Sequence Analysis 2

Lecture notes

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Preface

These lecture notes continue the "Sequence Analysis" series at Bielefeld University. They are based on earlier material by Robert Giegerich, Stefan Kurtz, Enno Ohlebusch, Sven Rahmann and myself, over the years supported by several helping hands: Eyla Willing, Peter Husemann, Roland Wittler, Katharina Klerx, Linda Sundermann, Karsten Wüllems, Tizian Schulz, Michel T. Henrichs, Daniel Dörr, Marília D. V. Braga and Leonard Bohnenkämper.

This second part, re-designed for the summer semester 2026, assumes that the material of the "Sequence Analysis 1" lecture notes is known. If you have not studied those, please do so first.

Jens Stoye, March 2026

Contents

1 Sequence Comparison Beyond Edit Distance

The edit distance (or alignment distance) is one of the most fundamental ways of quantifying the dissimilarity of two sequences x and y over a finite alphabet Σ. It is based on the **cost of an alignment** $A = (A_1, A_2, \ldots, A_n)$, defined as the sum of the costs of A's columns, i.e., $\text{cost}(A) = \sum_{i=1}^{n} \text{cost}(A_i)$, where the cost of alignment column $A_i = \binom{a_i}{b_i}$, $a_i, b_i \in \Sigma$, in the simplest (*unit cost*) case is 0 for a match column ($a_i = b_i$), and 1 for a mismatch column ($a_i \neq b_i$) or an indel column ($a_i =$ – or $b_i =$ –). Then we have:

Definition 1.1 The **alignment distance** of two sequences $x, y \in \Sigma^*$ is defined as

$$d(x, y) := \min\{\text{cost}(A) : A \in \mathcal{A}^* \text{ is an alignment of } x \text{ and } y\}.$$

The corresponding computational problem is as follows:

Problem 1.1 (Alignment Problem) For two given strings $x, y \in \Sigma^*$ and a given cost function, find the alignment distance of x and y and one or all optimal alignment(s).

From the "Sequence Analysis 1" lecture we know that this problem can be solved in $O(mn)$ time using a simple and elegant dynamic programming algorithm, where $m = |x|$ and $n = |y|$ are the two sequence lengths.

However, there are situations in which edit distance and alignments are not the perfect model to compare sequences. In the following two sections we will study two interesting alternatives.

1.1 The q-gram Distance

Edit distances emphasize the correct order of the characters in a string. If, however, a large block of a text is moved somewhere else (e.g. two paragraphs of a text are exchanged), the edit distance will be high, although from a certain standpoint, the texts are still quite similar. A more appropriate notion of distance can be defined in several ways. Here we introduce the q-gram distance d_q, which is actually a pseudo-metric: There exist strings $x \neq y$ with $d_q(x, y) = 0$.

Remember that for $q \in \mathbb{N}$, a ***q*-gram** is a string of length q.

Definition 1.2 Let $x \in \Sigma^m$ and choose $q \in [1, m]$. The **occurrence count** of a q-gram $z \in \Sigma^q$ in x is the number

$$\mathrm{Occ}(x, z) := |\{i \in [1, m-q+1] : x[i \ldots i+q-1] = z\}|.$$

Let $\sigma = |\Sigma|$. The ***q*-gram profile** of x is a σ^q-element vector $p_q(x) := (p_q(x)_z)_{z \in \Sigma^q}$, indexed by all q-grams, defined by $p_q(x)_z := \mathrm{Occ}(x, z)$.

The ***q*-gram distance** of $x \in \Sigma^m$ and $y \in \Sigma^n$ is defined for $1 \le q \le \min\{m, n\}$ as

$$d_q(x, y) := \sum_{z \in \Sigma^q} |p_q(x)_z - p_q(y)_z|.$$

Example 1.1 Consider $\Sigma = \{\mathtt{A}, \mathtt{B}\}$, $x = \mathtt{ABAA}$, $y = \mathtt{ABAB}$, $z = \mathtt{AABA}$, and $q = 2$. Using lexicographic order (`AA`, `AB`, `BA`, `BB`) among the q-grams, we have $p_2(x) = (1, 1, 1, 0)$, $p_2(y) = (0, 2, 1, 0)$ and $p_2(z) = (1, 1, 1, 0)$. Therefore we obtain $d_2(x, y) = 2$ and $d_2(x, z) = 0$ (although $x \neq z$). ◀

Theorem 1.1 The q-gram distance d_q is a pseudo-metric.

Proof. It fulfills nonnegativity, symmetry and the triangle inequality (exercise). □

1.1.1 Practical Computation

If the q-gram profile of a string $x \in \Sigma^m$ is dense (i.e., if it does not contain mostly zeros), it makes sense to implement it as an array $p[0 \ldots \sigma^q - 1]$, where $p[i]$ counts the number of occurrences of the i-th q-gram in some arbitrary but fixed (e. g. lexicographic) order.

