CSEP 590 B
Computational Biology
Autumn 2011

Lecture 2

Sequence Alignment

Tonight

Last week’s “quiz” & homework

Sequence alignment

Weekly “bio” interlude - DNA replication

More sequence alignment

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

Recognizable similarity after 10^8 –10^9 yr

BLAST Demo

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

Try it!

pick any protein, e.g. hemoglobin, insulin, exportin,… BLAST to find distant relatives.

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

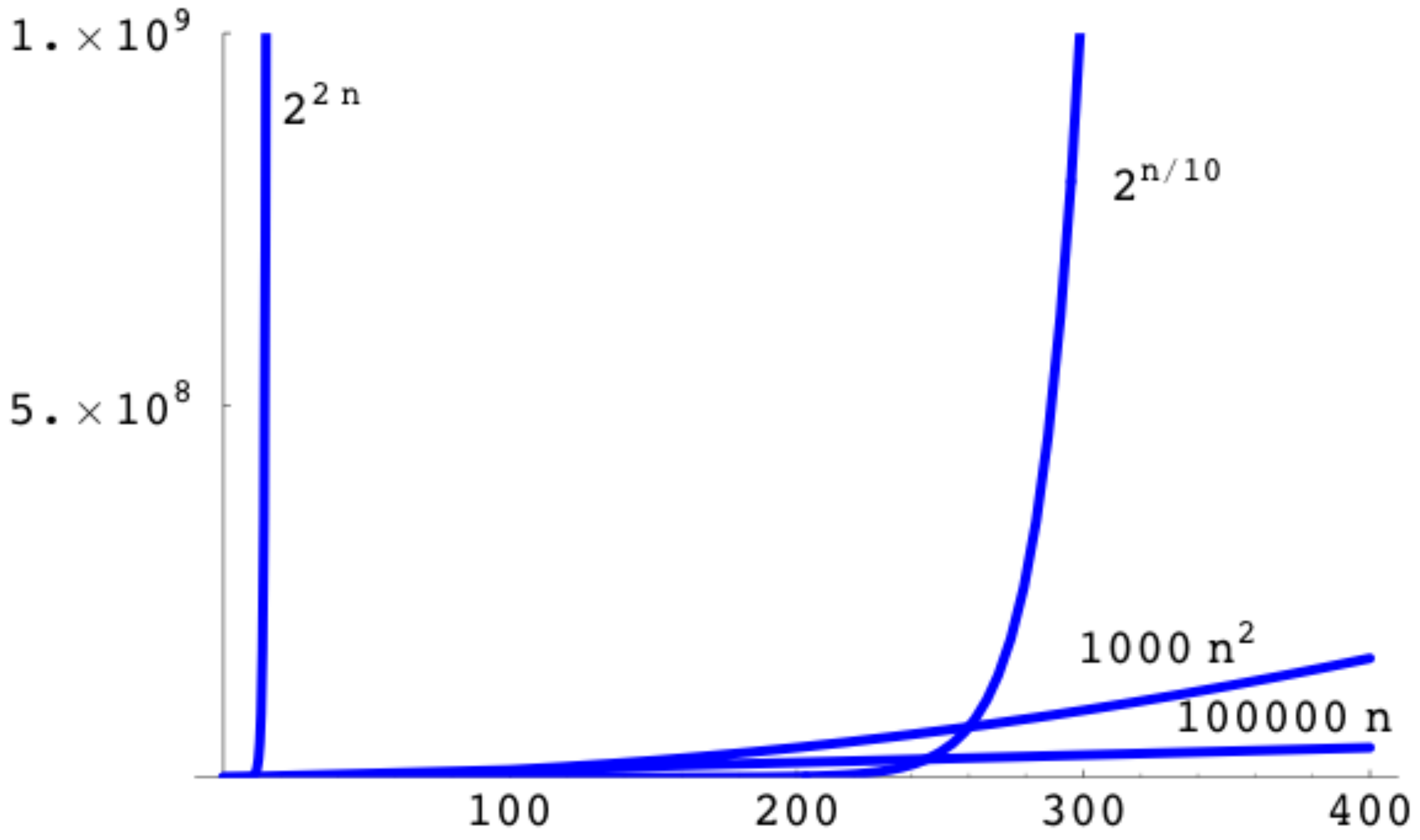
How many alignments are there: $\geq \binom{2n}{n}$

- 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: $\geq n\binom{2n}{n} > 2^{2n}, \text{ for } n > 3$

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 $100n^2 + 100n + 100$ 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.

