CSE 527
Computational Biology
Autumn 2006

Lectures 2-3

Sequence Alignment;

DNA Replication

This week

- Sequence alignment
- More sequence alignment
- Weekly "bio" interlude - DNA replication

Sequence Alignment

Part I

Motivation, dynamic programming, global alignment

Sequence Alignment

- What
- Why
- A Simple Algorithm
- Complexity Analysis
- A better Algorithm:
  "Dynamic Programming"

Sequence Similarity: What

G G A C C A

T A C T A A G

T C C A A T

Sequence Similarity: What

G G A C C A

```
T A C T A A G
| : | : | | :
T C C – A A T
```


Sequence Similarity: Why

- Most widely used comp. tools in biology
- New sequence always compared to sequence data bases
- **Similar sequences often have similar origin or function**
- Selection operates on system level, but mutation occurs at the sequence level
- Recognizable similarity after $10^8 – 10^9$ yr

BLAST Demo

http://www.ncbi.nlm.nih.gov/blast/

Taxonomy Report

```
root ................................   64 hits   16 orgs
. Eukaryota .........................   62 hits   14 orgs [cellular organisms]
. . Fungi/Metazoa group .............   57 hits   11 orgs
. . . Bilateria .....................   38 hits    7 orgs [Metazoa; Eumetazoa]
. . . . Coelomata ...................   36 hits    6 orgs
. . . . . Tetrapoda .................   26 hits    5 orgs [;;; Vertebrata;;;; Sarcopterygii]
. . . . . . Eutheria ................   24 hits    4 orgs [Amniota; Mammalia; Theria]
. . . . . . . Homo sapiens ..........   20 hits    1 orgs [Primates;; Hominidae; Homo]
. . . . . . . Murinae ...............    3 hits    2 orgs [Rodentia; Sciurognathi; Muridae]
. . . . . . . . Rattus norvegicus ....    2 hits    1 orgs [Rattus]
. . . . . . . . Mus musculus ........    1 hits    1 orgs [Mus]
. . . . . . . Sus scrofa ............    1 hits    1 orgs [Cetartiodactyla; Suina; Suidae; Sus]
. . . . . . Xenopus laevis ..........    2 hits    1 orgs [Amphibia;;;;;; Xenopodinae; Xenopus]
. . . . . Drosophila melanogaster ....   10 hits    1 orgs [Protostomia;;;; Drosophila;;;]
. . . . Caenorhabditis elegans .......    2 hits    1 orgs [; Nematoda;;;;;; Caenorhabditis]
. . . Ascomycota ....................   19 hits    4 orgs [Fungi]
. . . . Schizosaccharomyces pombe ....   10 hits    1 orgs [;;;; Schizosaccharomyces]
. . . . Saccharomycetales ...........    9 hits    3 orgs [Saccharomycotina; Saccharomycetes]
. . . . . Saccharomyces .............    8 hits    2 orgs [Saccharomycetaceae]
. . . . . . Saccharomyces cerevisiae .    7 hits    1 orgs
. . . . . . Saccharomyces kluyveri ...    1 hits    1 orgs
. . . . . Candida albicans ..........    1 hits    1 orgs [mitosporic Saccharomycetales;]
. . Arabidopsis thaliana ............    2 hits    1 orgs [Viridiplantae; …Brassicaceae;]
. . Apicomplexa .....................    3 hits    2 orgs [Alveolata]
. . . Plasmodium falciparum .........    2 hits    1 orgs [Haemosporida; Plasmodium]
. . . Toxoplasma gondii .............    1 hits    1 orgs [Coccidia; Eimeriida; Sarcocystidae;]
. synthetic construct ...............    1 hits    1 orgs [other; artificial sequence]
  lymphocystis disease virus ........    1 hits    1 orgs [Viruses; dsDNA viruses, no RNA …]
```

Terminology (CS, not necessarily Bio)

- *String*: ordered list of letters TATAAG
- *Prefix:* consecutive letters from front
  - empty, T, TA, TAT, ...
- Suffix: … from end
  - empty, G, AG, AAG, ...
- *Substring:* … from ends or middle
  - empty, TAT, AA, ...
- *Subsequence:* ordered, nonconsecutive
  - TT, AAA, TAG, ...

