BIOS 477/877
Bioinformatics and Molecular Evolution

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(School of Biological Sciences)

Spring 2026 | Lecture 11

Today's topics

- Statistical Significance of Alignment Scores
- Similarity Search
  - FASTA and BLAST

Significance of alignment scores

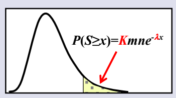
- $P(S \geq x|H_0)$: probability of getting the alignment score $S \geq x$ by chance

  Karlin-Altschul equation (Karlin and Altschul 1990)

  $$P(S \geq x) = 1 - \exp[-Kmne^{-\lambda x}] \approx Kmne^{-\lambda x}$$

  - K and λ: parameters based on a given scoring matrix and the amino acid composition of the sequences
  - m and n: lengths of sequences aligned

  → Solved for ungapped local alignments
  → Can be applied for gapped local alignments



EVD (Gumbel distribution):
$P(S \geq x) = 1 - \exp[-e^{-(x-\mu)/\beta}]$
$\lambda = 1/\beta$, $\mu = (\ln Kmn)/\lambda$

- E-value = $P(S \geq x|H_0) \times N$

  N: the number of sequences in the dataset

  Expected number of randomly selected sequences in the dataset that have alignment scores $\geq x$

E-value ≠ P-value

Estimation of K, λ, and P-value

- Estimation of K and λ from empirical distribution of random alignment scores (used in LALIGN and PRSS)
  1) The second sequence is shuffled many times
     → Simulates random sequences
  2) Smith-Waterman local alignment score is calculated from each alignment: $P(S \geq x|H_0)$
  3) The distribution is fitted to an extreme value distribution to obtain estimates of K and λ
  4) P-value is estimated based on the K and λ, and the original alignment score x: $P(S \geq x) \approx Kmne^{-\lambda x}$

Simulation of random score distribution
(example)

[Input sequences]
- RECA_ECOLI (P0A7G6; 353 amino acids)
- RAD51_YEAST (P25454; 400 amino acids)

Smith-Waterman local alignment score = 293
(BLOSUM50, gap opening: -10, gap extension: -1)

```
RECA_ECOLI        3 IDENK-QKALAAALGQIEKQFGKGSIMRLGEDRSMDVETISTGSLSLDIA     51
                    |.|.| .|.|..|...:...|...:...:   |..::..::|||.:||..
RAD51_YEAST     124 ISEAKADKLLNEAARLVPMGFVTAADFHM---RRSELICLTTGSKNLDTL    170

RECA_ECOLI       52 LGAGGLPMGRIVEIYGPESSGKTTL------TLQV-IAAAQREGKTCAFI     94
                    || ||:..|.|.|::|...:||:.|      |.|: :.....||| |.:|
RAD51_YEAST     171 LG-GGVETGSITELFGEFRTGKSQLCHTLAVTCQIPLDIGGGEGK-CLYI    218

RECA_ECOLI       95 DAEHALDPI----YARKLGVDID----NLLCSQPDTGEQALEICDALAR-    135
                    |.|..:.|:    .|:::|:|.|    |:..::....::.|.:.||.|:
RAD51_YEAST     219 DTEGTFRPVRLVSIAQRFGLDPDDALNNVAYARAYNADHQLRLLDAAAQM    268

RECA_ECOLI      136 --SGAVDVIVVDSVAALTPKAEI--EGEIGDSHMGLAARMMSQAMRKLAG    181
                      .....:||||||.||. :.:.  .||:...:|.||..|  :|:::||
RAD51_YEAST     269 MSESRFSLIVVDSVMALY-RTDFSGRGELSARQMHLAKFM--RALQRLA-    314

RECA_ECOLI      182 NLKQSNTLLIFINQIRMKI--GVMFG-NPETTTGGNALKFYASVRLDIRR    228
                      .|....:..||:..::  |:.|. :|:...|||.:....::.||
RAD51_YEAST     315 --DQFGVAVVVTNQVVAQVDGGMAFNPDPKKPIGGNIMAHSSTTRL----    358

RECA_ECOLI      229 IGAVKEGENVVGSETRVKVVKNKIAAPFKQAEFQILYGEGI    269
                      ..|:|:   |.:...||| :....|..:..|.| |.:|:
RAD51_YEAST     359 --GFKKGK---GCQRLCKVV-DSPCLPEAECVFAI-YEDGV    392
```

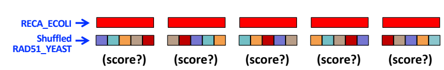
Using EMBOSS WATER @ EBI

Simulation of random score distribution
(example)

