COSC 348: Computing for Bioinformatics

Lecture 6: Sequence Alignment – Local Alignment

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Local sequence alignment

- By contrast to the global alignment, local alignments identify local regions of similarity between sequences of different lengths:

```
Global  FTFTALILLAVAV
        F--TAL-LLA-AV

Local   FTFTALILL-AVAV
        --FTAL-LLAAV--
```

- We distinguish two main approaches to the local alignment:
  - The **Smith-Waterman algorithm;**
  - **Word methods**, also known as **k-tuple methods**, implemented in the well-known families of programs FASTA and BLAST.

Smith-Waterman algorithm (SSEARCH)

- Variation of the Needleman-Wunsch algorithm. Thus, it is guaranteed to find the optimal local alignment (with respect to the scoring system being used).

- The difference to the Needleman-Wunsch algorithm is that *negative scoring matrix cells are set to zero*, which renders the local alignments visible. Backtracing ***starts at the highest scoring matrix cell and proceeds until a cell with score zero*** is encountered, yielding the highest scoring local alignment. We proceed with the second highest score, etc.

- The Smith-Waterman algorithm is costly: in order to align two sequences of lengths m and n, $O(mn)$ time and space are required.

Word (*k*-tuple) methods

- Word methods, also known as *k*-tuple methods, are heuristic methods that are not guaranteed to find an optimal alignment solution, but are significantly more efficient than Smith-Waterman algorithm.

- Word methods are especially useful in large-scale database searches where a large proportion of stored sequences will have essentially **no** significant match with the query sequence.

- Word methods are best known for their implementation in the database search tools **FASTA** and the **BLAST** family.

FASTA

EMBL-EBI

- FASTA (pronounced “fast A”) is a sequence alignment software package.

- The current FASTA package contains programs for protein:protein, DNA:DNA, protein:translated DNA (with frameshifts), and ordered or unordered peptide searches, etc.

- FASTA is one of the bioinformatics services of the The European Bioinformatics Institute (EBI) located in U.K., which is part of European Molecular Biology Laboratory (EMBL) (centered in Germany).

FASTA: how it works

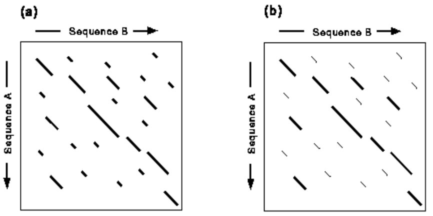
- Let us have a query sequence and a stored sequence.

- Identify a set of short non-overlapping strings (words, *k*-tuples) in the query sequence that will be matched against a stored sequence in the database.

- **Step1:** Initially the program stores word-to-word matches of a length *k* using a pattern search by the hash table. From the word hits that are returned, the program looks for segments that contain a cluster of nearby word hits. We have to define how many non-hits is allowed between nearby matching words so they form a cluster. *N* longest segments are stored.

FASTA – continuation

- **Step2:** Rescan the segments taken using the scoring matrix, while trimming the ends of the segments to include only those portions of segments that contribute highest to the segment score. A segment with the maximum score is identified. The highest score is referred to as **init1** score.



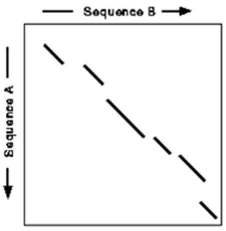
Find runs of identitical words
(Based on hash values)

Re-score using PAM matrix
Keep top scoring segments

FASTA – continuation

Step3:

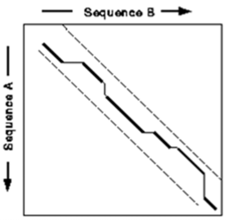
- Store segments with scores greater than a CUTOFF value. (This value is approximately one standard deviation above the average score expected from unrelated sequences in the database).



FASTA – continuation

Step3 (cont):

- Join these segments to form an approximate (global) alignment with gaps.
- Calculate the global alignment score that is the sum of the joined regions minus the penalties for gaps.



