CSEP 590A
Computational Biology
Summer 2006

Lecture 2

Sequence Alignment;

DNA Replication

Tonight

- Last week’s “quiz” & homework
- Sequence alignment
- Weekly “bio” interlude - DNA replication
- More sequence alignment

Week 1 (anonymous) “Quiz”

- In your own words, what is DNA? Its main role?
- What is RNA? What is its main role in the cell?
- How many amino acids are there? How many are used in proteins?
- Did human beings, as we know them, develop from earlier species of animals?
- What are stem cells?
- What did Viterbi invent?
- What is dynamic programming?
- What is a likelihood ratio test?
- What is the EM algorithm?
- How would you find the maximum of $f(x) = ax3 + bx2 + cx + d$ in the interval $-10<x<25$?

Don’t worry, we’ll talk about all this stuff before the course ends

Evolution & Scientific Literacy

- "Human beings, as we know them, developed from earlier species of animals"
  (avoiding the now politically charged word "evolution")
- from 1985 to 2005, the % of Americans
  – rejecting: declined from 48% to 39%
  – accepting: also declined 45% to 40
  – uncertain: increased 7% to 21%
- In a 2005 survey,the proportion of adults who accept evolution in 34 countries (US, Europe, Japan…), the United States ranked 33rd, just above/below Turkey.

- My interpretation: The public is surprisingly malleable in the face of political agendas…

http://biology.plosjournals.org/perlserv/?request=get-document&doi=10.1371/journal.pbio.0040167

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

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 ≤ i ≤ |A|
    align all other chars to spaces
    compute its value
    retain the max
end
output the retained alignment
```

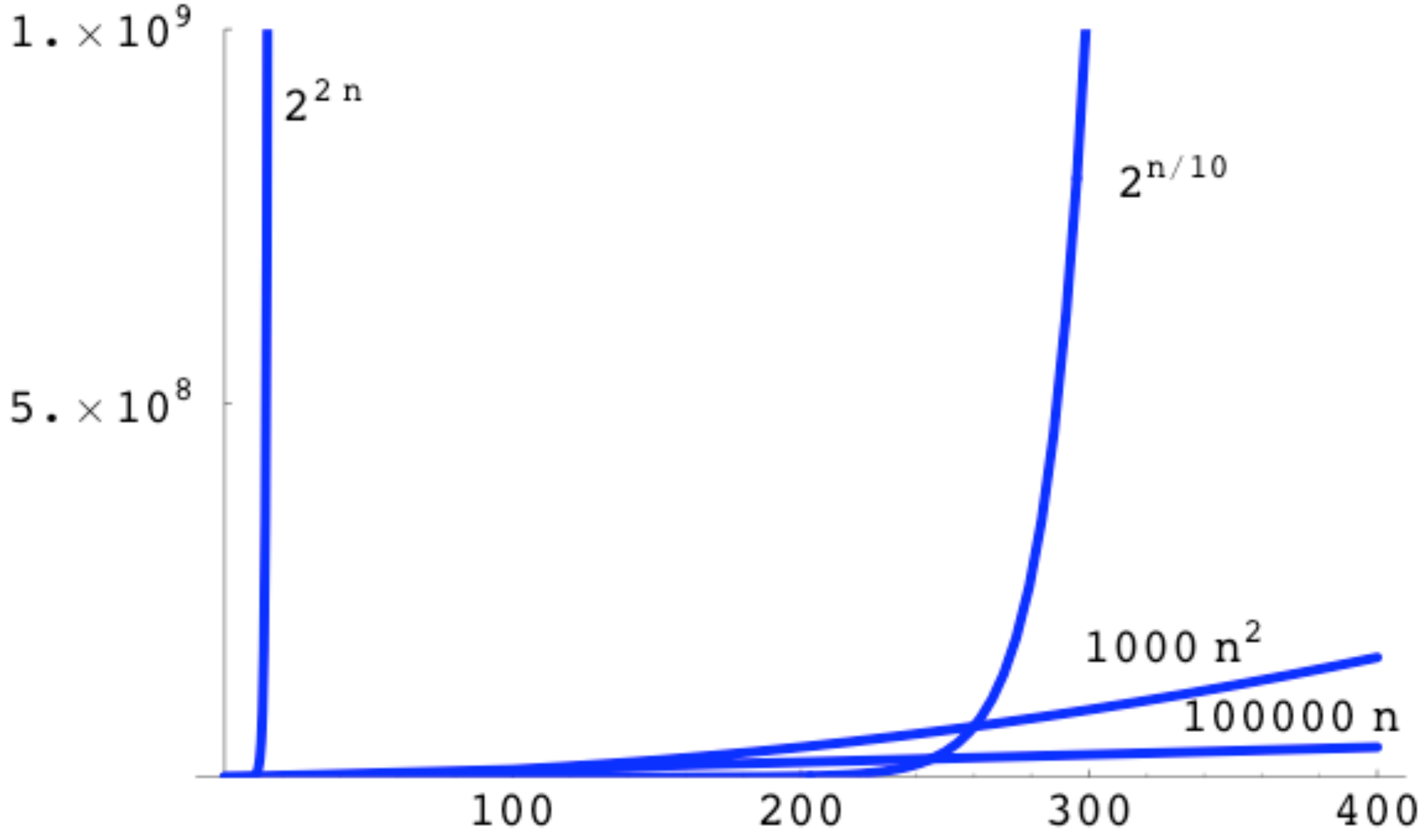
S = abcd   A = cd
T = wxyz   B = xz

