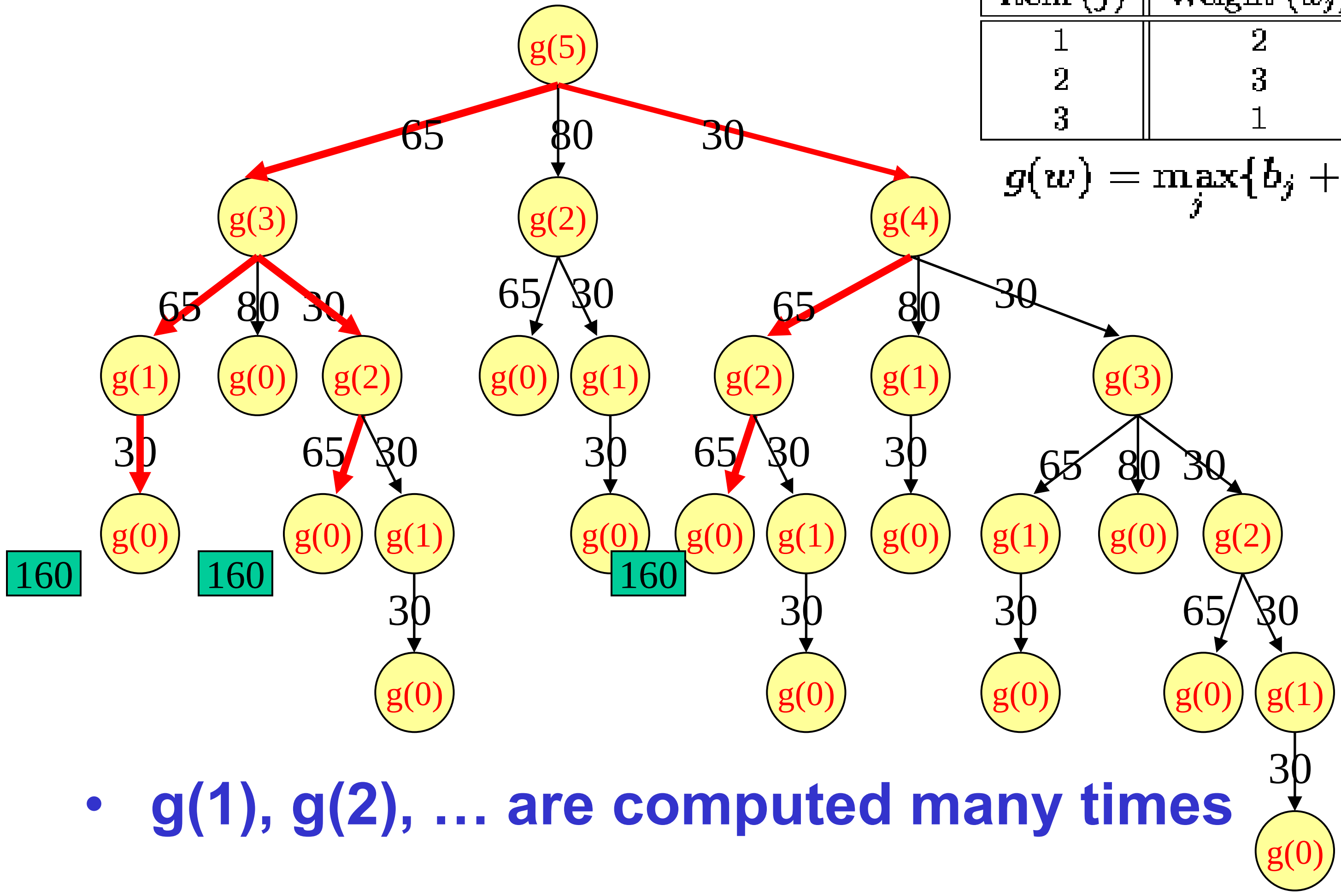
where

- w_j and b_j be weight and benefit for item j
- $g(w)$ is max benefit that can be gained from a w-pound knapsack

Direct recursive evaluation is inefficient

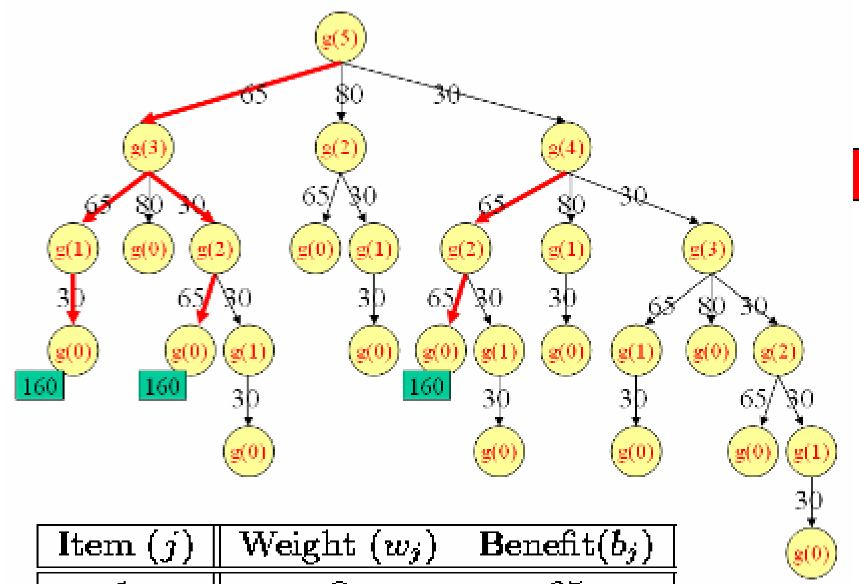
Item (j)	Weight (w_j)	Benefit(b_j)
1	2	65
2	3	80
3	1	30

$$g(w) = \max_j \{b_j + g(w - w_j)\}$$



- **g(1), g(2), … are computed many times**

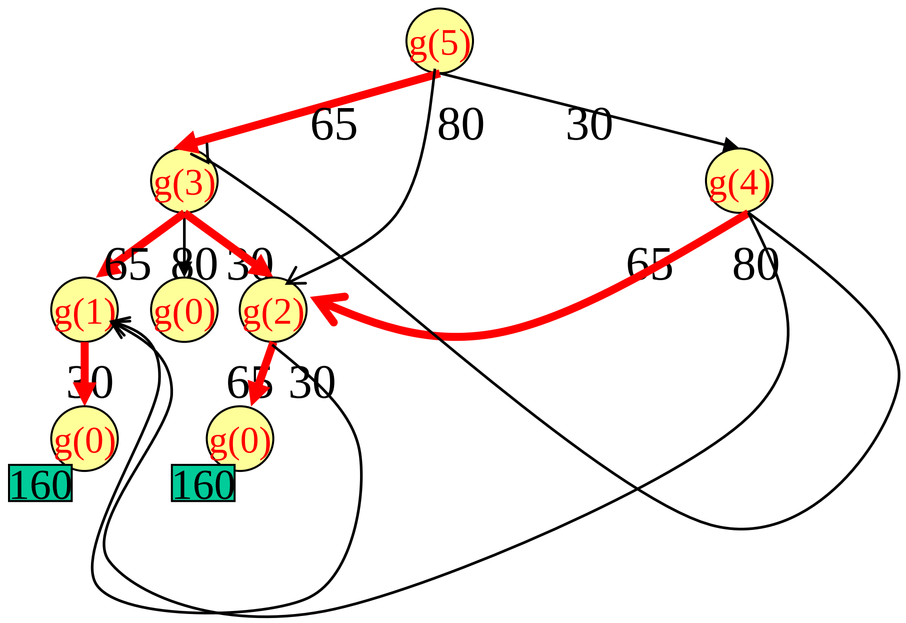
"Memoize" to avoid recomputation



Item (j)	Weight (w_j)	Benefit(b_j)
1	2	65
2	3	80
3	1	30

$$g(w) = \max_j\{b_j + g(w - w_j)\}$$

```
int s[]; s[0] := 0;
g'(w) = if s[w] is defined
    then return s[w];
    else {
        s[w] := max_j{b_j + g'(w – w_j)};
        return s[w]; }
```



Exercise #2

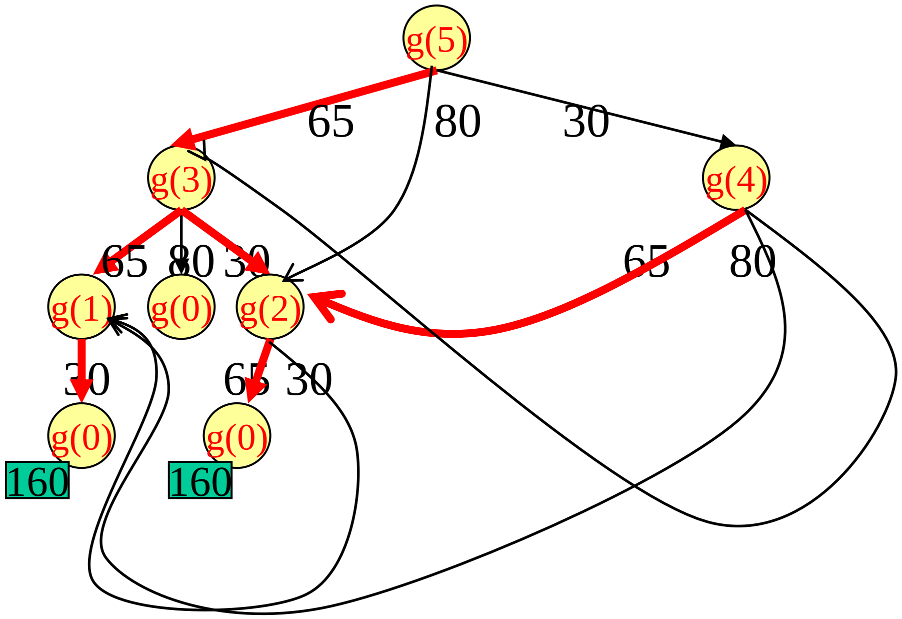
- In what order do s[0], s[1], … get defined?

```
int s[]; s[0] := 0;
g'(w) = if s[w] is defined
            then return s[w];
           else {
                      s[w] := max_j{b_j + g'(w – w_j)};
                       return s[w]; }
```

Remove recursion: Dynamic programming

```
int s[]; s[0] := 0;
g'(w) = if s[w] is defined
    then return s[w];
    else {
        s[w] := max_j{b_j + g'(w – w_j)};
        return s[w]; }
```

```
int s[]; s[0] := 0; s[1] := 30;
s[2] := 65; s[3] = 95;
for i := 4 .. w do
    s[i] := max_j{b_j + s[i – w_j]};
 return s[w];
```



g(0) = 0

g(1) = 30, item 3

g(2) = max{65 + g(0) =65, 30 + g(1) = 60} = 65, item 1

g(3) = max{65 + g(1) = 95, 80 + g(0) = 80, 30 + g(2) = 95} = 95, item 1/3

g(4) = max{65 + g(2) = 130, 80 + g(1) = 110, 30 + g(3) = 125} = 130, item 1

g(5) = max{65 + g(3) = 160, 80 + g(2) = 145, 30 + g(4) = 160} = 160, item 1/3

Protein evolution

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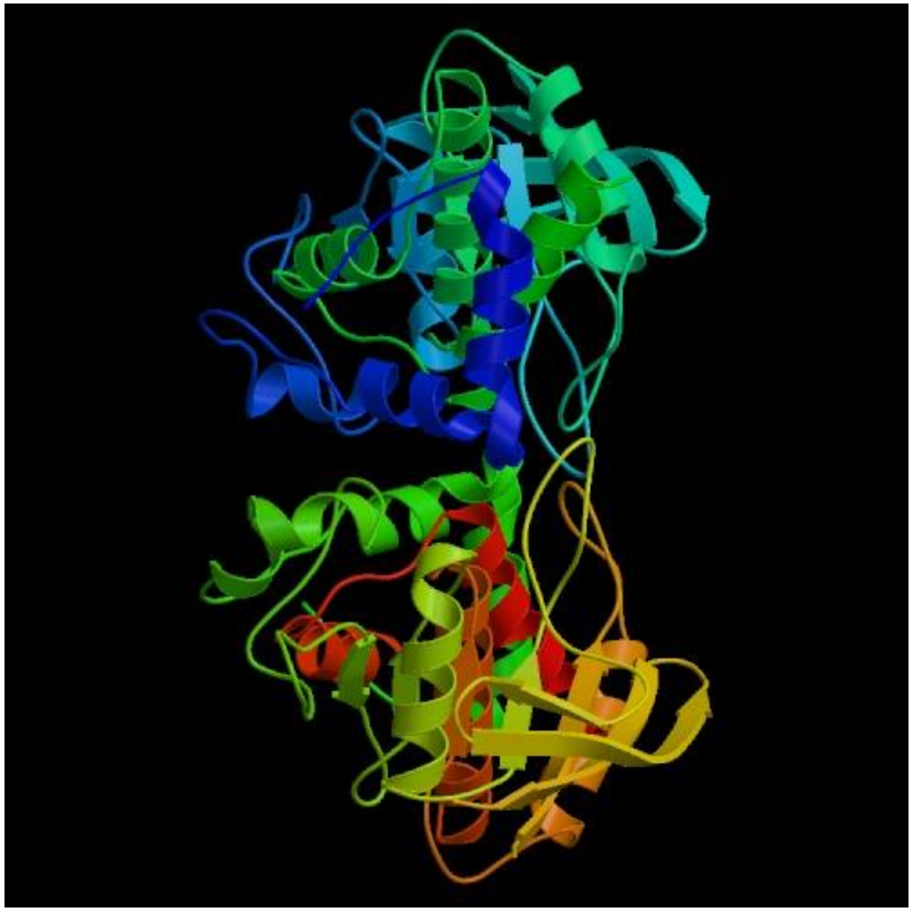
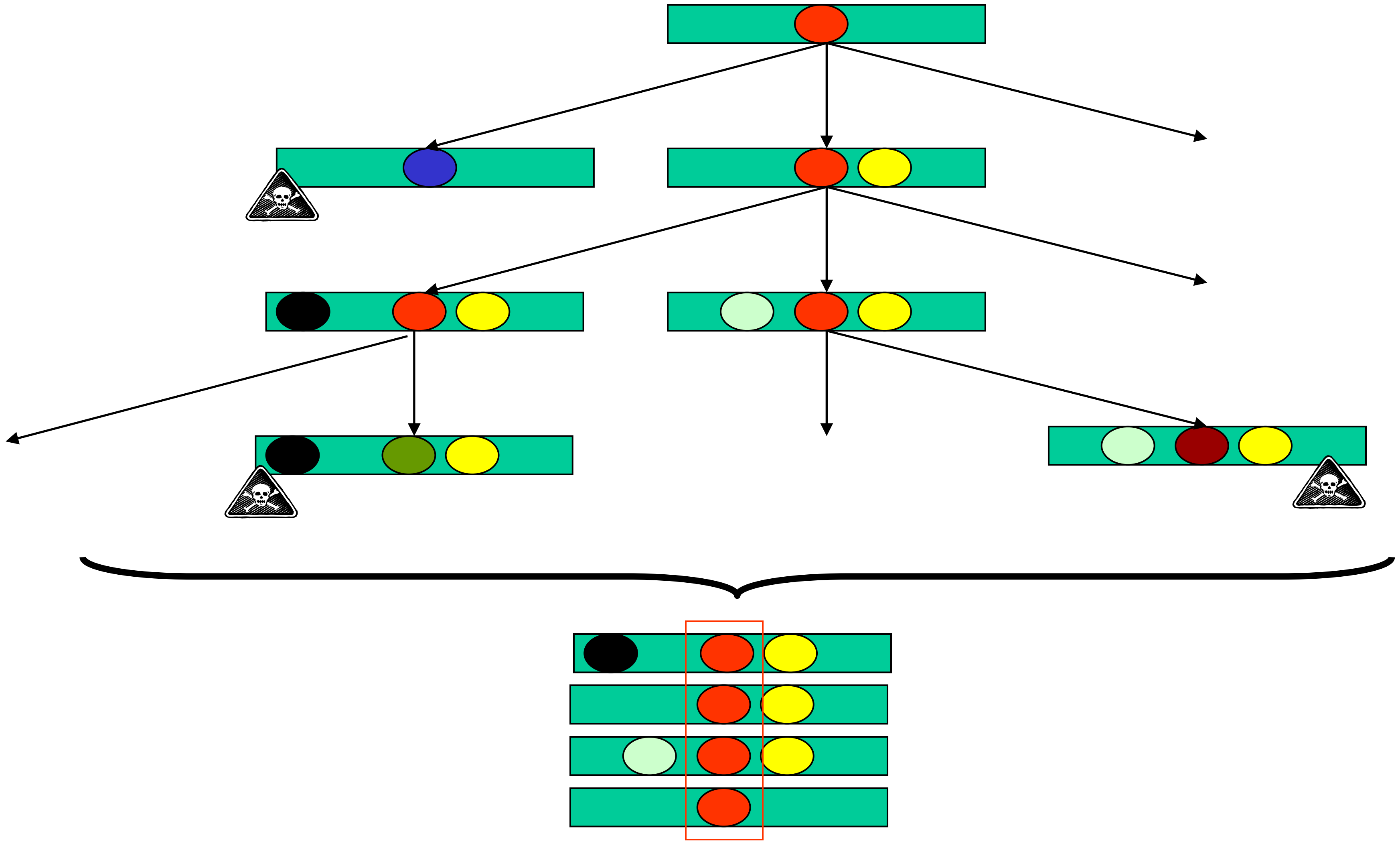
A protein is a ...

