For written notes on this lecture, please read chapter 10 of *The Practical Bioinformatician*

CS2220: Introduction to Computational Biology

Lecture 6: Essence of Sequence Comparison

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Plan

- **Dynamic Programming**
- **String Comparison**

- **Sequence Alignment**
  - Pairwise Alignment
    - **Needleman-Wunsch global alignment algorithm**
    - **Smith-Waterman local alignment algorithm**
  - Multiple Alignment

- **Popular tools**
  - FASTA, BLAST, Pattern Hunter

What is Dynamic Programming

The Knapsack Problem

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

- **The 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 so as to maximize the total benefit?
- **A dynamic programming solution**
  - Let w_j and b_j be weight and benefit for item *j.* Let *g*(*w*) be max benefit that can be gained from a *w*-pound knapsack. Then *g*(*w*) relates to previously calculated *g* values as follows:

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

Why is g(w) optimal?

An Example

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

- **Suppose the items are**

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

- **Recall that**

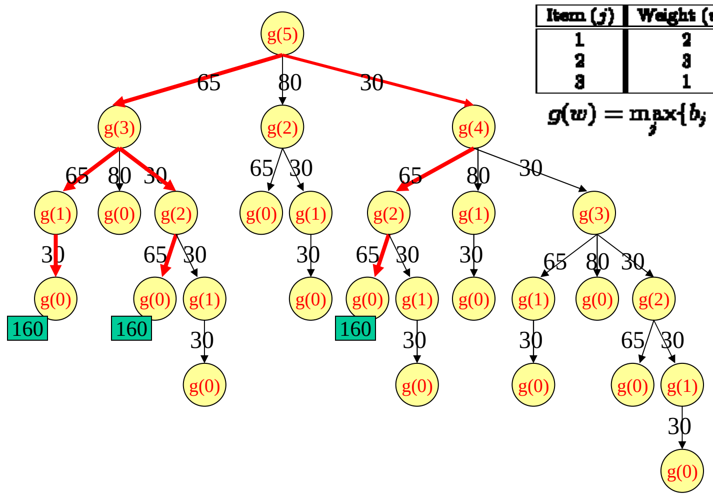
$$g(w) = \max_j \{b_j + g(w - w_j)\}$$

- **To fill a *w* pound knapsack, we must end off by adding some item. If we add item *j*, we end up with a knapsack of size *w – wj* to fill …**

- **To illustrate:**
  - 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**

$\Rightarrow$ **For knapsack of capacity 5, max benefit is 160, which is gained by adding 2 of item 1 and 1 of item 3**

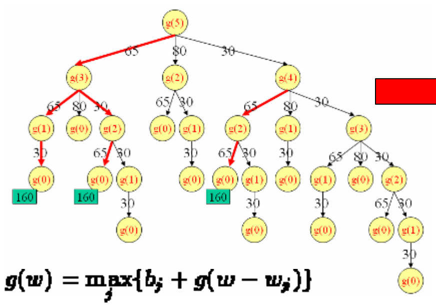
g(1), g(2), … are computed many times



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

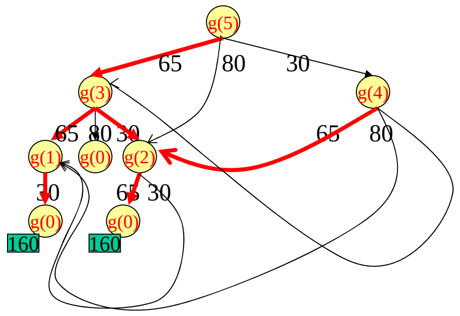
$$g(w) = \max_j \{b_j + g(w - w_j)\}$$

“Memoize” to avoid recomputation



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

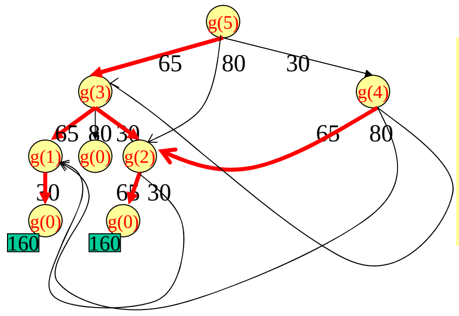
```
int s[]; s[0] := 0;
g'(w) = if s[w] is defined
    then return s[w];
    else {
        s[w] := $\max_j\{b_j + g'(w - w_j)\}$;
        return s[w]; }
```



Non-Recursive Version

```
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

Characteristics of Dynamic Programming

Source: http://mat.gsia.cmu.edu/classes/dynamic/node4.html

- The problem can be divided into *stages* with a *decision* required at each stage
- Each stage has a number of *states* associated
- The decision at one stage transforms one state into a state in the next stage
- Given current state, the optimal decision for each remaining states does not depend on previous states or decisions
- There is a recursive relationship that identifies the optimal decision for stage *j*, given stage *j*+1 has already been solved
- The final stage must be solvable by itself

Exercise: What is a stage in the Knapsack problem?

Exercise: What is a state in the Knapsack problem?

E.g., g(2) doesnt depends on g(3)

E.g., g(0) = 0

Sequence Alignment

Motivations for Sequence Comparison

- DNA is blue print for living organisms

⇒ Evolution is related to changes in DNA

⇒ By comparing DNA sequences we can infer evolutionary relationships between the sequences w/o knowledge of the evolutionary events themselves

- Foundation for inferring function, active site, and key mutations

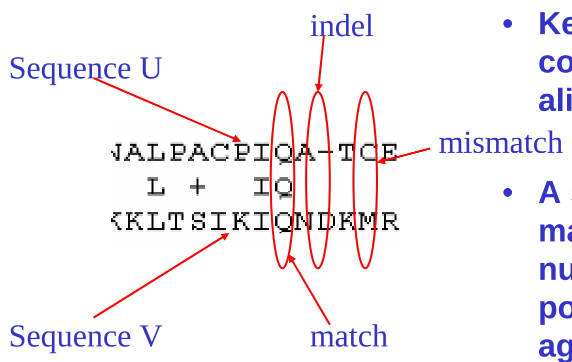
Earliest Research in Seq Comparison

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

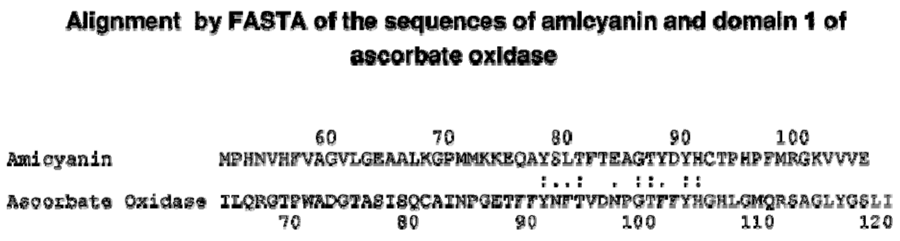


- **Key aspect of seq comparison is seq alignment**
- **A seq alignment maximizes the number of positions that are in agreement in two sequences**

Sequence Alignment: Poor Example

- **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

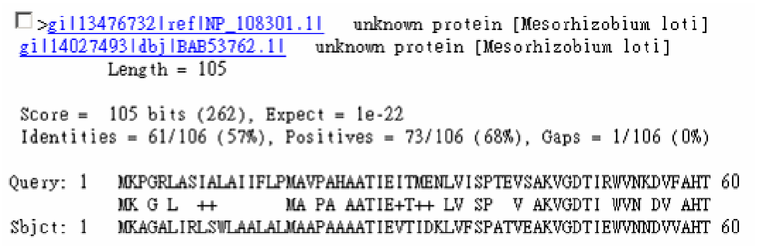
```
                           60        70        80        90       100
Amicyanin         MPHNVHFVAGVLGEAALKGPMMKKEQAYSLTFTEAGTYDYHCTPHPFMRGKVVVE
                                             :..:  . ::. ::
Ascorbate Oxidase ILQRGTPWADGTASISQCAINPGETFFYNFTVDNPGTFFYHGHLGMQRSAGLYGSLI
                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

