Notes on Bioinformatics

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- 1 - Introduction to Bioinformatics.

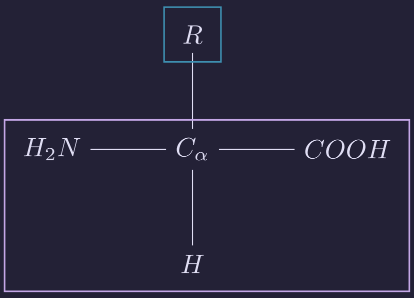
When we talk about bioinformatics, we refer to some sort of bridge between life sciences and informatics. The reason this kind of relationship is needed is due to the fact that biology, with Deoxyribonucleic Acid (DNA) sequencing, etc, is a source of big data; additionally, biology can benefit from informatics to ease some of its tasks, i.e., protein sequencing. In this notes we focus on various algorithms to menage both DNA and protein sequencing. Before doing so, in the remainder of this section we provide to the reader some notions of molecular biology; that is, we overview what aminoacids are, how these are related to proteins, the structure of DNA and RNAs and how these leadto proteins synthetisis.

- 1.1 - A brief introduction to molecular biology.

Human bodies, as well as any onther living thing, is constituted of several proteins which differ for their purpose. In this sense, we distinguish:

- **Structural proteins:** serves a building block for cells. An example of these is collagen.
- **Enzymes:** act as "cathalysts" for some chemical reaction. Some reactions are so slow that, in the case enzymes did not exist, life itself would not exist.
- **Transport proteins:** carry vital substunces through the body, i.e., hemoglobin.
- **Antibodies.**

Considering proteins in general, meaning without caring what's their task, these end up being a chain of smaller molecules: the *amino acids*. With reference the figure below, all amino acids share a common part (the one boxed in 🟪) and differ for the corresponding side chain (a.k.a the R-group, the one boxed in 🟦). Though one could argue the existence of



exceptions, we condider the classic 20 side chains, and consequentially the 20 classic amino acids (See Appendix A for the whole list).

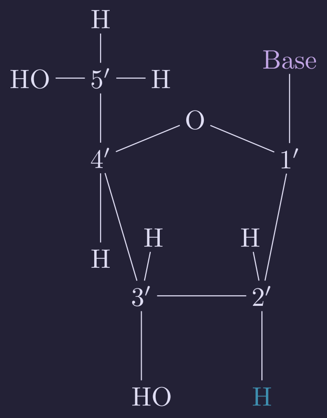
In truth proteins are not chains of amino acids, rather their residue: within a proteins, amino acids are bound to each other by petptide bonds, in which the COOH of an amino acid A_i binds with the H_2N of the amino acid A_{i+1}. This bond leads to the release of a water molecule, thus neither of the amino acids has its original molecular structure.

But how do we get proteins? To answer this question we need to understand what DNA and RNA are.

- 1.1.1 - From nucleic acids to proteins.

In nature there exist two types of nucleic acids: DNA and RNA. We present each one briefly.

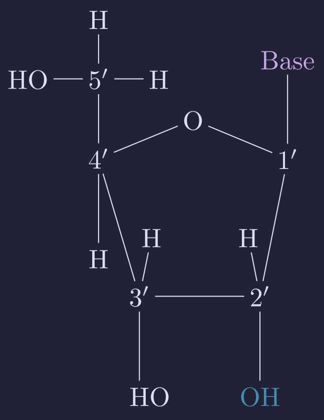
Starting with DNA, as for proteins, this is a chain of simpler molecules: the nucleotides. Structurally, it presents as a double strand chain in an helix. Chemically speaking (see the figure below), DNA it's a repetion of very similar units.



We distinguish four distinct bases: Adenine (A), Guanine (G), Cytosine (C) and Thymine (T). In DNA adenine bonds with thymine while guanine bonds with cytosine.