- **A protein is a large complex molecule made up of one or more chains of amino acids**
- **Proteins perform a wide variety of activities in the cell**



In the course of evolution…



Exercise #3

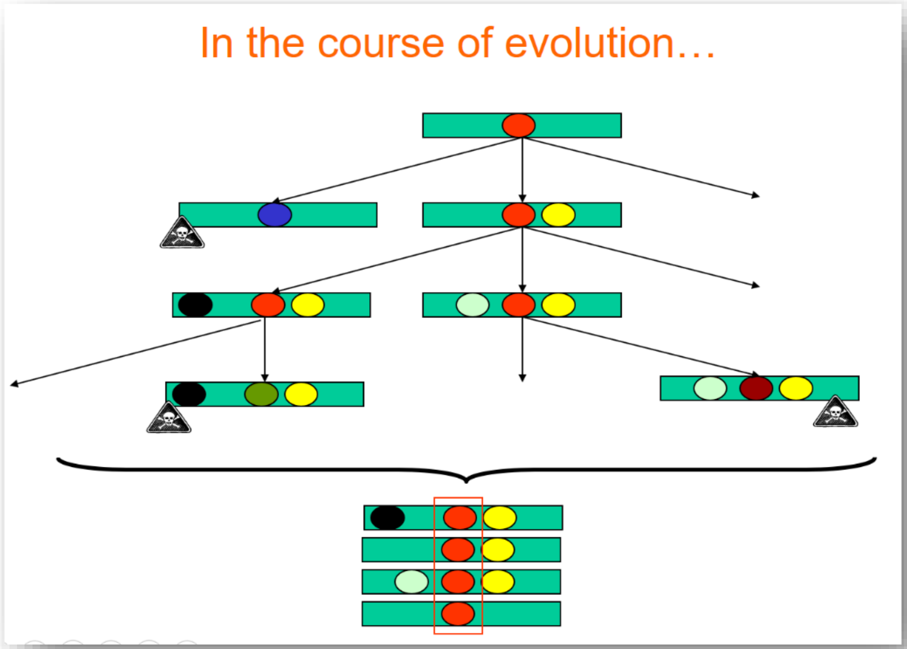
Let a = `AFPHQHRVP`
Let b = `PQVYNIMKE`

Suppose each generation differs from the previous by 1 residue

What is the max difference between the 2nd generation of a?

What is the min difference between the 2nd generation of a and b?

Therefore…


In the course of evolution…

Two proteins inheriting their function from a common ancestor have very similar amino acid sequences

Sequence alignment

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Why we compare sequences

- **The structure of a protein defines its function**
  - In order for a protein to have a specific function, it must satisfy specific structural constraints

"Law"

- **Protein evolves → amino acid seq changes → protein structure changes → breaks those structural constraints → protein loses function**

- **The more similar two proteins' amino acid sequences are, the more likely they come from the same ancestor → the more likely they have the same structure and function**

Abduction

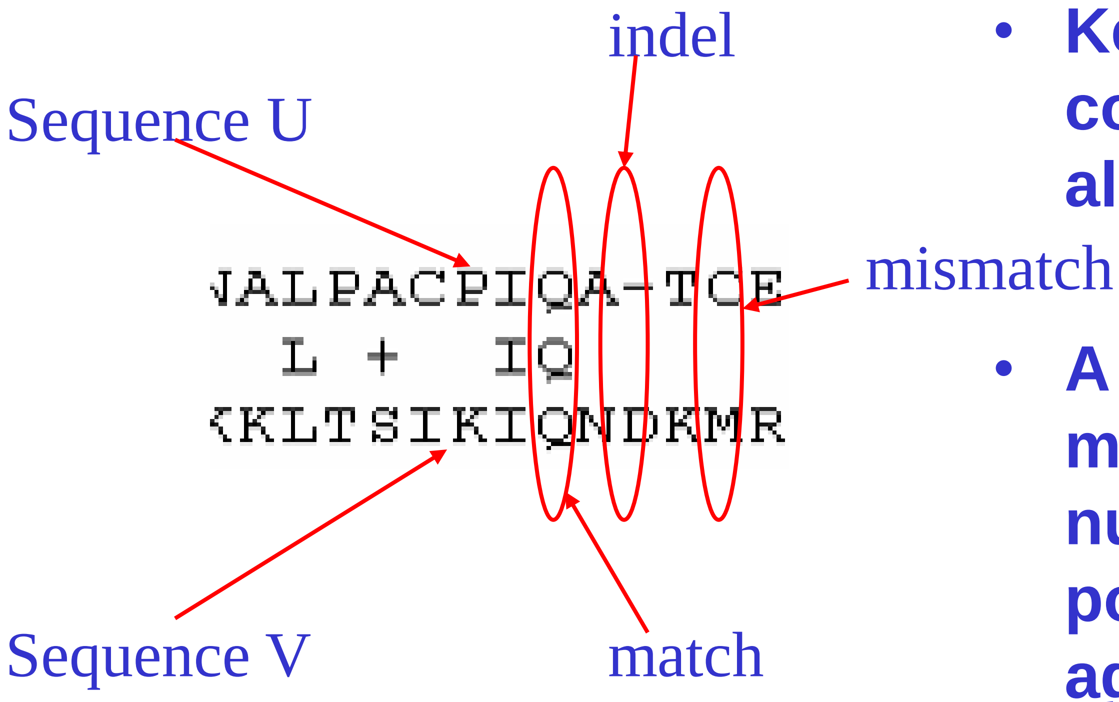
Earliest research in seq comparison

Source: Ken Sung

- **Doolittle et al. (*Science*, July 1983) searched for platelet-derived growth factor (PDGF) in his own DB. He found that PDGF is similar to v-sis oncogene**

```
PDGF-2   1       SLGSLTIAEPAMIAECKTREEVFCICRRL?DR?? 34
p28sis  61 LARGKRSLGSLSVAEPAMIAECKTRTEVFEISRRLIDRTN 100
```

Sequence alignment



- **Key aspect of seq comparison is seq alignment**
- **A seq alignment maximizes the number of positions that are in agreement in two sequences**

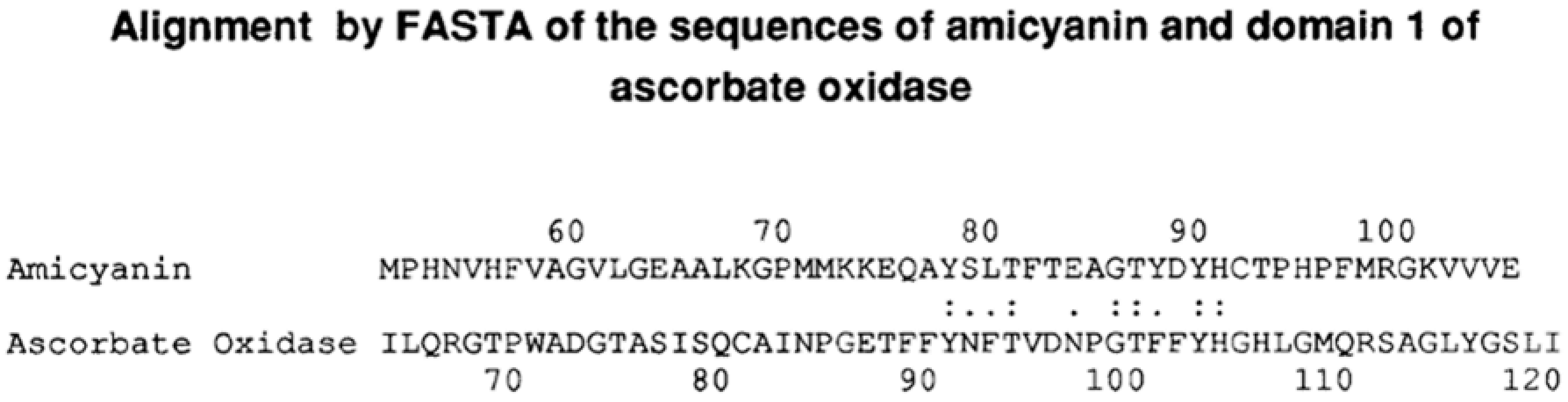
Applications of sequence comparison

- **Infer protein function**
  - When two protein look similar, we conjecture they come from the same ancestor and inherit the ancestor's function (i.e. they are homologous)
- **Find evolution distance between two species**
  - Evolution modifies the DNA of species → Similarity of their genome correlates with their evolutionary distance
- **Help genome assembly**
  - Human genome project reconstructs the whole genome based on overlapping info of a huge amount of short DNA pieces

Poor sequence alignment

- **Poor seq alignment shows few matched positions**

⇒ The two proteins are not likely to be homologous



Alignment by FASTA of the sequences of amicyanin and domain 1 of ascorbate oxidase

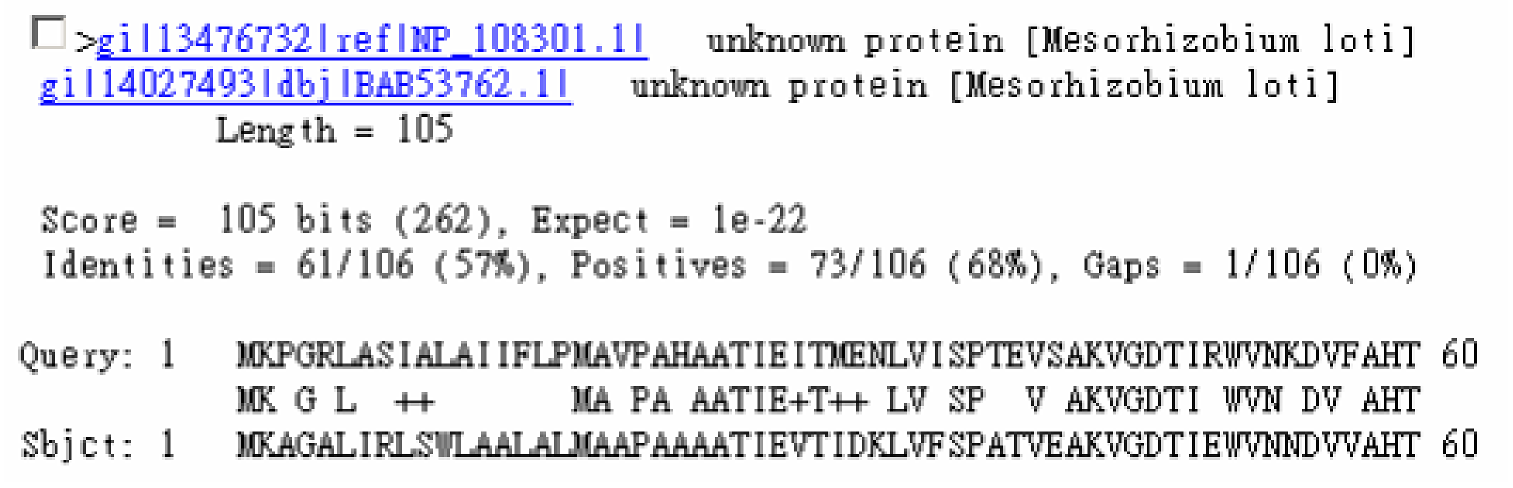
```
                          60        70        80        90       100
Amicyanin         MPHNVHFVAGVLGEAALKGPMMKKEQAYSLTFTEAGTYDYHCTPHPFMRGKVVVE
                                             :..:  . ::. ::
Ascorbate Oxidase ILQRGTPWADGTASISQCAINPGETFFYNFTVDNPGTFFYHGHLGMQRSAGLYGSLI
                       70        80        90       100       110       120
```

No obvious match between
Amicyanin and Ascorbate Oxidase

Good sequence alignment

- **Good alignment usually has clusters of extensive matched positions**

⇒ The two proteins are likely to be homologous



```
☐ >gi|13476732|ref|NP_108301.1|   unknown protein [Mesorhizobium loti]
 gi|14027493|dbj|BAB53762.1|   unknown protein [Mesorhizobium loti]
          Length = 105

 Score =  105 bits (262), Expect = 1e-22
 Identities = 61/106 (57%), Positives = 73/106 (68%), Gaps = 1/106 (0%)

Query: 1    MKPGRLASIALAIIFLPMAVPAHAATIEITMENLVISPTEVSAKVGDTIRWVNKDVFAHT 60
            MK G L  ++       MA PA AATIE+T++ LV SP  V AKVGDTI WVN DV AHT
Sbjct: 1    MKAGALIRLSWLAALALMAAPAAAATIEVTIDKLVFSPATVEAKVGDTIEWVNNDVVAHT 60
```

good match between
Amicyanin and unknown M. loti protein

Alignment: Simple-minded probability & score

Let p, q, r be respectively the probability of a match, a mismatch, and an indel. Then the probability of an alignment $A = (X, Y)$ is

$$prob(A) = p^m \cdot q^n \cdot r^h$$

where

$$\begin{aligned} m &= |\{i \mid x'_i = y'_i \neq -\}| \\ n &= |\{i \mid x'_i \neq y'_i, x'_i \neq -, y'_i \neq -\}| \\ h &= |\{i \mid x'_i = -, y'_i \neq -\} \cup \{i \mid x'_i \neq -, y'_i = -\}| \end{aligned}$$

- **Define score S(A) by simple log likelihood as**
  - S(A) = log(prob(A)) - [m log(s) + h log(s)], with log(p/s) = 1
- **Then S(A) = #matches - μ #mismatches - δ #indels**

Exercise: Derive μ and δ

Global pairwise alignment: Problem definition

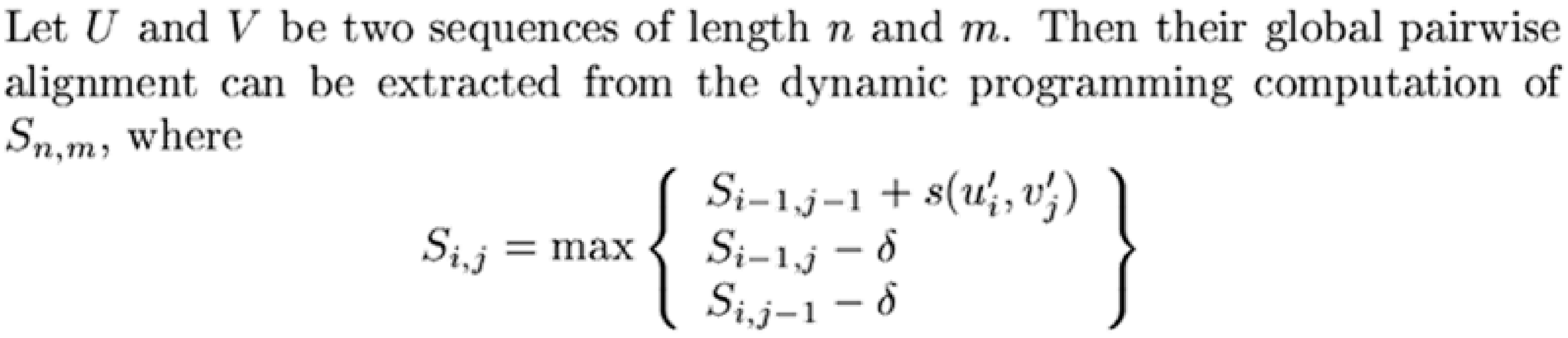
- **The problem of finding a global pairwise alignment is to find an alignment *A* so that *S(A)* is max among exponential number of possible alternatives**

- **Given sequences *U* and *V* of lengths *n* and *m*, then number of possible alignments is given by**
  - $f(n, m) = f(n-1,m) + f(n-1,m-1) + f(n,m-1)$
  - $f(n,n) \sim (1 + \sqrt{2})^{2n+1} n^{-1/2}$

Global pairwise alignment:

Dynamic programming solution

- **Define an indel-similarity matrix *s(.,.)*; e.g.,**
  - *s(x,x) = 2*
  - *s(x,y) = -μ, if x $\neq$ y*
- **Then**



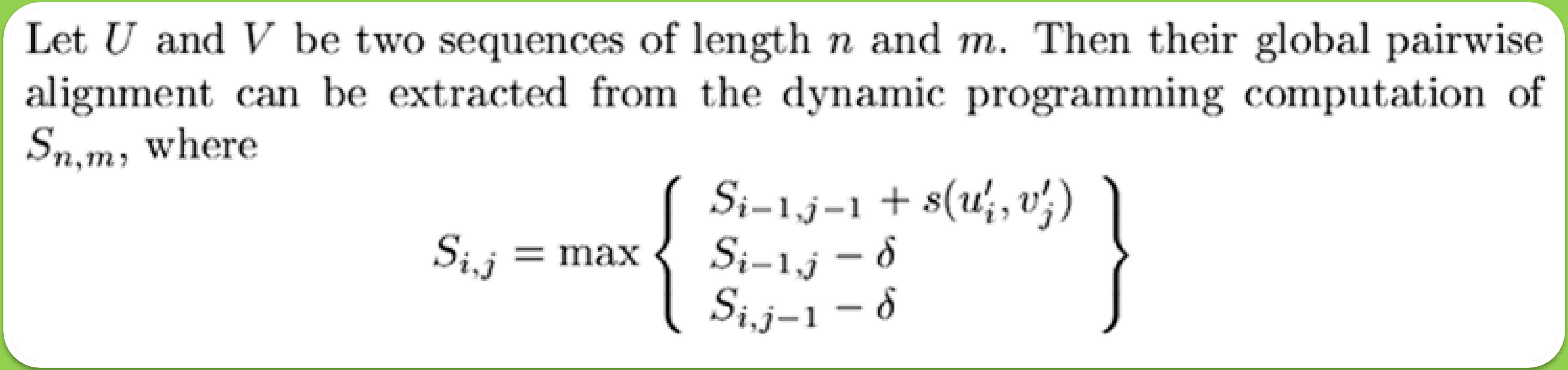
Let U and V be two sequences of length n and m. Then their global pairwise alignment can be extracted from the dynamic programming computation of $S_{n,m}$, where

$$S_{i,j} = \max \left\{ \begin{array}{l} S_{i-1,j-1} + s(u'_i, v'_j) \\ S_{i-1,j} - \delta \\ S_{i,j-1} - \delta \end{array} \right\}$$

This is the basic idea of the Needleman-Wunsch algorithm

Exercise #4

- **What happens when δ is large?**



Let U and V be two sequences of length n and m. Then their global pairwise alignment can be extracted from the dynamic programming computation of $S_{n,m}$, where

$$S_{i,j} = \max \left\{ \begin{array}{l} S_{i-1,j-1} + s(u'_i, v'_j) \\ S_{i-1,j} - \delta \\ S_{i,j-1} - \delta \end{array} \right\}$$

Needleman-Wunsch algorithm (I)

Source: Ken Sung

- **Consider two strings S[1..n] and T[1..m]**
- **Let V(i, j) be score of optimal alignment betw S[1..i] and T[1..j]**

- **Basis:**
  - $V(0, 0) = 0$
  - $V(0, j) = V(0, j-1) - \delta$
    - **Insert j times**
  - $V(i, 0) = V(i-1, 0) - \delta$
    - **Delete i times**