No obvious match between Amicyanin and Ascorbate Oxidase

Sequence Alignment: Good Example

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

$\Rightarrow$ **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

- **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)*
  - $f(n,n) \sim (1 + \sqrt{2})^{2n+1} n^{-1/2}$

Exercise: Explain the recurrence above

- **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**

Global Pairwise Alignment:

Dynamic Programming Solution

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

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

Exercise: What is the effect of a large δ ?

This is the basic idea of the Needleman-Wunsch algorithm

Needleman-Wunsch Algorithm (I)

Source: Ken Sung

- Consider two strings S[1..n] and T[1..m]
- Let V(i, j) be score of opt alignment betw S[1..i] and T[1..j]
- Basis:
  - V(0, 0) = 0
  - V(0, j) = V(0, j –1) – δ
    - Insert j times
  - V(i, 0) = V(i – 1, 0) – δ
    - Delete i times

Needleman-Wunsch Algorithm (II)

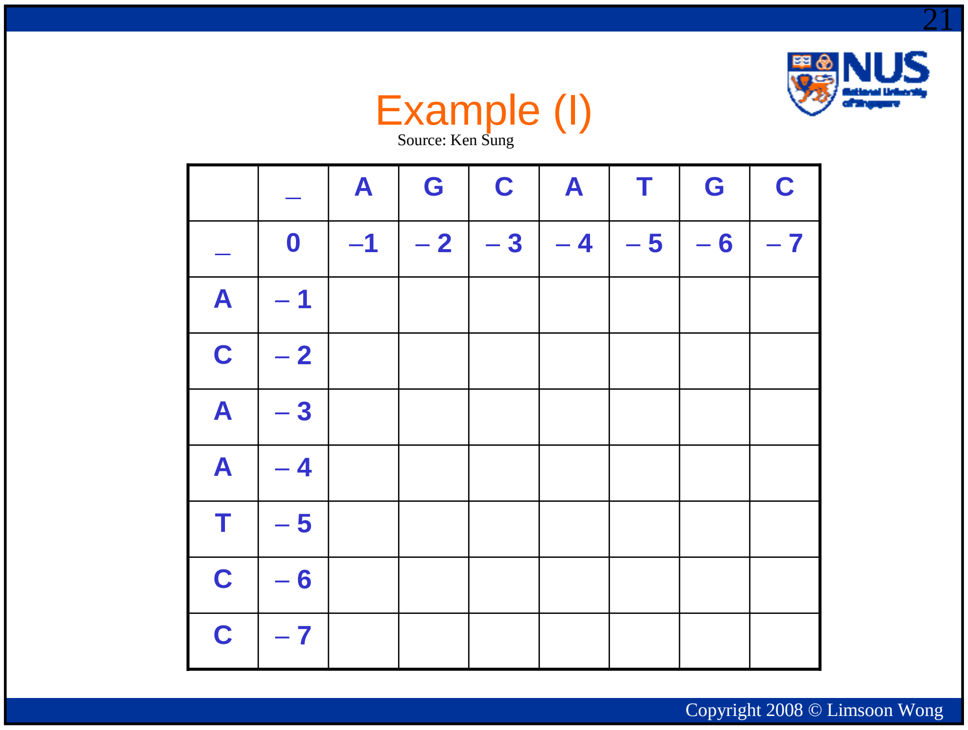
Source: Ken Sung

- Recurrence: For i>0, j>0

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

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

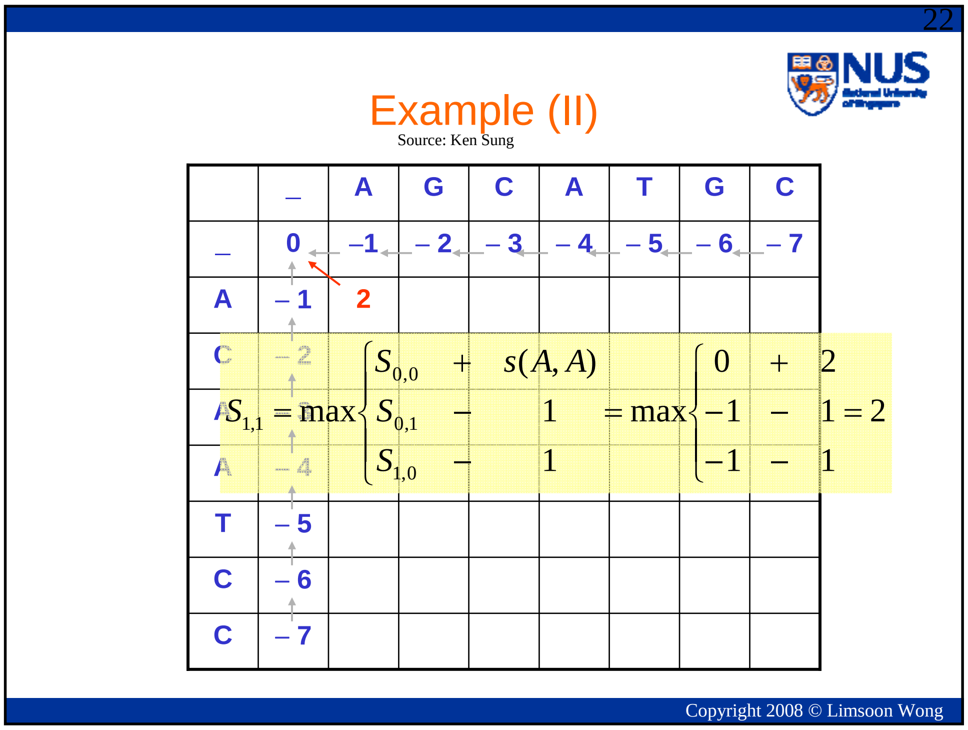
```
xxx…xx        xxx…xx      xxx…x_
     |             |           |
xxx…yy        yyy…y_      yyy…yy
Match/mismatch   Delete      Insert
```



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							

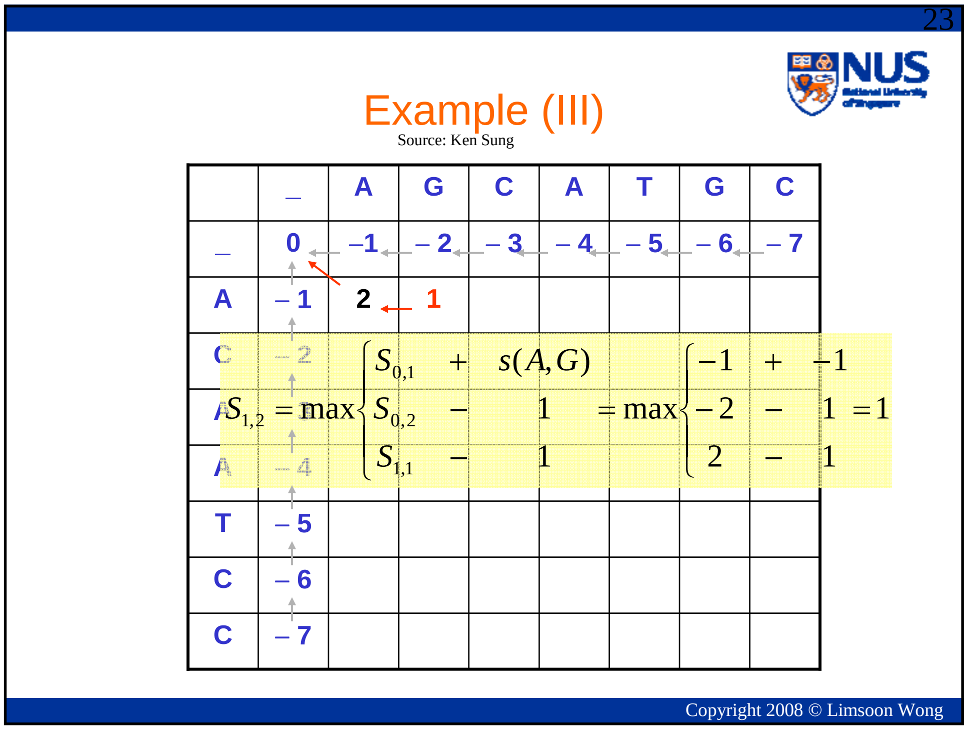


Example (II)

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							

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

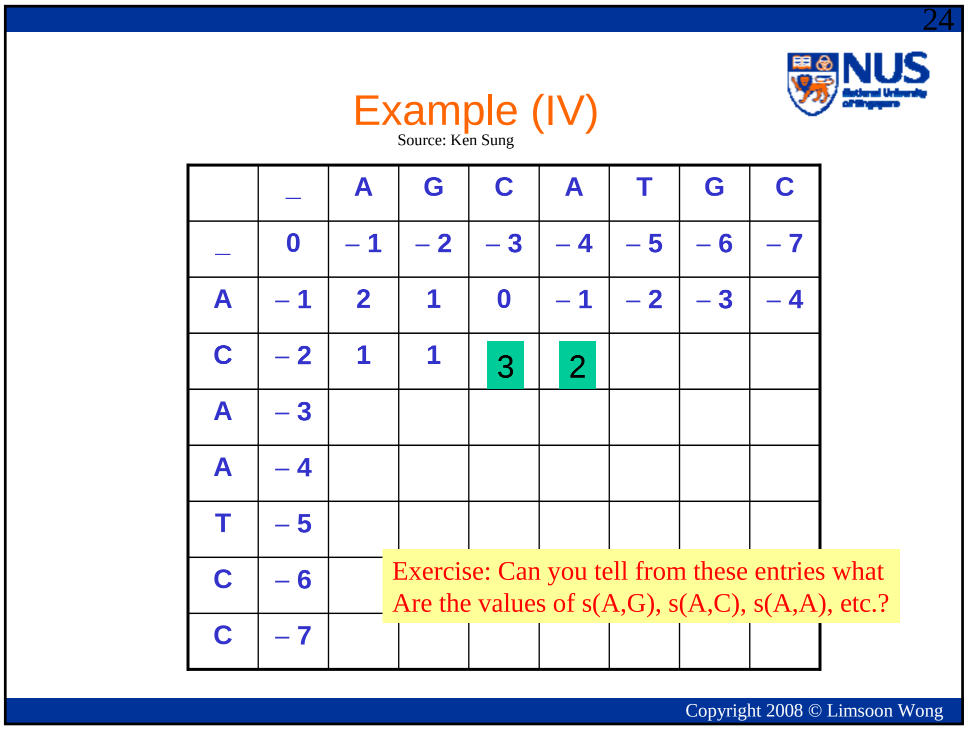


Example (III)