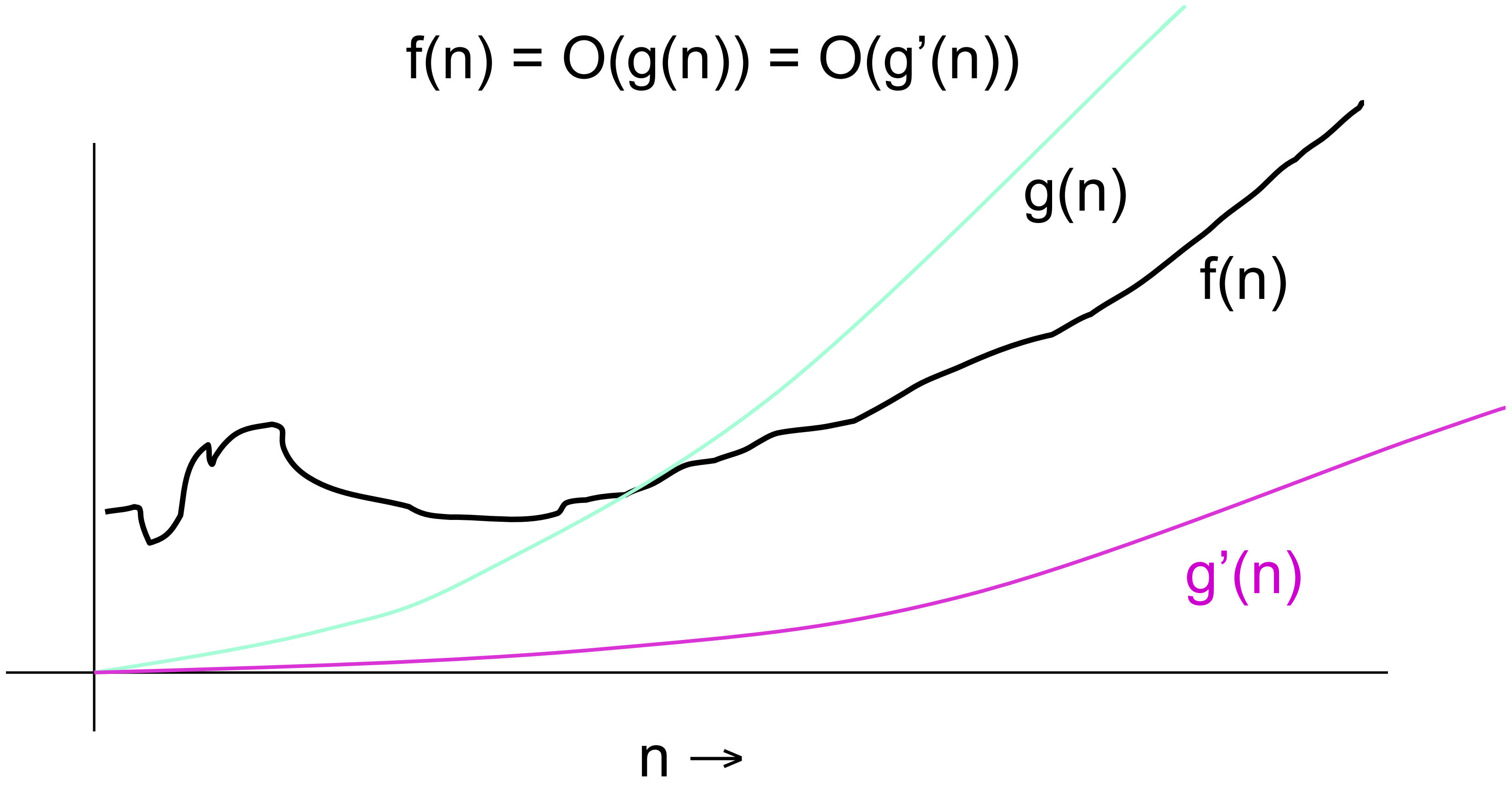
$100n^2 + 100n + 100 = O(n^2)$ [e.g. $c = 101$]

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

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.

(Yes, there are exceptions.)

Fibonacci Numbers (recursion)

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

Simple recursion, but many repeated subproblems!!

⇒

Time = $\Omega(1.61^n)$

Fibonacci, II (dynamic programming)

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

Avoid repeated subproblems by tabulating their solutions

$\Rightarrow$

Time = $O(n)$

(in this case)

Alignment by 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]:

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

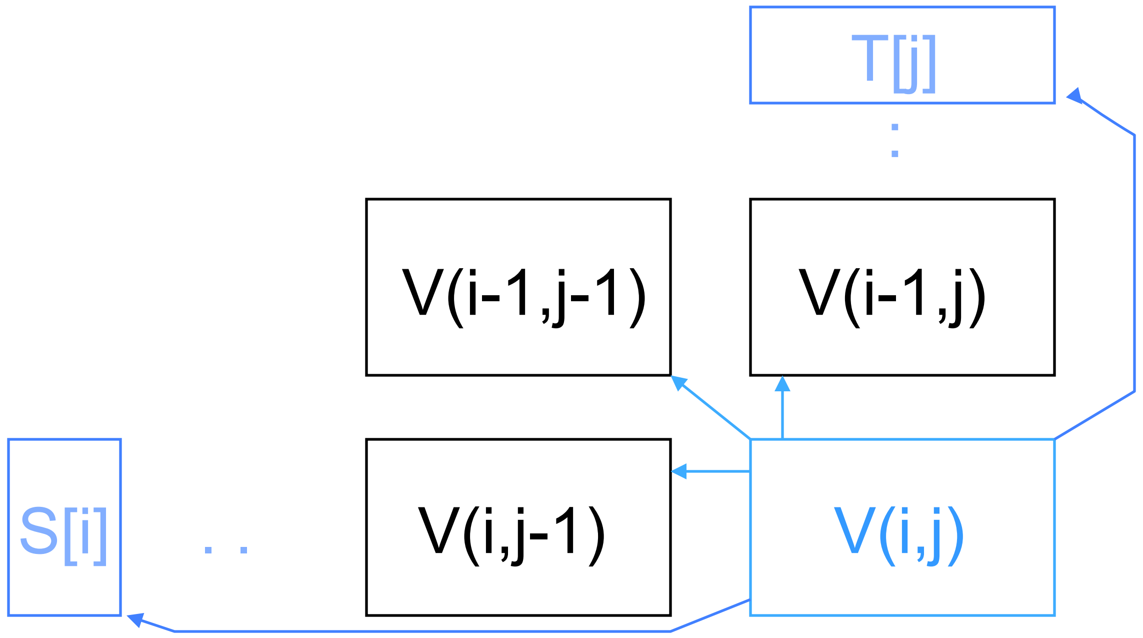
Opt align of $S_1 \ldots S_{i-1}$ & $T_1 \ldots T_{j-1}$