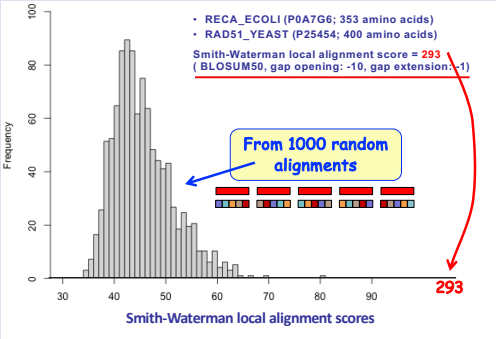
[Input sequences]
- RECA_ECOLI (P0A7G6; 353 amino acids)
- RAD51_YEAST (P25454; 400 amino acids)

Smith-Waterman local alignment score = 293
(BLOSUM50, gap opening: -10, gap extension: -1)

1. Shuffle RAD51_YEAST 1000 times
   (generate 1000 random sequences)
2. Align with RECA_ECOLI
   (generate 1000 random local alignments)



Simulation of random score distribution
(example)



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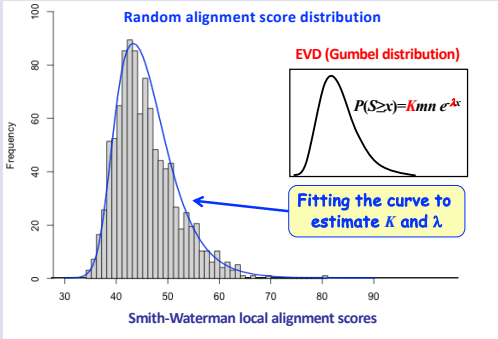
Simulation of random score distribution
(example)



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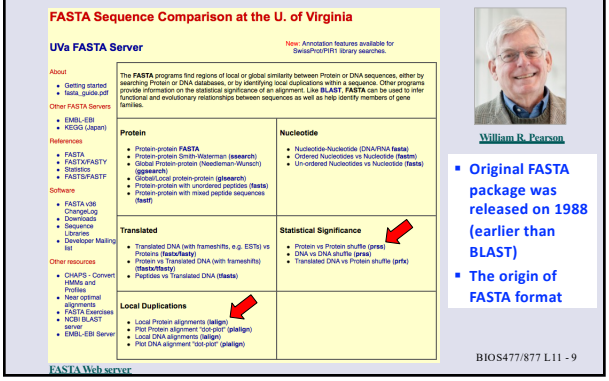
FASTA Web server by William Pearson



- Original FASTA package was released on 1988 (earlier than BLAST)
- The origin of FASTA format

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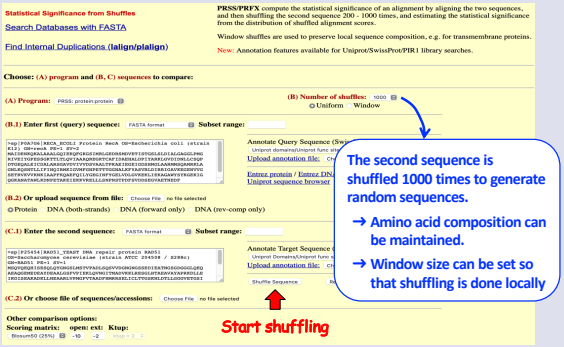
Statistical analysis of alignments by PRSS



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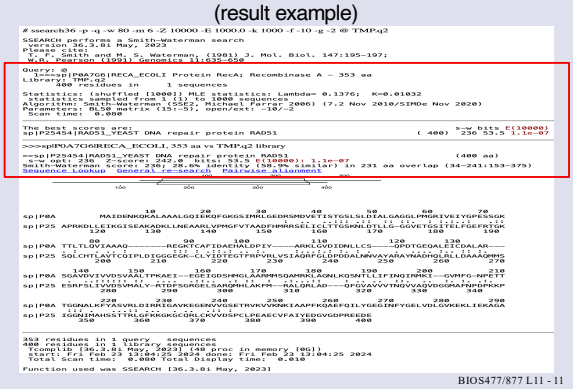
Statistical analysis of alignments by PRSS
(result example)



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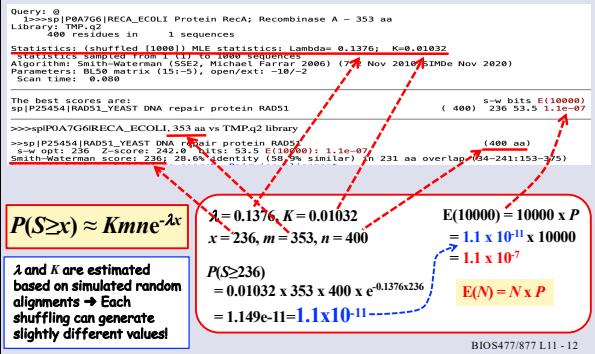
Statistical analysis of alignments by PRSS
(result example)