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)

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							

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

Example (V)

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] – δ ;
For i=1 to n, set V[i,0] := V[i – 1,0] – δ ;
For j=1 to m {
   For i = 1 to n {
       set V[i,j] := V[i,j – 1] – δ ;
       set P[i,j] := (0, – 1);
       if V[i,j] < V[i – 1,j] – δ then
              set V[i,j] := V[i – 1,j] – δ ;
              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!**

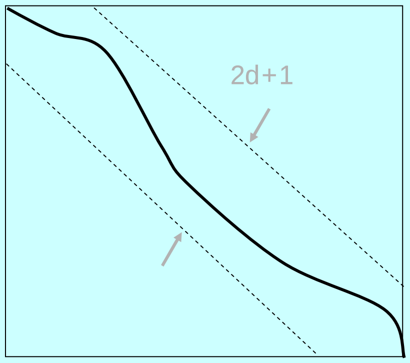
O(dn)-Time Algorithm

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

Recursive Equation for O(dn)-Time Algo

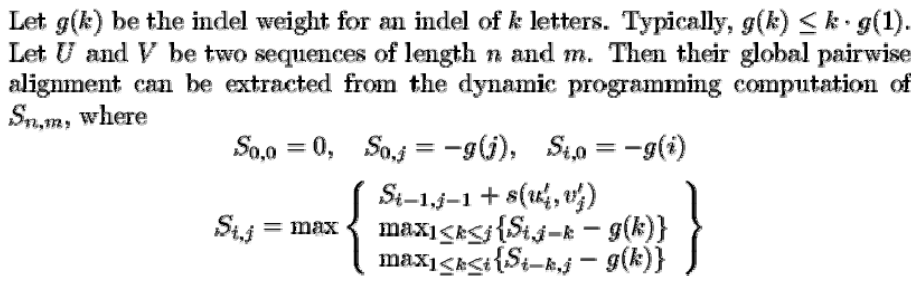
$$v(i,j,d) = \max\begin{cases} v(i-1,j-1,d) + s(S[i],S[j]) & \\ v(i-1,j,d-1) - \delta & if\ \ d > 0 \\ v(i,j-1,d-1) - \delta & if\ \ d > 0 \end{cases}$$

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

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, $g(p+q) \le g(p) + g(q)$**

- **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 Algorithm 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 Algorithm w/ General Gap Penalty (II)

Source: Ken Sung

- Recurrence for i>0 and j>0,

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

Analysis

Source: Ken Sung

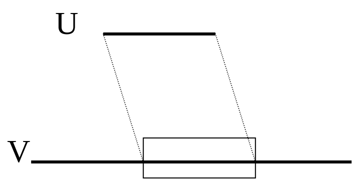
- We need to fill in all entries in the n×m table
- Each entry can be computed in O(max{n, m}) time

⇒ Time complexity = O(nm max{n, m})

⇒ Space complexity = O(nm)

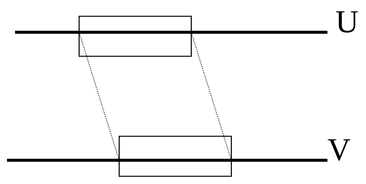
Variations of Pairwise Alignment

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



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

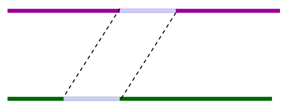
- **Find "local" alignment**



- **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

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:**

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

- 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)

Source: Ken Sung

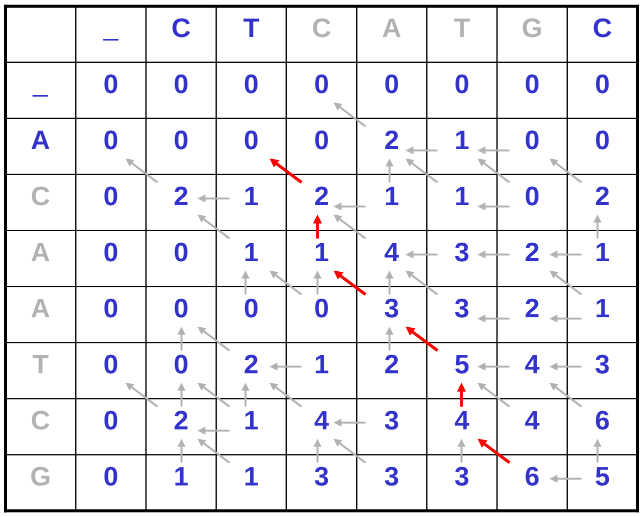
	_	C	T	C	A	T	G	C
_	0	0	0	0	0	0	0	0
A	0	0	0	0	2	1	0	0
C	0	2	1	2	1	1	0	2
A	0	0	1	1	4	3	2	1
A	0	0	0	0	3	3	2	1
T	0	0	2	1	2			
C								
G								

Example (III)

Source: Ken Sung

CAATCG

C_AT_G



	_	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	5	4	3
C	0	2	1	4	3	4	4	6
G	0	1	1	3	3	3	6	5

Analysis

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 = ??