```
-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: $\geq n\binom{2n}{n} > 2^{2n}, \text{ for } n > 3$
- 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. $|f(n)| \le cg(n)$ for all sufficiently large n.

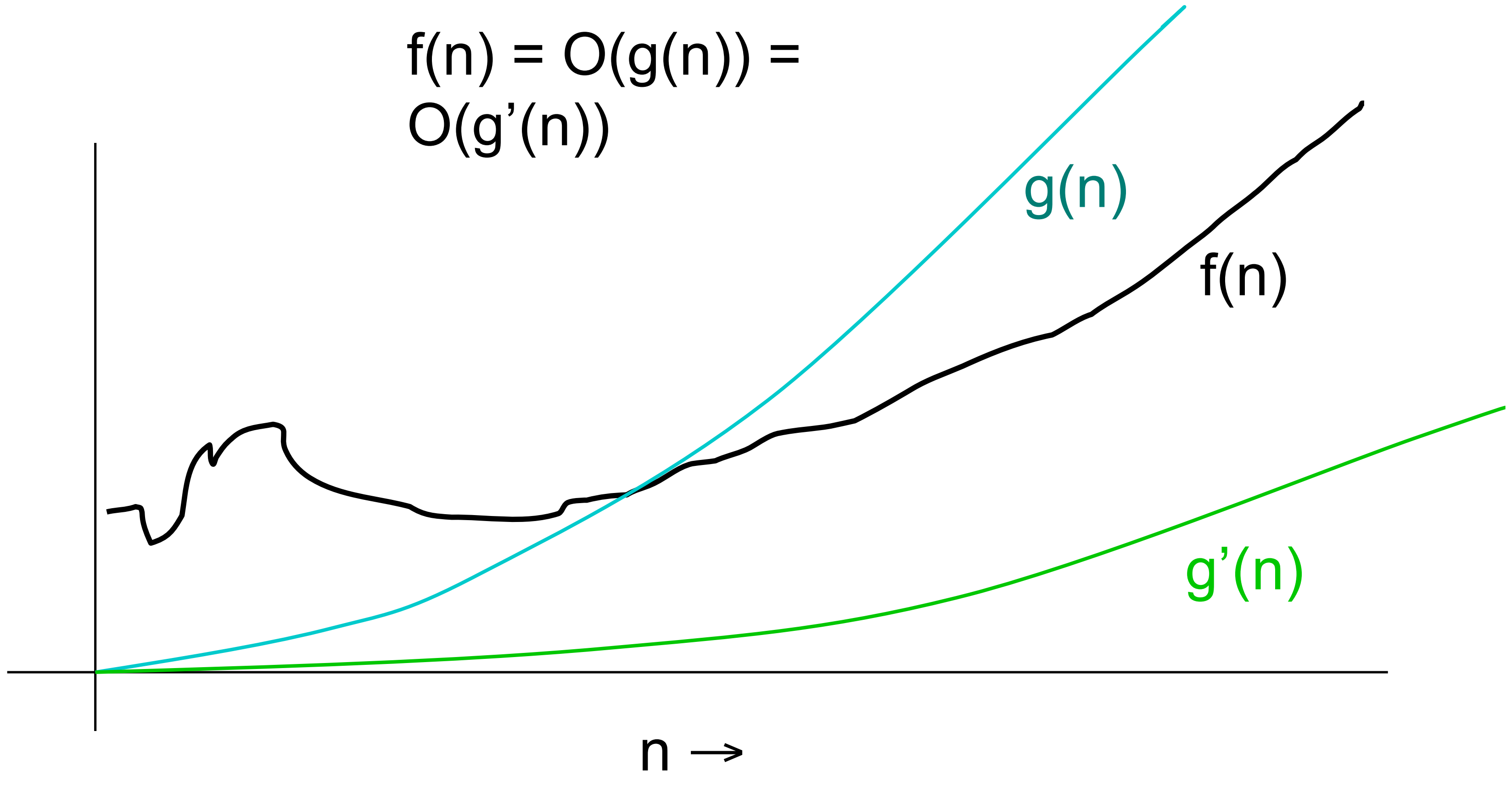
  $100\ n^2 + 100\ n + 100 = O(n^2)$ [e.g. c = 101]