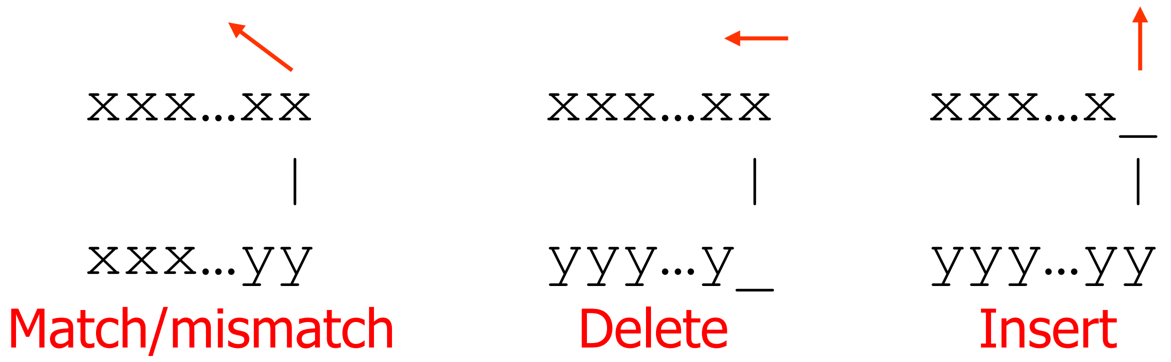
Needleman-Wunsch algorithm (II)

Source: Ken Sung

- **Recurrence: For i>0, j>0**

$$V(i,j) = \max \begin{cases} V(i-1, j-1) + s(S[i], T[j]) & \textbf{Match/mismatch} \\ V(i-1, j) - \delta & \textbf{Delete} \\ V(i, j-1) - \delta & \textbf{Insert} \end{cases}$$

- **In the alignment, the last pair must be either match/mismatch, delete, insert**



Example (I)

Source: Ken Sung

	_	A	G	C	A	T	G	C
_	0	–1	– 2	– 3	– 4	– 5	– 6	– 7
A	– 1							
C	– 2							
A	– 3							
A	– 4							
T	– 5							
C	– 6							
C	– 7							

Example (II)

Source: Ken Sung

	_	A	G	C	A	T	G	C
_	0	–1	– 2	– 3	– 4	– 5	– 6	– 7
A	– 1	2						
C	– 2							
A	– 3							
A	– 4							
T	– 5							
C	– 6							
C	– 7							

$$S_{1,1} = \max\begin{cases} S_{0,0} & + \quad s(A,A) \\ S_{0,1} & - \quad 1 \\ S_{1,0} & - \quad 1 \end{cases} = \max\begin{cases} 0 & + \quad 2 \\ -1 & - \quad 1 = 2 \\ -1 & - \quad 1 \end{cases}$$

Example (III)

Source: Ken Sung

	_	A	G	C	A	T	G	C
_	0	–1	– 2	– 3	– 4	– 5	– 6	– 7
A	– 1	2	1					
C	– 2							
A	– 3							
A	– 4							
T	– 5							
C	– 6							
C	– 7							

$$S_{1,2} = \max\begin{cases} S_{0,1} & + & s(A,G) \\ S_{0,2} & - & 1 \\ S_{1,1} & - & 1 \end{cases} = \max\begin{cases} -1 & + & -1 \\ -2 & - & 1 \\ 2 & - & 1 \end{cases} = 1$$

Example (IV) / Exercise #5

Source: Ken Sung

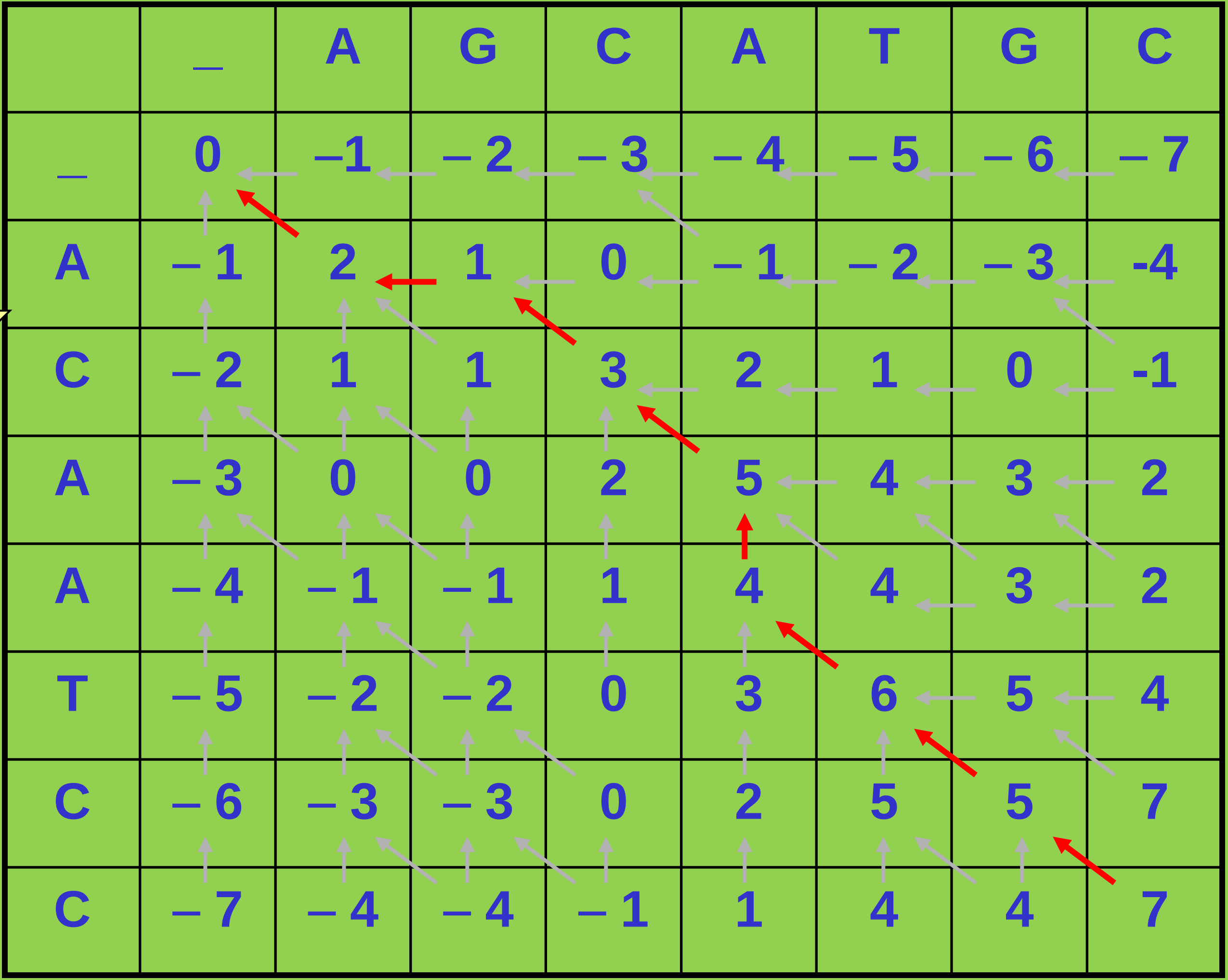
	_	A	G	C	A	T	G	C
_	0	– 1	– 2	– 3	– 4	– 5	– 6	– 7
A	– 1	2	1	0	– 1	– 2	– 3	– 4
C	– 2	1	1	3	2			
A	– 3							
A	– 4							
T	– 5							
C	– 6							
C	– 7							

Can you tell from these entries what are the values of s(A,G), s(A,C), s(A,A), etc.?

Example (V) / Exercise #6

Source: Ken Sung

What is the alignment corresponding to this?



	_	A	G	C	A	T	G	C
_	0	–1	– 2	– 3	– 4	– 5	– 6	– 7
A	– 1	2	1	0	– 1	– 2	– 3	-4
C	– 2	1	1	3	2	1	0	-1
A	– 3	0	0	2	5	4	3	2
A	– 4	– 1	– 1	1	4	4	3	2
T	– 5	– 2	– 2	0	3	6	5	4
C	– 6	– 3	– 3	0	2	5	5	7
C	– 7	– 4	– 4	– 1	1	4	4	7

Pseudo codes

Source: Ken Sung

```
Create the table V[0..n,0..m] and P[1..n,1..m];
V[0,0] = 0;
For j=1 to m, set V[0,j] := v[0,j – 1] – $\delta$ ;
For i=1 to n, set V[i,0] := V[i – 1,0] – $\delta$ ;
For j=1 to m {
   For i = 1 to n {
        set V[i,j] := V[i,j – 1] – $\delta$ ;
        set P[i,j] := (0, – 1);
        if V[i,j] < V[i – 1,j] – $\delta$ then
               set V[i,j] := V[i – 1,j] – $\delta$ ;
               set P[i,j] := (– 1, 0);
        if (V[i,j] < V[i – 1, j – 1] + s(S[i],T[j])) then
               set V[i,j] := V[i – 1, j – 1] + s(S[i],T[j]);
               set P[i,j] := (– 1, – 1);
   }
}
Backtracking P[n,m] to P[0,0] to find optimal alignment;
```

Analysis

Source: Ken Sung

- We need to fill in all entries in the n×m matrix
- Each entry can be computed in O(1) time

⇒ Time complexity = O(nm)

⇒ Space complexity = O(nm)

Exercise: Write down the memoized version of Needleman-Wunsch. What is its time/space complexity?

Problem on speed

Source: Ken Sung

- **Aho, Hirschberg, Ullman 1976**
  - If we can only compare whether two symbols are equal or not, the string alignment problem can be solved in $\Omega(nm)$ time
- **Hirschberg 1978**
  - If symbols are ordered and can be compared, the string alignment problem can be solved in $\Omega(n \log n)$ time
- **Masek and Paterson 1980**
  - Based on Four-Russian's paradigm, the string alignment problem can be solved in O(nm/log2 n) time
- **Let d be the total number of inserts and deletes. Thus $0 \leq d \leq n+m$. If d is smaller than n+m, can we get a better algorithm? Yes!**

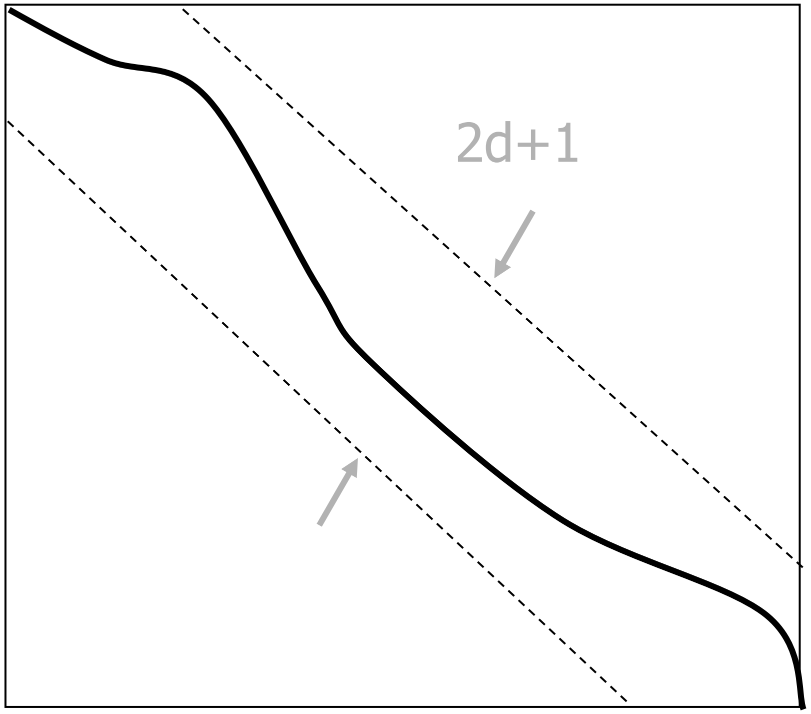
O(dn)-time algorithm

Source: Ken Sung

- **The alignment should be inside the 2d+1 band**

⇒ No need to fill-in the lower and upper triangle

⇒ Time complexity: O(dn)

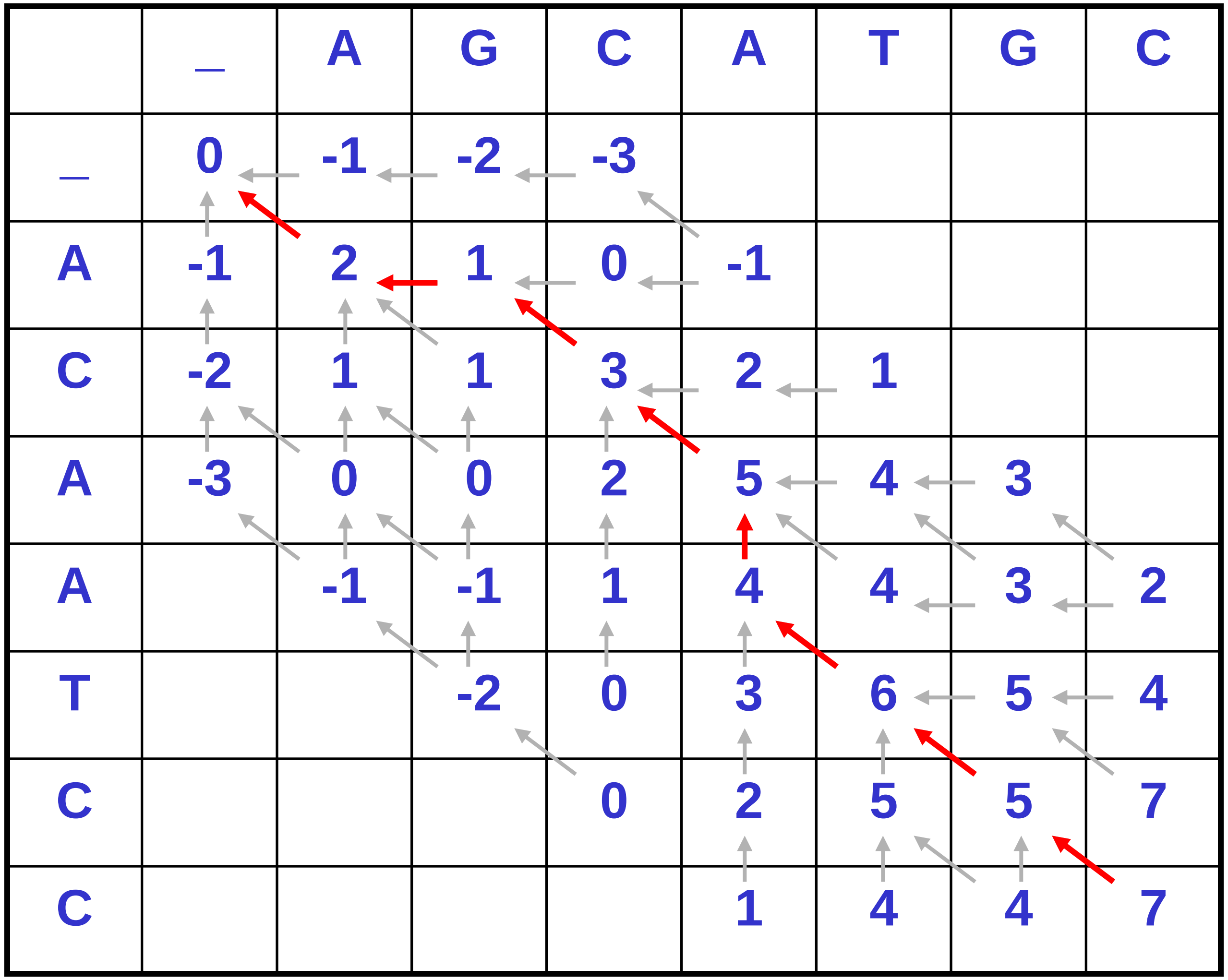


Example

- **d=3**

  A_CAATCC

  AGCA_TGC



	_	A	G	C	A	T	G	C
_	0	-1	-2	-3				
A	-1	2	1	0	-1			
C	-2	1	1	3	2	1		
A	-3	0	0	2	5	4	3	
A		-1	-1	1	4	4	3	2
T			-2	0	3	6	5	4
C				0	2	5	5	7
C					1	4	4	7

Exercise #7 / Recursive equation for O(dn)-time algo

$$v(i, j) = \max \begin{cases} v(i-1, j-1) + s(S[i], S[j]) & \\ v(i-1, j) - \delta, & \text{if } |i-j| < d \\ v(i, j-1) - \delta, & \text{if } |i-j| < d \end{cases}$$

Write down the base cases, the memoized version, and the non-recursive version.