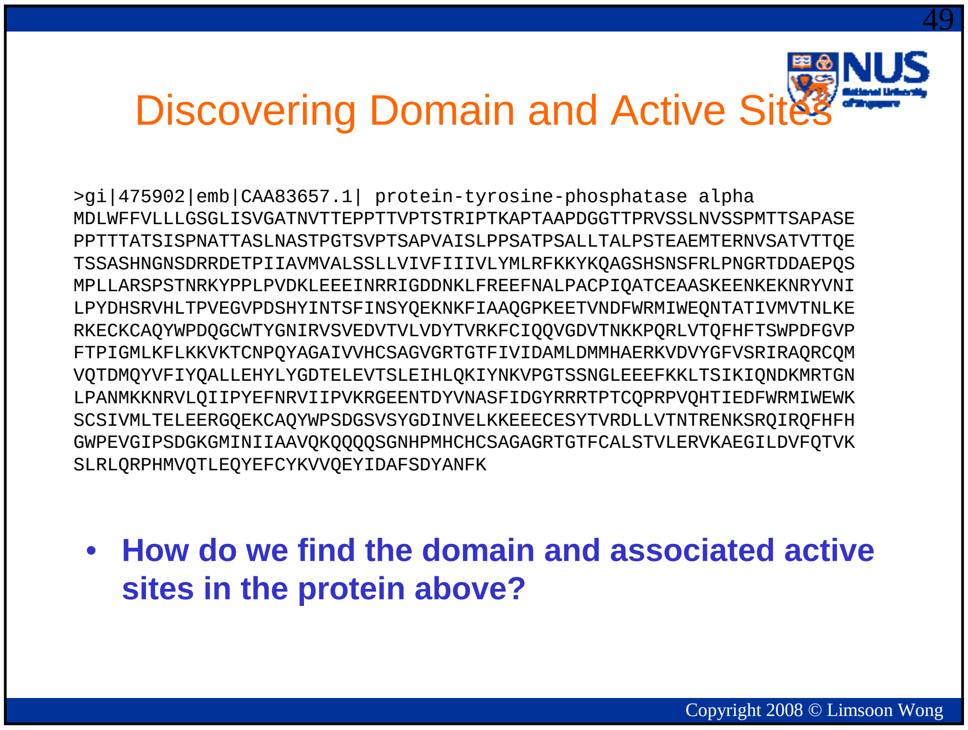
⇒Space complexity = O(nm)

Exercise: What is the time complexity?

Multiple Sequence Alignment

What is a domain

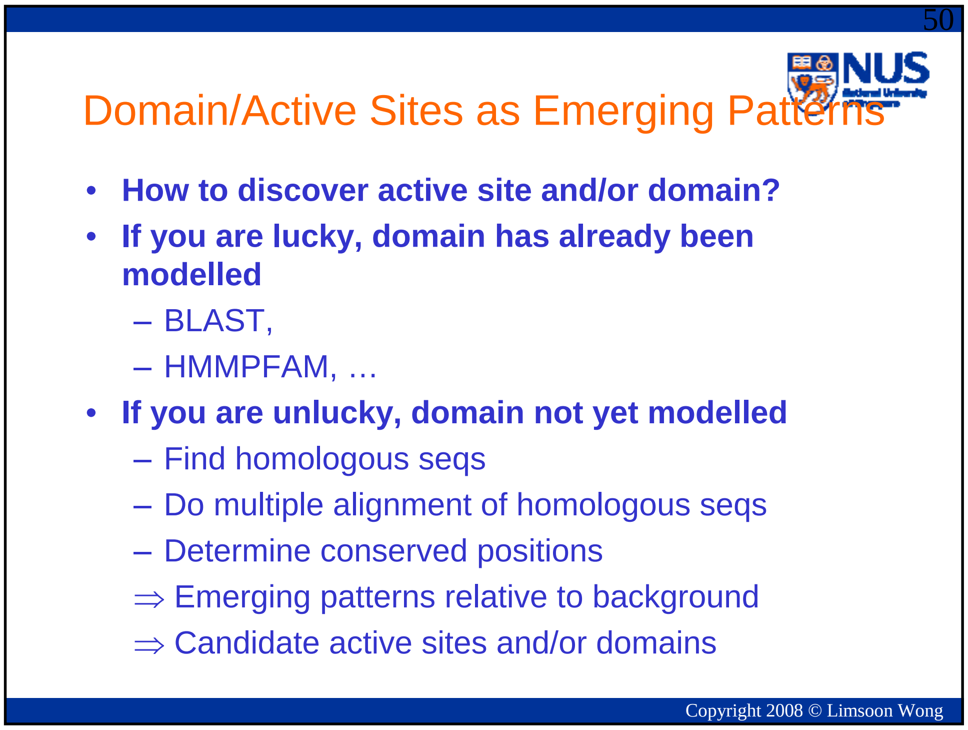
- **A domain is a component of a protein that is self-stabilizing and folds independently of the rest of the protein chain**
  - Not unique to protein products of one gene; can appear in a variety of proteins
  - Play key role in the biological function of proteins
  - Can be "swapped" by genetic engineering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

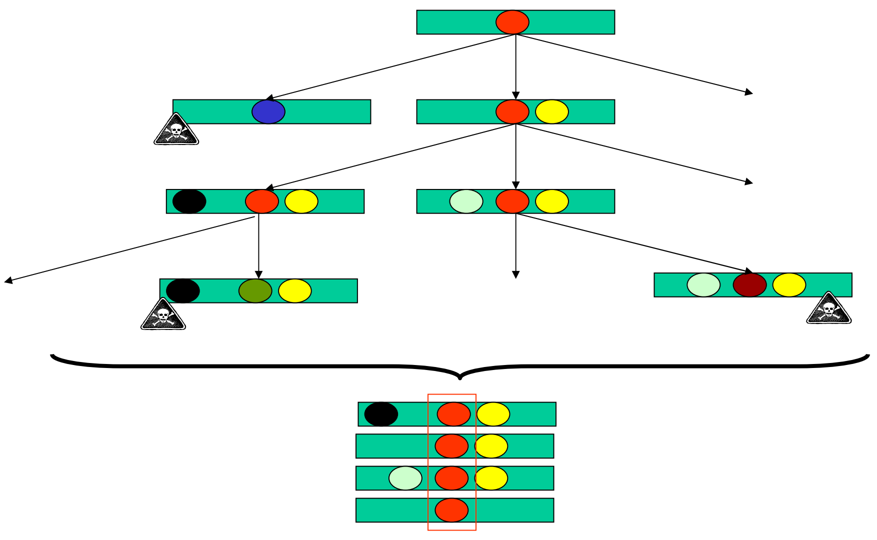
- **How do we find the domain and associated active sites in the protein above?**



