

# Deriving BLOSUM-matrices for protein-coding DNA

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## Abstract

Sequence Alignment algorithms rely on some form of substitution matrix, which can heavily influence their results. In this work, we try to derive a substitution matrix for the genes (DNA) of a given protein family akin to the BLOSUMx matrices, which are used for amino acid sequences.

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## 1 Introduction

Sequence alignment is the task of pairing up the elements of two (biological) sequences with the aim of finding conserved regions and homologous sequences which are anticipated to have some evolutionary relation. Sequence alignment algorithms are primarily used for constructing phylogenetic trees, searching for homologous sequences with BLAST, assembling full DNA strands from short reads, creating input features for AlphaFold2 and other.

Most alignment algorithms try to maximize a so called *scoring function*, which assigns to a given alignment some score. The classical methods score alignments by summing up scores assigned to each pair of letters in the given alignment. The scores for the pairs of letters can be represented by a symmetric matrix we call such matrices *scoring matrices*. Next, we formally define our vocabulary. W.l.o.g., let us restrict ourselves to pairwise alignments, i.e., alignments of two sequences.

**Definition 1** *The following will be used throughout the paper:*

- A sequence  $s$  is an ordered collection of letters from some alphabet of letters  $\mathcal{L}$ .
- The letter '-' is called indel and represents gaps in the alignment, which occur due to mutations or sequencing errors.
- A (pairwise) sequence alignment is a matrix  $\mathbf{A} \in (\mathcal{L} + \{-\})^{2 \times m}$ , where  $m$  is the length of the alignment. We symbolize the set of all alignments of alphabet  $\mathcal{L}$  of length  $m$
- A scoring function  $\sigma : (\mathcal{L} + \{-\})^{2 \times m} \rightarrow \mathbb{Z}$  maps an alignment to its score.

Most algorithms of concern here use a scoring function of the form  $\sigma(\mathbf{A}) = \sum_{j=0}^m s(\mathbf{A}_{1j}, \mathbf{A}_{2j})$ , where  $s : (\mathcal{L} + \{-\})^2 \rightarrow \mathbb{Z}$  is a symmetric function evaluating pairs of letters, which can be represented by a matrix  $\mathbf{S} \in \mathbb{Z}^{(\#\mathcal{L})+1 \times (\#\mathcal{L})+1}$ . Said matrix is the focus of this work.

**Example 1** Since we are talking about DNA, our alphabet of concern will be the 4 different nucleotide bases,  $\{A, C, G, T\}$ . Two exemplary DNA sequences are *ACA* and *AAGA*. An alignment between them is

$$\mathbf{A} = \begin{bmatrix} A & C & - & A \\ A & A & G & A \end{bmatrix}$$

Indexes where the two nucleotides are equal are called matches, where the two are not equal are called mismatches and where an indel is matched to a nucleotide are called gaps. An exemplary scoring matrix might be

$$\mathbf{S} = \begin{array}{c|ccccc} & A & C & G & T & - \\ \hline A & 1 & -2 & -3 & 0 & 0 \\ C & -2 & 0 & 0 & 0 & 0 \\ G & -3 & 0 & 0 & 0 & -4 \\ T & 0 & 0 & 0 & 0 & 0 \\ - & 0 & 0 & -4 & 0 & 0 \end{array}$$

Using the above matrix,

$$\sigma(\mathbf{A}) = s(A, A) + s(C, A) + s(-, G) + s(A, A) = 1 - 2 - 4 + 1 = -6.$$

## 2 Previous work

Most notably, the two ubiquitous families of scoring matrices are the PAM matrices and the BLOSUM matrices. However, and this is also true for most other research around sequence alignment: there is a strong emphasis on studying the alignment of proteins over studying the alignment of DNA. To the best of our knowledge, nearly all benchmarks concern *only protein alignments*, which we suspect to be one of the reasons that there is limited research focusing on DNA alignment. There have been attempts to create gold standards for RNA

alignment, however, even there, investigations suggest an overrepresentation of tRNA, thus leading to a suboptimal benchmark.

The literature for DNA scoring matrices derived from data is sparse. Hamada et al. [2] derive scoring matrices from specific organisms sequenced by specific sequencers. Further, as they claim, different genes in different organisms have significantly differing rates of mutation, which is why general-purpose DNA scoring matrices might be a bad idea. Their main focus lies on recovering and mitigating the sequencing errors and projecting the differing GC contents of different species in the resulting matrix. They evaluate their data on simulated data, which by definition makes some assumptions, thus is not too optimal. Their method performs slightly better than 2 manually created scoring matrices.

### 3 BLOSUM

As a successor to the PAM matrices, Henikoff and Henikoff [3] first constructed the BLOSUM matrices for protein alignments. A wonderful explanation thereof was written by Eddy [1], however, we shall dive into the theoretical underpinnings of BLOSUM here as well.

Underlying BLOSUM is the equation that, given the two paired letters  $a \in \mathcal{L}, b \in \mathcal{L}$ ,

$$s(a, b) = \lambda \log \frac{P(a, b)}{P(a)P(b)}.$$

Let us dissect this:

- $s(a, b)$  is the score of  $a$  and  $b$  being aligned.
- Our two hypotheses are that
  1.  $a$  and  $b$  are related evolutionarily and ought to be aligned and
  2.  $a$  and  $b$  are aligned due to random chance.
- We are interested in the odds of the 1 being true instead of 2 and encoding this into a score. Thus, we get the odds of their probability and scale them with a logarithm (as this makes the odds symmetric around 0).
- For numerical reasons, we would like the score to be an integer, which is why we scale all scores with  $\lambda$ .

### References

- [1] Sean R Eddy. “Where Did the BLOSUM62 Alignment Score Matrix Come From?” In: *Nature Biotechnology* 22.8 (Aug. 2004), pp. 1035–1036. ISSN: 1087-0156, 1546-1696. DOI: 10.1038/nbt0804-1035. (Visited on 08/05/2023).

- [2] Michiaki Hamada et al. “Training Alignment Parameters for Arbitrary Sequencers with LAST-TRAIN”. In: *Bioinformatics* 33.6 (Mar. 2017), pp. 926–928. ISSN: 1367-4803. DOI: 10.1093/bioinformatics/btw742. (Visited on 07/14/2023).
- [3] S Henikoff and J G Henikoff. “Amino Acid Substitution Matrices from Protein Blocks.” In: *Proceedings of the National Academy of Sciences* 89.22 (Nov. 1992), pp. 10915–10919. DOI: 10.1073/pnas.89.22.10915. (Visited on 08/05/2023).