Problem on space

- **Dynamic programming requires O(mn) space**
- **When we compare two very long sequences, space may be the limiting factor**
- **Can we solve the string alignment problem in linear space?**

Easy, if no need to recover alignment

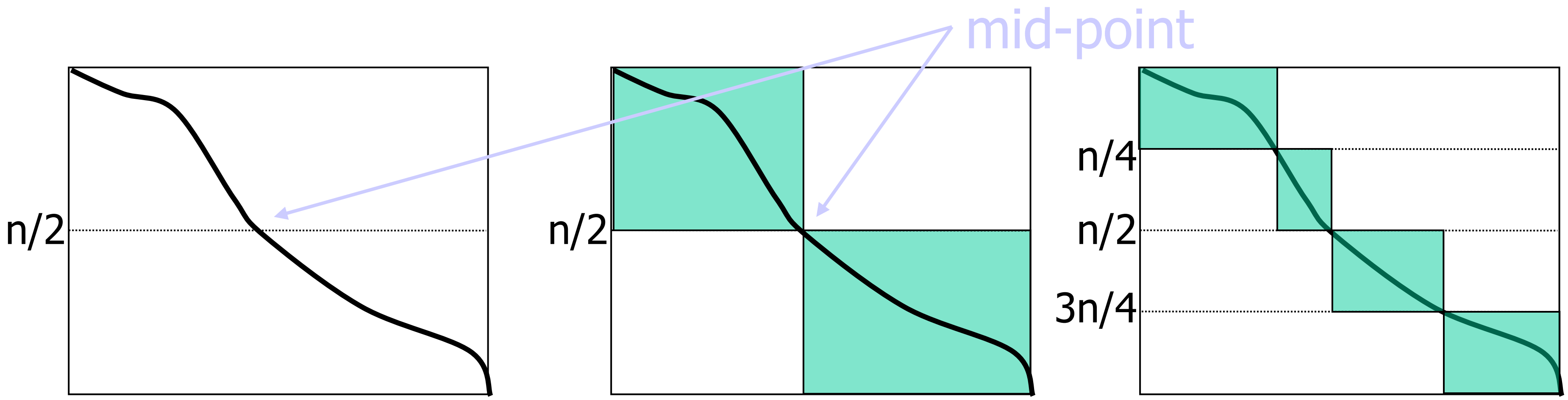
- **When filling row 4, it depends only on row 3**
  - No need to keep rows 1 and 2
- **I.e., we only need to keep two rows**

	_	A	G	C	A	T	G	C
_	0	-1	-2	-3	-4	-5	-6	-7
A	-1	2	1	0	-1	-2	-3	-4
C	-2	1	1	3	2	1	0	-1
A	-3	0	0	2	5	4	3	2
A	-4	-1	-1	1	4	4	3	2
T	-5	-2	-2	0	3	6	5	4
C	-6	-3	-3	0	2	5	5	7
C	-7	-4	-4	-1	1	4	4	7

⇒“Cost only” algo

Recovering alignment in O(n+m) space

- Use cost-only algo to find mid-point of alignment
- Divide the problem into two halves
- Recursively deduce alignments for the two halves



How to find mid-point

$$V(S[1..n],T[1..m]) =$$
$$\max_{0 \le j \le m} \left\{ V(S[1..\tfrac{n}{2}],T[1..j]) + V(S[\tfrac{n}{2}+1..n],T[j+1..m]) \right\}$$

- **Do cost-only dynamic programming for 1st half**
  - I.e., find V(S[1..n/2], T[1..j]) for all j

- **Do cost-only dynamic programming for 2nd half**
  - i.e., find V(S[n/2+1..n], T[j+1..m]) for all j

- **Determine j which maximizes the sum above**

Example

Step 1

	_	A	G	C	A	T	G	C	_
_	0	-1	-2	-3	-4	-5	-6	-7	
A	-1	2	1	0	-1	-2	-3	-4	
C	-2	1	1	3	2	1	0	-1	
A	-3	0	0	2	5	4	3	2	
A	-4	-1	-1	1	4	4	3	2	
T									
C									
C									
_									

Step 2

	_	A	G	C	A	T	G	C	_
_									
A									
C									
A									
A	-4	-1	-1	1	4	4	3	2	
T		-1	0	1	2	3	0	0	-3
C		-2	-1	1	-1	0	1	1	-2
C		-4	-3	-2	-1	0	1	2	-1
_		-7	-6	-5	-4	-3	-2	-1	0

Step 3

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

Step 4: Recursive on subproblems

	_	A	G	C	A	T	G	C	_
_	🟩	🟩	🟩	🟩	🟩				
A	🟩	🟩	🟩	🟩	🟩				
C	🟩	🟩	🟩	🟩	🟩				
A	🟩	🟩	🟩	🟩	🟩				
A	🟩	🟩	🟩	🟩	🟩				
T						🟩	🟩	🟩	🟩
C						🟩	🟩	🟩	🟩
C						🟩	🟩	🟩	🟩
_						🟩	🟩	🟩	🟩

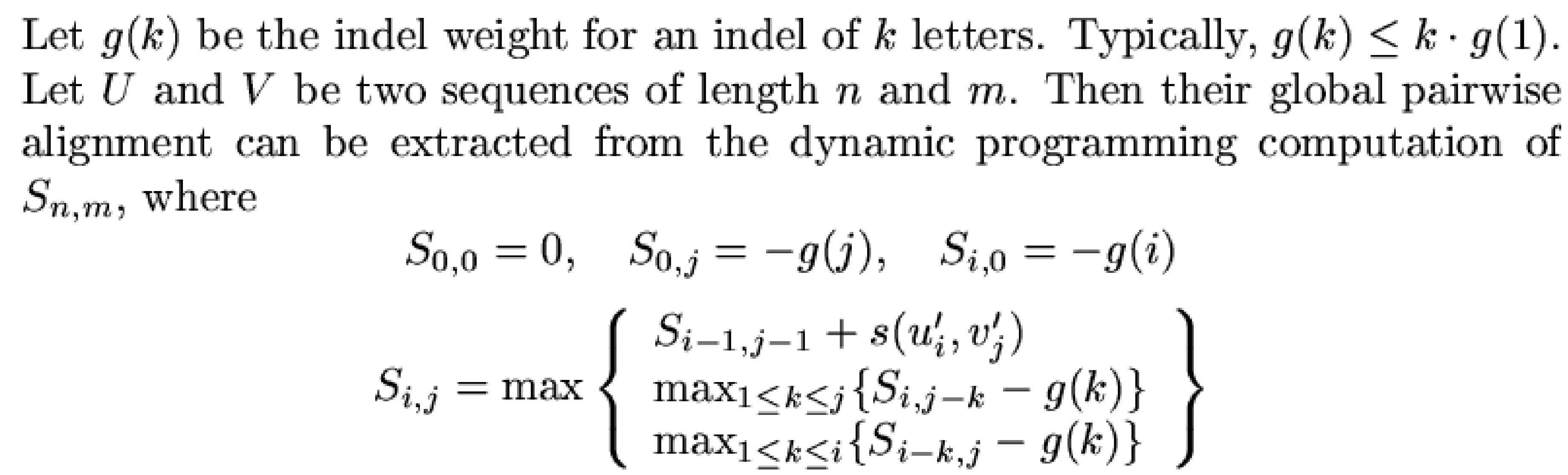
Complexity analysis

- **Space**
  - O(m) working memory for finding mid-point
  - Once mid-point is found, can free working memory → In each recursive call, we only need to store the alignment path
  - Alignment subpaths are disjoint → total space required is O(n+m)

- **Time?** This one is for you to think about ☺

Global pairwise alignment:
More Realistic Handling of Indels

- **In Nature, indels of several adjacent letters are not the sum of single indels, but the result of one event**
- **So reformulate as follows:**



Let $g(k)$ be the indel weight for an indel of k letters. Typically, $g(k) \leq k \cdot g(1)$. Let U and V be two sequences of length n and m. Then their global pairwise alignment can be extracted from the dynamic programming computation of $S_{n,m}$, where

$$S_{0,0} = 0, \quad S_{0,j} = -g(j), \quad S_{i,0} = -g(i)$$

$$S_{i,j} = \max \left\{ \begin{array}{l} S_{i-1,j-1} + s(u'_i, v'_j) \\ \max_{1 \leq k \leq j} \{S_{i,j-k} - g(k)\} \\ \max_{1 \leq k \leq i} \{S_{i-k,j} - g(k)\} \end{array} \right\}$$

Gap penalty

Source: Ken Sung

- **$g(q): \mathbb{N} \rightarrow \Re$ is the penalty of a gap of length q**
- **Note g() is subadditive, i.e, $g(p+q) \leq g(p) + g(q)$**

- **If $g(k) = \alpha + \beta k$, the gap penalty is called affine**
  - A penalty (α) for initiating the gap
  - A penalty (β) for the length of the gap

N-W algo w/ general gap penalty (I)

Source: Ken Sung

- **Global alignment of S[1..n] and T[1..m]:**
  - Denote V(i, j) be the score for global alignment between S[1..i] and T[1..j]
  - Base cases:
    - **V(0, 0) = 0**
    - **V(0, j) = g(j)**
    - **V(i, 0) = g(i)**

N-W algo w/ general gap penalty (II)

Source: Ken Sung

- **Recurrence for i>0 and j>0,**

$$V(i,j) = \max \begin{cases} V(i-1, j-1) + \delta(S[i], T[j]) & \textbf{Match/mismatch} \\ \max\limits_{0 \le k \le j-1} \{V(i,k) + g(j-k)\} & \textbf{Insert T[k+1..j]} \\ \max\limits_{0 \le k \le i-1} \{V(k,j) + g(i-k)\} & \textbf{Delete S[k+1..i]} \end{cases}$$

Analysis

Source: Ken Sung

- **We need to fill in all entries in the n×m table**

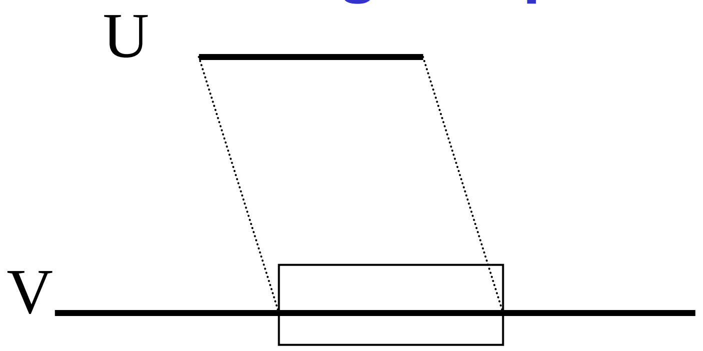
- **Each entry can be computed in O(max{n, m}) time**

$\Rightarrow$ **Time complexity = O(nm max{n, m})**

$\Rightarrow$ **Space complexity = O(nm)**

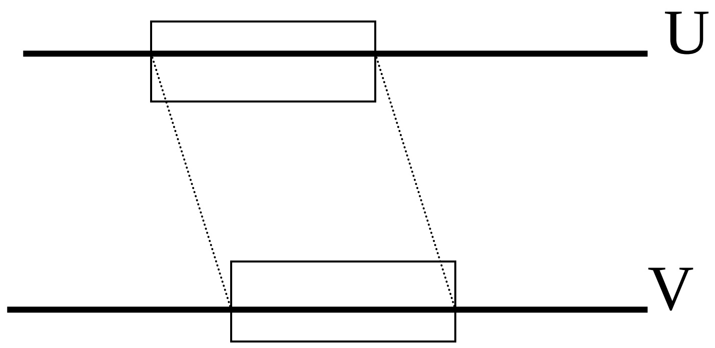
Variations of pairwise alignment

- **Fitting a "short" seq to a "long" seq**



- **Indels at beginning and end are not penalized**

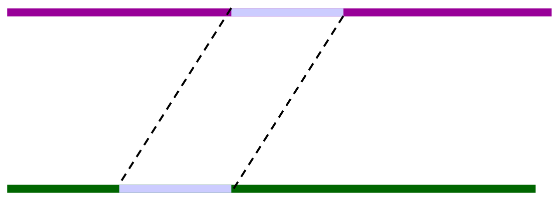
- **Find "local" alignment**



- **Find *i, j, k, l,* so that**
  - *S(A)* is maximized,
  - *A* is alignment of $u_i \ldots u_j$ and $v_k \ldots v_l$

Local alignment

Source: Ken Sung



- **Given two long DNAs, both of them contain the same gene or closely related gene**
  – Can we identify the gene?

- **Local alignment problem: Given two strings S[1..n] and T[1..m], among all substrings of S and T, find substrings A of S and B of T whose global alignment has the highest score**

Brute-force solution

Source: Ken Sung

- **Algorithm:**
  - For every substring A of S, for every substring B of T, compute the global alignment of A and B
  - Return the pair (A, B) with the highest score
- **Time:**
  - There are n^2 choices of A and m^2 choices of B
  - Global alignment computable in O(nm) time
  - In total, time complexity = $O(n^3m^3)$
- **Can we do better?**

Some background / Exercise #8

Source: Ken Sung

- **X is a suffix of S[1..n] if X=S[k..n] for some k≥1**
- **X is a prefix of S[1..n] if X=S[1..k] for some k≤n**

- **E.g.**
  - Consider S[1..7] = ACCGATT
  - ACC is a prefix of S, GATT is a suffix of S
  - Empty string is both prefix and suffix of S

Which other string is both a prefix and suffix of S?

Dynamic programming for local alignment problem

Source: Ken Sung

- **Define V(i, j) be max score of global alignment of A and B over**
  - all suffixes A of S[1..i] and
  - all suffixes B of T[1..j]

- **Then, score of local alignment is**
  - $\max_{i,j} V(i\ ,j)$

Smith-Waterman algorithm

Source: Ken Sung

- **Basis:**

  V(i, 0) = V(0, j) = 0

- **Recursion for i>0 and j>0:**

$$V(i,j) = \max \begin{cases} 0 & \text{Ignore initial segment} \\ V(i-1, j-1) + s(S[i], T[j]) & \text{Match/mismatch} \\ V(i-1, j) - \delta & \text{Delete} \\ V(i, j-1) - \delta & \text{Insert} \end{cases}$$

- Score for match = 2
- Score for insert, delete, mismatch = –1

Example (I)

Source: Ken Sung

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

- Score for match = 2
- Score for insert, delete, mismatch = –1

Example (II) / Exercise #9

Source: Ken Sung

	_	C	T	C	A	T	G	C
_	0	0	0	0	0	0	0	0
A	0	0	0	0	2	1	0	0
C	0	2	1	2	1	1	0	2
A	0	0	1	1	4	3	2	1
A	0	0	0	0	3	3	2	1
T	0	0	2	1	2			
C								
G								

Analysis / Exercise #10

Source: Ken Sung

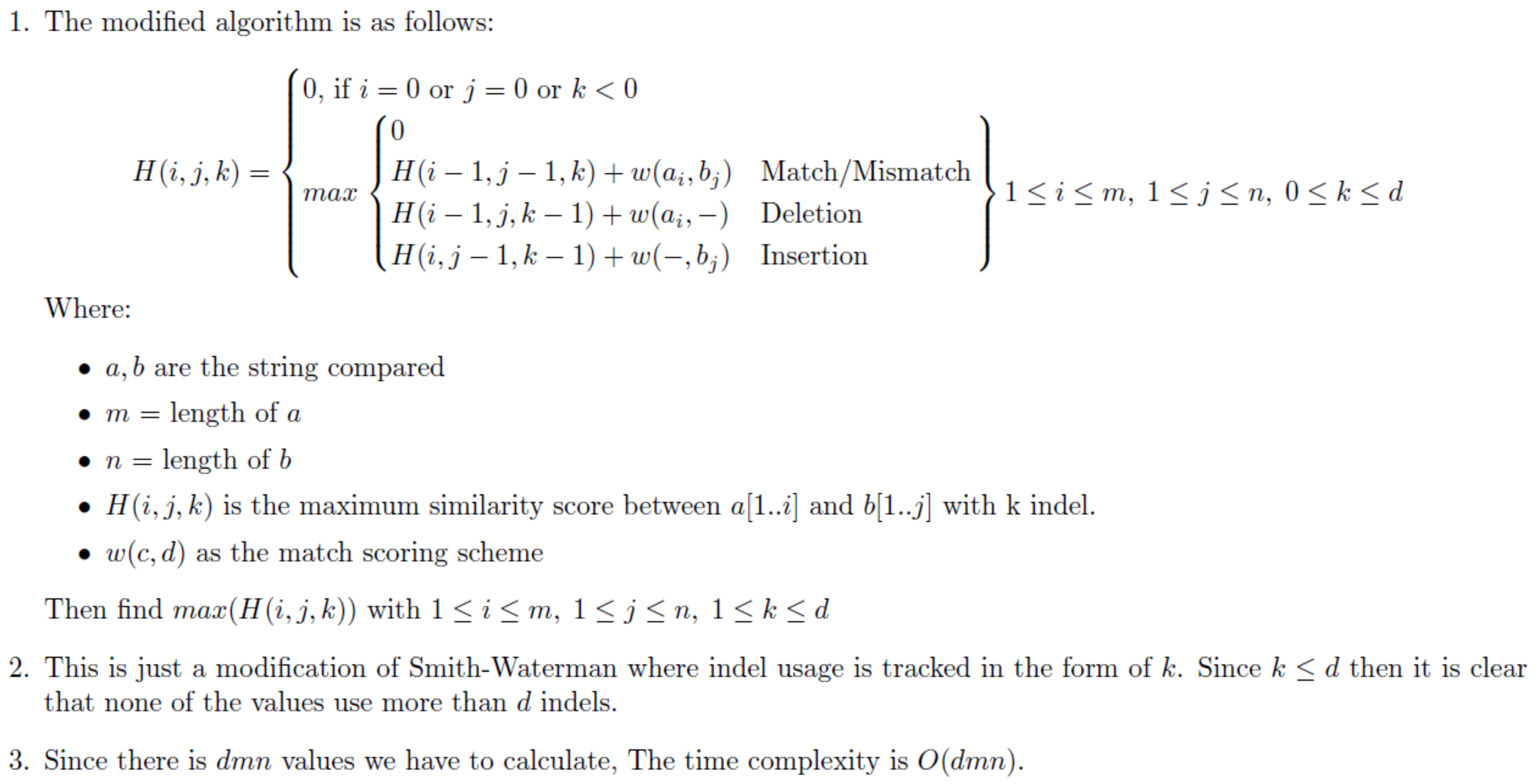
- **Need to fill in all entries in the n×m matrix**
- **Each entries can be computed in O(1) time**
- **Finally, finding the entry with the max value**

⇒ **Time complexity = ??**

⇒ **Space complexity = O(nm)**

What is the time complexity?