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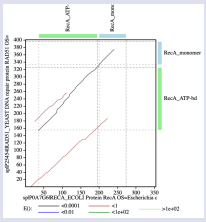
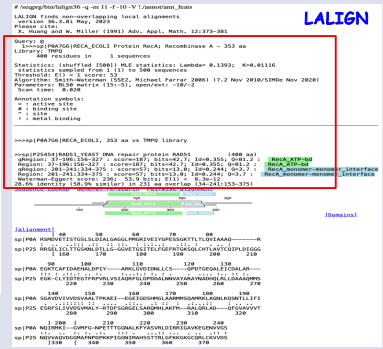
Pairwise alignment by LALIGN

PLALIGN (dot plot)



LALIGN



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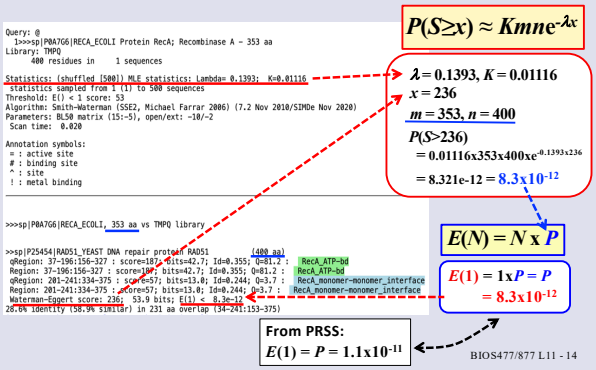
Pairwise alignment by LALIGN

$P(S \geq x) \approx Kmne^{-\lambda x}$



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Estimation of K and λ in BLAST

➢ K and λ ➔ from Altschul & Gish (1996) table.

a	b	λ	K	H (nats)	a	b	λ	K	H (nats)
∞	0–∞	0.232	0.11	0.34	11	8–11	0.197	0.05	0.21
					11	6, 7	0.190	0.04	0.19
16	4–16	0.222	0.08	0.31	11	5	0.184	0.04	0.17
16	3	0.213	0.06	0.27	11	4	0.177	0.031	0.15
16	2	0.207	0.05	0.24	11	3	0.167	0.028	0.11
16	1	0.180	0.024	0.15	11	2	0.130	0.009	0.06
					11	1		Linear	
15	8–15	0.222	0.09	0.31					
15	6, 7	0.219	0.08	0.29	10	8–10	0.183	0.04	0.17
15	4, 5	0.216	0.07	0.28	10	6, 7	0.178	0.035	0.16
15	3	0.210	0.06	0.25	10	5	0.168	0.026	0.13
15	2	0.202	0.05	0.22	10	4	0.156	0.020	0.10
15	1	0.166	0.018	0.11	10	3	0.139	0.013	0.07
					10	2	0.099	0.007	0.03
14	8–14	0.218	0.08	0.29	10	1		Linear	
14	5–7	0.214	0.07	0.27					
14	4	0.205	0.05	0.24	9	7–9	0.164	0.029	0.13
14	3	0.201	0.05	0.22	9	5, 6	0.152	0.021	0.10
14	2	0.188	0.034	0.17	9	4	0.134	0.014	0.07
14	1	0.140	0.009	0.07	9	3	0.107	0.008	0.04
					9	1, 2		Linear	
13	8–13	0.211	0.06	0.27					
13	5–7	0.205	0.05	0.24	8	8	0.139	0.017	0.08
13	4	0.202	0.05	0.22	8	7	0.134	0.015	0.07
13	3	0.188	0.034	0.18	8	6	0.127	0.013	0.06
13	2	0.174	0.025	0.13	8	5	0.117	0.011	0.05
13	1	0.114	0.006	0.04	8	4	0.101	0.009	0.03
					8	1–3		Borderline or linear	
12	7–12	0.205	0.06	0.24					
12	5, 6	0.197	0.05	0.21	7	7	0.100	0.010	0.04
12	4	0.192	0.04	0.18	7	6	0.094	0.010	0.03
12	3	0.178	0.028	0.15	7	1–5		Borderline or linear	
12	2	0.158	0.019	0.10					
12	1		Borderline		1–6	1–6		Linear	

← For BLOSUM50;
a: gap opening penalty
b: gap extension penalty
(affine gap penalty)

Based on 10,000 random sequence pairs,
➔ calculate optimal local alignment scores, S.
➔ estimate K and λ by fitting the distribution of S with $P(S \geq x) \approx Kmne^{-\lambda x}$

This is what BLAST does

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Significance of alignment scores

➢ Alignment scores cannot be compared directly because they depend on: scoring matrix, gap penalty, algorithms used

➢ Statistical significance of alignments can be tested
- Null hypothesis (H_0): the given alignment score is possible by aligning two randomly selected (unrelated) sequences
- If H_0 is rejected ($P <$ threshold),
  ➔ The given score is not possible simply by chance
  ➔ The alignment is meaningful (the two sequences must be related).