  $n^2 = O(2^{2n})$

  2^{2n} is *not* $O(n^2)$

Big-O Example

f(n) = O(g(n)) = O(g'(n))



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++) {
    fin[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; $\sigma(-, -) < 0$)
- In each case, the rest of S & T should be optimally aligned to each other

Optimal Alignment in $O(n^2)$ 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 $0 \le i \le n$, $0 \le j \le m$.

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]:

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

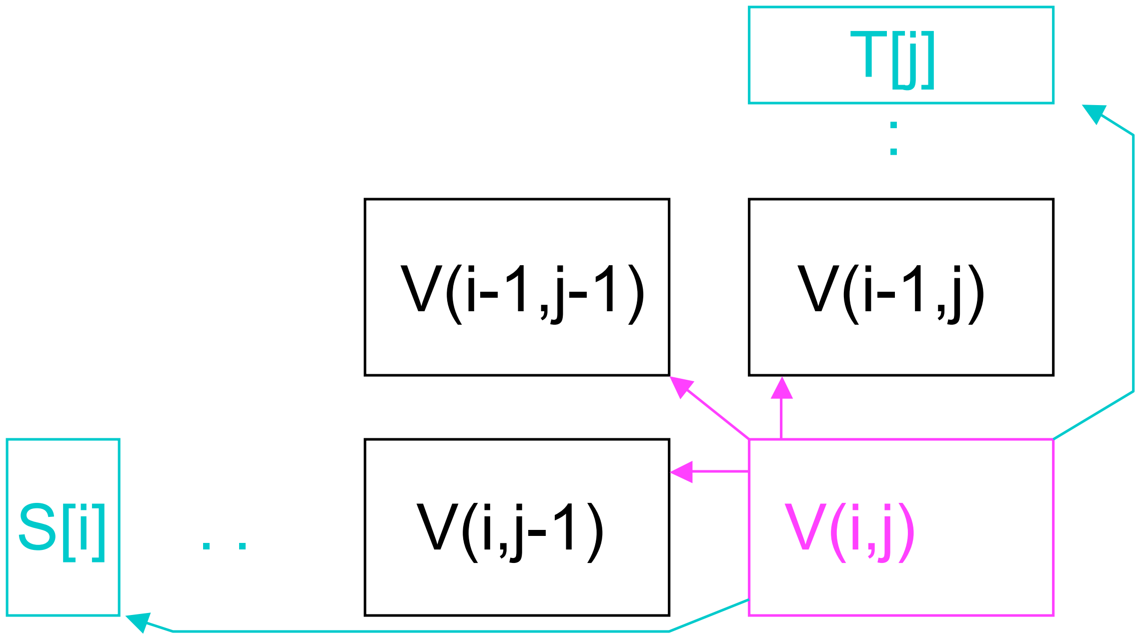
Opt align of $S_1 \ldots S_{i-1}$ & $T_1 \ldots T_{j-1}$

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

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

Calculating One Entry

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



Mismatch = -1
Match = 2

Example

i \ j		0	1	2	3	4	5
i			c	a	d	b	d
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					$\boxed{}$

