CMSC423: Bioinformatic Algorithms, Databases and Tools
Lecture 9

Sequence alignment: inexact alignment

dynamic programming, gapped alignment, heuristics

Play around with alignments

- USC alignment library (seqaln)

http://www.mhoenicka.de/software/cygwinports/seqaln.html

Global alignment recap

Score[i,j] is the maximum of:

1. Score[i-1, j-1] + Value[S1[i-1],S2[j-1]] (S1[i-1], S2[j-1] aligned)
2. Score[i – 1, j] + Value[S1[i], -] (S1[i] aligned to gap)
3. Score[i, j – 1] + Value[-, S2[j]] (S2[j] aligned to gap)

	-	A	G	C	G	T	A	G
-								
G								
T								
C								
A								
G								
A								
C								

```
AGCGTAG
GTCAGAC
```

Value(A,A) = 10
Value(A,G) = -5
Value(A,-) = -2

Global alignment recap

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3. Score[i, j – 1] + Value[-, S2[j]] (S2[j] aligned to gap)

	-	A	G	C	G	T	A	G
-	0	-4	-8	-12	-16	-20	-24	-28
G	-4	-5	6	2	-2	-6	-10	-14
T	-8	-9	2	1	-3	8	4	0
C	-12	-13	-2	12	8	4	3	-1
A	-16	-2	-6	8	7	3	14	10
G	-20	-6	8	4	18	14	10	24
A	-24	-10	4	3	14	13	24	20
C	-28	-14	0	14	10	9	20	19

```
AG-C-GTAG
-GTCAG-AC
```

Value(A,A) = 10
Value(A,G) = -5
Value(A,-) = -4

Local alignment recap

Score[i,j] is the maximum of:

0. 0
1. Score[i-1, j-1] + Value[S1[i-1],S2[j-1]] (S1[i-1], S2[j-1] aligned)
2. Score[i – 1, j] + Value[S1[i], -] (S1[i] aligned to gap)
3. Score[i, j – 1] + Value[-, S2[j]] (S2[j] aligned to gap)

	-	A	G	C	G	T	A	G
-								
G								
T								
C								
A								
G								
A								
C								

```
AGCGTAG
GTCAGAC
```

Value(A,A) = 10
Value(A,G) = -5
Value(A,-) = -2

How much do we pay for gaps?

- In the edit-distance/alignment framework

Cost(n gaps in a row) = n * Cost(gap)

- This doesn't work for e.g. RNA-DNA alignments

```
ACAGTTCGACTAGAGGACCTAGACCACTCTGT
    TTCGA----------TAGACCAC
```

- Affine gap penalties

Cost(n gaps in a row) = Cost(gap open) + n * Cost(gap)

- Gap opening penalty is high, gap extension penalty is low (once we start a gap we might as well pile more gaps on top)

Dynamic programming solution

- Traditional 1-table approach doesn't work anymore
- Instead, use 4 tables:
  - V – stores value of best alignment between S1[1..i], S2[1..j]
  - G – best alignment between S1[1..i], S2[1..j] s.t. S1[i] aligned with S2[j]
  - E – best alignment between S1[1..i], S2[1..j], s.t. alignment ends with gap in S1
  - F – best alignment between S1[1..i], S2[1..j], s.t. alignment ends with gap in S2
- V[i,j] = max(E[i,j], F[i,j], G[i,j])
- As in traditional approach, find box in V matrix where V[i,j] is maximal.

Affine gap recurrences

- V[i,j] = max[E[i,j], F[i,j], G[i,j]]
- G[i,j] = V[i-1, j-1] + Value(S1[i], S2[j])
  - irrespective how we got here (hence use of V), S1[i] and S2[j] are matched
- E[i,j] = max{E[i, j-1], V[i, j-1] – GapOpen} – GapExtend
  - either we add a gap in S1 to an existing one (E-GapExtend)
  - or we add a gap in S1 when there was none (V-GapOpen-GapExtend)
- F[i,j] = max{F[i-1, j], V[i-1, j] – GapOpen} – GapExtend
  - either we add a gap in S2 to an existing one (F–GapExtend)
  - or we add a gap in S2 when there was none (V-GapOpen-GapExtend)

Running times

- All these algorithms run in O(mn) – quadratic time
- Note – this is significantly worse than exact matching
- On Wednesday we'll talk about speed-up opportunities
- BTW, how much space is needed?
- If we only need to find the best score (not the exact alignment as well) – O(min(m,n))
- If we need to find the best alignment – elegant divide and conquer algorithm leads to linear space solution.

Where do the alignment scores come from?