➢ P-value or E-value (E=NP) can be compared regardless of the scoring systems used for alignments.
➔ $0 <$ P-value < 1 vs. $0 <$ E-value $< N$

NOTE: E-values change depending on the number of data used (if N is small, E-value becomes small)

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Similarity search

➢ What can you learn from similarity search?
- Related genes in another organisms
  ➔ Homologue candidates
- Possible functions of genes/protein

[From genomic sequence]
- Gene locations
- Against cDNA sequences
  ➔ exon-intron structure

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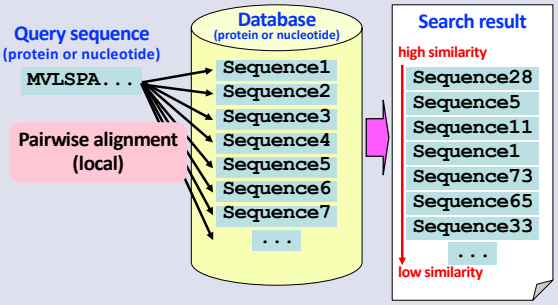
Similarity search



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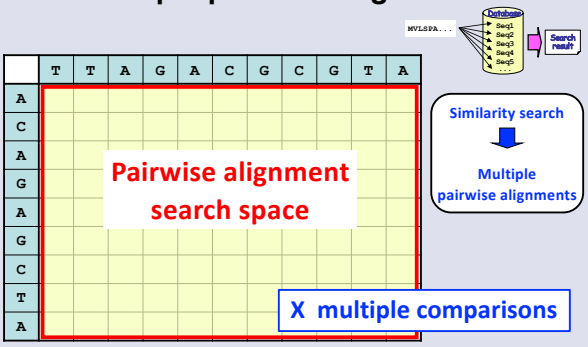
Similarity search as multiple pairwise alignments



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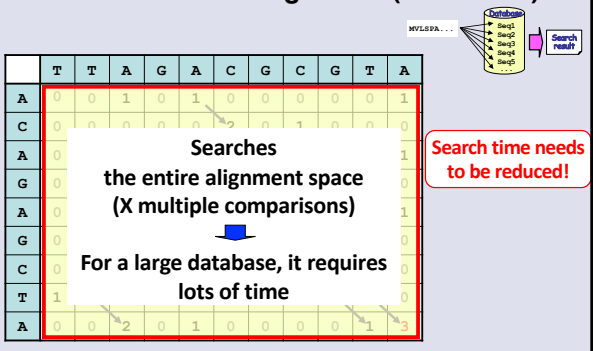
Similarity search using Smith-Waterman algorithm (SSEARCH)



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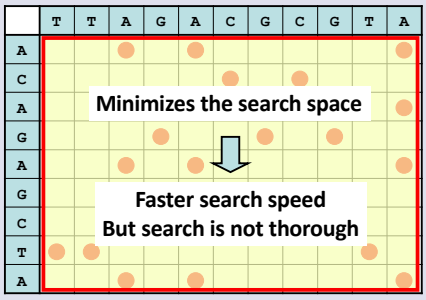
Heuristic algorithm (FASTA/BLAST)



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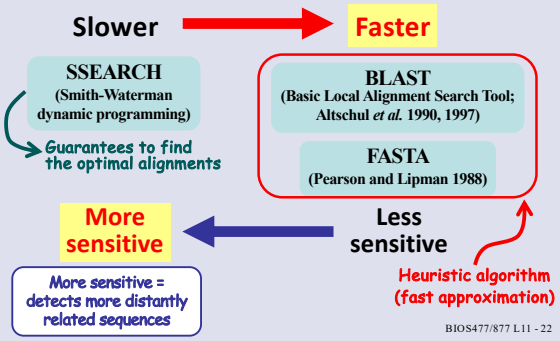
Search speed and sensitivity



