Bio-informatics Databases

GeneBank for nucleic acid data

Uniprot for protein sequences

Compositions and sources

Compositions

- UniProtKB/Swiss-Prot (manually annotated and reviewed)
- UniProtKB/TrEMBL (automatically annotated and not reviewed)

Sources

- Uniprot: all encapsulate
- UniRef: Non-redundant
- UniParc: Records previous status

Accession

> Accessions are stable identifiers and should be used to cite UniProtKB entries. Upon integration into UniProtKB, each entry is assigned a unique accession number, which is called 'Primary (citable) accession number'.
> We remind users that they should always use the primary accession number of an entry in any citation and link since it is the only unique stable identifier for an entry.

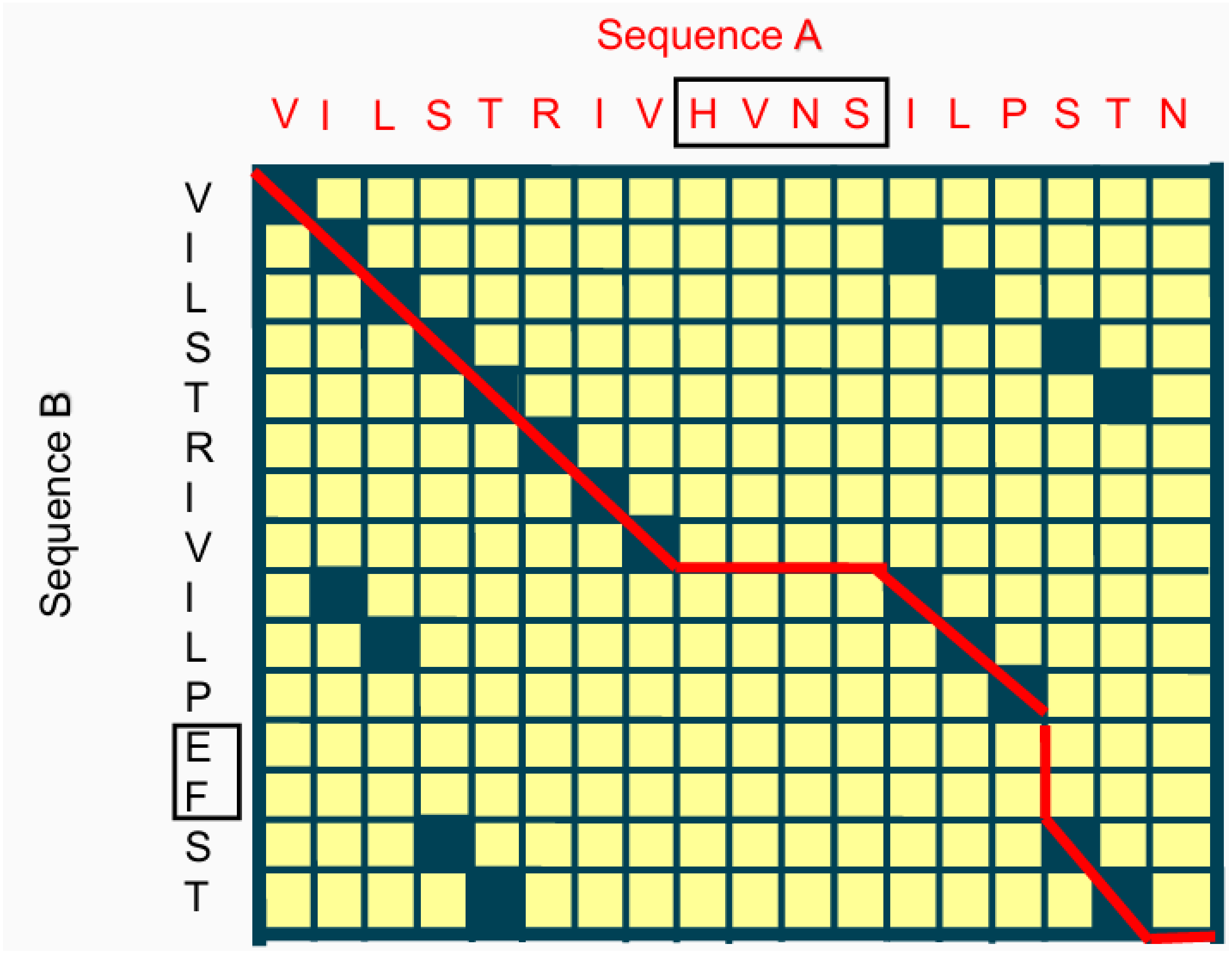
PDB for protein structures

Protein Sequence Alignment

> - Sequences longer than 150 amino acids with over 25% sequence identity are probably related.
> - With between only 15 and 25% sequence identity they may be related (TWILIGHT ZONE)

> - With less than 15% sequence identity, additional information such as structural and functional is required to determine if the proteins are related.