- PAM matrices
  - PAM1 – based on frequency of mutations between closely related proteins (within 1 "evolutionary step")
  - PAM 2 - ... within 2 evolutionary steps
  - ... PAM 250 – commonly used
- BLOSUM matrices
  - Frequency of mutations between proteins that are x% similar
  - BLOSUM100 – based on proteins that are exactly the same (e.g. score(A,A) is defined but not score(A,G))
  - BLOSUM62 – commonly used
- gap scores usually determined empirically

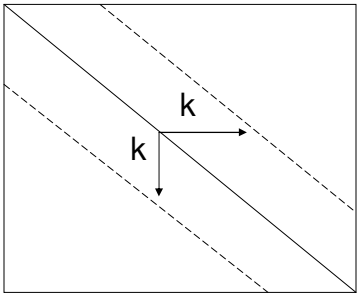
BLOSUM62

Table 2 - The log odds matrix for BLOSUM 62

	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
A	4	0	-2	-1	-2	0	-2	-1	-1	-1	-1	-2	-1	-1	-1	1	0	0	-3	-2
C		9	-3	-4	-2	-3	-3	-1	-3	-1	-1	-3	-3	-3	-3	-1	-1	-1	-2	-2
D			6	2	-3	-1	-1	-3	-1	-4	-3	1	-1	0	-2	0	-1	-3	-4	-3
E				5	-3	-2	0	-3	1	-3	-2	0	-1	2	0	0	-1	-2	-3	-2
F					6	-3	-1	0	-3	0	0	-3	-4	-3	-3	-2	-2	-1	1	3
G						6	-2	-4	-2	-4	-3	0	-2	-2	-2	0	-2	-3	-2	-3
H							8	-3	-1	-3	-2	1	-2	0	0	-1	-2	-3	-2	2
I								4	-3	2	1	-3	-3	-3	-3	-2	-1	3	-3	-1
K									5	-2	-1	0	-1	1	2	0	-1	-2	-3	-2
L										4	2	-3	-3	-2	-2	-2	-1	1	-2	-1
M											5	-2	-2	0	-1	-1	-1	1	-1	-1
N												6	-2	0	0	1	0	-3	-4	-2
P													7	-1	-2	-1	-1	-2	-4	-3
Q														5	1	0	-1	-2	-2	-1
R															5	-1	-1	-3	-3	-2
S																4	1	-2	-3	-2
T																	5	0	-2	-2
V																		4	-3	-1
W																			11	2
Y																				7

Heuristics

- What if limit the # of differences allowed? E.g. we expect the sequences to be very similar.
- Compute 'banded' alignment – stay within # of differences (k) from the diagonal.
- Optimal alignment cannot stray too far from diagonal



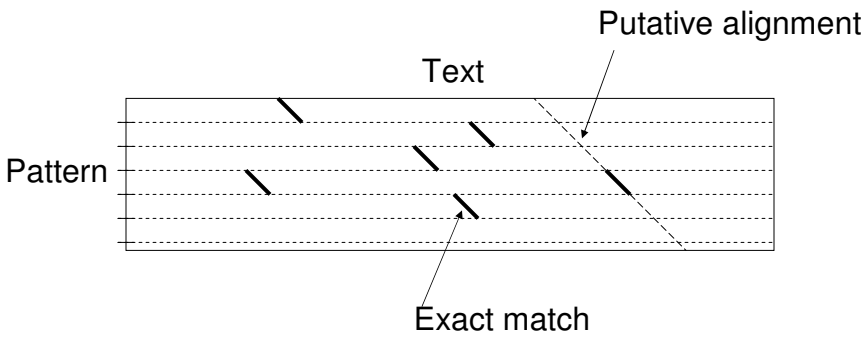
O(km) running time and space

- What if we do not know k? Do binary search to find it

Exclusion methods

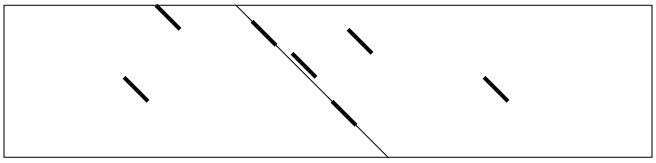
- Assume P must match T with at most k errors. Find places in T where P cannot match.
- Split P into **floor(n/k+1)**-sized chunks.
- If P matches T with less than k errors => at least one chunk matches with no errors
- Use any exact matching algorithm to find places where a chunk matches T, then run dynamic programming in that vicinity.
- Running time, on average O(m)

Exclusion methods



"Famous" approaches

- FASTA (Pearson et al.)
  - Take all k-mers (substrings of length k) from Pattern and identify whether and where they match in the Text
  - Assume the k-mer starting at pos'n i in Pattern matches at position j in Text, remember (j – i) – the diagonal on which the match occured
  - Identify "heavy" diagonals – diagonals where many k-mers match, then refine the diagonals with Smith Waterman
  - Also look for off-diagonal matches to account for gaps



"Famous" approaches

- BLAST (Altschul et al.)
  - Find short k-mer matches
  - Also search for possible inexact matches, e.g. all k-mers within 1 difference from current one.
  - Extend exact matches with Smith-Waterman algorithm
  - Assign probabilistic scores to matches: what is the probability of finding a match with the same S-W alignment score just by chance (e.g. matching a random string)?