FASTA – continuation

Step4:

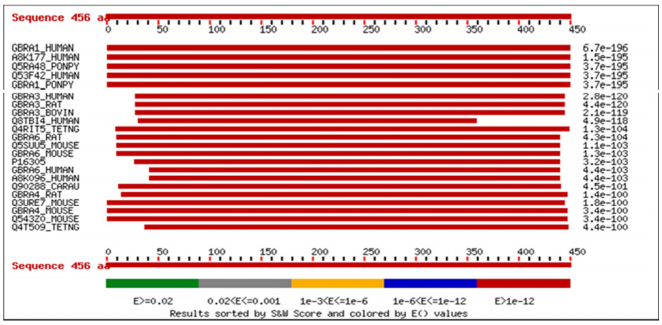
- This step uses a Smith-Waterman algorithm to create an optimised score (opt) for local alignment of query sequence to a each database sequence.
- It takes a band of 32 letters centered on the **init1** segment for calculating the optimal local alignment.
- After all sequences in the database are searched the program plots the scores of each database sequence in a histogram, and calculates the statistical significance of each.
- The so-called E-value represents the likelihood that the observed alignment is due to chance alone. It has to be < 0.05.

Interpretation of results

- very low E(.) values (~ E-100) are *homologues* (homologs)
- Homology is an evolutionary statement which means “similarity from common ancestry”
- long list of gradually declining E(.) values indicates a large sequence (gene, protein, RNA) family
- long regions of moderate similarity are more significant than short regions of high identity

Example of result from FASTA

Query sequence is GBR1_HUMAN and the list of the most similar ones:

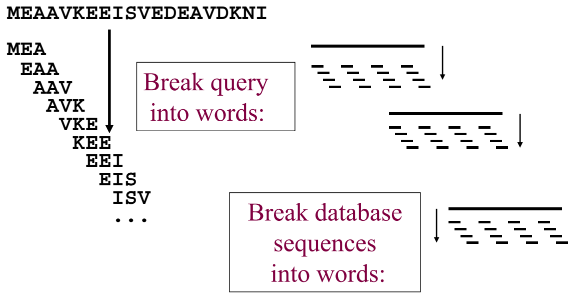


```
Sequence 456 aa
              0    50   100   150   200   250   300   350   400   450

GBRA1_HUMAN                                                          6.7e-196
A8K177_HUMAN                                                         1.5e-195
Q5RA48_PONPY                                                         3.7e-195
Q53F42_HUMAN                                                         3.7e-195
GBRA1_PONPY                                                          3.7e-195
GBRA3_HUMAN                                                          2.8e-120
GBRA3_RAT                                                            4.4e-120
GBRA3_BOVIN                                                          2.1e-119
Q8TBI4_HUMAN                                                         4.9e-118
Q4RIT5_TETNG                                                         1.3e-104
GBRA6_RAT                                                            4.3e-104
Q5SUU5_MOUSE                                                         1.1e-103
GBRA6_MOUSE                                                          1.3e-103
P16305                                                               3.2e-103
GBRA6_HUMAN                                                          4.4e-103
A8K096_HUMAN                                                         4.4e-103
Q90288_CARAU                                                         4.5e-101
GBRA4_RAT                                                            1.4e-100
Q3URE7_MOUSE                                                         1.8e-100
GBRA4_MOUSE                                                          3.4e-100
Q543Z0_MOUSE                                                         3.4e-100
Q4T509_TETNG                                                         4.4e-100

              0    50   100   150   200   250   300   350   400   450
Sequence 456 aa

        E>=0.02   0.02<E<=0.001   1e-3<E<=1e-6   1e-6<E<=1e-12   E>1e-12
               Results sorted by S&W Score and colored by E() values
```

BLAST (**B**asic **L**ocal **A**lignment **S**earch **T**ool)