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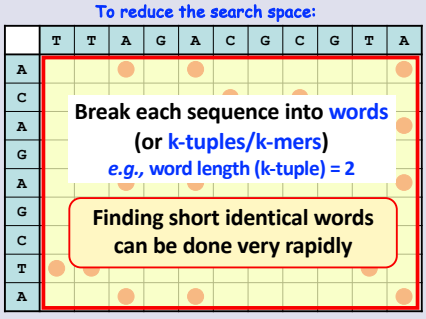
Word/k-tuple matching



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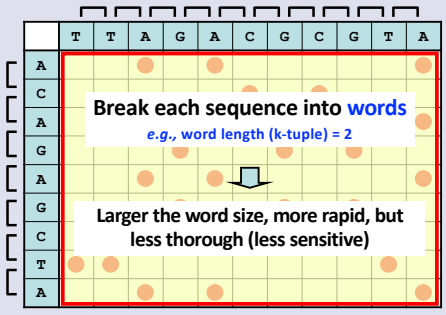
Word/k-tuple matching



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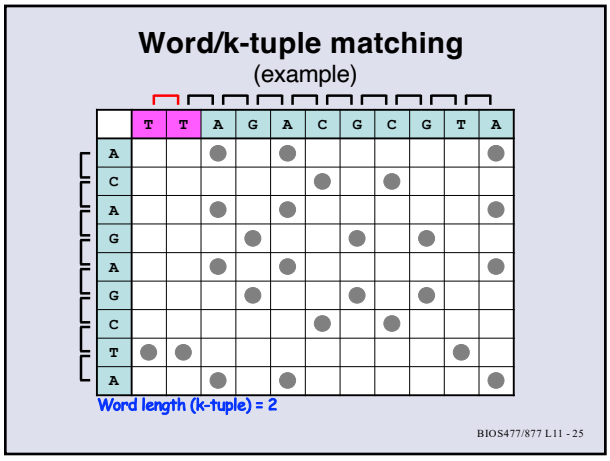

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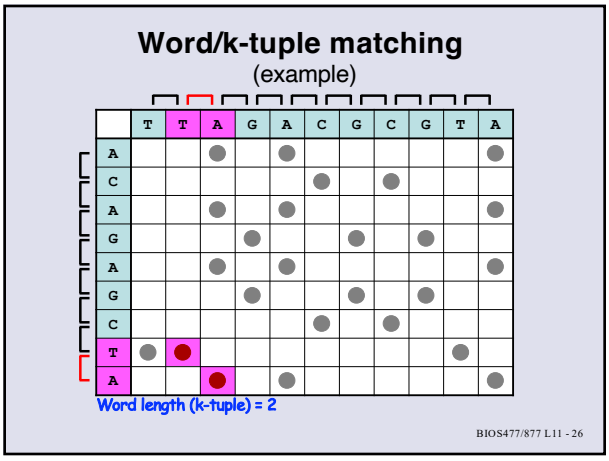

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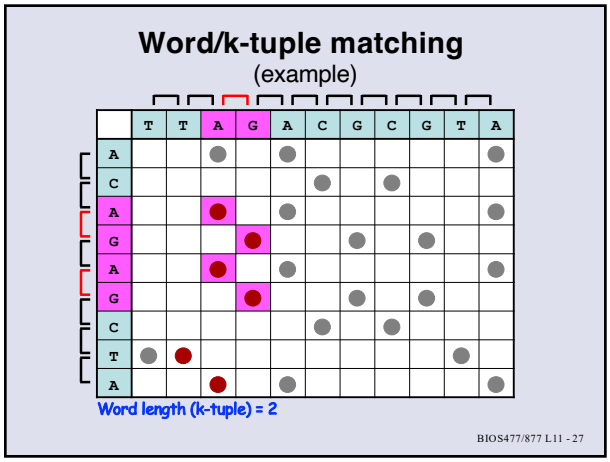

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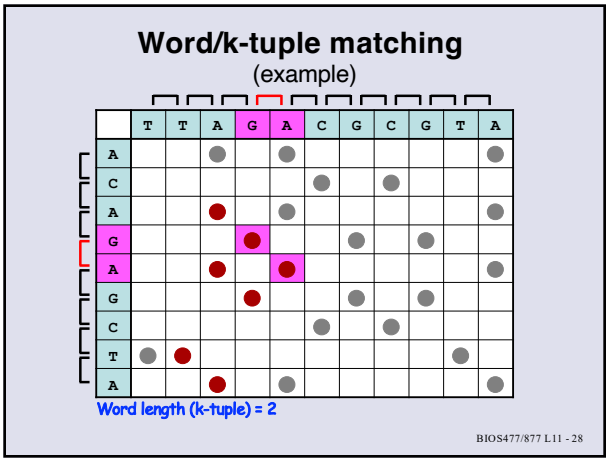

