Lecture outline

- Sequence alignment
  - Why do we need to align sequences?
  - Evolutionary relationships
- Gaps and scoring matrices
- Dynamic programming
  - Global alignment (Needleman & Wunsch)
  - Local alignment (Smith & Waterman)
- Database searches
  - BLAST
  - FASTA

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Dot Plot

Put 1's where characters are identical.

	A	B	C	N	Y	R	Q	C	L	C	R	P	M
A	1												
Y					1								
C			1					1		1			
Y					1								
N				1									
R						1					1		
C			1					1		1			
K													
C			1					1		1			
R						1					1		
B		1											
P												1	

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A More Interesting Dot Matrix

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Edit Distance

Levenshtein (1966) introduced edit distance between two strings as the minimum number of elementary operations (insertions, deletions, and substitutions) to transform one string into the other

d(**v**,**w**) = MIN number of elementary operations to transform **v** → **w**

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Edit Distance: Example

TGCATAT → ATCCGAT in 5 steps

TGCATAT → (delete last T)
TGCATA → (delete last A)
TGCAT → (insert A at front)
ATGCAT → (substitute C for 3rd G)
ATCCAT → (insert G before last A)
ATCCGAT (Done)

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Edit Distance: Example

TGCATAT → ATCCGAT in 5 steps

TGCATAT → (delete last T)
TGCATA → (delete last A)
TGCAT → (insert A at front)
ATGCAT → (substitute C for 3rd G)
ATCCAT → (insert G before last A)
ATCCGAT (Done)

What is the edit distance? 5?

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Edit Distance: Example (cont'd)

TGCATAT → ATCCGAT in 4 steps

TGCATAT → (insert A at front)
ATGCATAT → (delete 6th T)
ATGCATA → (substitute G for 5th A)
ATGCGTA → (substitute C for 3rd G)
ATCCGAT (Done)

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Edit Distance: Example (cont'd)

TGCATAT → ATCCGAT in 4 steps

TGCATAT → (insert A at front)
ATGCATAT → (delete 6th T)
ATGCATA → (substitute G for 5th A)
ATGCGTA → (substitute C for 3rd G)
ATCCGAT (Done)

Can it be done in 3 steps???

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Types of alignment

- Global (Needleman & Wunsch)
  - Strings of similar size
    - Genes with a similar structure
    - Larger regions with a preserved order (syntenic regions)
- Local (Smith & Waterman)
  - Finding similar regions among
    - Dissimilar regions
    - Sequences of different lengths

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Dynamic programming

- Instead of evaluating every possible alignment, we can create a table of partial scores by breaking the alignment problem into subproblems.
- Consider two sequences CACGA and CGA
  - we have three possibilities for the first position of the alignment

First position	Score	Remaining seqs.
C C	+1	ACGA GA
- C	-1	CACGA GA
C -	-1	ACGA CGA

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Example

score(H,P) = -2, gap penalty=-8 (linear)

	-	H	E	A	G	A	W	G	H	E	E
-	0	-8	-16	-24	-32	-40	-48	-56	-64	-72	-80
P	-8	-2									
A	-16										
W	-24										
H	-32										
E	-40										
A	-48										
E	-56										

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The DP recurrence relation

- s(a,b) = score of aligning a and b
- S(i,j) = optimal similarity of A(1:i) and B(1:j)
- Recurrence relation
  - $S(i,0) = \Sigma\, s(A(k),-),\ 0 <= k <= i$
  - $S(0,j) = \Sigma\, s(-,B(k)),\ 0 <= k <= j$
  - $S(i,j) = \max [S(i,j-1) + s(-,B(j)),\ S(i-1,j) + s(A(i),-),\ S(i-1,j-1) + s(A(i),B(j))]$
  - Assume linear gap penalty

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Example contd.