Sequence Alignment

```
a c b c d b        a c – – b c d b
c a d b d          – c a d b – d –
```

Defn: An *alignment* of strings S, T is a pair of strings S', T' (with spaces) s.t.

(1) |S'| = |T'|, and (|S| = "length of S")

(2) removing all spaces leaves S, T

Alignment Scoring

Mismatch = -1
Match = 2

```
a c b c d b      a  c  –  –  b  c  d  b
c a d b d        –  c  a  d  b  –  d  –
                -1  2 -1 -1  2 -1  2 -1
                Value = 3*2 + 5*(-1) = +1
```

- The *score* of aligning (characters or spaces) x & y is $\sigma(x,y)$.
- *Value* of an alignment $\sum_{i=1}^{|S'|}\sigma(S'[i],T'[i])$
- An *optimal alignment:* one of max value

Optimal Alignment: A Simple Algorithm

for all subseqs A of S, B of T s.t. |A| = |B| **do**
  align A[i] with B[i], $1 \le i \le |A|$
  align all other chars to spaces
  compute its value
  retain the max
end
output the retained alignment

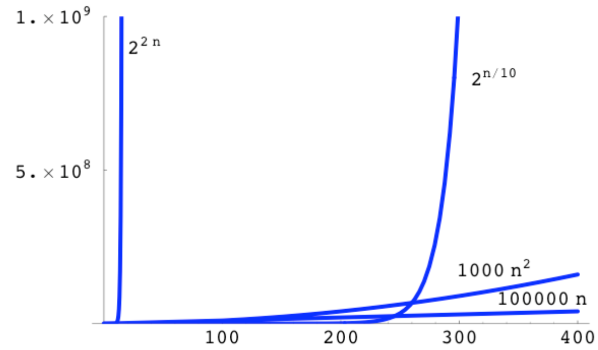
S = abcd A = cd
T = wxyz B = xz

```
-abc-d   a-bc-d
w--xyz   -w-xyz
```

Analysis

- Assume |S| = |T| = n
- Cost of evaluating one alignment: ≥ n
- How many alignments are there: $\geq \binom{2n}{n}$
  - pick n chars of S,T together
  - say k of them are in S
  - match these k to the k *un*picked chars of T
- Total time: $\geq n\binom{2n}{n} > 2^{2n}$, for $n > 3$
- E.g., for n = 20, time is > 2^{40} operations

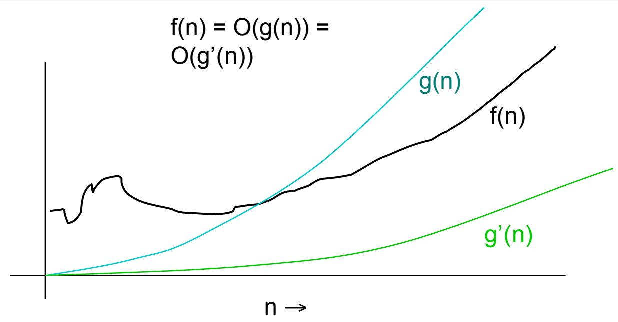
Polynomial vs Exponential Growth



Asymptotic Analysis

- How does run time grow as a function of problem size?
  - n^2 or $100\ n^2 + 100\ n + 100$ vs 2^{2n}
- **Defn:** f(n) = O(g(n)) iff there is a constant c s.t. |f(n)| ≤ cg(n) for all sufficiently large n.
  - $100\ n^2 + 100\ n + 100 = O(n^2)$ [e.g. c = 101]
  - $n^2 = O(2^{2n})$
  - 2^{2n} is *not* $O(n^2)$

Big-O Example



Utility of Asymptotics

- "All things being equal," smaller asymptotic growth rate is better
- All things are never equal
- Even so, big-O bounds often let you quickly pick most promising candidates among competing algorithms
- Poly time algs often practical; non-poly algs seldom are.