$\leftarrow$T

$\uparrow$ S

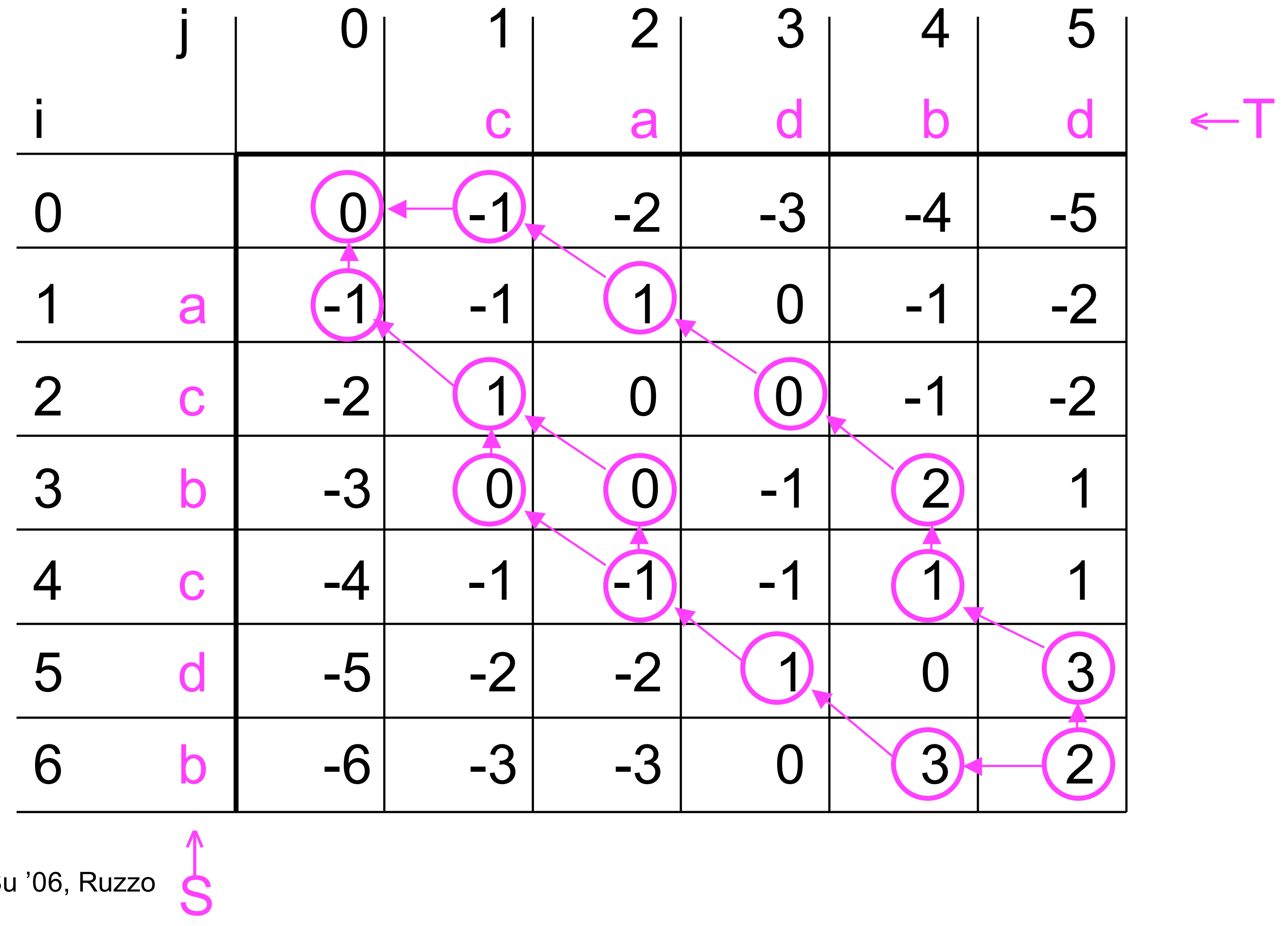
Time = O(mn)

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



i	j	0	1	2	3	4	5
			c	a	d	b	d
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