Local alignment with at most d indels



1. The modified algorithm is as follows:

$$H(i,j,k) = \begin{cases} 0, \text{ if } i = 0 \text{ or } j = 0 \text{ or } k < 0 \\ max \begin{Bmatrix} 0 & \\ H(i-1,j-1,k) + w(a_i, b_j) & \text{Match/Mismatch} \\ H(i-1,j,k-1) + w(a_i, -) & \text{Deletion} \\ H(i,j-1,k-1) + w(-, b_j) & \text{Insertion} \end{Bmatrix} 1 \leq i \leq m,\ 1 \leq j \leq n,\ 0 \leq k \leq d \end{cases}$$

Where:

- a, b are the string compared
- m = length of a
- n = length of b
- $H(i,j,k)$ is the maximum similarity score between $a[1..i]$ and $b[1..j]$ with k indel.
- $w(c,d)$ as the match scoring scheme

Then find $max(H(i,j,k))$ with $1 \leq i \leq m,\ 1 \leq j \leq n,\ 1 \leq k \leq d$

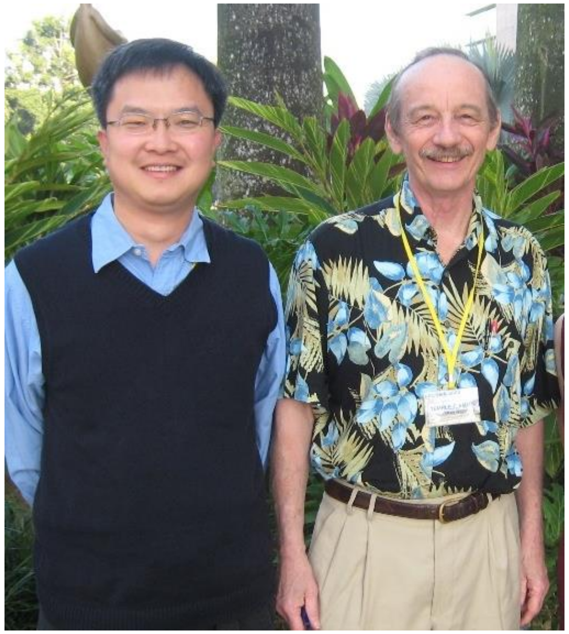
2. This is just a modification of Smith-Waterman where indel usage is tracked in the form of k. Since $k \leq d$ then it is clear that none of the values use more than d indels.

3. Since there is dmn values we have to calculate, The time complexity is $O(dmn)$.

- **Cf. global alignment with at most d index has time complexity O(dn)**

Photos

Limsoon & Temple Smith



Ken & Michael Waterman

Scoring function

Scoring function for DNA

- **For DNA, since we only have 4 nucleotides, the score function is simple**
  - BLAST matrix
  - Transition-transversion matrix: Give mild penalty for replacing purine by purine. Similar for replacing pyrimadine by pyrimadine

	A	C	G	T
A	5	-4	-4	-4
C	-4	5	-4	-4
G	-4	-4	5	-4
T	-4	-4	-4	5

BLAST Matrix

	A	C	G	T
A	1	-5	-1	-5
C	-5	1	-5	-1
G	-1	-5	1	-5
T	-5	-1	-5	1

Transition-Transversion Matrix

Scoring function for protein

- **Commonly, it is devised based on two criteria:**
  – Chemical/physical similarity
  – Observed substitution frequencies

Scoring function for protein using physical/chemical properties

- An amino acid is more likely to be substituted by another if they have similar property [Karlin & Ghandour, PNAS, 82:8597, 1985]

- The score matrices can be derived based on hydrophobicity, charge, electronegativity, & size

- E.g., give higher score for substituting nonpolar amino acid to another nonpolar amino acid

Scoring function for protein based on statistical model

- **Most often used approaches**

- **Two popular matrices:**
  - Point Accepted Mutation (PAM) matrix
  - BLOSUM

- **Both methods define the score as the log-odds ratio between the observed substitution rate and the expected substitution rate**

- **https://en.wikipedia.org/wiki/Substitution_matrix**

Point Accepted Mutation (PAM)

- **PAM was developed by Dayhoff (1978)**
- **A point mutation means substituting one residue by another**
  - It is called an accepted point mutation if the mutation does not change the protein's function or is not fatal
- **Two sequence S1 and S2 are said to be 1 PAM diverged if a series of accepted point mutations can convert S1 to S2 with an average of 1 accepted point mutation per 100 residues**

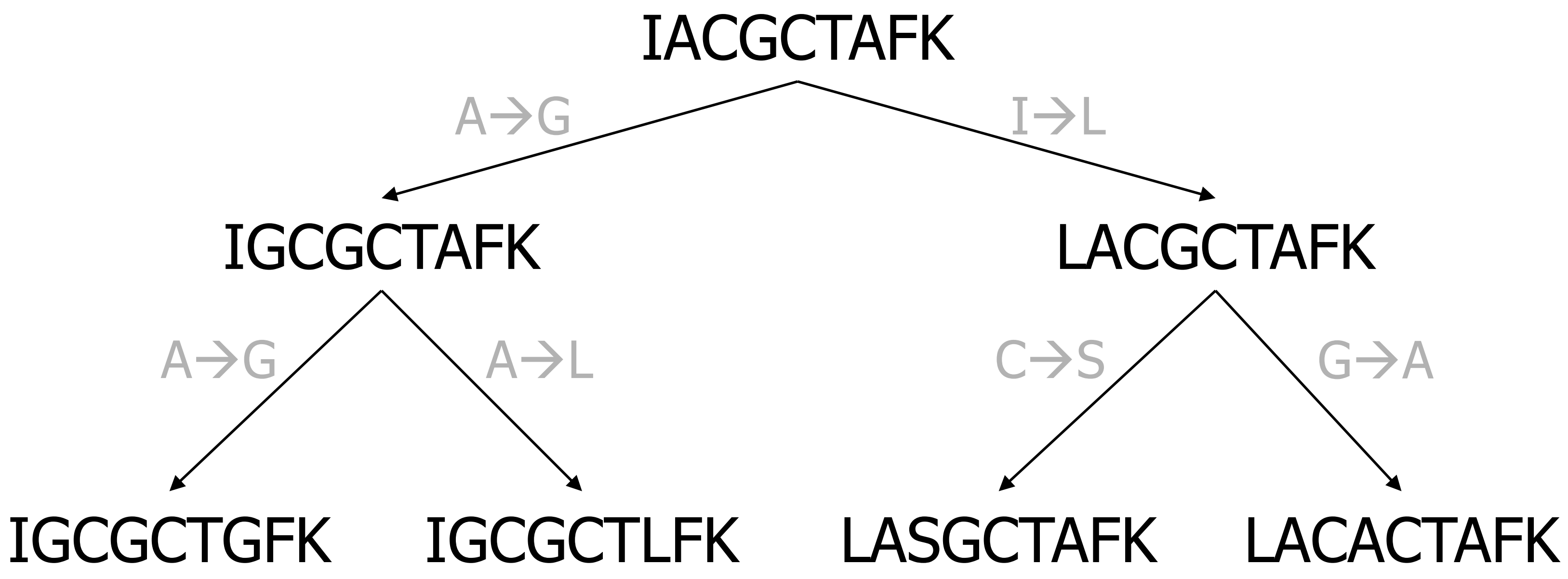
PAM matrix by example (I)

- **Ungapped alignment is constructed for high similarity amino acid sequences (usually >85%)**

- **Below is a simplified gap-free global multiple alignment of some highly similar amino acid seqs**
  - IACGCTAFK
    IGCGCTAFK
    LACGCTAFK
    IGCGCTGFK
    IGCGCTLFK
    LASGCTAFK
    LACACTAFK

PAM matrix by example (II)

- **Build the phylogenetic tree for the sequences**



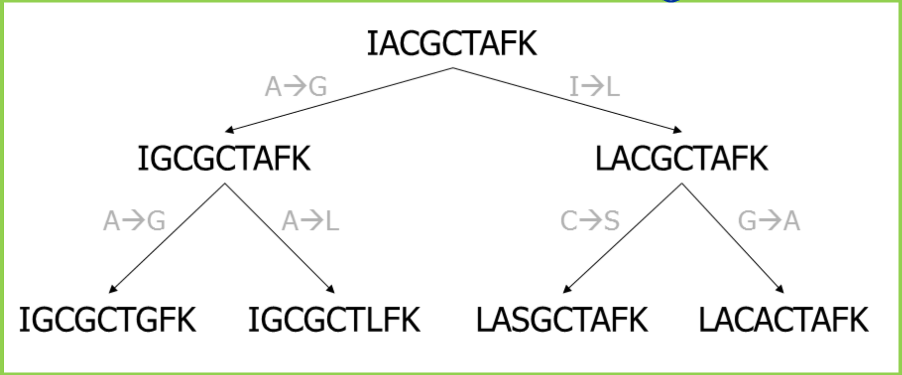
PAM-1 matrix

$$\delta(a,b) = \log_{10} \frac{O_{a,b}}{E_{a,b}}$$

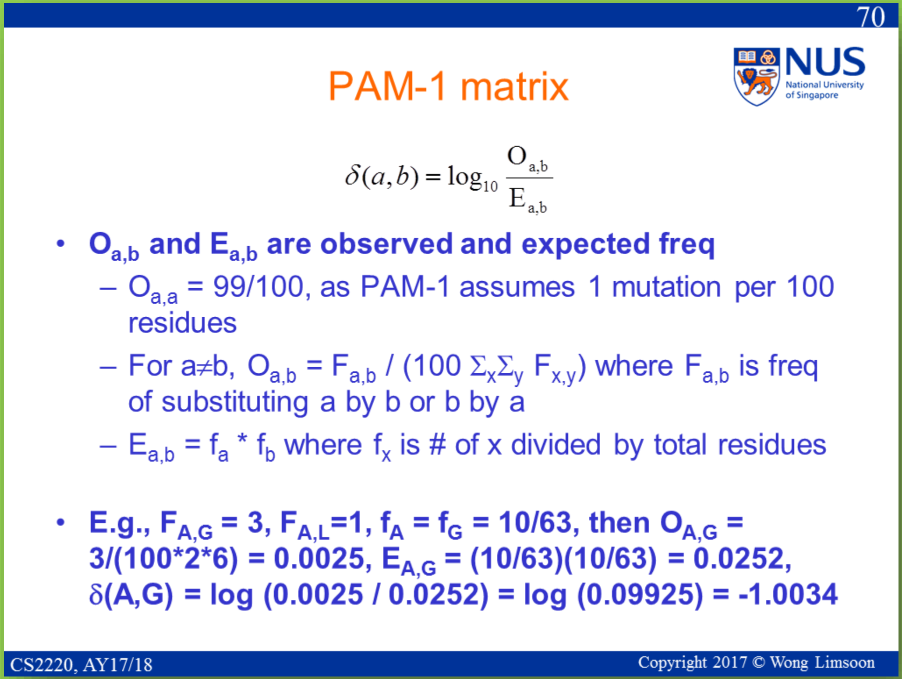
- **$O_{a,b}$ and $E_{a,b}$ are observed and expected freq**
  - $O_{a,a}$ = 99/100, as PAM-1 assumes 1 mutation per 100 residues
  - For $a \neq b$, $O_{a,b} = F_{a,b} / (100\ \Sigma_x \Sigma_y\ F_{x,y})$ where $F_{a,b}$ is freq of substituting a by b or b by a
  - $E_{a,b} = f_a * f_b$ where f_x is # of x divided by total residues

- **E.g., $F_{A,G}$ = 3, $F_{A,L}$=1, $f_A = f_G$ = 10/63, then $O_{A,G}$ = 3/(100*2*6) = 0.0025, $E_{A,G}$ = (10/63)(10/63) = 0.0252, δ(A,G) = log (0.0025 / 0.0252) = log (0.09925) = -1.0034**

Exercise #11



- **$O_{A,G}$ = 3/(100 * 2 * 6)**
- **Where do the 2 and 6 come from?**



70

PAM-1 matrix

$$\delta(a,b) = \log_{10} \frac{O_{a,b}}{E_{a,b}}$$

- **$O_{a,b}$ and $E_{a,b}$ are observed and expected freq**
  - $O_{a,a}$ = 99/100, as PAM-1 assumes 1 mutation per 100 residues
  - For a≠b, $O_{a,b} = F_{a,b} / (100\ \Sigma_x \Sigma_y\ F_{x,y})$ where $F_{a,b}$ is freq of substituting a by b or b by a
  - $E_{a,b} = f_a * f_b$ where f_x is # of x divided by total residues
- **E.g., $F_{A,G}$ = 3, $F_{A,L}$=1, $f_A = f_G$ = 10/63, then $O_{A,G}$ = 3/(100*2*6) = 0.0025, $E_{A,G}$ = (10/63)(10/63) = 0.0252, δ(A,G) = log (0.0025 / 0.0252) = log (0.09925) = -1.0034**

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PAM-n matrix

- **Let $M_{a,b} = O_{a,b} / f_a$ be prob that a is mutated to b**
- **$M^n(a,b)$ is prob that a is mutated to b after n mutations**
- **PAM-n matrix is created by extrapolating PAM-1**
- **PAM-n matrix is computed as follows.**
  - At time t, suppose the residue is a
  - At time t+1, prob that it becomes j is M(a,b)
  - At time t+2, prob that it becomes j is $M^2(a,b)$
  - …
  - At time t+n, prob that it becomes j is $M^n(a,b)$

$\Rightarrow$ **(a,b) entry of PAM-n matrix is $\log(f_a M^n(a,b)/f_a f_b) = \log(M^n(a,b)/f_b)$**

BLOSUM (BLOck SUbstition Matrix)

- PAM did not work well for aligning evolutionarily divergent sequences since the matrix is generated by extrapolation
- Henikoff and Henikoff (1992) proposed BLOSUM
- Unlike PAM, BLOSUM matrix is constructed directly from the observed alignment (instead of extrapolation)

Generating conserved blocks

- In BLOSUM, the input is a set of multiple alignments for nonredundant groups of protein families

- Based on PROTOMAT, blocks of nongapped local aligments are derived

- Each block represents a conserved region of a protein family

Extract frequencies from blocks

- From all blocks, we count the frequency f_a for each amino acid residue a.
- For any two amino acid residues a and b, we count the frequency p_{ab} of aligned pair of a and b.

- For example,

  -
    ```
    ACGCTAFKI
    GCGCTAFKI
    ACGCTAFKL
    GCGCTGFKI
    GCGCTLFKI
    ASGCTAFKL
    ACACTAFKL
    ```
- There are 7*9=63 residues, including 9's A and 10's G. Hence, F_A = 9/63, F_G = 10/63.
- There are $9 * \binom{7}{2}$ = 189 aligned residue pairs, including 23 (A,G) pairs. Hence, p_{AG} = 23 / 189.

BLOSUM scoring function

- **For each pair of aligned residues a and b, the alignment score $\delta(a,b) = (1/\lambda)(\ln p_{ab}/(p_a p_b))$**
  – p_{ab} is prob that a and b are observed to align together
  – p_a and p_b are freq of residues a and b
  – λ is a normalization constant

- **Example: p_L=0.099, p_A=0.074, p_{AL} = 0.0044. With λ=0.347, $\delta(A,L)$ = -1.47**

What is BLOSUM 62?

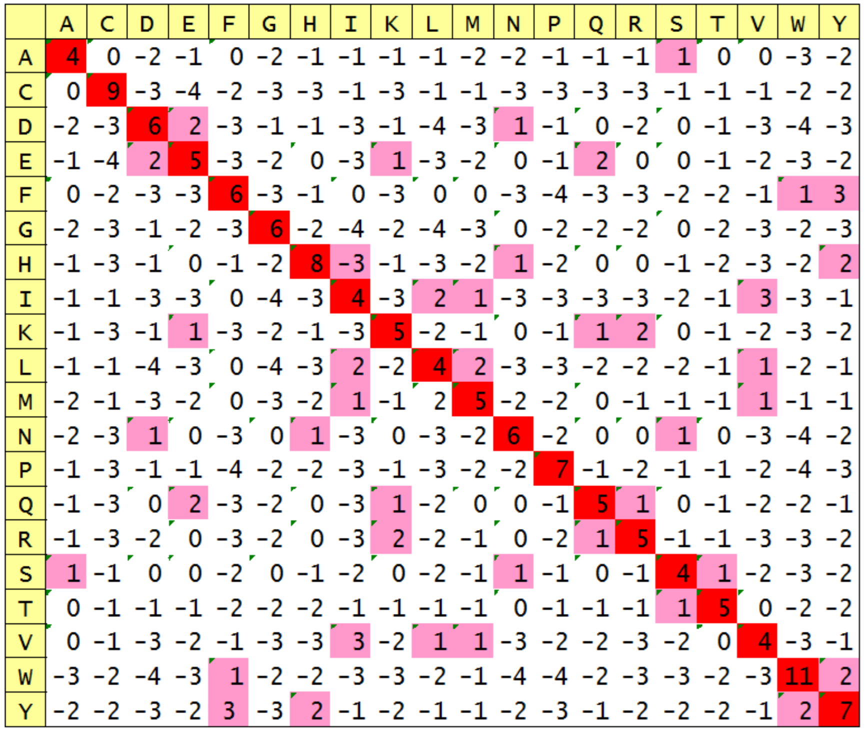
- **To reduce multiple contributions to amino acid pair freq from the most closely related members of a family, similar seqs are merged within block**
- **BLOSUM p matrix is created by merging seqs with ≥p% similarity**

- **Example**
  - AVAAA, AVAAA, AVAAA, AVLAA, VVAAL
  - First 4 seqs have ≥80% similarity. Similarity of last seq with the other 4 sequences is <62%
  - For BLOSUM 62, we group first 4 seqs and get $AV[A_{0.75}L_{0.25}]AA$, VVAAL. Then $p_{AV} = 1/5$, $p_{AL} = (0.25 + 1)/5$.

BLOSUM vs PAM

- **BLOSUM 80 ≈ PAM 1**
- **BLOSUM 62 ≈ PAM 120**
- **BLOSUM 45 ≈ PAM 250**

- **BLOSUM 62 is the default matrix for BLAST 2.0**



	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
A	4	0	-2	-1	0	-2	-1	-1	-1	-1	-2	-2	-1	-1	-1	1	0	0	-3	-2
C	0	9	-3	-4	-2	-3	-3	-1	-3	-1	-1	-3	-3	-3	-3	-1	-1	-1	-2	-2
D	-2	-3	6	2	-3	-1	-1	-3	-1	-4	-3	1	-1	0	-2	0	-1	-3	-4	-3
E	-1	-4	2	5	-3	-2	0	-3	1	-3	-2	0	-1	2	0	0	-1	-2	-3	-2
F	0	-2	-3	-3	6	-3	-1	0	-3	0	0	-3	-4	-3	-3	-2	-2	-1	1	3
G	-2	-3	-1	-2	-3	6	-2	-4	-2	-4	-3	0	-2	-2	-2	0	-2	-3	-2	-3
H	-1	-3	-1	0	-1	-2	8	-3	-1	-3	-2	1	-2	0	0	-1	-2	-3	-2	2
I	-1	-1	-3	-3	0	-4	-3	4	-3	2	1	-3	-3	-3	-3	-2	-1	3	-3	-1
K	-1	-3	-1	1	-3	-2	-1	-3	5	-2	-1	0	-1	1	2	0	-1	-2	-3	-2
L	-1	-1	-4	-3	0	-4	-3	2	-2	4	2	-3	-3	-2	-2	-2	-1	1	-2	-1
M	-2	-1	-3	-2	0	-3	-2	1	-1	2	5	-2	-2	0	-1	-1	-1	1	-1	-1
N	-2	-3	1	0	-3	0	1	-3	0	-3	-2	6	-2	0	0	1	0	-3	-4	-2
P	-1	-3	-1	-1	-4	-2	-2	-3	-1	-3	-2	-2	7	-1	-2	-1	-1	-2	-4	-3
Q	-1	-3	0	2	-3	-2	0	-3	1	-2	0	0	-1	5	1	0	-1	-2	-2	-1
R	-1	-3	-2	0	-3	-2	0	-3	2	-2	-1	0	-2	1	5	-1	-1	-3	-3	-2
S	1	-1	0	0	-2	0	-1	-2	0	-2	-1	1	-1	0	-1	4	1	-2	-3	-2
T	0	-1	-1	-1	-2	-2	-2	-1	-1	-1	-1	0	-1	-1	-1	1	5	0	-2	-2
V	0	-1	-3	-2	-1	-3	-3	3	-2	1	1	-3	-2	-2	-3	-2	0	4	-3	-1
W	-3	-2	-4	-3	1	-2	-2	-3	-3	-2	-1	-4	-4	-2	-3	-3	-2	-3	11	2
Y	-2	-2	-3	-2	3	-3	2	-1	-2	-1	-1	-2	-3	-1	-2	-2	-2	-1	2	7

Multiple sequence alignment

What is a domain

- **A domain is a component of a protein that is self-stabilizing and folds independently of the rest of the protein chain**
  – Not unique to protein products of one gene; can appear in a variety of proteins
  – Play key role in the biological function of proteins
  – Can be "swapped" by genetic engineering betw one protein and another to make chimeras

- **May be composed of one, more than one, or not any structural motifs (often corresponding to active sites)**

Discovering domain and active sites