Global Alignment Algorithm Using Dynamic Programming: Needleman-Wunsch Algorithm


Sequence A
V I L S T R I V H V N S I L P S T N
Sequence B
V I L S T R I V I L P E F S T

Some very basic concepts behind this algorithm is *match*, *mismatch*, and *gap*. In above figure, what we can see is some slashed lines and some horizontal/vertical lines, with the horizontal/vertical lines denoting *gaps*, the coloured slashes denoting *matched residues*.

According to this figure, the horizontal line indicates that to maximise the overall score, four gaps are introduced into Sequence B to align with residue `HVNS` in Sequence A. The verticle line indicates two gaps are introduced into Sequence A in contrast.

Python Implementation

Following is a Needleman-Wunsch algorithm implemented in Python:

```
import numpy as np

class NW():
    def __init__(
            self, seqa:str, seqb:str,
            match, mismatch, gap
    ):
        self.seqa = seqa
        self.seqb = seqb
        self.match_score = match
        self.mismatch_score = mismatch
        self.gap_score = gap
        self.matrix = np.zeros((len(seqa)+1, len(seqb)+1))
        self.matrix.fill(np.inf)
        self.matrix[:, 0] = [self.gap_score * i for i in range(self.matrix.shape[
        self.matrix[0, :] = [self.gap_score * j for j in range(self.matrix.shape[


    def _scoring(self):
        for row_idx, i in enumerate(self.matrix):
            for col_idx, j in enumerate(i):

                if j != np.inf:
                    pass
                else:
                    '''
                    lt: right top (diagonal)
                    lt_: rt + match or mismatch
                    rb: right bottom
                    lt: left top
                    '''
                    lt = self.matrix[row_idx-1, col_idx-1]
                    lt_ = lt + self.match_score if self.seqa[row_idx-1] == self.s

                    lb = self.matrix[row_idx, col_idx-1]
                    rt = self.matrix[row_idx-1, col_idx]

                    score = np.max([lt_, lb+self.gap_score, rt+self.gap_score])

                    self.matrix[row_idx, col_idx] = score

        return self.matrix
    def _traceback(self,):
        seqa_output, seqb_output = [], []
        row = self.matrix.shape[0] - 1
        col = self.matrix.shape[1] - 1

        while row > 0 or col > 0:

            # How N&W gets rid of local optimum

            #print(f'row: {row}, col: {col}')
            current_score = self.matrix[row, col]

            if row > 0 and col >0:
                #match_val = self.match_score if self.seqa[row-1] == self.seqb[co
                match_val = self.match_score if self.seqa[row-1] == self.seqb[col
                #print(match_val)
                if current_score == self.matrix[row-1, col - 1] + match_val:
                    seqa_output.append(self.seqa[row-1])
                    seqb_output.append(self.seqb[col-1])

                    row -= 1
                    col -= 1
                    continue

            if col>0 and current_score == self.matrix[row, col - 1] + self.gap_sc
                seqa_output.append('-')
                seqb_output.append(self.seqb[col-1])
                col -= 1
                continue

            if row > 0 and current_score == self.matrix[row - 1, col] + self.gap_

                seqa_output.append(self.seqa[row-1])
                seqb_output.append('-')
                row -= 1

                continue


        seqa_output.reverse(), seqb_output.reverse()
        return seqa_output, seqb_output

    def align(self):
        score_matrix = self._scoring()
        #print(score_matrix)
        seqa_output, seqb_output = self._traceback()
        return seqa_output, seqb_output

nw = NW(seqa, seqb, match=1, mismatch=-1, gap=-2)
seqa_, seqb_ = nw.align()
```

As what the code shows, there are some major flaws in this algorithm:

Some Problems to be Solved

- The scoreing procedure is not flexible enough. To resolve this problem, substitution matrix might be an option.
- High time complexity $O_{(mn)}$. So heuristic search algorithms are needed.
- Considering sequence similarity only. Proteins have closely related sequences are less universal situations in the evolution process.

Local Alignment Algorithm and its derivation

Smith-Waterman algorithm

Heuristic Search Algorithm

The minimal searching unit is k-mer tuple, instead of a single residue.

To do this, the first thing is constructing a k-mer table [1].