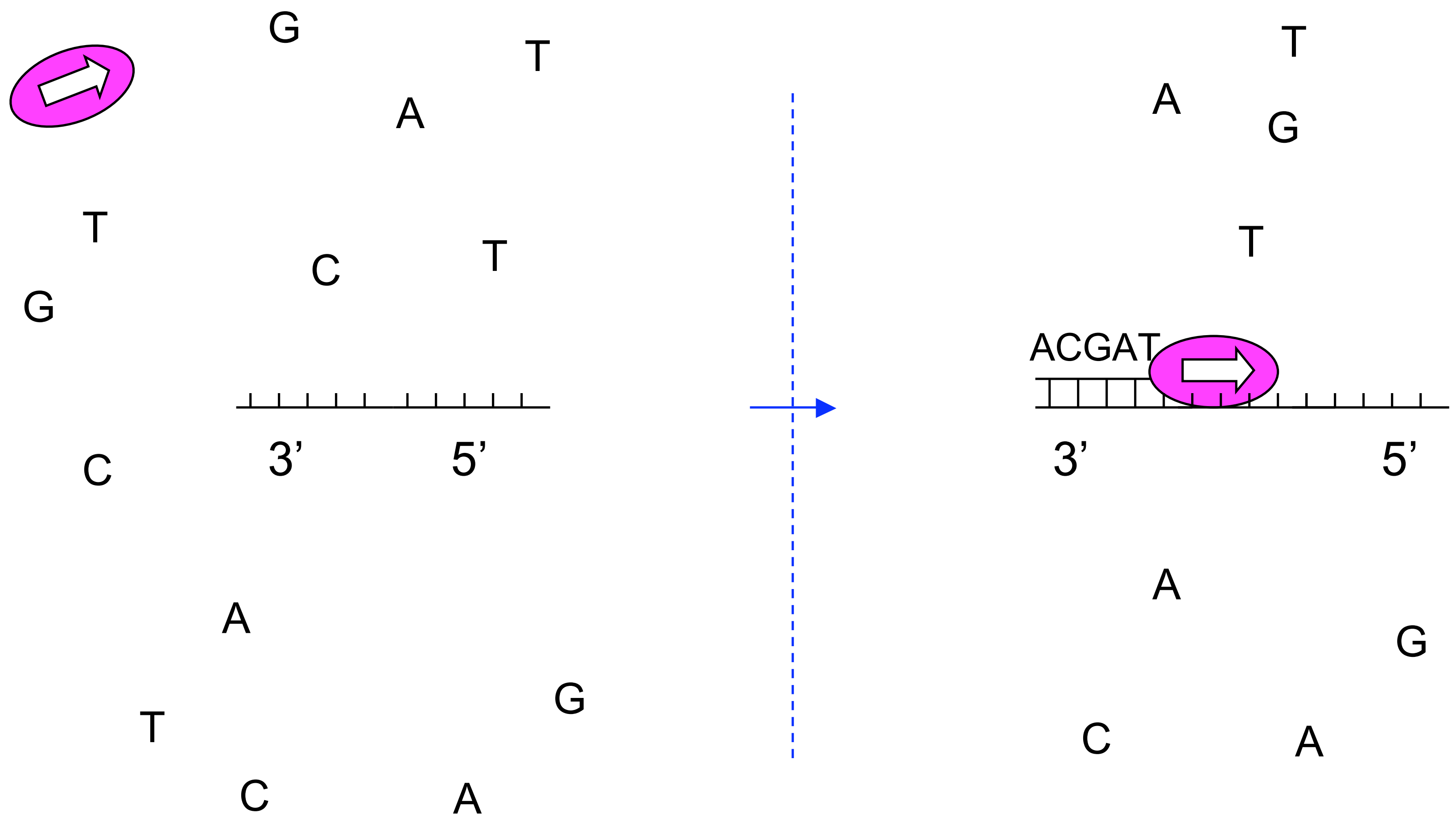
Complexity Notes

- 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.