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

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

Calculating One Entry

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



Mismatch = -1
Match = 2

Example

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

$\boxed{\begin{matrix} c \\ - \end{matrix}}$ Score(c,-) = -1

Mismatch = -1
Match = 2

Example

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

$\begin{array}{|c|}- \\ a\end{array}$ Score(-,a) = -1

Mismatch = -1
Match = 2

Example

	j	0	1	2	3	4	5	
i			c	a	d	b	d	←T
0		0	-1	-2	-3	-4	-5	
1	a	-1						
2	c	$\boxed{-2}$						
3	b	-3						
4	c	-4						
5	d	-5						
6	b	-6						
	↑ S							

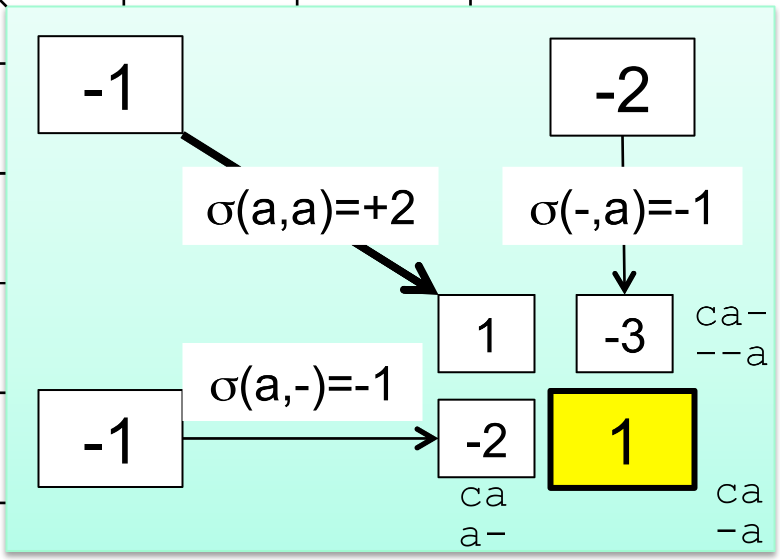
$$\begin{array}{|c|c|} - & - \\ a & c \\ \hline \end{array}$$
-1

Score(-,c) = -1

Mismatch = -1
Match = 2

Example

	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						
3	b	-3						
4	c	-4						
5	d	-5						
6	b	-6						
	↑S							



Mismatch = -1
Match = 2

Example

	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					$\boxed{}$

←T

↑
S

Time = O(mn)