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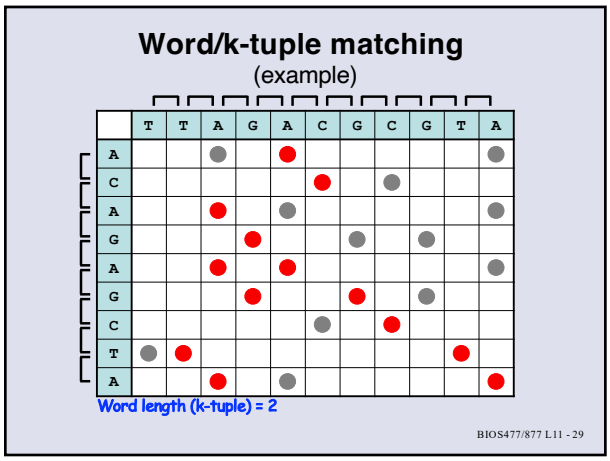

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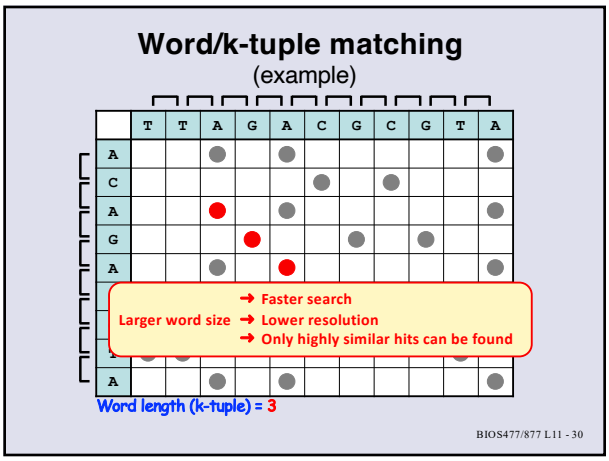

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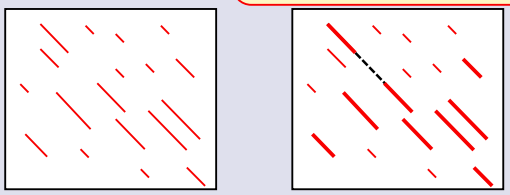
FASTA algorithm

1. Find identities using k-tuples
   (k-tuples = words)
2. Join diagonals without gaps
3. Choose top 10 diagonals using a scoring matrix (e.g., BLOSUM62)
   (init1: the top diagonal score)



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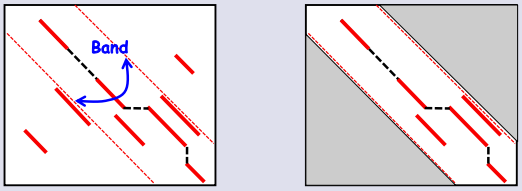
FASTA algorithm

4. Join again with gaps
   (initn: score of the longer diagonal)
5. A diagonal band is defined
   (width: 32 if k=1, 16 if k=2 for protein)
6. Find optimal local alignment using dynamic programming algorithm within the band
   (opt: the final score)



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FASTA similarity search

- Ranking
  - Database sequences are ranked based on z-values or OPT scores
    → z-value = the number of standard deviations from the mean (standardized score)
- Alignment
  - The high scored sequence pairs are aligned using the full Smith-Waterman dynamic programming algorithm
    → Not just from the diagonal band → better alignment
- FASTA/SSEARCH website (more on CANVAS)
  Original FASTA server @ U Virginia (By W. Pearson)
  FASTA website @ EBI

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BLAST similarity search

https://blast.ncbi.nlm.nih.gov/Blast.cgi

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BLAST resources

- BLAST Help
  https://blast.ncbi.nlm.nih.gov/doc/blast-help/
  - Guide to BLAST home and search pages
  - BLAST Command Line User Manual
  - BLAST Tutorials on YouTube
- BLAST Statistics
  https://www.ncbi.nlm.nih.gov/BLAST/tutorial/Altschul-1.html
- "BLAST" (online version)
  (see more on CANVAS)

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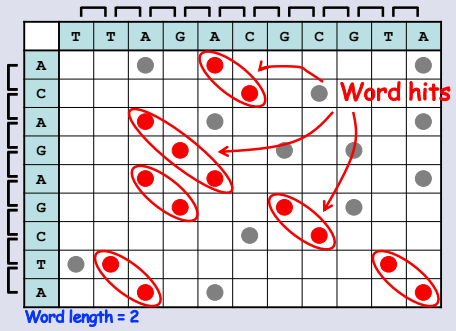
BLAST algorithm: seeding



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BLAST algorithm: seeding
(including neighborhood)

- Using words reduces the search space
- Neighborhood increases the sensitivity

Match = 2
Mismatch (Ts) = -1
Mismatch (Tv) = -5

TC / TC = 2 + 2 = 4

TC / TT = 2 - 1 = 1

TC / CC = -1 + 2 = 1

...