Domain/Active Sites as Emerging Patterns

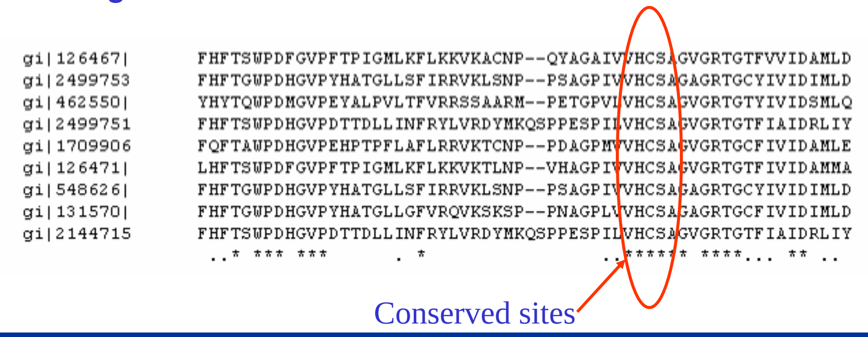
- **How to discover active site and/or domain?**
- **If you are lucky, domain has already been modelled**
  - BLAST,
  - HMMPFAM, …
- **If you are unlucky, domain not yet modelled**
  - Find homologous seqs
  - Do multiple alignment of homologous seqs
  - Determine conserved positions
  - ⇒ Emerging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