Fibonacci Numbers

```
fib(n) {
   if (n <= 1) {
     return 1;
   } else {
     return fib(n-1) + fib(n-2);
   }
}
```

Simple recursion, but many repeated subproblems!!

=>

Time = $\Omega(1.61^n)$

Fibonacci, II

```
int fib[n];
fib[0] = 1;
fib[1] = 1;
for(i=2; i<=n; i++) {
   fib[i] = fib[i-1] + fib[i-2];
}
return fib[n];
```

Avoid repeated subproblems by tabulating them

=>

Time = O(n)

Candidate for Dynamic Programming?

- Common Subproblems?
  - Plausible: probably re-considering alignments of various small substrings unless we're careful.
- Optimal Substructure?
  - Plausible: left and right "halves" of an optimal alignment probably should be optimally aligned (though they obviously interact a bit at the interface).
- (Both made rigorous below.)

Optimal Substructure
(In More Detail)

- Optimal alignment ends in 1 of 3 ways:
  - last chars of S & T aligned with each other
  - last char of S aligned with space in T
  - last char of T aligned with space in S
  - (never align space with space; σ(–, –) < 0)
- In each case, the rest of S & T should be optimally aligned to each other

Optimal Alignment in O(n²) via "Dynamic Programming"

- Input: S, T, |S| = n, |T| = m
- Output: value of optimal alignment

Easier to solve a "harder" problem:

V(i,j) = value of optimal alignment of S[1], …, S[i] with T[1], …, T[j] for all 0 ≤ i ≤ n, 0 ≤ j ≤ m.

Base Cases

- V(i,0): first i chars of S all match spaces

$$V(i,0) = \sum_{k=1}^{i} \sigma(S[k], -)$$

- V(0,j): first j chars of T all match spaces

$$V(0,j) = \sum_{k=1}^{j} \sigma(-, T[k])$$

General Case

Opt align of S[1], …, S[i] vs T[1], …, T[j]:

$$\begin{bmatrix} \sim\sim\sim\sim & S[i] \\ \sim\sim\sim\sim & T[j] \end{bmatrix}, \begin{bmatrix} \sim\sim\sim\sim & S[i] \\ \sim\sim\sim\sim & - \end{bmatrix}, \text{ or } \begin{bmatrix} \sim\sim\sim\sim & - \\ \sim\sim\sim\sim & T[j] \end{bmatrix}$$

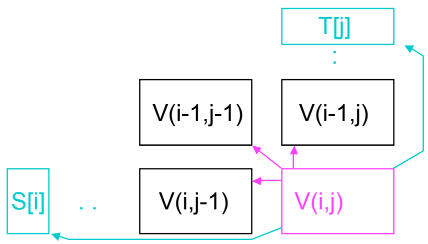
Opt align of $S_1 \ldots S_{i-1}$ & $T_1 \ldots T_{j-1}$

$$V(i,j) = \max \begin{Bmatrix} V(i\text{-}1,j\text{-}1) + \sigma(S[i],T[j]) \\ V(i\text{-}1,j) + \sigma(S[i], -) \\ V(i,j\text{-}1) + \sigma(-, T[j]) \end{Bmatrix},$$

for all $1 \le i \le n,\ 1 \le j \le m.$

Calculating One Entry

$$V(i,j) = \max \begin{cases} V(i\text{-}1,j\text{-}1) + \sigma(S[i],T[j]) \\ V(i\text{-}1,j) \quad + \sigma(S[i],\ -\ ) \\ V(i,j\text{-}1) \quad + \sigma(\ -\ ,\ T[j]) \end{cases}$$



Example

Mismatch = -1
Match = 2

j		0	1	2	3	4	5	
i			c	a	d	b	d	←T
0		0	-1	-2	-3	-4	-5	
1	a	-1	-1	1				
2	c	-2	1					
3	b	-3						
4	c	-4						
5	d	-5						
6	b	-6						

↑S

Time = O(mn)