```
>gi|475902|emb|CAA83657.1| protein-tyrosine-phosphatase alpha
MDLWFFVLLLGSGLISVGATNVTTEPPTTVPTSTRIPTKAPTAAPDGGTTPRVSSLNVSSPMTTSAPASE
PPTTTATSISPNATTASLNASTPGTSVPTSAPVAISLPPSATPSALLTALPSTEAEMTERNVSATVTTQE
TSSASHNGNSDRRDETPIIAVMVALSSLLVIVFIIIVLYMLRFKKYKQAGSHSNSFRLPNGRTDDAEPQS
MPLLARSPSTNRKYPPLPVDKLEEEINRRIGDDNKLFREEFNALPACPIQATCEAASKEENKEKNRYVNI
LPYDHSRVHLTPVEGVPDSHYINTSFINSYQEKNKFIAAQGPKEETVNDFWRMIWEQNTATIVMVTNLKE
RKECKCAQYWPDQGCWTYGNIRVSVEDVTVLVDYTVRKFCIQQVGDVTNKKPQRLVTQFHFTSWPDFGVP
FTPIGMLKFLKKVKTCNPQYAGAIVVHCSAGVGRTGTFIVIDAMLDMMHAERKVDVYGFVSRIRAQRCQM
VQTDMQYVFIYQALLEHYLYGDTELEVTSLEIHLQKIYNKVPGTSSNGLEEEFKKLTSIKIQNDKMRTGN
LPANMKKNRVLQIIPYEFNRVIIPVKRGEENTDYVNASFIDGYRRRTPTCQPRPVQHTIEDFWRMIWEWK
SCSIVMLTELEERGQEKCAQYWPSDGSVSYGDINVELKKEEECESYTVRDLLVTNTRENKSRQIRQFHFH
GWPEVGIPSDGKGMINIIAAVQKQQQQSGNHPMHCHCSAGAGRTGTFCALSTVLERVKAEGILDVFQTVK
SLRLQRPHMVQTLEQYEFCYKVVQEYIDAFSDYANFK
```

- **How do we find the domain and associated active sites in the protein above?**

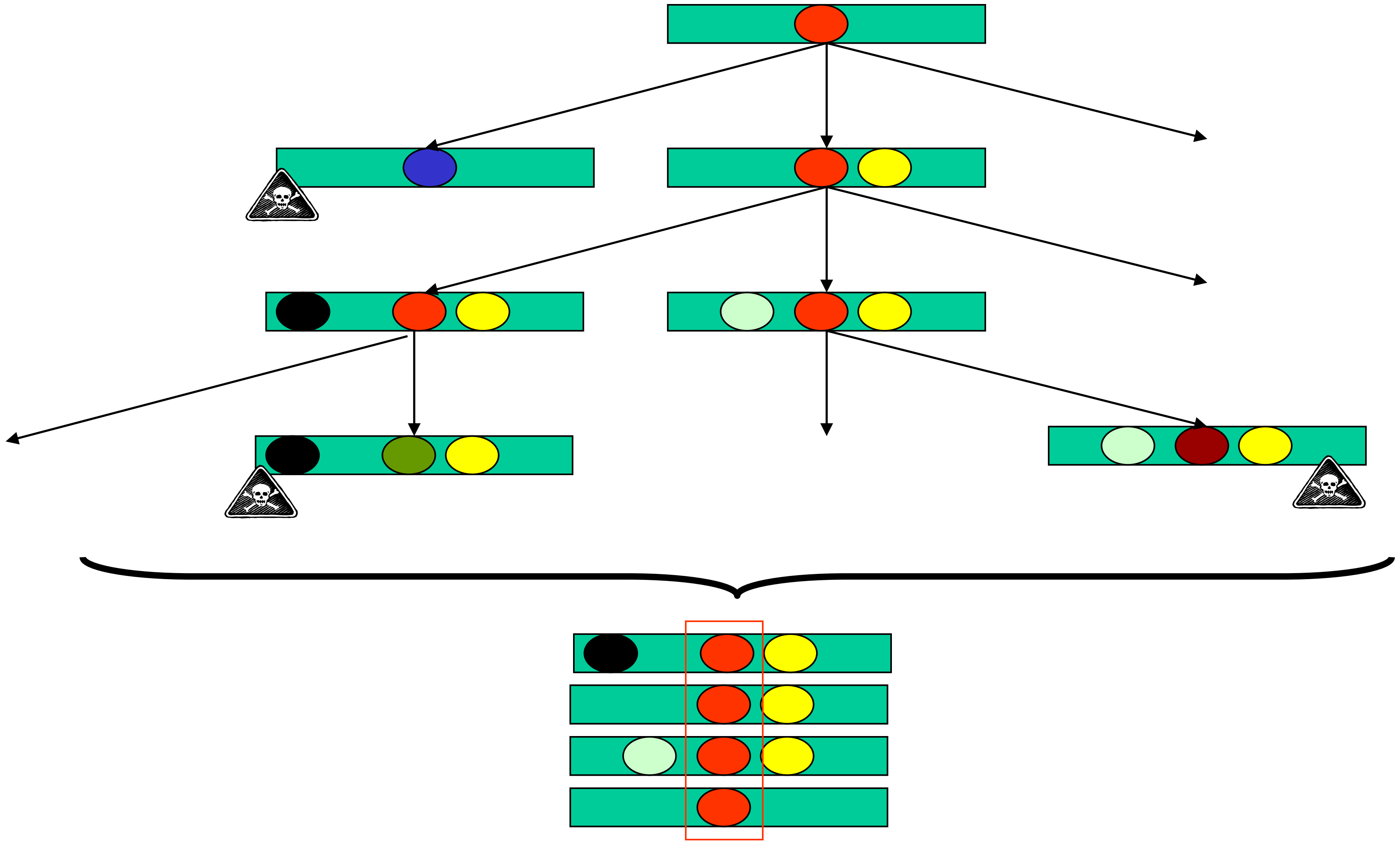
Domain/active sites as emerging patterns

- **How to discover active site and/or domain?**
- **If you are lucky, domain has already been modelled**
  - BLAST, HMMPFAM, …
- **If you are unlucky, domain not yet modelled**
  - Find homologous seqs
  - Do multiple alignment of homologous seqs
  - Determine conserved positions

  $\Rightarrow$ Emerging patterns relative to background

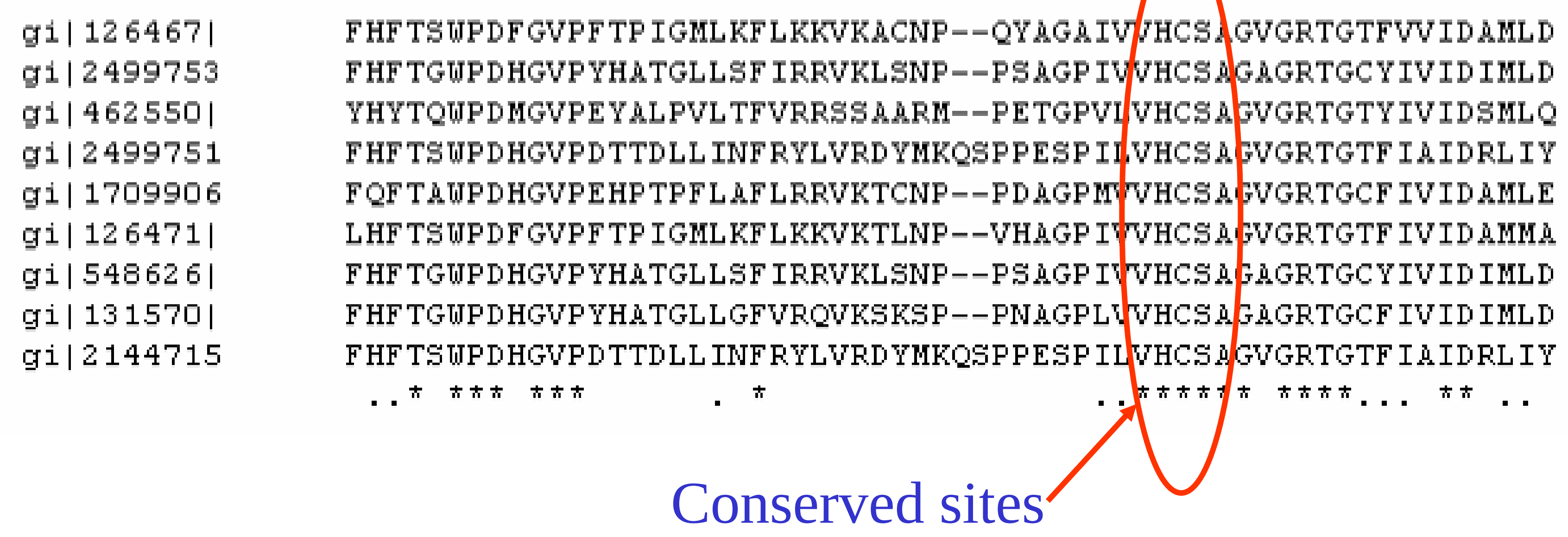
  $\Rightarrow$ Candidate active sites and/or domains

In the course of evolution…



Multiple alignment: Example

- **Multiple seq alignment maximizes number of positions in agreement across several seqs**
- **Seqs belonging to same “family” usually have more conserved positions in a multiple seq alignment**