Mismatch = -1
Match = 2

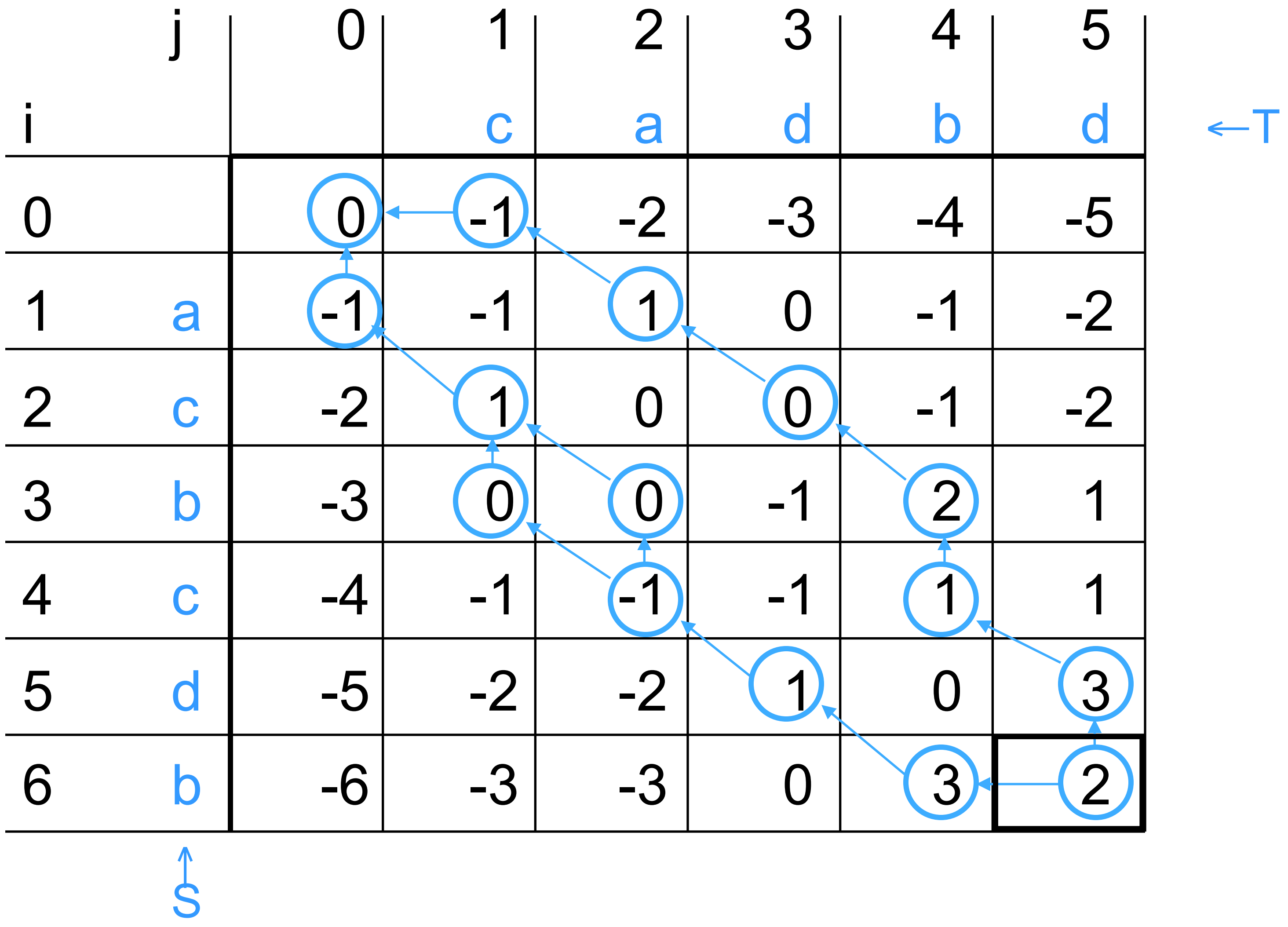
Example

	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	$\boxed{2}$

↑
S

Finding Alignments: Trace Back

Arrows = (ties for) max in V(i,j); 3 LR-to-UL paths = 3 optimal alignments



	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.

Significance of Alignments

Is “42” a good score?

Compared to what?

Usual approach: compared to a specific “null model”, such as “random sequences”

More on this next time; a taste today, for use in next HW

Overall Alignment Significance, II
Empirical (via randomization)

You just searched with x, found “good” score for x:y

Generate N random “y-like” sequences (say $N = 10^3 - 10^6$)

Align x to each & score

If k of them have better score than alignment of x to y, then the (empirical) probability of a chance alignment as good as observed x:y alignment is $(k+1)/(N+1)$

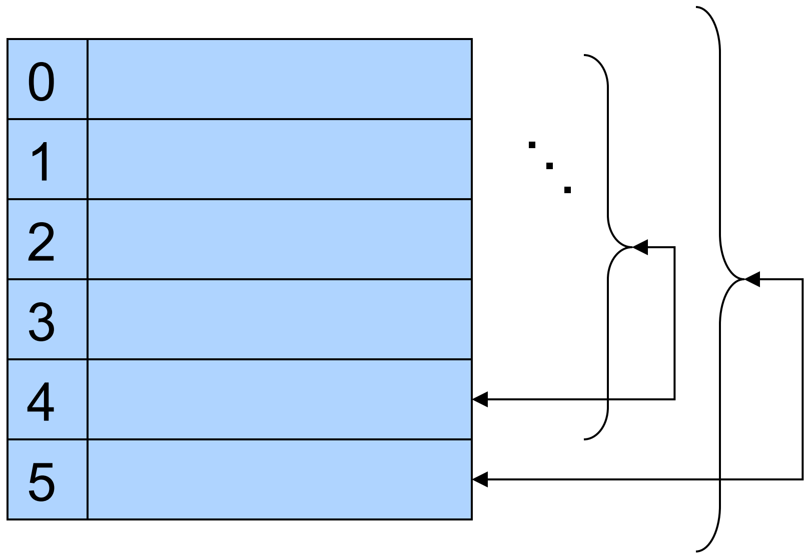
- e.g., if 0 of 99 are better, you can say “estimated $p < .01$”

How to generate “random y-like” seqs? Scores depend on:

- Length, so use same length as y
- Sequence composition, so uniform 1/20 or 1/4 is a bad idea; even background p_i can be dangerous
- Better idea: *permute* y N times

Generating Random Permutations

```
for (i = n-1; i > 0; i--){
    j = random(0..i);
    swap X[i] <-> X[j];
}
```