Example

Mismatch = -1
Match = 2

j		0	1	2	3	4	5	
i			c	a	d	b	d	←T
0		0	-1	-2	-3	-4	-5	
1	a	-1	-1	1	0	-1	-2	
2	c	-2	1	0	0	-1	-2	
3	b	-3	0	0	-1	2	1	
4	c	-4	-1	-1	-1	1	1	
5	d	-5	-2	-2	1	0	3	
6	b	-6	-3	-3	0	3	2	

↑S

Finding Alignments: Trace Back

j		0	1	2	3	4	5	
i			c	a	d	b	d	←T
0		0	-1	-2	-3	-4	-5	
1	a	-1	-1	1	0	-1	-2	
2	c	-2	1	0	0	-1	-2	
3	b	-3	0	0	-1	2	1	
4	c	-4	-1	-1	-1	1	1	
5	d	-5	-2	-2	1	0	3	
6	b	-6	-3	-3	0	3	2	

↑S

Complexity Notes

- Time = O(mn), (value and alignment)
- Space = O(mn)
- Easy to get value in Time = O(mn) and Space = O(min(m,n))
- Possible to get value *and alignment* in Time = O(mn) and Space = O(min(m,n)) but tricky.

Sequence Alignment

Part II

Local alignments & gaps

Variations

- Local Alignment
  - Preceding gives *global* alignment, i.e. full length of both strings;
  - Might well miss strong similarity of part of strings amidst dissimilar flanks
- Gap Penalties
  - 10 adjacent spaces cost 10 x one space?
- Many others

Local Alignment:Motivations

- "Interesting" (evolutionarily conserved, functionally related) segments may be a small part of the whole
  - "Active site" of a protein
  - Scattered genes or exons amidst "junk", e.g. retroviral insertions, large deletions
  - Don't have whole sequence
- Global alignment might miss them if flanking junk outweighs similar regions

Local Alignment

Optimal *local alignment* of strings S & T:
Find substrings A of S and B of T
having max value global alignment

S = abcxdex   A = c x d e
T = xxxcde   B = c - d e   value = 5

The "Obvious" Local Alignment Algorithm

for all substrings A of S and B of T
  Align A & B via dynamic programming
  Retain pair with max value
end ;
Output the retained pair

Time: $O(n^2)$ choices for A, $O(m^2)$ for B, $O(nm)$ for DP, so $O(n^3m^3)$ total.

[Best possible? Lots of redundant work…]

Local Alignment in O(nm) via Dynamic Programming

- Input: S, T, $|S| = n$, $|T| = m$
- Output: value of optimal local alignment

Better to solve a "harder" problem
for all $0 \le i \le n$, $0 \le j \le m$:
  V(i,j) = max value of opt (global) alignment of a suffix of S[1], …, S[i] with a suffix of T[1], …, T[j]
  Report best i,j

Base Cases

- Assume $\sigma(x,-) \le 0$, $\sigma(-,x) \le 0$
- V(i,0): some suffix of first i chars of S; all match spaces in T; best suffix is empty
  V(i,0) = 0
- V(0,j): similar
  V(0,j) = 0

General Case Recurrences

Opt suffix align S[1], …, S[i] vs T[1], …, T[j]:

$$\begin{bmatrix} \sim\sim\sim\sim & S[i] \\ \sim\sim\sim\sim & T[j] \end{bmatrix}, \begin{bmatrix} \sim\sim\sim\sim & S[i] \\ \sim\sim\sim\sim & - \end{bmatrix}, \begin{bmatrix} \sim\sim\sim\sim & - \\ \sim\sim\sim\sim & T[j] \end{bmatrix}, \text{or} \begin{bmatrix} \ \end{bmatrix}$$