In the course of evolution…



Multiple Alignment: An 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|       FHFTSWPDFGVPFFTPIGMLKFLKKVKACNP--QYAGAIVVHCSAGVGRTGTFVVIDAMLD
gi|2499753       FHFTGWPDHGVPYHATGLLSFIRRVKLSNP--PSAGPIVVHCSAGAGRTGCYIVIDIMLD
gi|462550|       YHYTQWPDMGVPEYALPVLTFVRRSSAARM--PETGPVLVHCSAGVGRTGTYIVIDSMLQ
gi|2499751       FHFTSWPDHGVPDTTDLLINFRYLVRDYMKQSPPESPILVHCSAGVGRTGTFIAIDRLIY
gi|1709906       FQFTAWPDHGVPEHPTPFLAFLRRVKTCNP--PDAGPMVVHCSAGVGRTGCFIVIDAMLE
gi|126471|       LHFTSWPDFGVPFFTPIGMLKFLKKVKTLNP--VHAGPIVVHCSAGVGRTGTFIVIDAMMA
gi|548626|       FHFTGWPDHGVPYHATGLLSFIRRVKLSNP--PSAGPIVVHCSAGAGRTGCYIVIDIMLD
gi|131570|       FHFTGWPDHGVPYHATGLLGFVRQVKSKSP--PNAGPLVVHCSAGAGRTGCFIVIDIMLD
gi|2144715       FHFTSWPDHGVPDTTDLLINFRYLVRDYMKQSPPESPILVHCSAGVGRTGTFIAIDRLIY
                  ..* *** ***      . *                 ..****** ****... ** ..
```

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

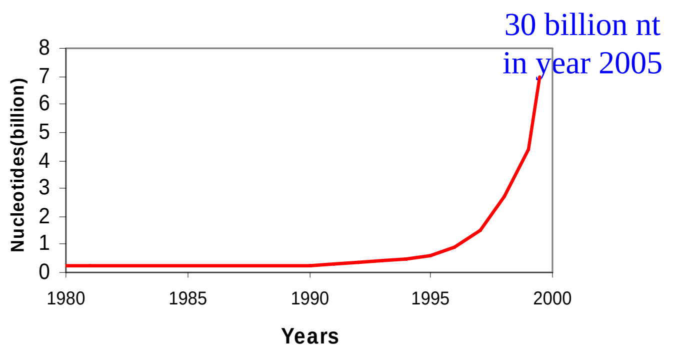
$$\epsilon_i \cdot a = \begin{cases} a & \text{if } \epsilon_i = 1 \\ - & \text{if } \epsilon_i = 0 \end{cases}$$

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

Exercise for the Brave:
Propose a practical approximation

Popular Tools for Sequence Comparison: FASTA, BLAST, Pattern Hunter

Scalability of Software



- **Increasing number of sequenced genomes: yeast, human, rice, mouse, fly, …**
- **S/w must be "linearly" scalable to large datasets**

Need Heuristics for Sequence Comparison

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

⇒ **Given current size of sequence databases, use of optimal algorithms is not practical for database search**