- One of the tools of the NCBI - The U.S. **N**ational **C**enter for **B**iotechnology **I**nformation.
- Uses word matching like FASTA
- Similarity matching of words (3 AA's, 11 bases/nucleotides)
  - does not require identical words.
- If no words are similar, then there is no alignment
  - won't find matches for very short sequences

BLAST word matching



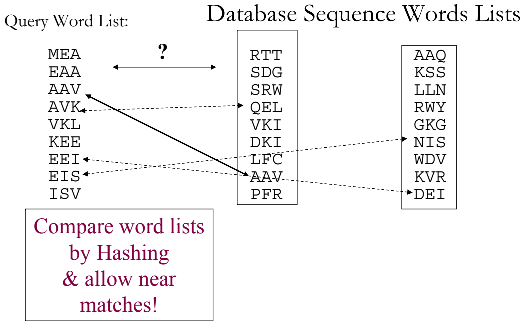
MEAAVKEEISVEDEAVDKNI

MEA
EAA
AAV
AVK
VKE
KEE
EEI
EIS
ISV
...

Break query into words:

Break database sequences into words:

Compare word lists

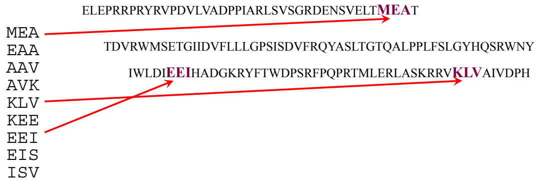


Query Word List:

Database Sequence Words Lists

Compare word lists by Hashing & allow near matches!

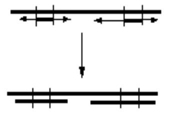
Find locations of matching words in all sequences



ELEPRRPRYRVPDVLVADPPIARLSVSGRDENSVELT**MEA**T
TDVRWMSETGIIDVFLLLGPSISDVFRQYASLTGTQALPPLFSLGYHQSRWNY
IWLDI**EEI**HADGKRYFTWDPSRFPQPRTMLERLASKRRV**KLV**AIVDPH
MEA
EAA
AAV
AVK
KLV
KEE
EEI
EIS
ISV

Extend hits one base at a time

- Then BLAST extends the matches in both directions, starting at the seed. The un-gapped alignment process extends the initial seed match of length W in each direction in an order to boost the alignment score. Indels are not considered during this stage.



Maximal Segment Pairs (MSPs)

- In the last stage, BLAST performs a gapped alignment between the query sequence and the database sequence using a variation of the *Smith-Waterman algorithm*. Statistically significant alignments are then displayed to the user.