Opt align of suffix of $S_1 \ldots S_{i-1}$ & $T_1 \ldots T_{j-1}$

$$V(i,j) = \max \begin{cases} V(i\text{-}1,j\text{-}1) + \sigma(S[i], T[j]) \\ V(i\text{-}1,j) \quad + \sigma(S[i], -) \\ V(i,j\text{-}1) \quad + \sigma(-, T[j]) \\ 0 \end{cases},$$

for all $1 \le i \le n,\ 1 \le j \le m.$

opt suffix alignment has: 2, 1, 1, 0 chars of S/T

Scoring Local Alignments

	j	0	1	2	3	4	5	6	
i			x	x	x	c	d	e	←T
0		0	0	0	0	0	0	0	
1	a	0							
2	b	0							
3	c	0							
4	x	0							
5	d	0							
6	e	0							
7	x	0							
	↑S								

Finding Local Alignments

	j	0	1	2	3	4	5	6	
i			x	x	x	c	d	e	←T
0		0	0	0	0	0	0	0	
1	a	0	0	0	0	0	0	0	
2	b	0	0	0	0	0	0	0	
3	c	0	0	0	0	2	1	0	
4	x	0	2	2	2	1	1	0	
5	d	0	1	1	1	1	3	2	
6	e	0	0	0	0	0	2	5	
7	x	0	2	2	2	1	1	4	
	↑S								

Notes

- Time and Space = O(mn)
- Space O(min(m,n)) possible with time O(mn), but finding alignment is trickier
- Local alignment: "Smith-Waterman"
- Global alignment: "Needleman-Wunsch"

Alignment With Gap Penalties

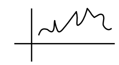
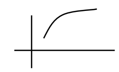
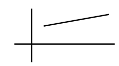
- *Gap:* maximal run of spaces in S’ or T’

```
ab----c-d
a-ddddcbd
```

2 gaps in S’, 1 in T’

- Motivations, e.g.:
  - mutation might insert/delete several or even many residues at once
  - matching cDNA (no introns) to genomic DNA (exons and introns)

Gap Penalties

- Score = f(gap length)
- Kinds, & best known alignment time
  - general  $O(n^3)$
  - convex  $O(n^2 \log n)$
  - affine  O(mn)

Global Alignment with Affine Gap Penalties

V(i,j) = value of opt alignment of
S[1], …, S[i] with T[1], …, T[j]

G(i,j) = …, s.t. last pair matches S[i] & T[j]

F(i,j) = …, s.t. last pair matches S[i] & –

E(i,j) = …, s.t. last pair matches – & T[j]

Time: O(mn) [calculate all, O(1) each]

Affine Gap Algorithm

Gap penalty = g + s*(gap length), g,s ≥ 0

V(i,0) = E(i,0) = V(0,i) = F(0,i) = -g-i*s

V(i,j) = max(G(i,j), F(i,j), E(i,j))

G(i,j) = V(i-1,j-1) + σ(S[i],T[j])

F(i,j) = max(F(i-1,j)-s , V(i-1,j)-g-s)

E(i,j) = max(E(i,j-1)-s , V(i,j-1)-g-s)

old gap — new gap

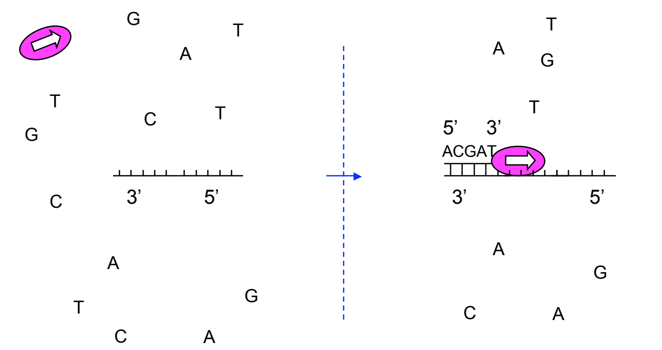
Summary

- Functionally similar proteins/DNA often have recognizably similar sequences even after eons of divergent evolution
- Ability to find/compare/experiment with "same" sequence in other organisms is a huge win
- Surprisingly simple scoring model works well in practice: score each position separately & add, possibly w/ fancier gap model like affine
- Simple "dynamic programming" algorithms can find *optimal* alignments under these assumptions in poly time (product of sequence lengths)
- This, and heuristic approximations to it like BLAST, are workhorse tools in molecular biology