- **Heuristic techniques:**
  - BLAST
  - FASTA
  - Pattern Hunter
  - MUMmer, ...
- **Speed up:**
  - 20 min (optimal alignment)
  - 2 min (FASTA)
  - 20 sec (BLAST)

Exercise: Describe MUMer

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**

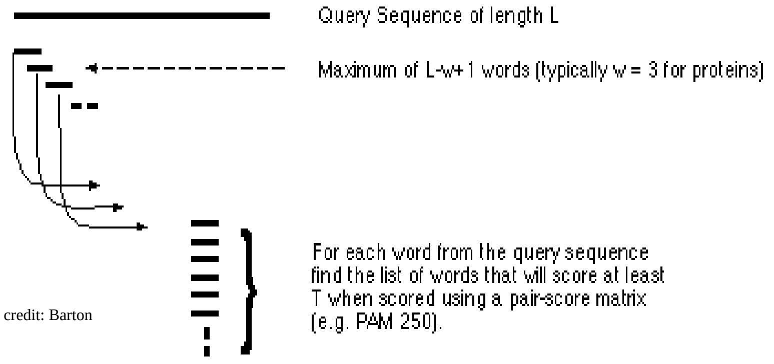

Query Sequence of length L
Maximum of L-w+1 words (typically w = 3 for proteins)
For each word from the query sequence find the list of words that will score at least T when scored using a pair-score matrix (e.g. PAM 250).

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**

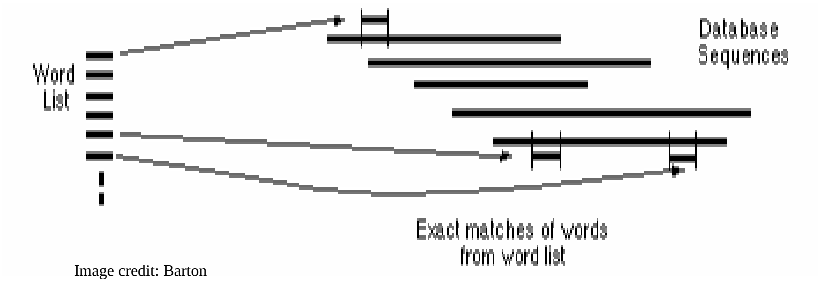


Image credit: Barton

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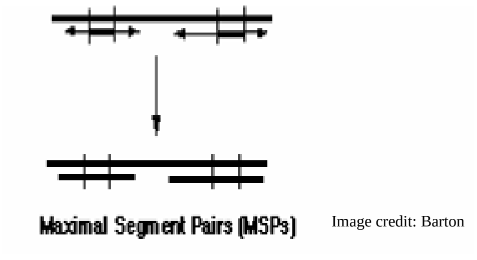
BLAST in 3 Steps

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**



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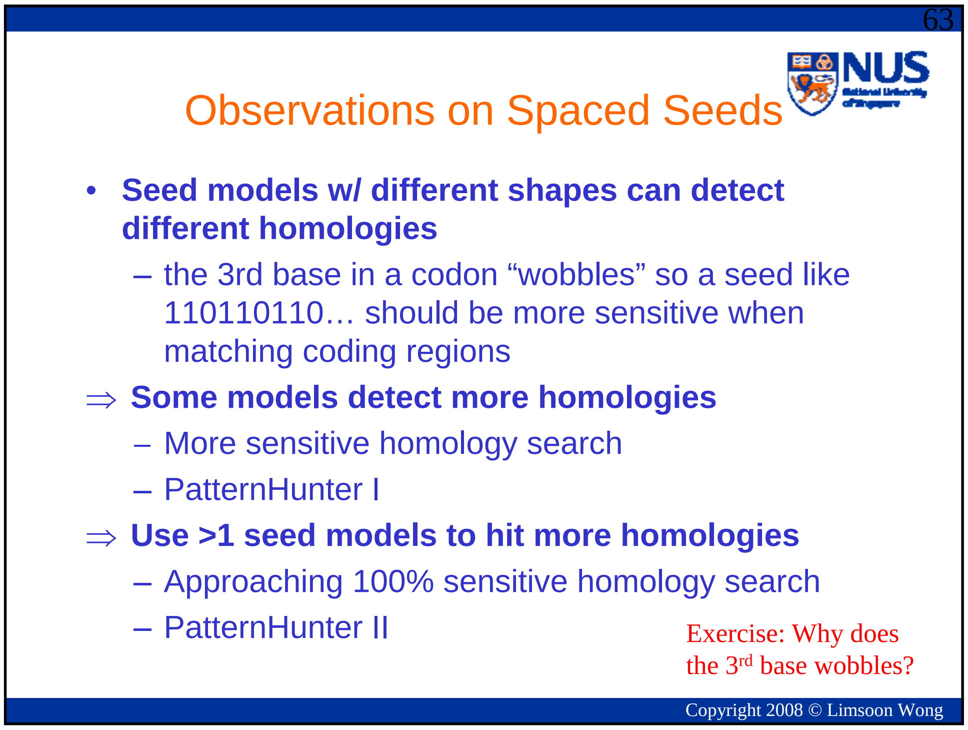
Spaced Seeds

- **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**



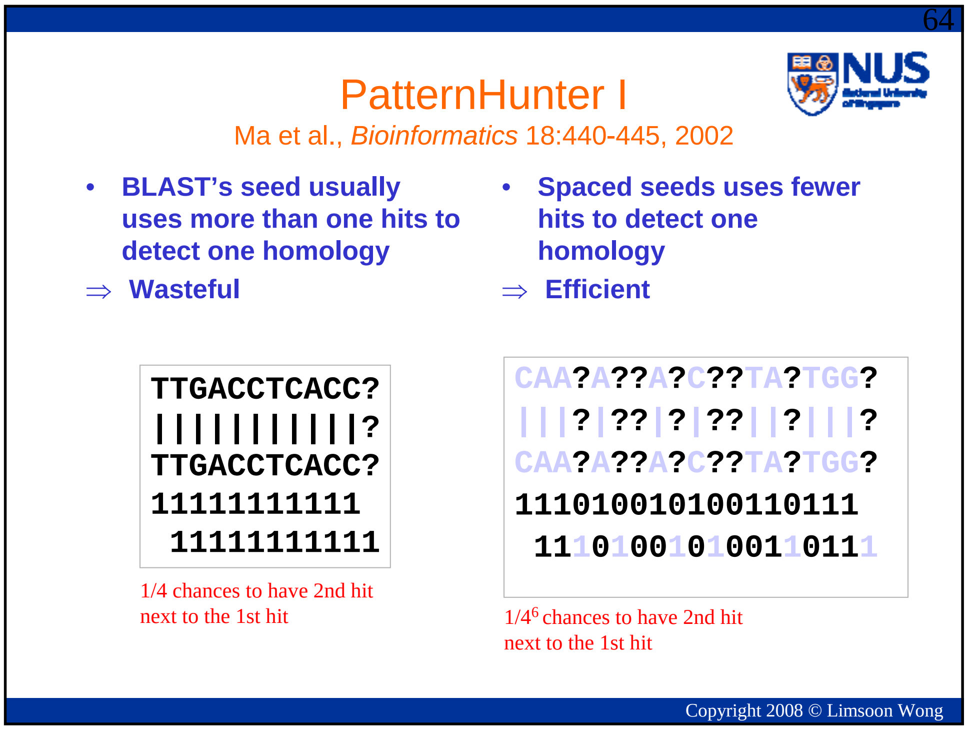
Observations on Spaced Seeds

- **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*

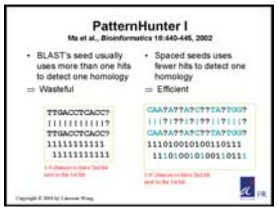
Proof.

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

⇒ Wasteful

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

[illegible]

- Spaced seeds uses fewer hits to detect one homology

⇒ Efficient

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

[illegible]

Copyright © 2004 by Limsoon Wong

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.

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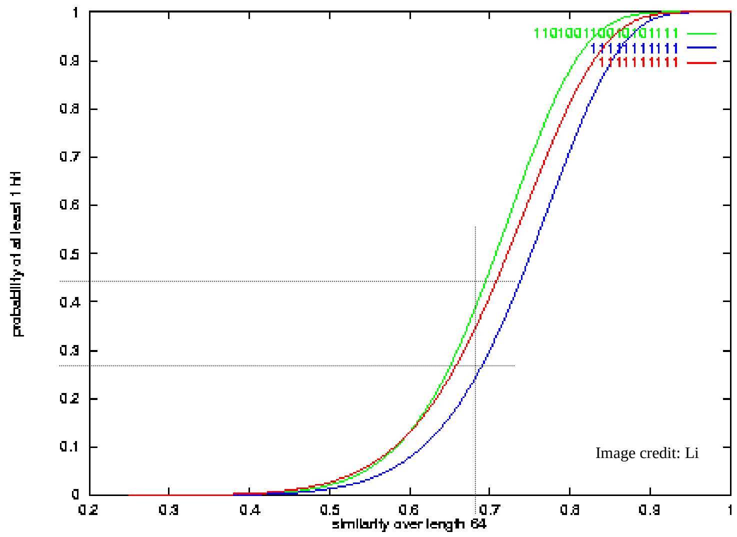
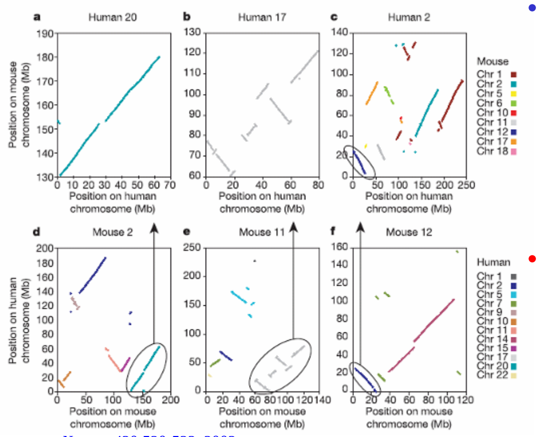
- **For *L = 1017***