score(E,P) = 0, score(E,A) = -1, score(H,A) = -2

	-	H	E	A	G	A	W	G	H	E	E
-	0	-8	-16	-24	-32	-40	-48	-56	-64	-72	-80
P	-8	-2	-8								
A	-16	-10	-3								
W	-24										
H	-32										
E	-40										
A	-48										
E	-56										

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Optimal alignment:

H E A G A W G H E - E
- P - - A W - H E A E

		H	E	A	G	A	W	G	H	E	E
	0	-8	-16	-24	-32	-40	-48	-56	-64	-72	-80
P	-8	-2	-8	-16	-24	-33	-42	-49	-57	-65	-73
A	-16	-10	-3	-4	-12	-19	-28	-36	-44	-52	-60
W	-24	-18	-11	-6	-7	-15	-4	-12	-21	-29	-37
H	-32	-14	-18	-13	-8	-9	-12	-6	-2	-11	-19
E	-40	-22	-8	-16	-16	-9	-12	-14	-6	-4	-5
A	-48	-30	-16	-3	-11	-11	-12	-12	-14	-4	2
E	-56	-38	-24	-11	-6	-12	-14	-15	-12	-8	2

The value in the final cell is the best score for the alignment

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More examples

- Sequence alignment applet at:

  http://www.iro.umontreal.ca/~casagran/baba.html

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Semi-global alignment

- In Needleman&Wunsch DP algorithm the gap penalty is assessed regardless of whether gaps are located internally or at the terminal ends.
- Terminal gaps may not be biologically significant

```
AATCTATA
--TCT---
```

- Treat terminal gaps differently than internal gaps → semi-global alignment
- What modifications should be made to the original DP?

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Local sequence alignment

- Suppose, we have a long DNA sequence (e.g., 4000 bp) and we want to compare it with the complete yeast genome (12.5M bp).
- What if only a portion of our query, say 200 bp length, has strong similarity to a gene in yeast.
  - Can we find this 200 bp portion using (semi) global alignment?

    Probably not. Because, we are trying to align the complete 4000 bp sequence, thus a random alignment may get a better score than the one that aligns 200 bp portion to the similar gene in yeast.

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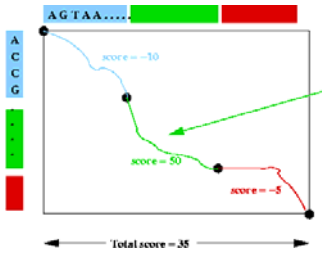
Local alignment



local alignment may have higher score than overall global alignment

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Local sequence alignment (Smith-Waterman)

- S(i,j) = optimal local similarity among suffixes of A(1:i) and B(1:j)
- Recurrence relation
  - S(i,0) = 0
  - S(0,j) = 0
  - S(i,j) = max [0,
    S(i,j-1) + s(-,B(j)),
    S(i-1,j) + s(A(i),-),
    S(i-1,j-1) + s(A(i),B(j))]
  - Assume linear gap model

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Example

Q: E Q L L K A L E F K L
P: K V L E F G Y

Linear gap model
Gap = -1
Match = 4
Mismatch = -2

	-	E	Q	L	L	K	A	L	E	F	K	L
-												
K												
V												
L												
E												
F												
G												
Y												

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Example

Q: E Q L L K A L E F K L
P: K V L E F G Y

Linear gap model
Gap = -1
Match = 4
Mismatch = -2

	-	E	Q	L	L	K	A	L	E	F	K	L
-	0	0	0	0	0	0	0	0	0	0	0	0
K	0											
V	0											
L	0											
E	0											
F	0											
G	0											
Y	0											

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Example

Q: E Q L L K A L E F K L
P: K V L E F G Y

Linear gap model
Gap = -1
Match = 4
Mismatch = -2

	-	E	Q	L	L	K	A	L	E	F	K	L
-	0	0	0	0	0	0	0	0	0	0	0	0
K	0	0	0	0	0	4	3	2	1	0	4	3
V	0	0	0	0	0	3	2	1	0	0	3	2
L	0	0	0	4	4	3	2	6	5	4	3	7
E	0	4	3	3	3	2	1	5	10	9	8	7
F	0	3	2	2	2	1	0	4	9	14	13	12
G	0	2	1	1	1	0	0	3	8	13	12	11
Y	0	1	0	0	0	0	0	2	7	12	11	10