Each DNA strand, though bound in pair, follows an orientation: either from 5' to 3' or viceversa. In the following we use the former. This mechanism is what allows DNA replication. Essentially, since hydrogen bonds are not covalent (strong) they can be easily broken; in addition, since each nucleotide bonds only with a different one, from a single DNA strand we can get a copy of it. More precisely, given a DNA and a *primer*, that is a segment of DNA that triggers the replication, and the four nucleotides, we can synthesize a new complementary strand.

RNA is very similar to DNA (see figure below), and differs from it for the following reasons:



- Structure: RNA is single-stranded;
- Thymine is replaced with Uracile (U);
- We have different types of RNA.

RNA shows its importance in DNA duplication, as explained in the next section.

- 1.1.2 - Synthesis.

We have seen that proteins are residues of amino acids, thus, to identify each proteins we can consider the amino acids in its primiry structure. This is exactly the role of DNA. Precisesly, each triplet of nucleotide (codone) encodes an amino acid. Let us observe that 4 nucleotides should produce 64 distinct amino acids, but as we have said, nature only provides us only 20. We can conclude that some codones identify the same acid. Additionally, some codones (UAG, UAA, UGA) do not encode any amino acid, rather they are used to signal the end of a gene.

The process that leads to proteins synthesis begins with a phase know as *transcription*. In this phase, a codone AUG identifies the begin of a gene which is copied onto an RNA molecule. We obtain this way the messanger RNA, or mRNA. Now, the process just described works only for procaryotes; for eucaryotes things are a bit more complex. Essentially, eucaryotes genes are composed of aternating parts: the *introns* and the *exons*. We care only of exons. To solve this issue, once we get the mRNA the parts corresponding to introns are removed.

Synthesis is done in a special cellular structure: the ribosome. Ribosomes are composed by proteins and a new type of RNA, the ribosomal

RNA. Here mRNA is read sequentially and a special type of RNA, the tRNA, carries the amino acid associated with the codone being read.

To summarize: within DNA we produce copies of genes, these are then transcibed via RNA and within ribosomes we synthesize the proteins.

What we just described assumes no error occurs during DNA replication. As easily understood this is rarely the case. In fact, if errors did not occure, evolution would impossible. More in general, when dealing with DNA sequences we have to consider mutations, i.e, nucleotide X becomes nucleotide Y, and/or gaps, some nucleotide could get added/removed from the original sequence.

- 2 - Alignment algorithms.

We are interested into serching for similarities within genes; that is, given two sequences of DNA or proteins, we want to find subsequences that are common to both. There are essentially two approches we can take: alignment and alignment-free based algorithms. The remainder of this section covers the former, in addition to some notions needed to work with such algorithms.

- 2.1 - Alignment and similarity measures.

Sequence alignment is the process trough which we search for patterns within the given sequences. The process can be either applied locally, we discuss in details [2], or globally, we present a variant of [1]. Before doing so, we consider the naïve approach; that is, we just take in consideration mismatches and matches.

Considering two sequences, essentially, we consider all the possible alignments and choose the one with the highest number of matches.

Example

Let $s_1 = ACCAC$ and $s_2 = ACGGCC$. Consider all possible alignments:

```
     ACCAC              ACCAC
ACGGCC                  ACGGCC

     ACCAC             ACCAC
 ACGGCC                 ACGGCC

     ACCAC            ACCAC
  ACGGCC                ACGGCC

     ACCAC           ACCAC
   ACGGCC               ACGGCC

     ACCAC          ACCAC
    ACGGCC              ACGGCC
```

It's easy to observe that the best one is the top-right one.

This approach has a severe issue: it does not take in account gaps, which, as we have said, are extremely frequent in nature. Therefore, though easy to inplement, we should consider something different.

The necessity to consider gaps lead to a question: how do we define “the best alignment“ when gaps are involved? To solve the problem we define the so called *similarity measure*; i.e., a measure that takes in account both matches/mismatches and gaps. The one we use is the following

$$S_{AB} = \sum_{i=1}^{L} s(a_i, b_i) - \sum_{k=1}^{NG} \delta + \gamma[l(k) - 1]$$

where δ is said to be the *opening penalty*, $\gamma \leq \delta$ is called *extention penalty* and $l(k)$ is the length of the k-th gap.