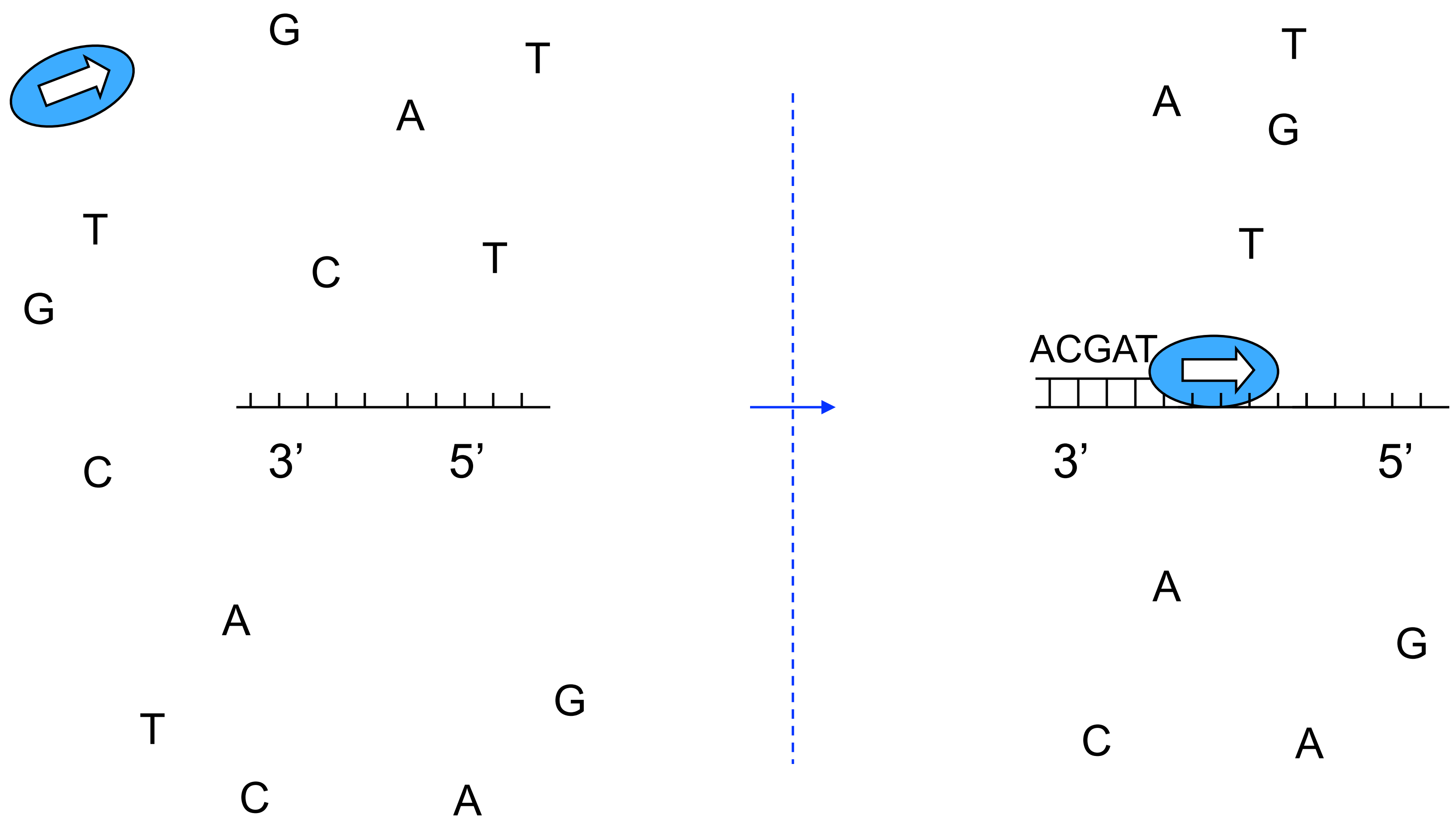


All n! permutations of the original data equally likely: A specific element will be last with prob 1/n; given that, a specific other element will be next-to-last with prob 1/(n-1), …; overall: 1/(n!)

Weekly Bio Interlude

DNA Replication

DNA Replication: Basics



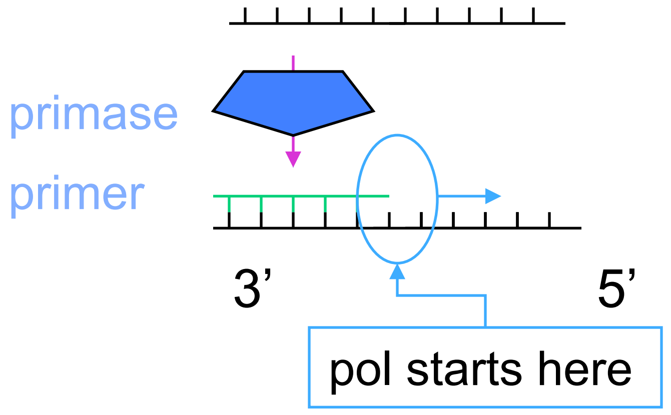
Issues & Complications, I

1st ~10 nt's added are called the *primer*

In simple model, DNA pol has 2 jobs: prime & extend

Priming is error-prone

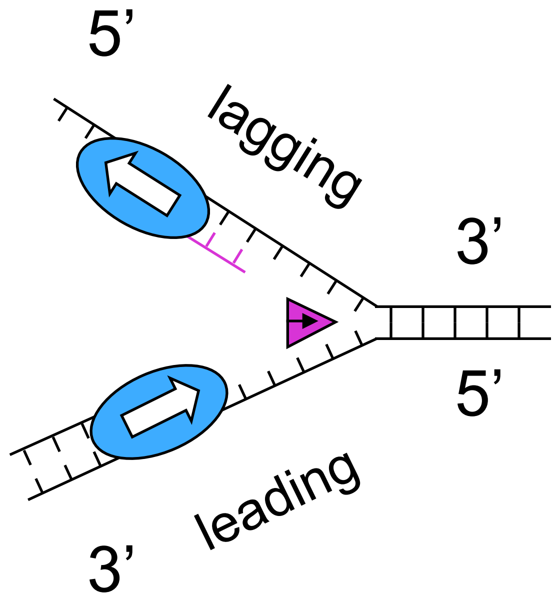
So, specialized *primase* does the priming; pol specialized for fast, accurate extension



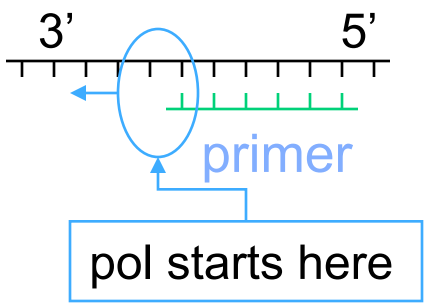
Still doesn't solve the accuracy problem (hint: primase makes an *RNA* primer)

Issue 2: Rep Forks & Helices

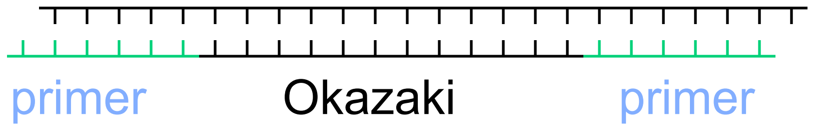
- “Replication Fork”: DNA double helix is progressively unwound by a DNA helicase, and both resulting single strands are duplicated
- DNA polymerase synthesizes new strand 5’ -> 3’(reading its template strand 3’ -> 5’)
- That means on one (the “leading”) strand, DNA pol is chasing/pushing the replication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