Weekly Bio Interlude

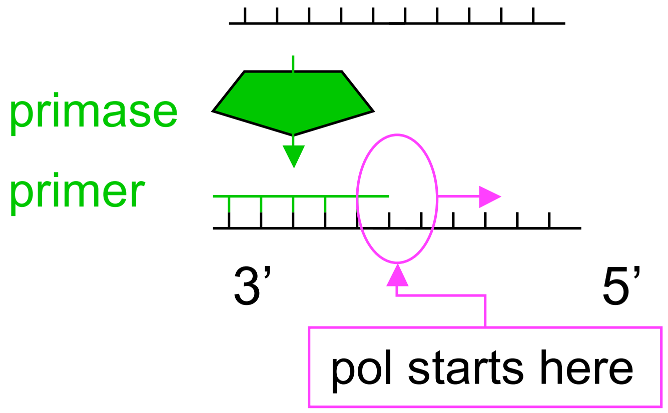
DNA Replication

DNA Replication: Basics



Issues & Complications, I

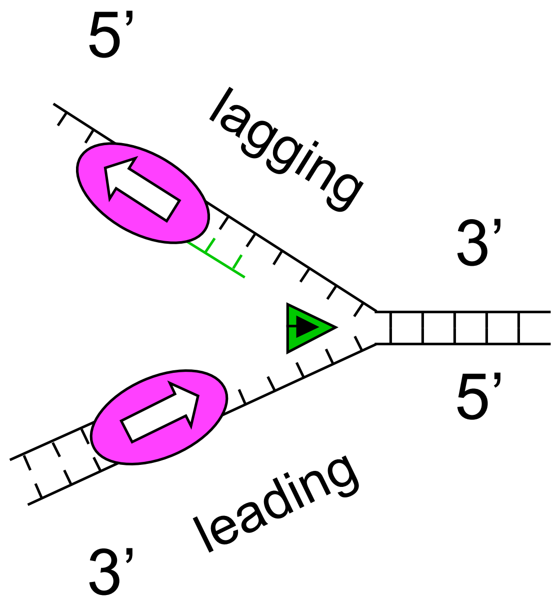
- 1st ~10 nt's added are called the *primer*
- In simple model, DNA pol has 2 jobs: prime & extend
- Priming is error-prone
- So, specialized *primase* does the priming; pol specialized for fast, accurate extension



- Still doesn't solve the accuracy problem (hint: primase makes an *RNA* primer)

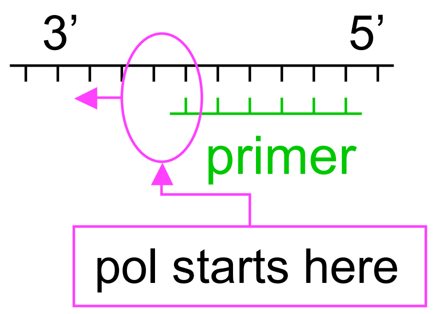
Issue 2: Rep Forks & Helices

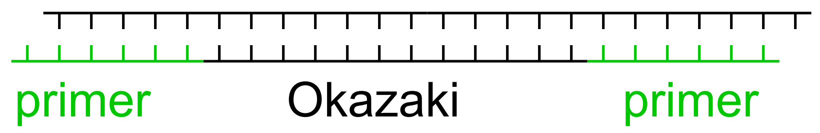
- "Replication Fork": DNA double helix is progressively unwound by a DNA helicase, and both resulting single strands are duplicated
- DNA polymerase synthesizes new strand 5' -> 3'(reading its template strand 3' -> 5')
- That means on one (the "leading") strand, DNA pol is chasing/pushing the replication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