TC / TA = 2 - 5 = -3

TC / TG = 2 - 5 = -3

TC / AC = -5 + 2 = -3

TC / AT = -5 - 1 = -6

Neighborhood

Neighborhood Threshold (T) = 1
[minimum score allowed to be the neighborhood]

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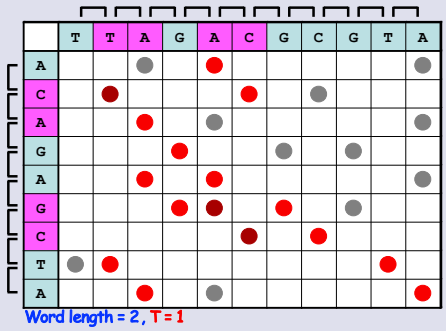
BLAST algorithm: seeding
(including neighborhood)



Word length = 2, T = 1

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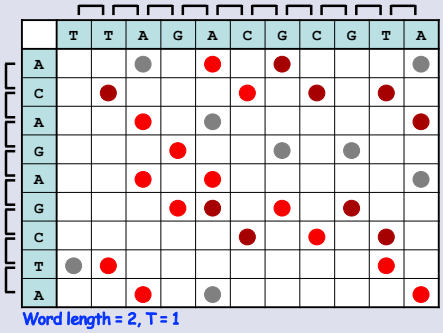
BLAST algorithm: seeding
(including neighborhood)



Word length = 2, T = 1

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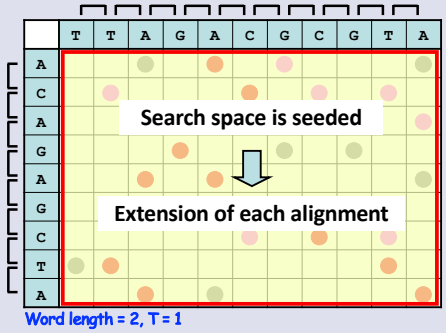
BLAST algorithm



Word length = 2, T = 1

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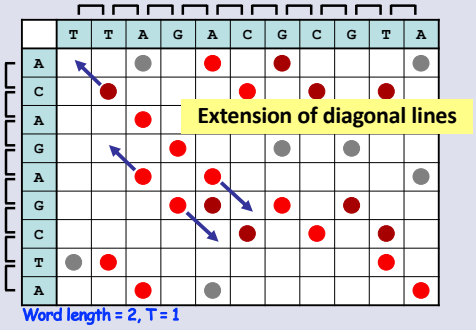
BLAST algorithm: extension



Word length = 2, T = 1

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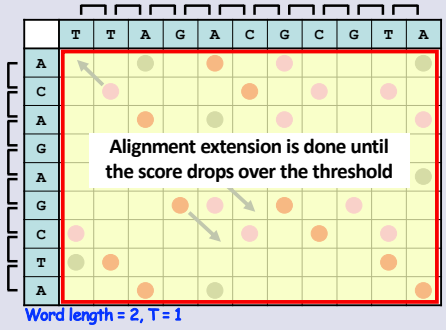
BLAST algorithm: extension



Word length = 2, T = 1

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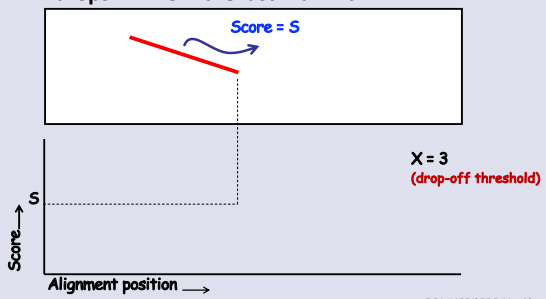
BLAST algorithm: extension

- Alignment extension is done until its score drops ≥ X from the last maximum



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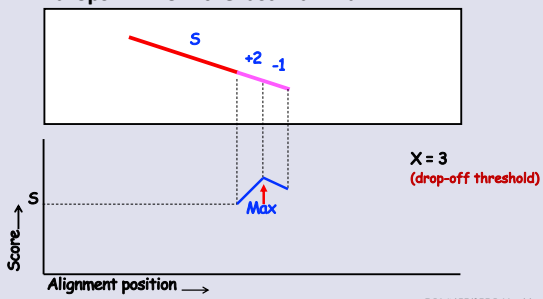
BLAST algorithm: extension