Issue 3: Fragments



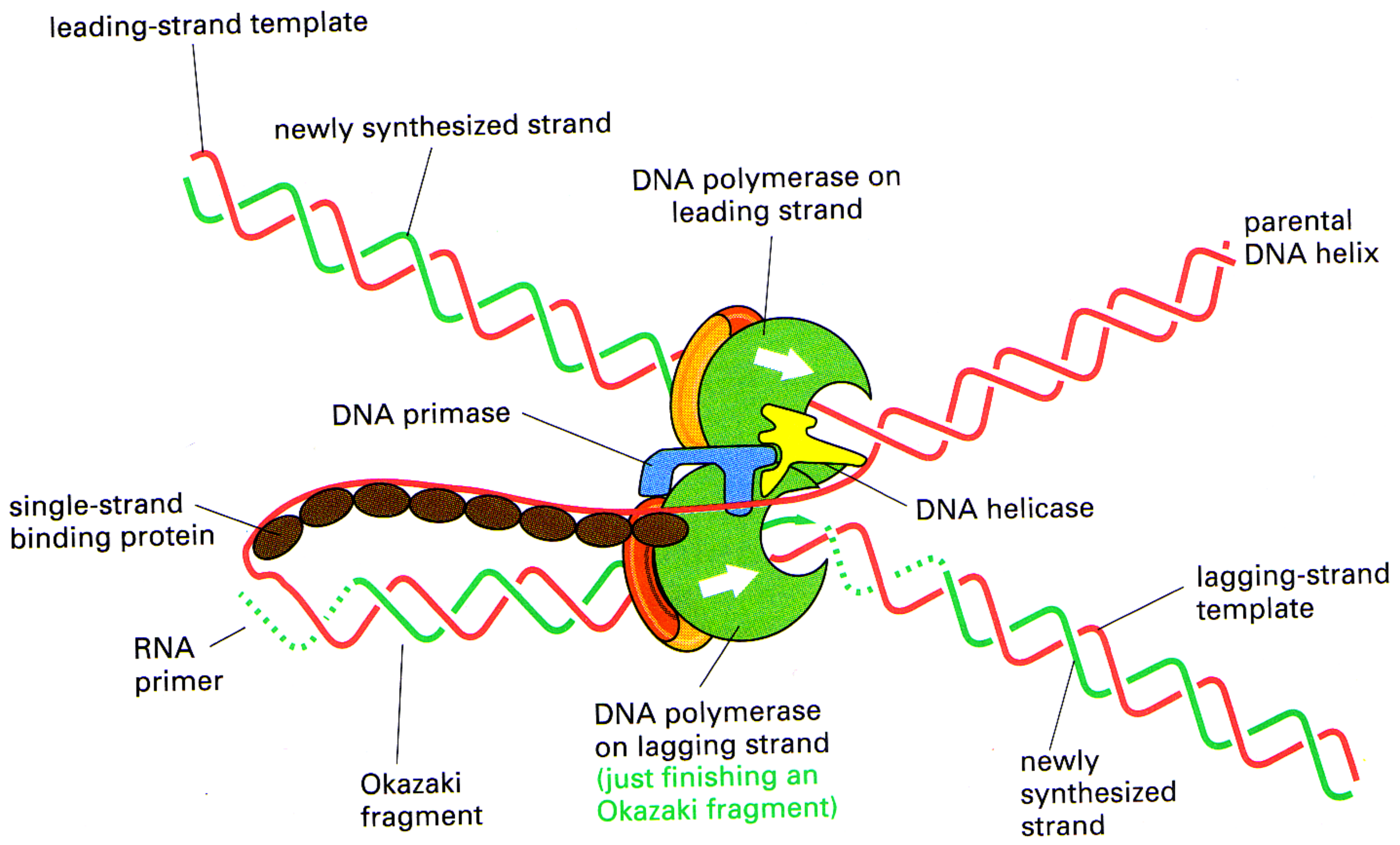
Lagging strand gets a series of "Okazaki fragments" of DNA (~200nt in eukaryotes) following each primer



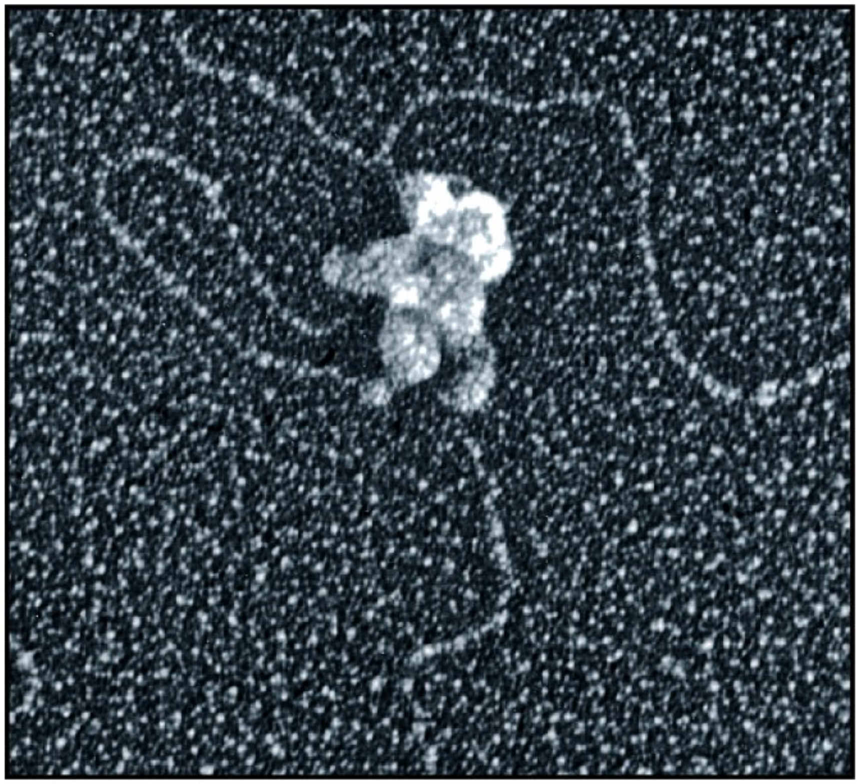
The RNA primers are later removed by a *nuclease* and *DNA* pol fills gaps (more accurate than primase; primed by *DNA* from adjacent Okazaki frag