```
gi|126467|      FHFTSWPDFGVPFTPIGMLKFLKKVKACNP--QYAGAIVVHCSAGVGRTGTFVVIDAMLD
gi|2499753      FHFTGWPDHGVPYHATGLLSFIRRVKLSNP--PSAGPIVVHCSAGAGRTGCYIVIDIMLD
gi|462550|      YHYTQWPDMGVPEYALPVLTFVRRSSAARM--PETGPVLVHCSAGVGRTGTYIVIDSMLQ
gi|2499751      FHFTSWPDHGVPDTTDLLINFRYLVRDYMKQSPPESPILVHCSAGVGRTGTFIAIDRLIY
gi|1709906      FQFTAWPDHGVPEHPTPFLAFLRRVKTCNP--PDAGPMVVHCSAGVGRTGCFIVIDAMLE
gi|126471|      LHFTSWPDFGVPFTPIGMLKFLKKVKTLNP--VHAGPIVVHCSAGVGRTGTFIVIDAMMA
gi|548626|      FHFTGWPDHGVPYHATGLLSFIRRVKLSNP--PSAGPIVVHCSAGAGRTGCYIVIDIMLD
gi|131570|      FHFTGWPDHGVPYHATGLLGFVRQVKSKSP--PNAGPLVVHCSAGAGRTGCFIVIDIMLD
gi|2144715      FHFTSWPDHGVPDTTDLLINFRYLVRDYMKQSPPESPILVHCSAGVGRTGTFIAIDRLIY
                 ..* *** ***      . *                ..****** ****... ** ..
```

Conserved sites

Multiple alignment: Naïve approach

- **Let S(A) be the score of a multiple alignment A. The optimal multiple alignment A of sequences $U_1, \ldots, U_r$ can be extracted from the following dynamic programming computation of $S_{m1,\ldots,mr}$:**

$$S_{m_1,\ldots,m_r} = \max_{\epsilon_1 \in \{0,1\},\ldots,\epsilon_r \in \{0,1\}} \left\{ \begin{array}{l} S_{m_1-\epsilon_1,\ldots,m_r-\epsilon_r} + \\ s(\epsilon_1 \cdot u'_{1,m_1}, \ldots, \epsilon_r \cdot u'_{r,m_r}) \end{array} \right\}$$

where

$$\epsilon_i \cdot a = \begin{cases} a & \text{if } \epsilon_i = 1 \\ - & \text{if } \epsilon_i = 0 \end{cases}$$

- **This requires $O(2^r)$ steps**

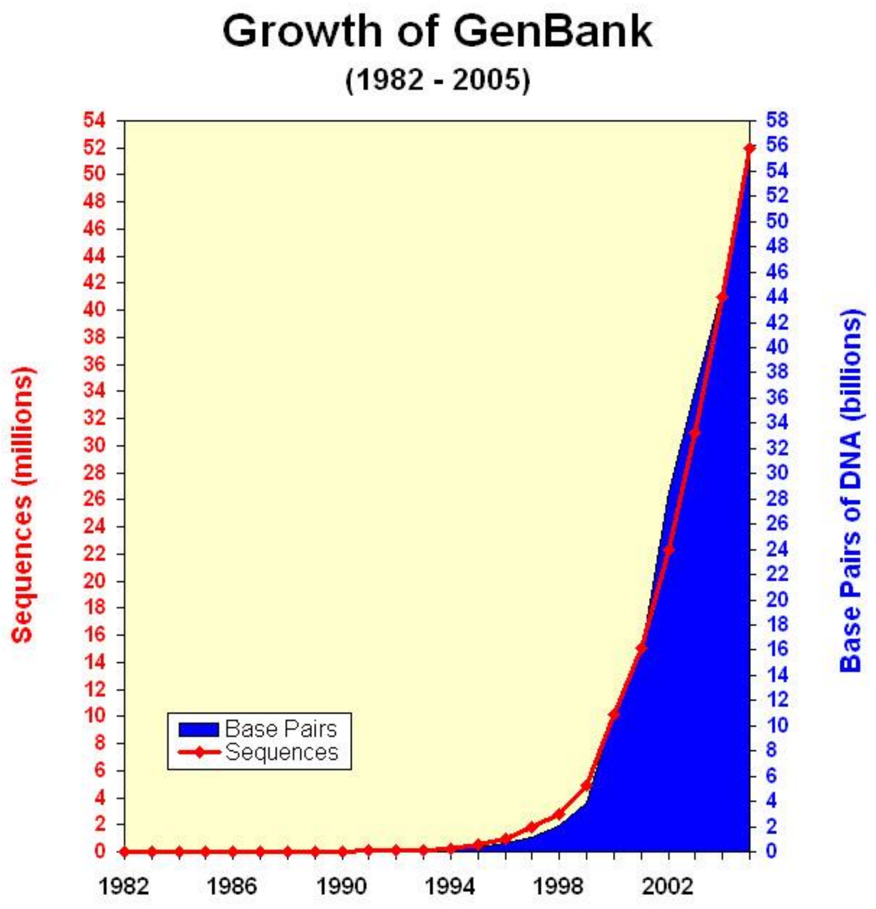
Exercise for the Brave:
Propose a practical approximation

Popular tools for sequence comparison: FASTA, BLAST, Pattern Hunter

National University of Singapore

Scalability

- Increasing # of sequenced genomes: yeast, human, rice, mouse, fly, …
- S/w must be “linearly” scalable to large datasets



Database search

- **Consider a database D of genomic sequences (or protein sequences)**

- **Given a query string Q,**
  - – Look for string S in D which is the closest match to the query string Q
  - – Two meanings for closest match:
    - **S and Q has a semi-global alignment (forgive the spaces at the two ends of Q)**
    - **S and Q have a local alignment**

Goodness of a search algorithm

- **Sensitivity**
  - Ability to detect "true positive"
  - Measured as the probability of finding the match given the query and the database sequence has only x% similarity

- **Specificity**
  - Ability to reject "false positive"

- **A good search algorithm should be both sensitive and specific**

Need heuristics for sequence comparison

- **Time complexity for optimal alignment is O(n^2), where n is seq length**

$\Rightarrow$ **Given current size of seq databases, use of optimal algorithms is not practical for database search**

Exercise: Describe MUMer

- **Heuristic techniques:**
  - BLAST
  - FASTA
  - Pattern Hunter
  - MUMmer, ...

- **Speed up:**
  - 20 min (optimal alignment)
  - 2 min (FASTA)
  - 20 sec (BLAST)

Basic idea: Indexing & filtering

- **Good alignment includes short identical, or similar fragments**

⇒ Break entire string into substrings, index the substrings

⇒ Search for matching short substrings and use as seed for further analysis

⇒ Extend to entire string find the most significant local alignment segment

BLAST in 3 steps

Altschul et al, *JMB* 215:403-410, 1990

- **Similarity matching of words (3 aa's, 11 bases)**
  - No need identical words
- **If no words are similar, then no alignment**
  - Won't find matches for very short sequences
- **MSP: Highest scoring pair of segments of identical length. A segment pair is locally maximal if it cannot be improved by extending or shortening the segments**
- **Find alignments w/ optimal max segment pair (MSP) score**
- **Gaps not allowed**
- **Homologous seqs will contain a MSP w/ a high score; others will be filtered out**

BLAST in 3 steps

Altschul et al, *JMB* 215:403-410, 1990

Step 1

- **For the query, find the list of high scoring words of length w**

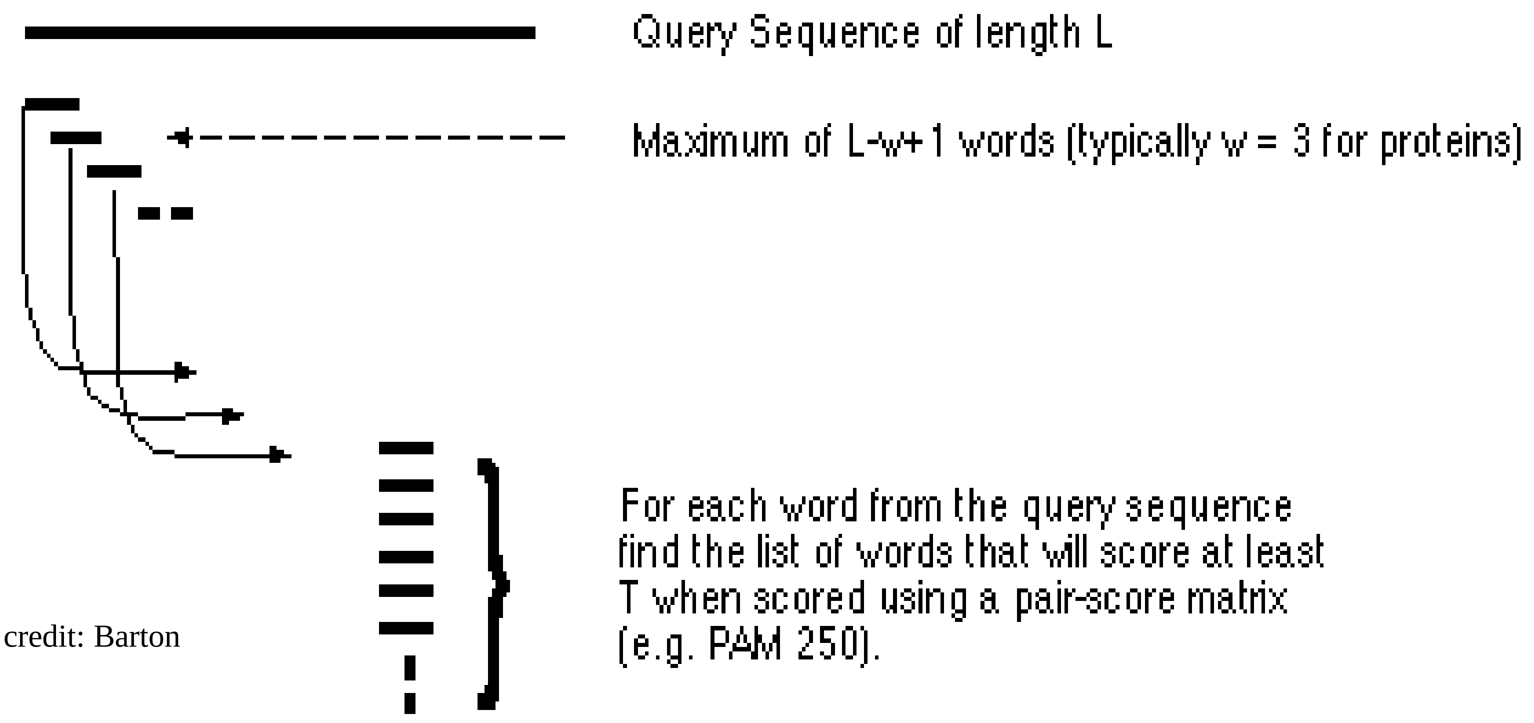

Query Sequence of length L
Maximum of L-w+1 words (typically w = 3 for proteins)
For each word from the query sequence find the list of words that will score at least T when scored using a pair-score matrix (e.g. PAM 250).

Image credit: Barton

BLAST in 3 steps

Altschul et al, *JMB* 215:403-410, 1990

Step 2

- Compare word list to db & find exact matches

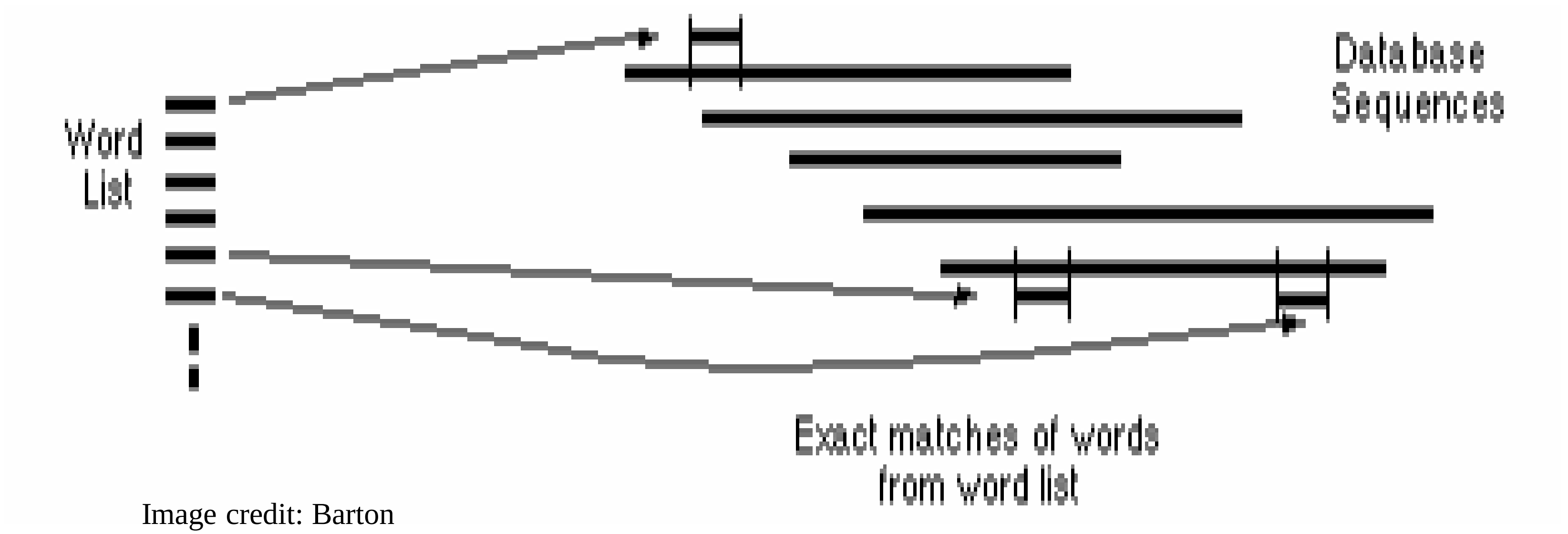


Image credit: Barton

BLAST in 3 steps

Altschul et al, *JMB* 215:403-410, 1990

Step 3

- **For each word match, extend alignment in both directions to find alignment that score greater than a threshold s**

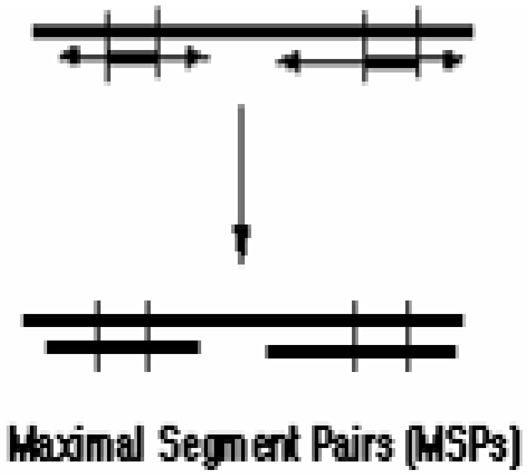


Image credit: Barton

Spaced seeds

- **111010010100110111 is an example of a spaced seed model with**
  - 11 required matches (weight=11)
  - 7 "don't care" positions

```
GAGTACTCAACACCAACATTAGTGGCAATGGAAAAT...
|| ||||||||| ||||| || |||||   ||||||
GAATACTCAACAGCAACACTAATGGCAGCAGAAAAT...
       111010010100110111
```

- **11111111111 is the BLAST seed model for comparing DNA seqs**

Observations on spaced seeds

- **Seed models w/ different shapes can detect different homologies**
  - the 3rd base in a codon "wobbles" so a seed like 110110110… should be more sensitive when matching coding regions

⇒ **Some models detect more homologies**

  - More sensitive homology search
  - PatternHunter I

⇒ **Use >1 seed models to hit more homologies**

  - Approaching 100% sensitive homology search
  - PatternHunter II

Exercise: Why does the 3rd base wobbles?

PatternHunter I

Ma et al., *Bioinformatics* 18:440-445, 2002

- **BLAST's seed usually uses more than one hits to detect one homology**

⇒ **Wasteful**

- **Spaced seeds uses fewer hits to detect one homology**

⇒ **Efficient**

```
TTGACCTCACC?
|||||||||||?
TTGACCTCACC?
11111111111
 11111111111
```

1/4 chances to have 2nd hit next to the 1st hit

```
CAA?A??A?C??TA?TGG?
|||?|??|?|??||?|||?
CAA?A??A?C??TA?TGG?
111010010100110111
 111010010100110111
```

1/4^6 chances to have 2nd hit next to the 1st hit

PatternHunter I

Ma et al., *Bioinformatics* 18:440-445, 2002

Proposition. The expected number of hits of a weight-*W* length-*M* model within a length-*L* region of similarity *p* is $(L - M + 1) * p^W$

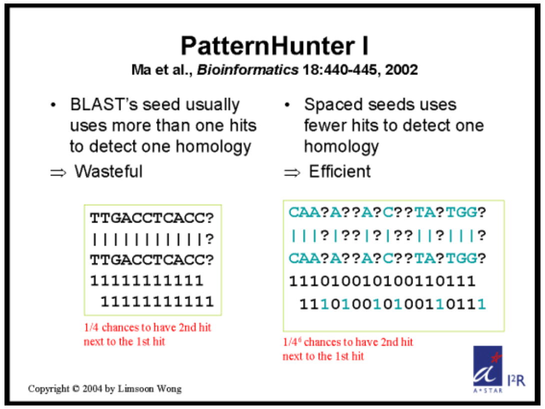
Proof.

For any fixed position, the prob of a hit is p^W.

There are $L - M + 1$ candidate positions.

The proposition follows.

Implication



PatternHunter I

Ma et al., *Bioinformatics* 18:440-445, 2002

- BLAST's seed usually uses more than one hits to detect one homology

$\Rightarrow$ Wasteful

```
TTGACCTCACC?
|||||||||||?
TTGACCTCACC?
11111111111
 11111111111
```

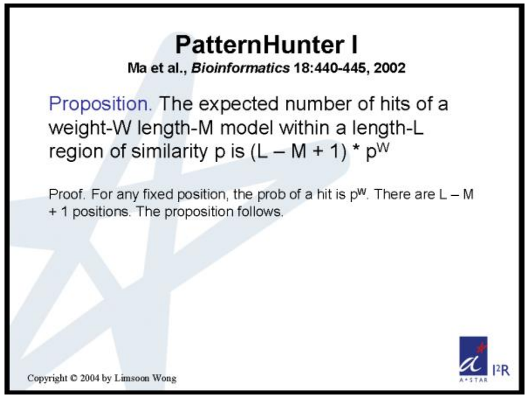
1/4 chances to have 2nd hit next to the 1st hit

- Spaced seeds uses fewer hits to detect one homology

$\Rightarrow$ Efficient