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Example

Q: E Q L L K A L E F K L
P: K V L E F G Y

Linear gap model
Gap = -1
Match = 4
Mismatch = -2

	-	E	Q	L	L	K	A	L	E	F	K	L
-	0	0	0	0	0	0	0	0	0	0	0	0
K	0	0	0	0	0	4	3	2	1	0	4	3
V	0	0	0	0	0	3	2	1	0	0	3	2
L	0	0	0	4	4	3	2	6	5	4	3	7
E	0	4	3	3	3	2	1	5	10	9	8	7
F	0	3	2	2	2	1	0	4	9	14	13	12
G	0	2	1	1	1	0	0	3	8	13	12	11
Y	0	1	0	0	0	0	0	2	7	12	11	10

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Example

Q: E Q L L K A L E F K L
P: K V L E F G Y

Linear gap model
Gap = -1
Match = 4
Mismatch = -2

	-	E	Q	L	L	K	A	L	E	F	K	L
-	0	0	0	0	0	0	0	0	0	0	0	0
K	0	0	0	0	0	4	3	2	1	0	4	3
V	0	0	0	0	0	3	2	1	0	0	3	2
L	0	0	0	4	4	3	2	6	5	4	3	7
E	0	4	3	3	3	2	1	5	10	9	8	7
F	0	3	2	2	2	1	0	4	9	14	13	12
G	0	2	1	1	1	0	0	3	8	13	12	11
Y	0	1	0	0	0	0	0	2	7	12	11	10

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Example

Q: E Q L L K A L E F K L
P: K V L E F G Y

Alignment

```
Q:  K A - L E F
P:  K - V L E F
```

	-	E	Q	L	L	K	A	L	E	F	K	L
-	0	0	0	0	0	0	0	0	0	0	0	0
K	0	0	0	0	0	4	3	2	1	0	4	3
V	0	0	0	0	0	3	2	1	0	0	3	2
L	0	0	0	4	4	3	2	6	5	4	3	7
E	0	4	3	3	3	2	1	5	10	9	8	7
F	0	3	2	2	2	1	0	4	9	14	13	12
G	0	2	1	1	1	0	0	3	8	13	12	11
Y	0	1	0	0	0	0	0	2	7	12	11	10

Example

Q: E Q L L K A L E F K L
P: K V L E F G Y

Alignment

Q: K - A L E F
P: K V - L E F

	-	E	Q	L	L	K	A	L	E	F	K	L
-	0	0	0	0	0	0	0	0	0	0	0	0
K	0	0	0	0	0	4	3	2	1	0	4	3
V	0	0	0	0	0	3	2	1	0	0	3	2
L	0	0	0	4	4	3	2	6	5	4	3	7
E	0	4	3	3	3	2	1	5	10	9	8	7
F	0	3	2	2	2	1	0	4	9	14	13	12
G	0	2	1	1	1	0	0	3	8	13	12	11
Y	0	1	0	0	0	0	0	2	7	12	11	10

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Example

Q: E Q L L K A L E F K L
P: K V L E F G Y

Alignment

Q: K A L E F
P: K V L E F

	-	E	Q	L	L	K	A	L	E	F	K	L
-	0	0	0	0	0	0	0	0	0	0	0	0
K	0	0	0	0	0	4	3	2	1	0	4	3
V	0	0	0	0	0	3	2	1	0	0	3	2
L	0	0	0	4	4	3	2	6	5	4	3	7
E	0	4	3	3	3	2	1	5	10	9	8	7
F	0	3	2	2	2	1	0	4	9	14	13	12
G	0	2	1	1	1	0	0	3	8	13	12	11
Y	0	1	0	0	0	0	0	2	7	12	11	10