BLAST : example of result

```
•  Job Title: P14867|GBRA1_HUMAN Gamma-aminobutyric acid...
   Show Conserved Domains
   Putative conserved domains have been detected, click on the image below for detailed results.
       *
   BLASTP 2.2.18 (Mar-02-2008) protein-protein BLAST

   Database: Non-redundant SwissProt sequences
             309,621 sequences; 115,465,120 total letters

   Query=  P14867|GBRA1_HUMAN Gamma-aminobutyric acid receptor subunit alpha-1 - Homo sapiens
   (Human).
   Length=456

   Sequences producing significant alignments:                      (Bits)  Value

   sp|P14867.3|GBRA1_HUMAN  Gamma-aminobutyric acid receptor subu...   948    0.0    Gene info
   sp|Q5R6B2.1|GBRA1_PONPY  Gamma-aminobutyric acid receptor subu...   944    0.0
   sp|Q4R534.1|GBRA1_MACFA  Gamma-aminobutyric acid receptor subu...   944    0.0
   sp|P08219.1|GBRA1_BOVIN  Gamma-aminobutyric acid receptor subu...   939    0.0    Gene info
   sp|P62813.1|GBRA1_RAT  Gamma-aminobutyric acid receptor subuni...   908    0.0    Gene info
   sp|P19150.1|GBRA1_CHICK  Gamma-aminobutyric acid receptor subu...   882    0.0    Gene info
   sp|P47869.2|GBRA2_HUMAN  Gamma-aminobutyric acid receptor subu...   670    0.0    Gene info
   sp|P26048.1|GBRA2_MOUSE  Gamma-aminobutyric acid receptor subu...   669    0.0    Gene info
   sp|P23576.1|GBRA2_RAT  Gamma-aminobutyric acid receptor subuni...   669    0.0    Gene info
   sp|P10063.1|GBRA2_BOVIN  Gamma-aminobutyric acid receptor subu...   667    0.0    Gene info
•  sp|Q08E50.1|GBRA5_BOVIN  Gamma-aminobutyric acid receptor subu...   641    0.0    Gene info
   sp|Q8BHJ7.1|GBRA5_MOUSE  Gamma-aminobutyric acid receptor subu...   640    0.0    Gene info
   sp|P31644.1|GBRA5_HUMAN  Gamma-aminobutyric acid receptor subu...   638    0.0    Gene info
   sp|P19969.1|GBRA5_RAT  Gamma-aminobutyric acid receptor subuni...   636    0.0    Gene info
   sp|P34903.1|GBRA3_HUMAN  Gamma-aminobutyric acid receptor subu...   632    0.0    Gene info
   sp|P26049.1|GBRA3_MOUSE  Gamma-aminobutyric acid receptor subu...   630    6e-180 Gene info
   sp|P10064.1|GBRA3_BOVIN  Gamma-aminobutyric acid receptor subu...   628    2e-179 Gene info
   sp|P20236.1|GBRA3_RAT  Gamma-aminobutyric acid receptor subuni...   627    3e-179 Gene info
   sp|P30191.1|GBRA6_RAT  Gamma-aminobutyric acid receptor subuni...   520    6e-147 Gene info
   sp|P16305.2|GBRA6_MOUSE  Gamma-aminobutyric acid receptor subu...   518    2e-146 Gene info
   sp|Q90845.1|GBRA6_CHICK  Gamma-aminobutyric acid receptor subu...   518    3e-146 Gene info
```

BLAST is approximate but fast

- BLAST makes similarity searches very quickly, but also makes errors
  - misses some important similarities
  - makes many incorrect matches
- The NCBI **BLAST** web server lets you compare your query sequence to various sequences stored in the GenBank;
- This is a VERY fast and powerful computer.
- The speed and relatively good accuracy of BLAST are the key why the tool is the most popular bioinformatics search tool.

What program to use for alignment?

1) **BLAST** is the fastest
- limited sets of databases
- nice translation tools, i.e. BLASTX (automatic translation of DNA query sequence to compare with protein databanks)
- TBLASTN (automatic translation of an entire DNA database to compare with your protein query sequence)

2) **FASTA** works best
- precise choice of databases
- more sensitive for DNA-DNA comparisons
- **FASTX** and **TFASTX** can find similarities in sequences with frameshifts

3) Smith-Waterman is slower, but even more sensitive
- **SSEARCH** in **FASTA**

Multiple sequence alignment (MSA)

- Multiple sequence alignment (MSA) is an alignment of **> 2** sequences at a time; usually a query sequence and the database (library of sequences).
- MSA is used to identify conserved sequence regions across a group of sequences. Such conserved *sequence motifs* can be used for instance, to locate the catalytic sites of enzymes, promoter regions in DNA, etc.
- MSA is also used to find evolutionary relationships by constructing *phylogenetic trees* based on similarity of sequences.
- MSA is computationally difficult to produce and rigorous formulations of the problem lead to *NP-complete* combinatorial optimisation problems.