Fragments joined by *ligase*

Issue 4: Coord Lead/Lag



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


(B)

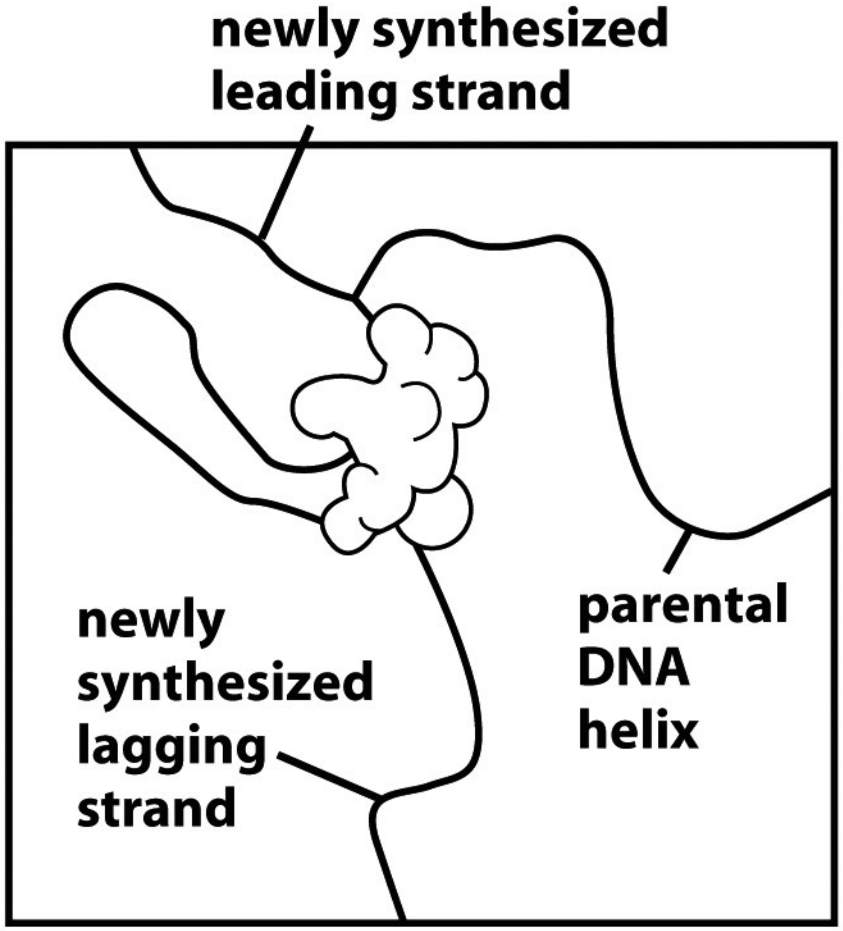
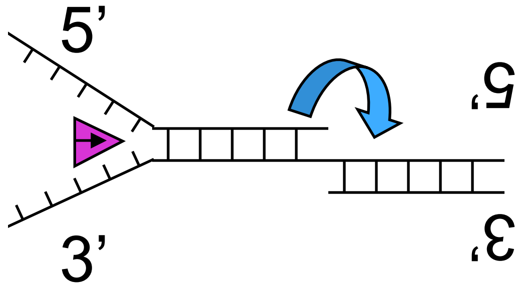

newly synthesized leading strand
newly synthesized lagging strand
parental DNA helix
(C)

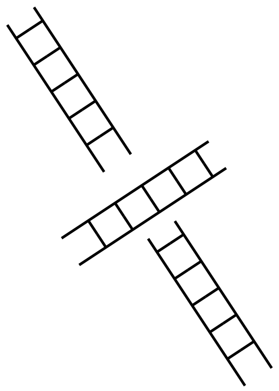
Figure 5-19bc Molecular Biology of the Cell 5/e (© Garland Science 2008)

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? Bacteria: “methylate” some A’s, eventually. Euks: strand nicking

Replication Summary

Speed: 50 (eukaryotes) to
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

Local Alignment: “Obvious” 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 $\sigma(x,-) \leq 0$, $\sigma(-,x) \leq 0$

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

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

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

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

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

Again, arrows follow max

	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”

Sequence Evolution

"Nothing in Biology Makes Sense Except in the Light of Evolution" – Theodosius Dobzhansky, 1973

Changes happen at random

Deleterious/neutral/advantageous changes unlikely/ possibly/likely spread widely in a population

Changes are less likely to be tolerated in positions involved in many/close interactions, e.g.