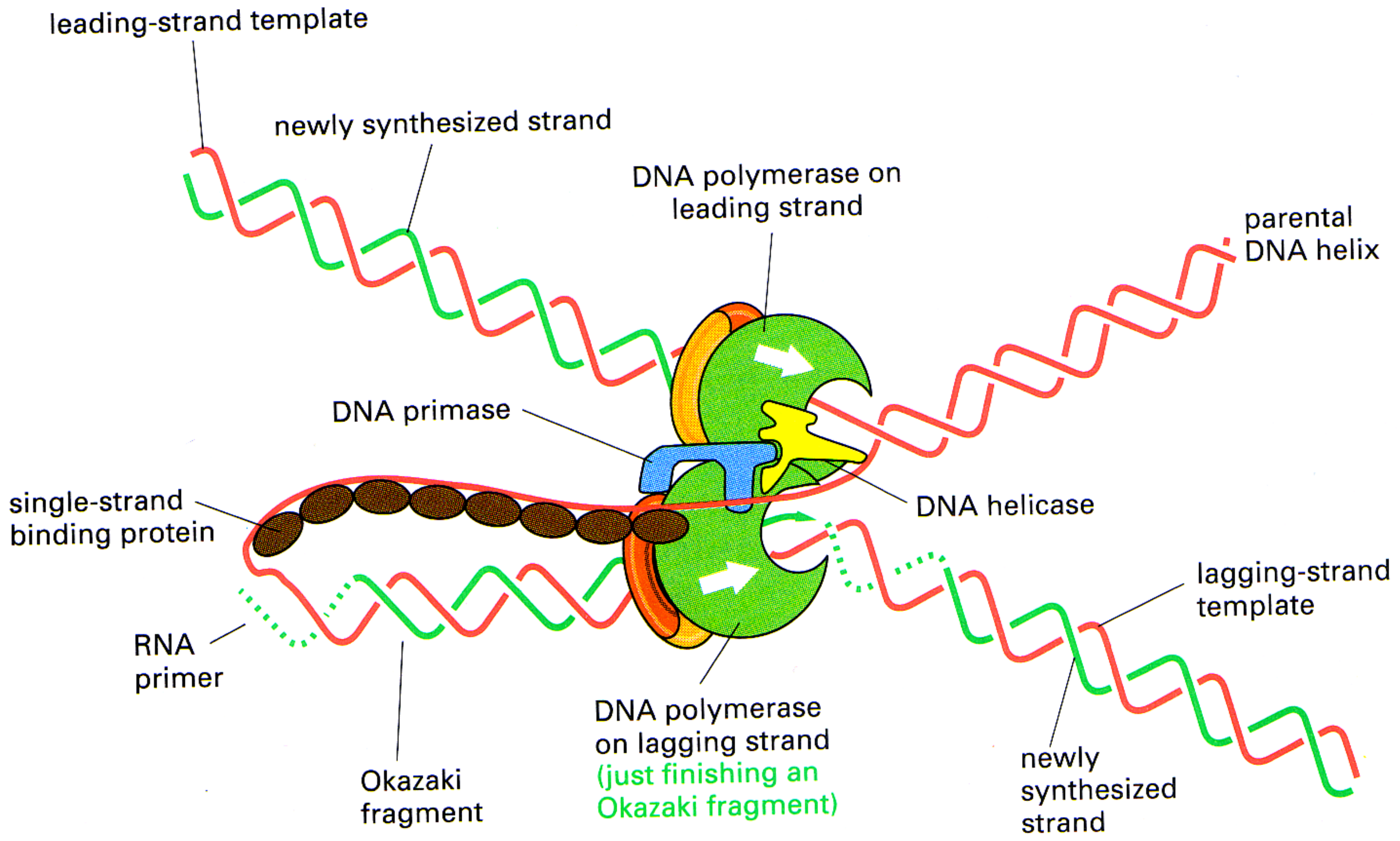
Issue 3: Fragments

- 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*





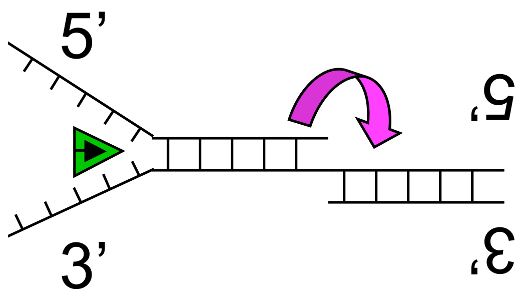
Issue 4: Coord Lead/Lag

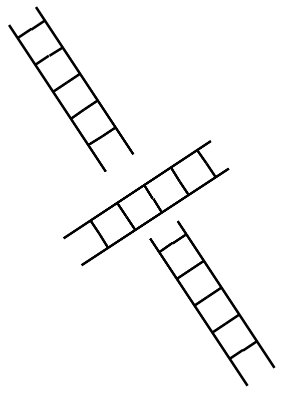


Alberts et al., Mol. Biol. of the Cell, 3rd ed, p258

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

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

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

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

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

Scoring Local Alignments

i	j	0	1	2	3	4	5	6	
			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”

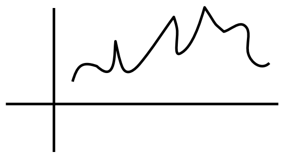
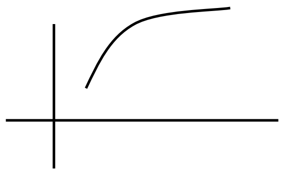
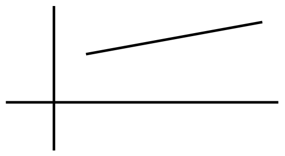
Alignment With Gap Penalties

- *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[i], …, 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 = $g + s*(\text{gap length}),\ g,s \geq 0$

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

$$\begin{aligned}
V(i,j) &= \max(G(i,j), F(i,j), E(i,j)) \\
G(i,j) &= V(i-1,j-1) + \sigma(S[i],T[j]) \\
F(i,j) &= \max(\boxed{F(i-1,j)-s}, \boxed{V(i-1,j)-g-s}) \\
E(i,j) &= \max(\boxed{E(i,j-1)-s}, \boxed{V(i,j-1)-g-s})
\end{aligned}$$

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