Weekly Bio Interlude

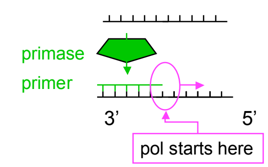
DNA Replication

DNA Replication: Basics



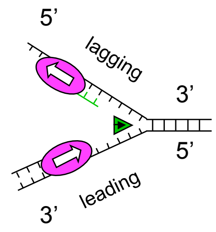
Issues & Complications, I

- 1st ~10 nt's added are called the *primer*
- In simple model, DNA pol has 2 jobs: prime & extend
- Priming is error-prone
- So, specialized *primase* does the priming; pol specialized for fast, accurate extension
- Still doesn't solve the accuracy problem (hint: primase makes an *RNA* primer)



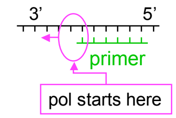
Issue 2: Rep Forks & Helices

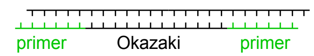
- "Replication Fork": DNA double helix is progressively unwound by a DNA helicase, and both resulting single strands are duplicated
- DNA polymerase synthesizes new strand 5' -> 3'(reading its template strand 3' -> 5')
- That means on one (the "leading") strand, DNA pol is chasing/pushing the replication fork
- But on the other "lagging" strand, DNA pol is running away from it.



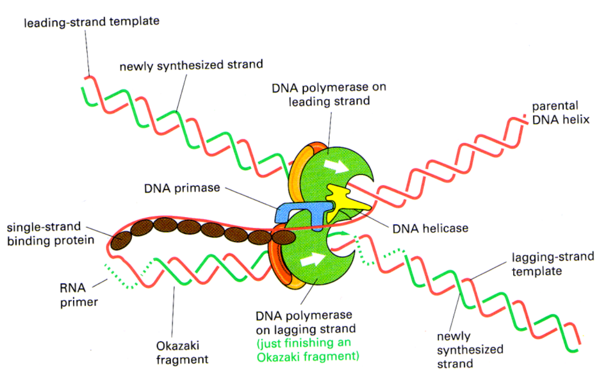
Issue 3: Fragments

- Lagging strand gets a series of "Okazaki fragments" of DNA (~200nt in eukaryotes) following each primer
- The RNA primers are later removed by a *nuclease* and *DNA* pol fills gaps (more accurate than primase)
- Fragments joined by *ligase*





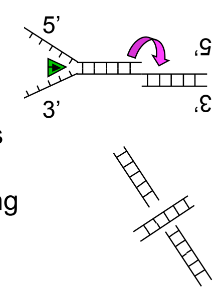
Issue 4: Coord Lead/Lag



Alberts et al., Mol. Biol. of the Cell, 3rd ed, p258

Issue 5: Twirls & Tangles

- Unwinding helix (~10 nucleotides per turn) would cause stress. *Topoisomerase I* cuts DNA backbone on *one* strand, allowing it to spin about the remaining bond, relieving stress
- *Topoisomerase II* can cut & rejoin *both* strands, after allowing another double strand to pass through the gap, de-tangling it.



Issue 6: Proofreading

- Error rate of pol itself is ~10^{-4}, but overall rate is 10^{-9}, due to proofreading & repair, e.g.
  - pol itself can back up & cut off a mismatched base if one happens to be inserted
  - priming the new strand is hard to do accurately, hence RNA primers, later removed & replaced
  - other enzymes scan helix for “bulges” caused by base mismatch, figure out which strand is original, cut away new (faulty) copy; DNA pol fills gap
  - which strand is original? In bacteria, some A’s are “methylated”, but not immediately after replication

Replication Summary

- Speed: 50 (eukaryotes) - 500 (prokaryotes) bp/sec
- Accuracy: 1 error per 10^9 bp
- Complex & highly optimized
- Highly similar across all living cells

- More info:
  Alberts et al., Mol. Biol. of the Cell