Basic Local Alignment Search Tool (BLAST) alignment

Methods: Heuristic Search Algorithm

The minimal searching unit is k-mer tuple, instead of a single residue.

To do this, the first thing is constructing a k-mer table [1:1].

Methods: Local Alignment

The local alignment strategy in BLAST can be described by a *Seeding-Scanning-Extension* workflow.

Seeding process is based on aforementioned heuristic search method. The expected outcome of *seeding* is obtaining a bunch of "similar" k-mers.

Scanning tries to locate the k-mers obtained in *seeding* among sequences in databases.

Subsequently, sequence elements at two ends of seeds compare with the corresponding amino acids (or nucleic acids) in searched sequences along with scoring elementwisely. This process, *extension*, terminates as the score starting to decrease and attains high-scoring segment pair, **HSP**.

For both protein sequence alignment and nucleotide sequence alignment. BLASTN is the platform to perform a nucleotide alignment, while BLASTP is the platform to perform a protein alignment.

BLAST Setup

Max target sequences

Max matches in a query range

Database

- Non-redundant (nr) db is the default database used in BLAST search.
- Clustered db like **UniRef90** and **UniRef50** are more prefered, where UniRef90 clusters sequences have 90% sequence identity. UniRef50 clusters sequences have 50% sequence identity.
- RefSeq and Swiss-Prot are databases smaller in scale, but composed of manually annotated entries.

Since the major difference between clustered db and other non-clustered dbs is **entries with a similarity larger than the specified value** will be merged into one entry, the searching time can be greatly reduced by adapting clustered dbs, while at a cost of slightly resolution reduction.

Scoring matrix

Changing the substitution matrix causes different searching results.

Read about substitution matrices on Wikipedia and NCBI BLAST.

Result Interpretation: GenBank and Graphview

View external information for each hit from the link to **GenBank** or **Graphview** (legend: NCBI Graphview legend interpretation)

Result Interpretation: Layout

Organism	Blast Name	Score	Number of Hits
Relevant close to far, the most distantly-related taxon locates at the last second row		Descending	Descending

Result Interpretation: Parameters

Query Coverage: What proportion of the queried sequence is involved in the match

$$Coverage = \frac{Alignment \quad length}{Query \quad sequence \quad length} \times 100\%$$

Percentile Identity: What proportion of amino acids are identical in the compared region

$$Identity = \frac{Alignment \quad length}{min(A, B)} \times 100\%$$

A is the length of reference sequence. B is the length of mobile sequence.

Bit Score

Bit scores are normalised raw scores making raw scores obtained through different algorithms comparable.

> The bit score tells you the alignment score that BLAST calculated between your query sequence and the hit. This is a measure of how good the hit is. Take care when interpreting this as the maximum score possible is a function of the length of the query sequence and the hit. Bit scores between BLAST searches with different query sequences are not comparable.

E-value: The significance of the match

> The e-value tells us how many hits with this score or better we would expect to find at random given the size of the sequence database we searched.

Formula to calculate

$$E = K \times m \times n \times e^{-lambda \times S}$$

Where *S* denotes the homolog scores between two sequences, *m* denotes the length of the target sequence, *n* denotes the size of database, and *K* and *n* are parameters depending on algorithm and database.

Values

- E-value < 0.01: commonly considered significant, but there are features of biological sequences which can confound this so care is advised
- E-value < 1e-5: usually homologues
- E-value > 0.01: doesn't necessarily mean a hit is false
- E-values are normalized basing on each database, only E-values from the same db are comparable.
- Bit scores between BLAST searches with different query sequences are not comparable.

Length of hit

> It is possible to get good (i.e. small) e-values even if only a small regions of your query sequence is matched by a hit. In such instances you might consider very short matches as False Positives, even when the e-values are below the values you're looking for.

Other BLAST algorithms

Search Programme	Query	Hit
BLASTX	Input nucleotide, translate/ map to protein	Protein db
TBLASTN	Input protein	Nucleotide db, map to protein
TBLASTX	Input nucleotide, map to protein	Nucleotide db, map to protein

Result inspection

Check the organism of query and hit.

Hit has high bit score but presents in a irrelevant organism might be an FP.

Also be aware of **Low complexity regions**. Low complexity regions are regions composed of highly duplicated regions, which are commonly observed in hydrophobic regions of transmembrane proteins.

BLAST search may hit these sequences and draw high scores but devoid of biological meanings.

MSA

1. the usually case is that k=3, which means the whole table contains 20^3 trigram residue tuples. ↩ ↩