```
CAA?A??A?C??TA?TGG?
|||?|??|?|??||?|||?
CAA?A??A?C??TA?TGG?
111010010100110111
 111010010100110111
```

$1/4^6$ chances to have 2nd hit next to the 1st hit



PatternHunter I

Ma et al., *Bioinformatics* 18:440-445, 2002

Proposition. The expected number of hits of a weight-W length-M model within a length-L region of similarity p is $(L – M + 1) * p^W$

Proof. For any fixed position, the prob of a hit is p^W. There are L – M + 1 positions. The proposition follows.

- **For *L = 1017***
  - BLAST seed expects *(1017 – 11 + 1) * p^{11}* = *1007 * p^{11}* hits
  - But ~*1/4* of these overlap each other. So likely to have only ~*750 * p^{11}* distinct hits
  - Our example spaced seed expects *(1017 – 18 + 1) * p^{11}* = *1000 * p^{11}* hits
  - But only *$1/4^6$* of these overlap each other. So likely to have ~*1000 * p^{11}* distinct hits

Spaced seeds likely to be more sensitive & more efficient

Sensitivity of PatternHunter I

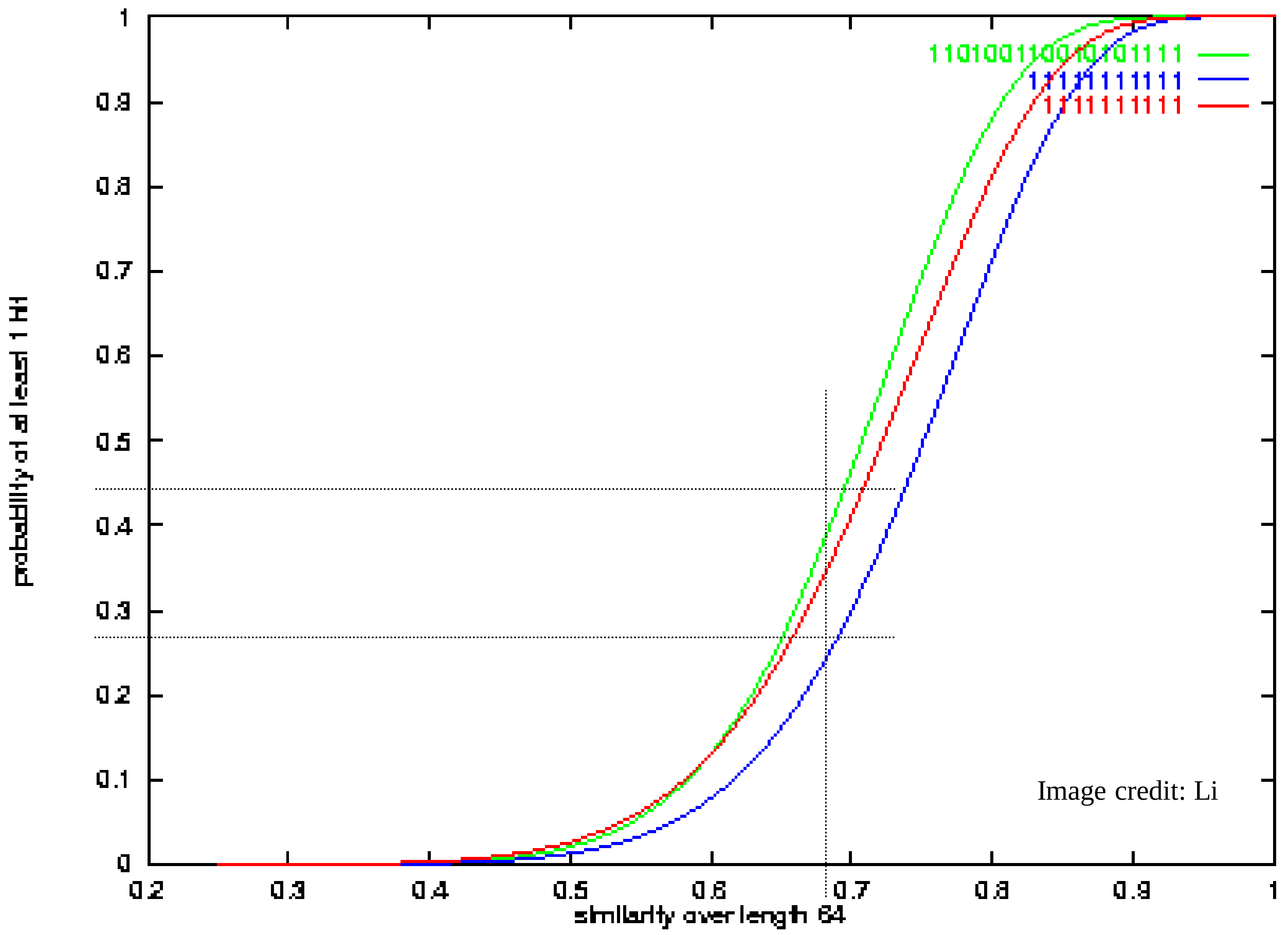
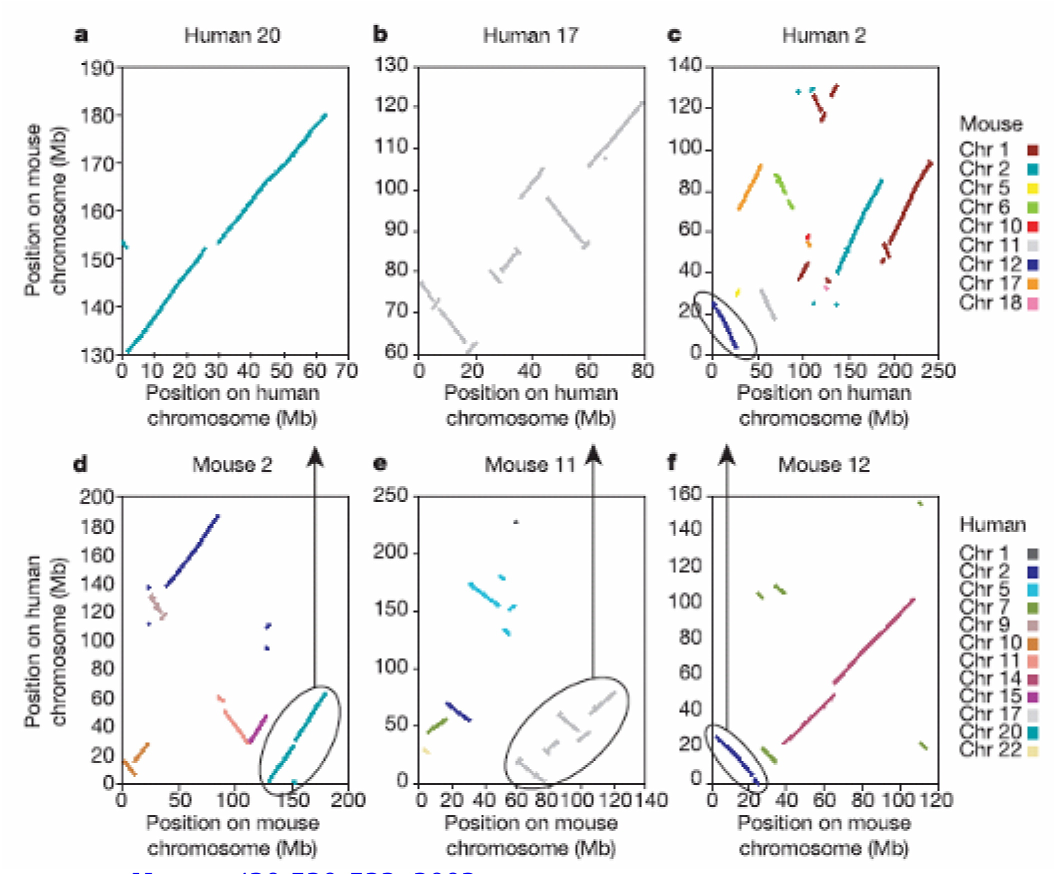


Image credit: Li

Speed of PatternHunter I



Nature, 420:520-522, 2002

- **Mouse Genome Consortium used PatternHunter to compare mouse genome & human genome**

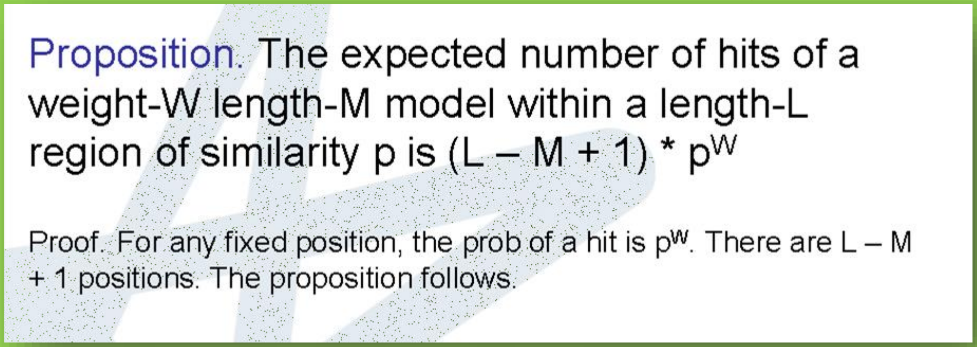
- **PatternHunter did the job in a 20 CPU-days --- it would have taken BLAST 20 CPU-years!**

How to increase sensitivity?

- **Ways to increase sensitivity:**
  - "Optimal" seed
  - Reduce weight by 1
  - Increase number of spaced seeds by 1
- **Intuitively, for DNA seq,**
  - Reducing weight by 1 will increase number of matches 4 folds
  - Doubling number of seeds will increase number of matches 2 folds
- **Is this really so?**

How to increase sensitivity?

- **Ways to increase sensitivity:**
  - “Optimal” seed
  - Reduce weight by 1
  - Increase number of spaced seeds by 1



Proposition. The expected number of hits of a weight-W length-M model within a length-L region of similarity p is $(L – M + 1) * p^W$

Proof. For any fixed position, the prob of a hit is p^W. There are L – M + 1 positions. The proposition follows.

- **For *L = 1017 & p = 50%***
  - 1 weight-11 length-18 model expects $1000/2^{11}$ hits
  - 2 weight-12 length-18 models expect $2 * 1000/2^{12} = 1000/2^{11}$ hits

$\Rightarrow$ When comparing regions w/ >50% similarity, using 2 weight-12 spaced seeds together is more sensitive than using 1 weight-11 spaced seed!

Exercise #12: Proof this claim

PatternHunter II

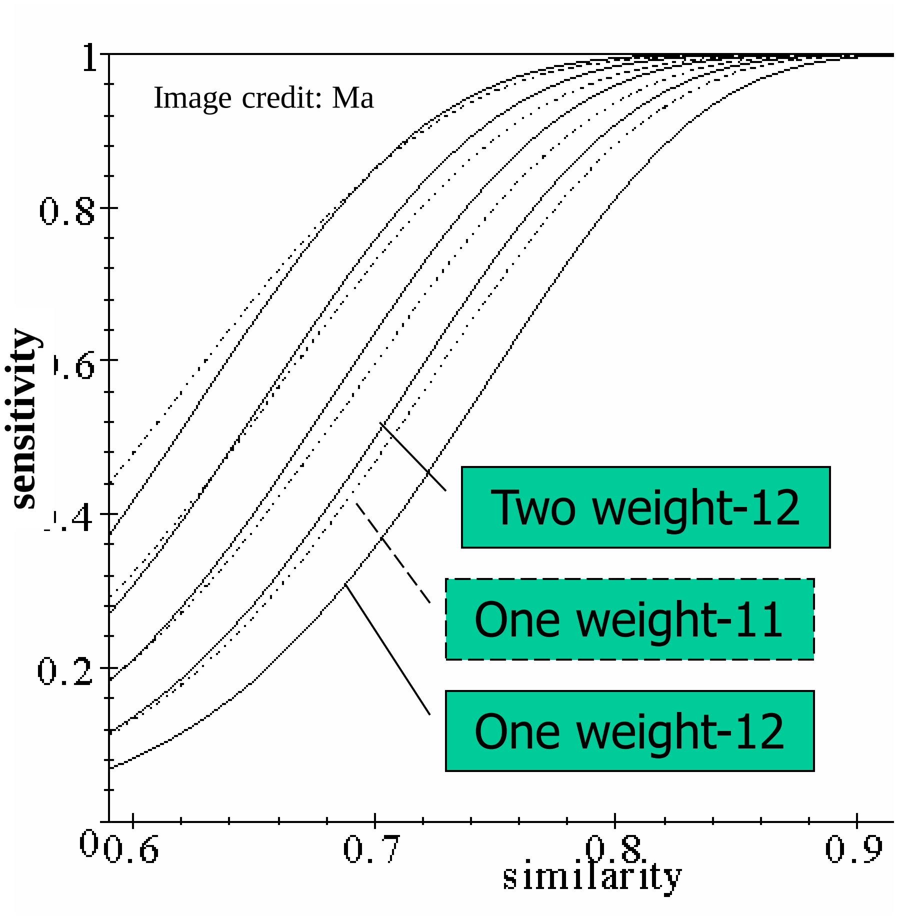
Li et al, *GIW*, 164-175, 2003

- **Idea**
  - Select a group of spaced seed models
  - For each hit of each model, conduct extension to find a homology
- **Selecting optimal multiple seeds is NP-hard**

- **Algorithm to select multiple spaced seeds**
  - Let A be an empty set
  - Let s be the seed such that A U {s} has the highest hit probability
  - A = A U {s}
  - Repeat until |A| = K
- **Computing hit probability of multiple seeds is NP-hard**

But see also Ilie & Ilie, "Multiple spaced seeds for homology search", *Bioinformatics*, 23(22):2969-2977, 2007

Sensitivity of PatternHunter II



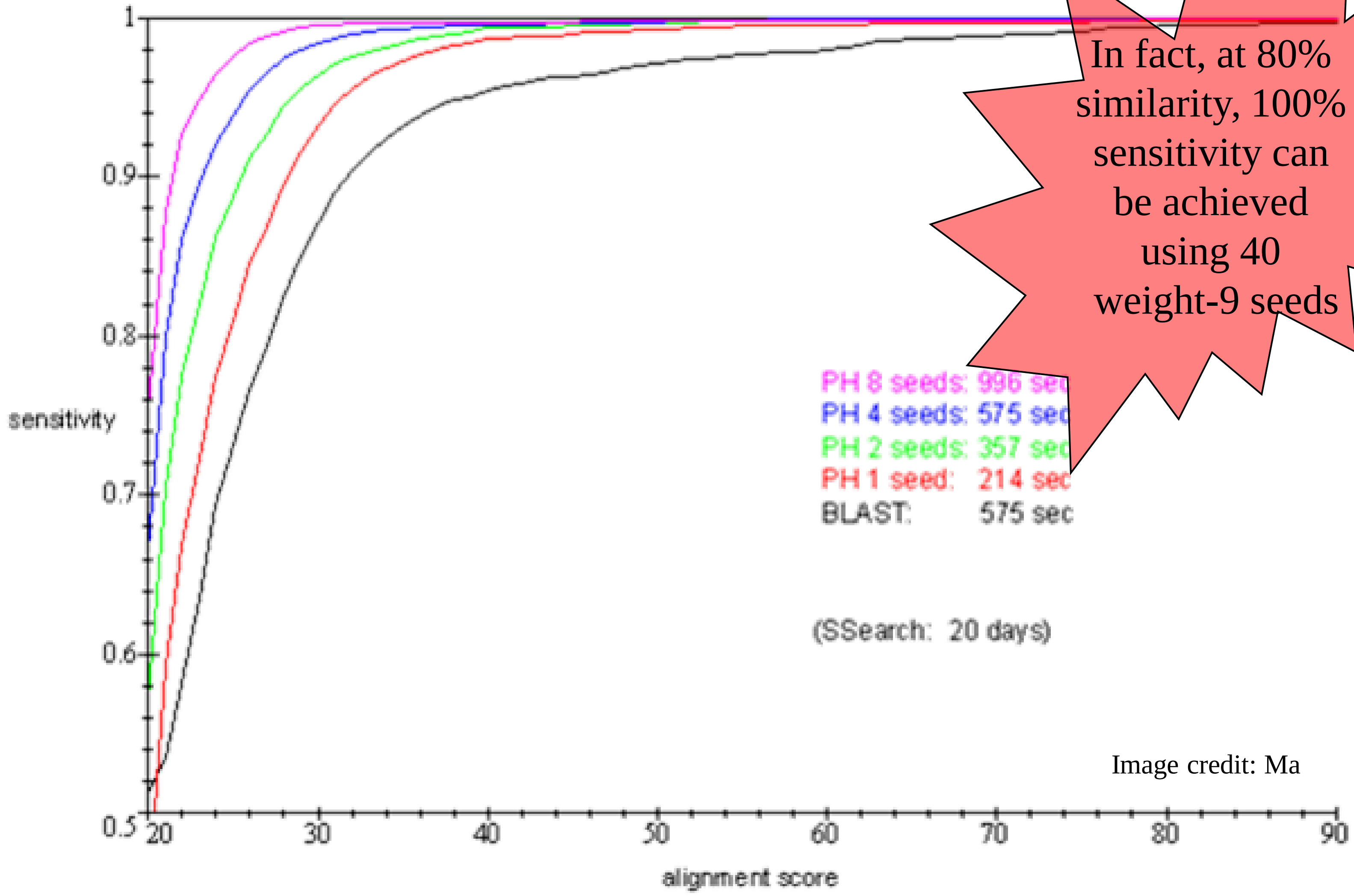
- **Solid curves: Multiple (1, 2, 4, 8,16) weight-12 spaced seeds**
- **Dashed curves: Optimal spaced seeds with weight = 11,10, 9, 8**

$\Rightarrow$ **"Double the seed number" gains better sensitivity than "decrease the weight by 1"**

Expts on real data

- **30k mouse ESTs (25Mb) vs 4k human ESTs (3Mb)**
  - downloaded from NCBI genbank
  - "low complexity" regions filtered out
- **SSearch (Smith-Waterman method) finds "all" pairs of ESTs with significant local alignments**
- **Check how many percent of these pairs can be "found" by BLAST and different configurations of PatternHunter II**

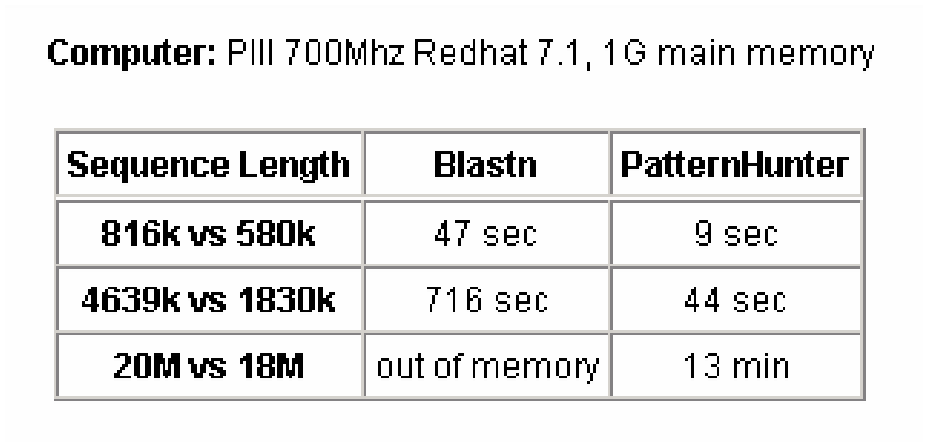
Results



In fact, at 80% similarity, 100% sensitivity can be achieved using 40 weight-9 seeds

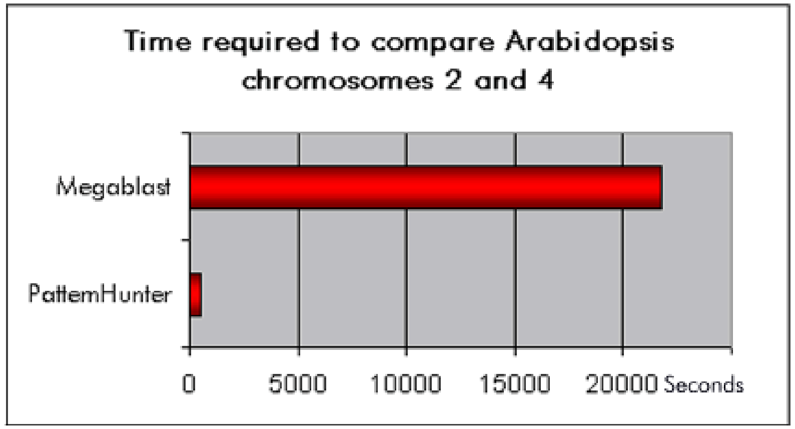
Image credit: Ma

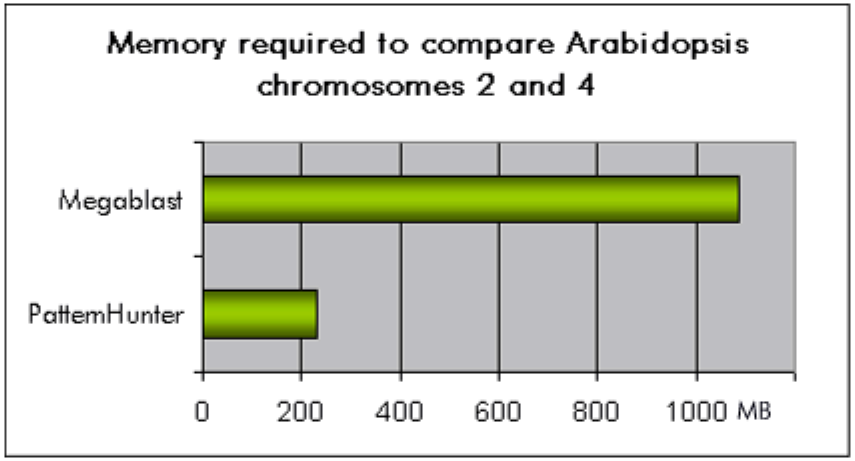
Farewell to Supercomputer Age of sequence comparison!



Computer: PIII 700Mhz Redhat 7.1, 1G main memory

Sequence Length	Blastn	PatternHunter
816k vs 580k	47 sec	9 sec
4639k vs 1830k	716 sec	44 sec
20M vs 18M	out of memory	13 min





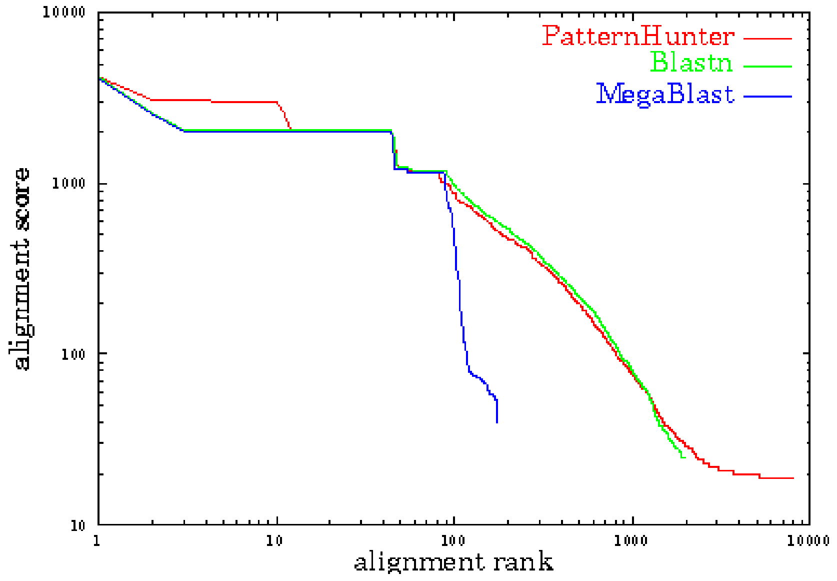


Image credit: Bioinformatics Solutions Inc

About the inventor: Ming Li

- **Ming Li**
  - Canada Research Chair Professor of Bioinformatics, University Professor, Univ of Waterloo
  - Fellow, Royal Society of Canada. Fellow, ACM. Fellow, IEEE

Concluding remarks

What have we learned?

- **General methodology**
  - Dynamic programming
- **Dynamic programming applications**
  - Pairwise Alignment
    - **Needleman-Wunsch global alignment algorithm**
    - **Smith-Waterman local alignment algorithm**
  - Multiple Alignment
- **Important tactics**
  - Indexing & filtering (BLAST)
  - Spaced seeds (Pattern Hunter)

Any question?

Acknowledgements

- Some slides on popular sequence alignment tools are based on those given to me by Bin Ma and Dong Xu

- Some slides on Needleman-Wunsch, Smith-Waterman, and scoring functions are based on those given to me by Ken Sung

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