Sequence Alignment

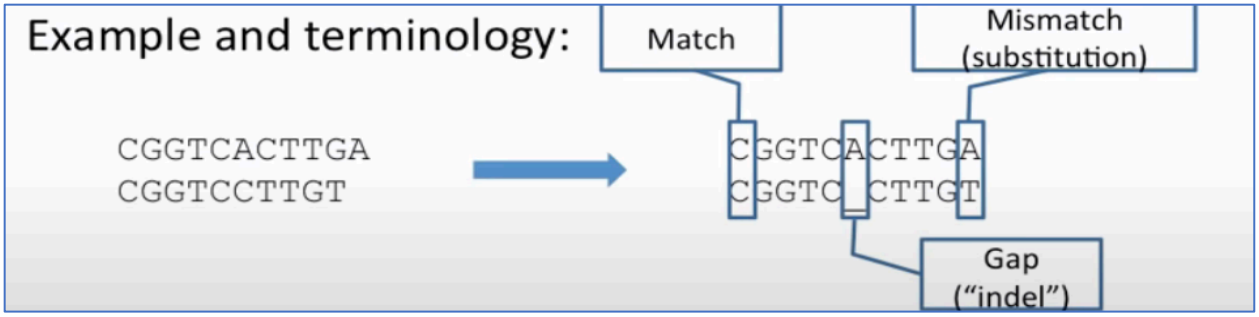
Many types of biological objects can be represented as sequences such as DNA, RNA, or proteins. To study for example, either:

- Similarity between the genome of humans or chimpanzees.
- The causing gene of obesity in mice is the same as in humans.
- Determining the mutations causing sickle cell anemia in 100 patients and 100 normal people.
- Determining the conserved sequences of a protein for the purpose of assessing the degree of similarity and the possibility of homology.

→ Preforming an **alignment** makes it easy to compute the similarity between sequences.

Alignment

The process of matching up the nucleotide or amino acid residues of two or more biological sequences to achieve maximal levels of identity.



A gap is the space introduced into an alignment to compensate for insertions and deletions in one sequence relative to another.

→To prevent the accumulation of too many gaps in an alignment, introduction of a gap causes the deduction of a fixed amount (the gap score) from the alignment score. Extension of the gap to encompass additional nucleotides or amino acid is also penalized in the scoring of an alignment.

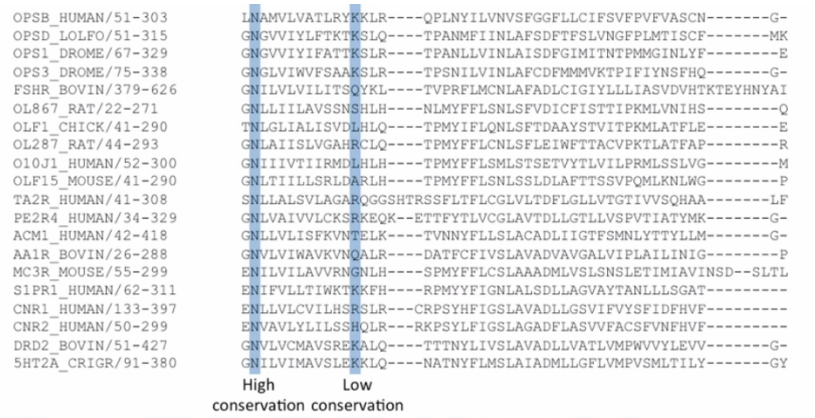
→ So, a good alignment is one with few substitutions and indels.

Types of alignments:

1) **Depending on the number of sequences:**
   a) **Pairwise sequence alignment:** the alignment of two biological sequences.
   b) **Multiple sequence alignment:** the alignment of three or more biological sequences.

2) **Depending on the part of sequences aligned:**
   a) **Local alignment** identify **regions** of similarity within long sequences that are often widely divergent overall. Most common tool used is BLAST (Basic Local Alignment Search Tool).
   →It is useful for example in finding motifs common to two unrelated sequences.

   b) **Global alignment** is a form of global optimization that "forces" the alignment to span **the entire length** of all query sequences. One of the most common tools used is **CLUSTALW.**
   → it is useful for example to compare sequences in cases where we have reason to believe that the sequences are related along their entire length to find conserved sequences, or finding mutations in closely related gene or protein sequences and identification of single nucleotide polymorphisms (SNPs).



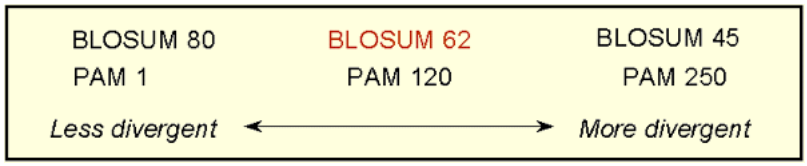
Alignment matrices and scoring

Given a pair of aligned sequences, we want to assign a score to the alignment that gives a measure of the relative likelihood that the sequences are related as opposed to being unrelated. We do this by having models that assign a probability to the alignment in each of the two cases, and then we compare these two probabilities.

1) **PAM-matrices:**
    - PAM (point accepted mutation) matrices are based on global alignments of closely related sequences.
    - The PAM1 is the matrix calculated from comparisons of **sequences with no more than 1% divergence.**
    - Other PAM matrices are extrapolated from PAM1. The number with the matrix (e.g. PAM40, PAM100) refers to the evolutionary distance; the greater numbers mean greater distances.
2) **BLOSUM matrices**:
    - **BLOSUM** (BLOcks SUbstitution Matrix) are based on local alignments. It is used to score alignments between evolutionarily **divergent** sequences.
    - The number after "BLOSUM" refers to the **minimum percentage identity** of the blocks used to construct the matrix; hence greater numbers mean smaller distances. For example, BLOSUM 62 is a matrix calculated from comparisons of sequences with no less than 62% similarity.
    - All BLOSUM matrices are based on observed alignments; **they are not extrapolated from comparisons of closely related proteins.**

→ **BLOSUM matrices with higher numbers and PAM matrices with low numbers** are both designed for comparisons of closely related sequences.

→ **BLOSUM matrices with low numbers and PAM matrices with high numbers** are designed for comparisons of distantly related proteins.



BLAST (Basic Local Alignment Search Tool):

It is an algorithm for comparing biological sequences, such as the amino-acid sequences of different proteins or the DNA sequences. It is one of the most widely used bioinformatics programs, probably because it addresses a fundamental problem emphasizing speed over sensitivity.

There are many different ftypes of BLAST searches:

1) **BLASTN** preforms nucleotide-nucleotide sequence comparison.
2) **BLASTP** performs protein-protein sequence comparison.
3) **BLASTX** searches a nucleotide "query" (refers to the term used in the search) against a protein database, translating the query on the fly.
4) **TBLASTN** searches a protein query against a nucleotide database, translating the database on the fly.

The Statistics of BLAST

From a search with BLAST we obtain a series of sequence alignments, each one with a similarity score. However, an important issue is how to know the statistical significance of such a score.

E-value: The Expectation value or Expect value represents the number of different alignments with scores equivalent to.

→ **The lower the E value, the more significant the score and the alignment.**

Note:

Most biochemists consider **25% identity the cutoff for sequence homology**, meaning that if two proteins are less than 25% identical in sequence, more evidence is needed to determine whether they are homologs.