Let us note that the concept of match/mismatch holds just for DNA and RNA sequences; in the context of amino acids, and therefore proteins, a different criterion is needed. Throuhgout this notes we use the *amino acid interconvertibility* criterion. In this case we make use of two special matrices (more correctly, substitution matrices) which tells us the value to assign to any given pair of amino acid. The ones we consider are PAM-1 and BLOSOM-62.

- 2.2 - Needleman-Wunsh algorithm.

Proposed in 1970, the Needleman-Wunsh algorithm allows one to compute the best global alignment in $\mathcal{O}(n^2)$ time (we assume the two sequences to have approximately the same length). The version we discuss is a slight variant of the algorithm proposed in [1].

Let s_1 and s_2 be the sequences to align, also let n, m be the respective lengths. The algorithm proceeds as follow:

1. Create an $n \times m$ matrix M: each entry represents the intersection of the i-th symbol of s_1 with the j-th symbol of s_2.

2. Set the inital value of each entry of the matrix accordingly to the type of sequences in analisis:

   - nucleotides: use the match/mismatch score; or
   - amino acids: use the value provided by the chosen substitution matrix.

3. Update the values of each entry as follow:

$$M(i,j) = \max \begin{cases} M(i-1, j-1) + M(i,j), \\ M(i-1, j) - \delta, \\ M(i, j-1) - \delta. \end{cases}$$

   If any of the index is negative, that entry has value equal to zero.

4. Within the last row, or column, dind the cell with the maximum score/s; from it track the best path/s proceeding backwards.

- 2.3 - Smith-Waterman algorithm.

Proposed in [2], the Smith-Waterman algorithm allows one to find the best local alignment in $\mathcal{O}(n^2)$ time (we assume the two sequences to have approximately the same length).

Let s_1 and s_2 be the sequences to align, and let n, m be their lengths. We proceed as follow:

1. Create an $n \times m$ matrix: each entry represents the intersection of the i-th symbol of s_1 with the j-th symbol of s_2.

2. Set the inital value of each entry of the chosen substitution matrix; either PAM or BLOSUM.

3. Update the value of each entry using the following relation:

$$M(i,j) = \max \begin{cases} 0, \\ M(i-1, j-1) + M(i,j), \\ M(i-1, j) - \delta \text{ or } \gamma, \\ M(i, j-1) - \delta \text{ or } \gamma. \end{cases}$$

   If $M(k,l)$ does not exists, i.e., either k or l are negative, assume that entry to be zero.

4. Within the whole matrix, find the maximum[1]score/s and from it track back the best path/s.

In the above relation, the choice between δ and γ depends on the gap; i.e., if the gap just opened use δ, use γ otherwise.

See that if we limit the search of the maximum score to the last row and column, we get the Needleman-Wunsh algorithm discussed previously; that is, the Needleman-Wunsh algorithm we described is a particular case of the Smith-Waterman algorithm.

[1] Alternatively, one could set a threshold and consider the path starting at each entry above said threshold.

References.

[1] Saul B. Needleman and Christian D. Wunsch. "A general method applicable to the search for similarities in the amino acid sequence of two proteins". In: *Journal of Molecular Biology* 48.3 (1970), pp. 443–453. DOI: 10.1016/0022-2836(70)90057-4.

[2] T.F. Smith and M.S. Waterman. "Identification of common molecular subsequences". In: *Journal of Molecular Biology* 147.1 (1981), pp. 195–197. DOI: 10.1016/0022-2836(81)90087-5.

Acronyms.

A Adenine. 2

C Cytosine. 2

DNA Deoxyribonucleic Acid. 1

G Guanine. 2

T Thymine. 2

U Uracile. 3

Appendix A.

Amino Acid	3-letter	1-letter
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V