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Another Example

Find the local alignment between:

Q: G C T G G A A G G C A T
P: G C A G A G C A C G

Linear gap model
Gap = -4
Match = +5
Mismatch = -4

Q

P	--	G	C	T	G	G	A	A	G	G	C	A	T
--	0	0	0	0	0	0	0	0	0	0	0	0	0
G	0												
C	0												
A	0												
G	0												
A	0												
G	0												
C	0												
A	0												
C	0												
G	0												

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Another Example

Q's subsequence: G A A G - G C A
P's subsequence: G C A G A G C A

Q

P	--	G	C	T	G	G	A	A	G	G	C	A	T
--	0	0	0	0	0	0	0	0	0	0	0	0	0
G	0	5	1	0	5	5	1	0	5	5	1	0	0
C	0	1	10	6	2	1	1	0	1	1	10	6	2
A	0	0	6	6	2	0	6	6	2	0	6	15	11
G	0	5	2	2	11	7	3	2	11	7	3	11	11
A	0	1	1	0	7	7	11	8	7	7	3	8	7
G	0	5	1	0	5	11	7	7	13	12	8	4	4
C	0	0	10	6	2	7	7	3	9	8	17	13	9
A	0	0	6	6	2	3	11	12	8	5	13	22	18
C	0	0	5	2	2	0	7	8	8	4	18	18	18
G	0	5	1	1	7	7	5	4	13	13	14	14	14

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Local vs. Global alignment

PIR Entry				Similarity Score: Global, End Penalty	Global, No End Penalty	Local
HBHU	vs	HBHU	Hemoglobin beta-chain—human	725	725	725
		HAHU	Hemoglobin alpha-chain—human	314	320	322
		MYHU	Myoglobin—Human	121	164	166
		GPYL	Leghemoglobin—Yellow lupin	8	28	43
		LZCH	Lysozyme precursor—Chicken	−107	16	32
		NRBO	Pancreatic ribonuclease—Bovine	−124	16	31
		CCHU	Cytochrome c—Human	−160	10	26
MCHU	vs	MCHU	Calmodulin—Human	671	671	671
		TPHUCS	Troponin C, skeletal muscle	395	430	438
		PVPK2	Parvalbumin beta—Pike	−57	103	115
		CBHUH	Calpain heavy chain—Human	−2085	89	100
		AQJFNV	Aequorin precursor—Jelly fish	−65	48	76
		KLSWM	Calcium binding protein—Scallop	−89	45	52
QRHULD	vs	EGMSMG	Epidermal growth factor precursor	−591	475	655

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Complexity

- O(mn) time
- O(mn) space
  - O(max(m,n)) if only distance value is needed
- More complicated "divide-and-conquer" algorithm that doubles time complexity and uses O(min(m,n)) space [Hirschberg, JACM 1977]

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Time and space bottlenecks

- Comparing two one-megabase genomes.
- Space:
  An entry: 4 bytes;
  Table: 4 * 10^6 * 10^6= 4 T bytes memory.
- Time:
  1000 MHz CPU: 1M entries/second;
  10^{12} entries: 1M seconds = 10 days.

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Affine Gap Penalties

- -d for gap opening
- -e for gap extension
- *H(i,j)* for gaps in horizontal seq.
- *V(i,j)* for gaps in vertical seq.

$$S(i,j) = \max\begin{cases} S(i-1,j-1) + s(x_i, y_j), \\ H(i-1,j-1), \\ V(i-1,j-1). \end{cases}$$

$$H(i,j) = \max\begin{cases} F(i-1,j) - d, \\ H(i-1,j) - e. \end{cases}$$

$$V(i,j) = \max\begin{cases} F(i,j-1) - d, \\ V(i,j-1) - e. \end{cases}$$

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