Dynamic programming methods

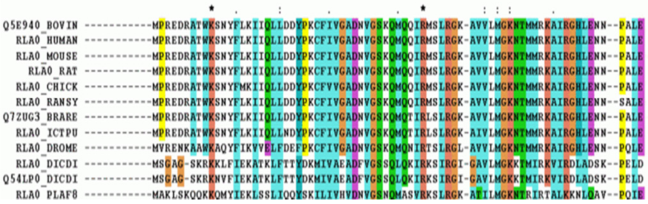
- Programs first perform pair-wise alignment on each pair of sequences (*using any of the pair-wise alignment methods*).
- Then, they perform local re-arrangements on these results, in order to optimise overlaps between multiple sequences. **The goal is to optimise *multiple* local alignments.**
- The so-called **"sum of pairs"** method has been implemented as a **scoring** method to evaluate these multiple alignments.
- The sum-of-pairs criterion means that the score of a multiple alignment of *N* sequences *is the sum of the N created pair-wise alignments*.

Progressive methods (ClustalW)

- Progressive, also known as hierarchical or tree methods, generate MSA by first aligning pair-wise the most similar sequences and then adding successively less related sequences.
- The initial tree describing the sequence relatedness is based on pair-wise comparisons for instance by FASTA or BLAST.
- Local re-arrangements are performed in order to optimise multiple overlaps. Scoring is based on sum of pairs.
- Progressive techniques automatically construct a phylogenetic tree as well as MSA (ClustalW).

Example of MSA by ClustalW

- Colours denote different chemical groups of amino acids, i.e. hydrophobic, acidic, etc.   Symbols: "*" means identical character, ":" means conserved substitutions, "." means semi-conserved substitution, and blank means a non-conserved substitution:



```
                                 *   .      :       .          .   *         : : :      .
Q5E940_BOVIN -----------MPREDRATWKSNYFLKIIQLLDDYPKCFIVGADNVGSKQMQQIRMSLRGK-AVVLMGKNTMMRKAIRGHLENN--PALE
 RLA0_HUMAN -----------MPREDRATWKSNYFLKIIQLLDDYPKCFIVGADNVGSKQMQQIRMSLRGK-AVVLMGKNTMMRKAIRGHLENN--PALE
 RLA0_MOUSE -----------MPREDRATWKSNYFLKIIQLLDDYPKCFIVGADNVGSKQMQQIRMSLRGK-AVVLMGKNTMMRKAIRGHLENN--PALE
   RLA0_RAT -----------MPREDRATWKSNYFLKIIQLLDDYPKCFIVGADNVGSKQMQQIRMSLRGK-AVVLMGKNTMMRKAIRGHLENN--PALE
 RLA0_CHICK -----------MPREDRATWKSNYFMKIIQLLDDYPKCFVVGADNVGSKQMQQIRMSLRGK-AVVLMGKNTMMRKAIRGHLENN--PALE
 RLA0_RANSY -----------MPREDRATWKSNYFLKIIQLLDDYPKCFIVGADNVGSKQMQQIRMSLRGK-AVVLMGKNTMMRKAIRGHLENN--SALE
Q7ZUG3_BRARE -----------MPREDRATWKSNYFLKIIQLLDDYPKCFIVGADNVGSKQMQTIRLSLRGK-AVVLMGKNTMMRKAIRGHLENN--PALE
 RLA0_ICTPU -----------MPREDRATWKSNYFLKIIQLLNDYPKCFIVGADNVGSKQMQTIRLSLRGK-AIVLMGKNTMMRKAIRGHLENN--PALE
 RLA0_DROME -----------MVRENKAAWKAQYFIKVVELFDEFPKCFIVGADNVGSKQMQNIRTSLRGL-AVVLMGKNTMMRKAIRGHLENN--PQLE
 RLA0_DICDI -----------MSGAG-SKRKKLFIEKATKLFTTYDKMIVAEADFVGSSQLQKIRKSIRGI-GAVLMGKKTMIRKVIRDLADSK--PELD
Q54LP0_DICDI -----------MSGAG-SKRKNVFIEKATKLFTTYDKMIVAEADFVGSSQLQKIRKSIRGI-GAVLMGKKTMIRKVIRDLADSK--PELD
 RLA0_PLAF8 -----------MAKLSKQQKKQMYIEKLSSLIQQYSKILIVHVDNVGSNQMASVRKSLRGK-ATILMGKNTRIRTALKKNLQAV--PQIE
```