  - BLAST seed expects *(1017 – 11 + 1) * p^{11} = 1007 * p^{11}* hits
  - But *~1/4* of these overlap each other. So likely to have only *~750 * p^{11}* distinct hits
  - Our example spaced seed expects *(1017 – 18 + 1) * p^{11} = 1000 * p^{11}* hits
  - But only *$1/4^6$* of these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



Speed of PatternHunter I



Nature, 420:520-522, 2002

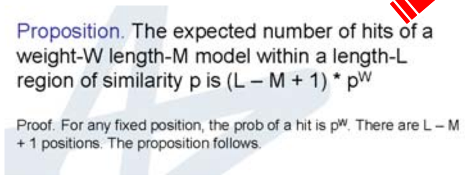
- Mouse Genome Consortium used PatternHunter to compare mouse genome & human genome
- PatternHunter did the job in a 20 CPU-days --- it would have taken BLAST 20 CPU-years!

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: Proof this claim

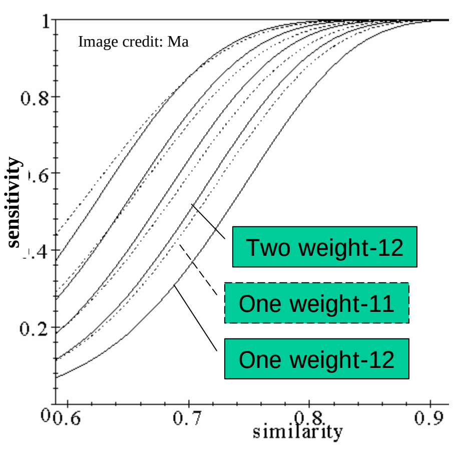
PatternHunter II

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 ∪ {s} has the highest hit probability
  - A = A ∪ {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



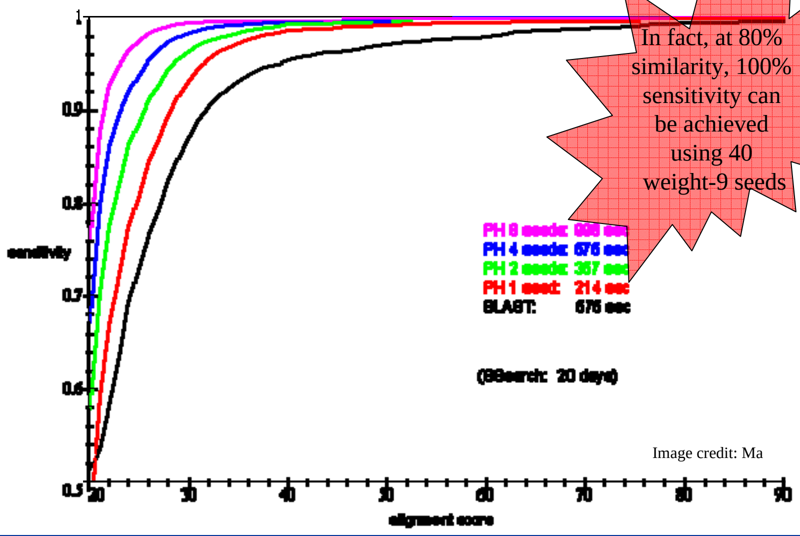
- **Solid curves: Multiple (1, 2, 4, 8,16) weight-12 spaced seeds**
- **Dashed curves: Optimal spaced seeds with weight = 11,10, 9, 8**

⇒ **"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



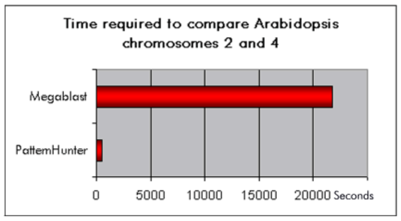
In fact, at 80% similarity, 100% sensitivity can be achieved using 40 weight-9 seeds

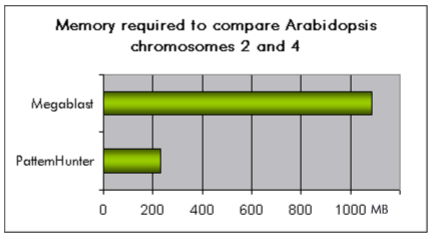
Image credit: Ma

Farewell to the 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





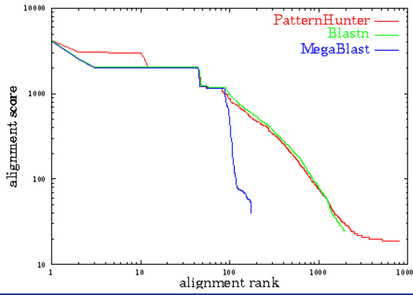


Image credit: Bioinformatics Solutions Inc

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 and Smith-Waterman are based on those given to me by Ken Sung**

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