- Alignment extension is done until its score drops ≥ X from the last maximum



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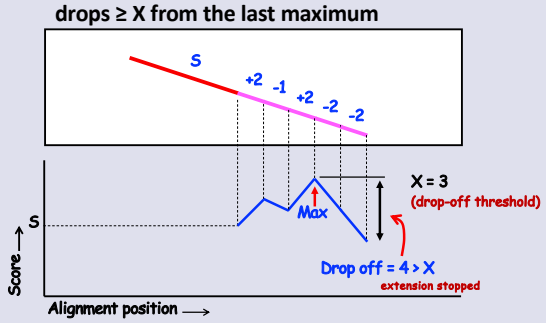
BLAST algorithm: extension

- Alignment extension is done until its score drops ≥ X from the last maximum



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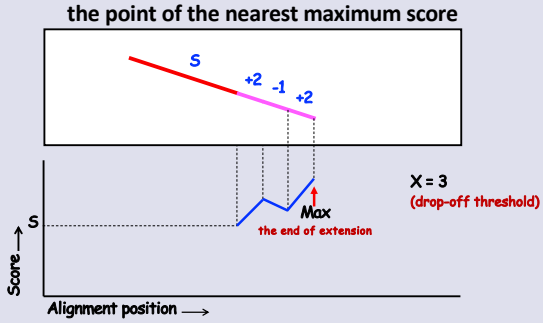
BLAST algorithm: extension

- Alignment extension is trimmed back to the point of the nearest maximum score



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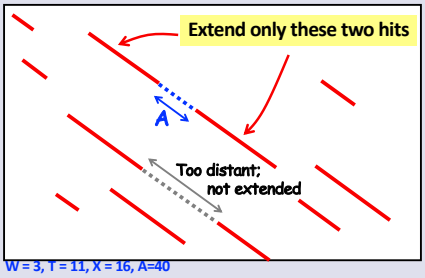
BLAST algorithm: extension
(two-hit method)

- Two-hit method: Extension is invoked only when two hits are found within distance A and on the same diagonal



W = 3, T = 11, X = 16, A=40

Many thresholds are set!

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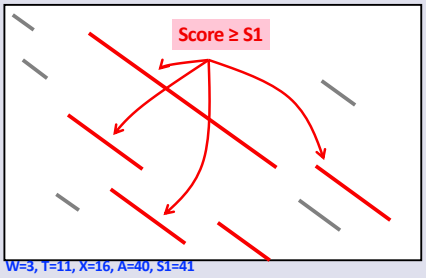
BLAST algorithm: HSP selection

- HSPs (High-scoring Segment Pairs) scoring higher than the threshold (S1) are chosen



W=3, T=11, X=16, A=40, S1=41

Even more thresholds!

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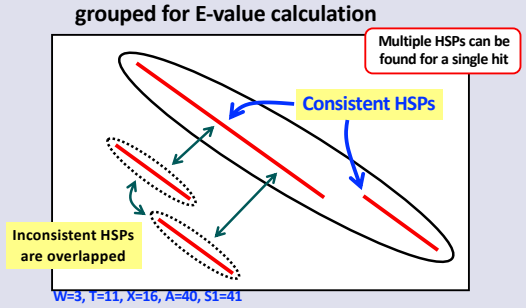
BLAST algorithm: HSP selection

- Consistent (non-overlapping) HSPs are grouped for E-value calculation



W=3, T=11, X=16, A=40, S1=41

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BLAST algorithm: gapped extension

- Gapped extension is done only for selected HSPs
- Gapped alignments scoring higher than the threshold (S2) is chosen as the final set

W=3, T=11, X=16, A=40, S1=41, S2=70

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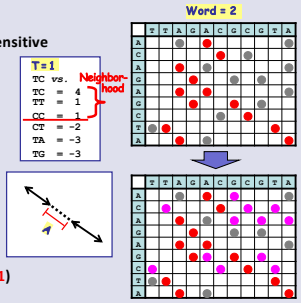
BLAST algorithm: summary

- Word-matching size (W)
  - → Longer words: faster but less sensitive
- Neighborhood threshold (T)
  - → Lower T:
    - ○ detects weaker similarities
    - ○ slower but more sensitive
- Extension
  - Drop-off score threshold (X)
  - Two-hit method (A: distance b/w 2 hits)
- HSP selection (ungapped alignment threshold: S1)
- Gapped HSP extension (gapped alignment threshold: S2)



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