- enzyme binding pocket
- protein/protein interaction surface
- …

Alignment With Gap Penalties

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

`ab--ddc-d`	2 gaps in S’
`a---ddcbd`	1 gap in T’

Motivations, e.g.:

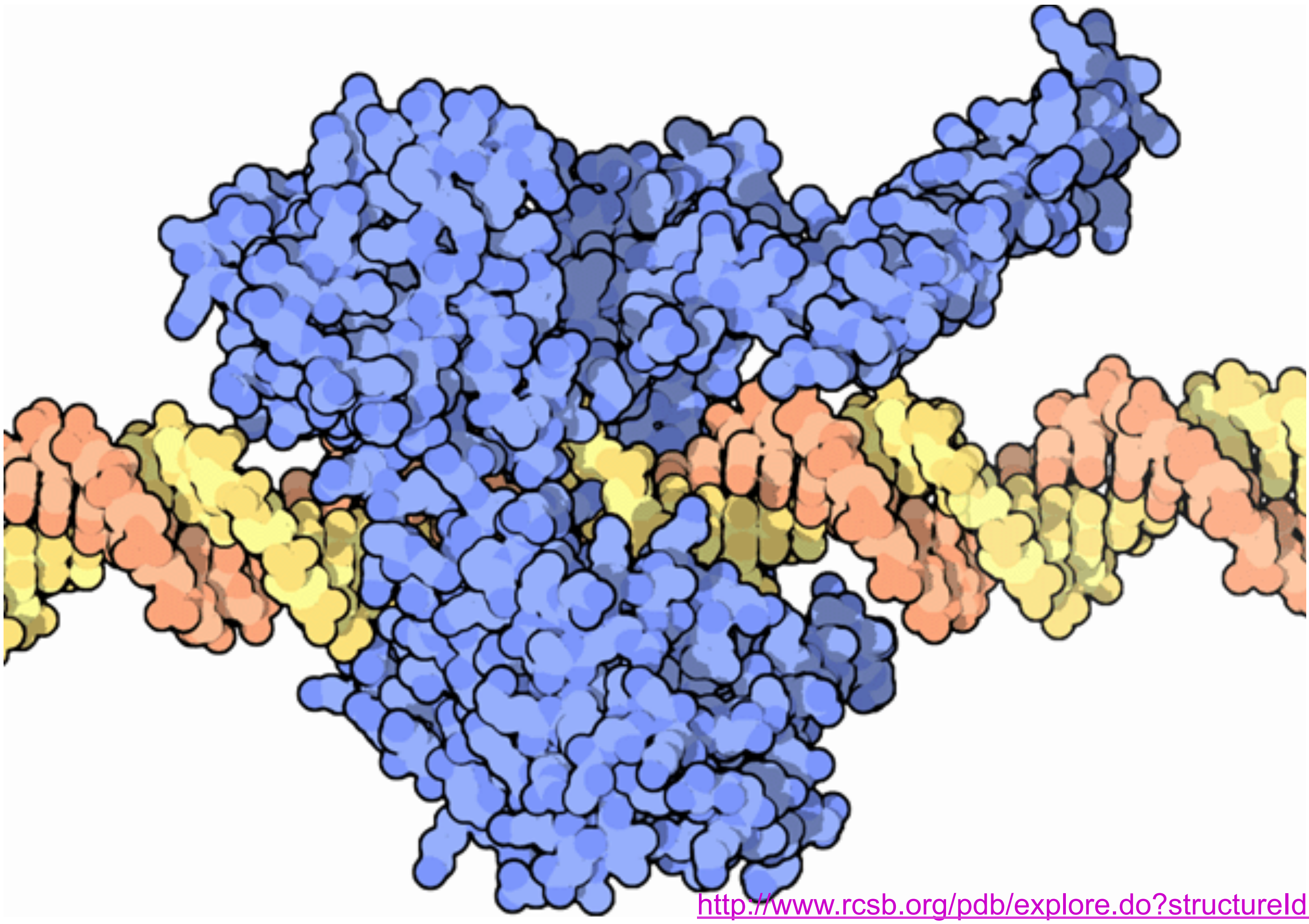
mutation might insert/delete several or even many residues at once

matching mRNA (no introns) to genomic DNA (exons and introns)

some parts of proteins less critical

A Protein Structure: (Dihydrofolate Reductase)

Topoisomerase I

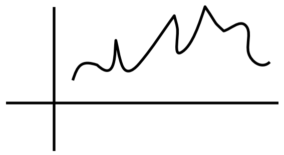
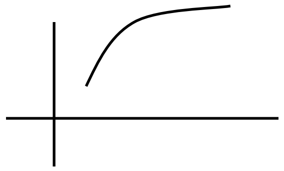
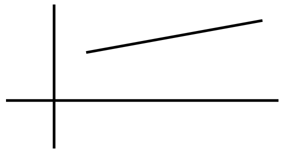


http://www.rcsb.org/pdb/explore.do?structureId=1a36

Gap Penalties

Score = f(gap length)

Kinds, & best known alignment time

general		O(n^3)
convex		O(n^2log n)
affine		O(n^2) [really, 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 open penalty

gap extend penalty

$$\text{Gap penalty} = \overset{\text{gap open penalty}}{g} + \overset{\text{gap extend penalty}}{e}*(\text{gaplen}-1),\ g \geq e \geq 0$$

$$V(i,0) = E(i,0) = V(0,i) = F(0,i) = -g-(i-1)*e$$

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

$$G(i,j) = V(i-1,j-1) + \sigma(S[i],T[j])$$

$$F(i,j) = \max(\boxed{F(i-1,j)-e}, \boxed{V(i-1,j)-g})$$

$$E(i,j) = \max(\underbrace{\boxed{E(i,j-1)-e}}_{\text{old gap}}, \underbrace{\boxed{V(i,j-1)-g}}_{\text{new gap}})$$

Summary: Dynamic Programming

Keys to D.P. are to

a) identify the subproblems (usually repeated/overlapping)
b) solve them in a careful order so all small ones solved before they are needed by the bigger ones, and
c) build table with solutions to the smaller ones so bigger ones just need to do table lookups (*no* recursion, despite recursive formulation implicit in (a))
d) Implicitly, optimal solution to whole problem devolves to optimal solutions to subproblems

Summary: Alignment

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 works well in practice: score positions separately & add, usually 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