Definition 1.3 For a finite set $\mathcal{X}$, a bijective function $r : \mathcal{X} \to [0, |\mathcal{X}|-1]$ is called **ranking function**, an algorithm that implements it is called **ranking algorithm**. The inverse of a ranking function is an **unranking function**, its implementation an **unranking algorithm**.

Of course, we are interested in an *efficient* (un-)ranking algorithm for the set of all q-grams over Σ. A classical one is to first define a ranking function r_Σ on the characters (alphabet encoding) and then extend it to a ranking function r on the q-grams by interpreting them as base-σ numbers. There are two possibilities to number the positions of a q-gram: From q to 1 falling or from 1 to q rising, as shown in the Example 1.2.

Example 1.2 Assume the alphabet $\Sigma = \{\mathtt{A}, \mathtt{C}, \mathtt{G}, \mathtt{T}\}$ and the alphabet encoding $r_\Sigma(\mathtt{A}) = 0$, $r_\Sigma(\mathtt{C}) = 1$, $r_\Sigma(\mathtt{G}) = 2$, and $r_\Sigma(\mathtt{T}) = 3$. For $q = 5$, the two different possibilities to rank the q-gram $z = \mathtt{ATACG}$ are:

(a) Falling ranking

$$\begin{aligned} r(\mathtt{ATACG}) &= \underbrace{r_\Sigma(z[1]) \cdot \sigma^4}_{0 \cdot 4^4} + \underbrace{r_\Sigma(z[2]) \cdot \sigma^3}_{3 \cdot 4^3} + \underbrace{r_\Sigma(z[3]) \cdot \sigma^2}_{0 \cdot 4^2} + \underbrace{r_\Sigma(z[4]) \cdot \sigma^1}_{1 \cdot 4^1} + \underbrace{r_\Sigma(z[5]) \cdot \sigma^0}_{2 \cdot 4^0} \\ &= 0 + 192 + 0 + 4 + 2 = \mathbf{198} \end{aligned}$$

(b) Rising ranking

$$\begin{aligned} r(\texttt{ATACG}) &= \underbrace{r_\Sigma(z[1])\cdot\sigma^0}_{0\cdot 4^0} + \underbrace{r_\Sigma(z[2])\cdot\sigma^1}_{3\cdot 4^1} + \underbrace{r_\Sigma(z[3])\cdot\sigma^2}_{0\cdot 4^2} + \underbrace{r_\Sigma(z[4])\cdot\sigma^3}_{1\cdot 4^3} + \underbrace{r_\Sigma(z[5])\cdot\sigma^4}_{2\cdot 4^4} \\ &= 0+12+0+64+512 = \mathbf{588} \end{aligned}$$

◀

The first numbering of positions in (a) corresponds naturally to the ordering of positional number systems; in the decimal number 23, the first 2 has positional value (weight) $10^1=10$ whereas the 3 has the lower weight of $10^0=1$. For texts, however, the second numbering in (b) is more natural since we expect lower position numbers on the left side of higher position numbers.

In general, we see that $z \in \Sigma^q$ has rank

$$\text{(a): } r(z) = \sum_{i=1}^{q} r_\Sigma\big(z[i]\big)\cdot\sigma^{q-i}, \qquad \text{(b): } r(z) = \sum_{i=1}^{q} r_\Sigma\big(z[i]\big)\cdot\sigma^{i-1}.$$

Whichever numbering system we use, we have to do it consistently.

For each of the two ways to rank a q-gram, there is a corresponding unranking method. Both methods work with integer division and remainder. The one for the falling ranking function uses a dynamic divisor and determines the characters of the q-gram based on the integer results, the other one for the rising ranking function uses a fixed divisor and determines the characters based on the remainder.

Example 1.3 Unranking the rank values 198 and 588 from Example 1.2. (Again, we have $\Sigma = \{\texttt{A},\texttt{C},\texttt{G},\texttt{T}\}$ and use the alphabet encoding $r_\Sigma(\texttt{A}) = 0$, $r_\Sigma(\texttt{C}) = 1$, $r_\Sigma(\texttt{G}) = 2$, and $r_\Sigma(\texttt{T}) = 3$.)

(a) Unranking for falling ranking function

$$\begin{aligned} \frac{198}{4^4} &= 0,\ \text{R } 198 \rightarrow\ \texttt{A} \\ \frac{198}{4^3} &= 3,\ \text{R } 6 \ \ \rightarrow\ \texttt{T} \\ \frac{6}{4^2} &= 0,\ \text{R } 6 \ \ \rightarrow\ \texttt{A} \\ \frac{6}{4^1} &= 1,\ \text{R } 2 \ \ \rightarrow\ \texttt{C} \\ \frac{2}{4^0} &= 2,\ \text{R } 0 \ \ \rightarrow\ \texttt{G} \end{aligned}$$

(b) Unranking for rising ranking function

$$\begin{aligned}\frac{588}{4} &= 147, \text{ R } 0 \to \texttt{A}\\ \frac{147}{4} &= 36, \quad \text{R } 3 \to \texttt{T}\\ \frac{36}{4} &= 9, \quad \text{R } 0 \to \texttt{A}\\ \frac{9}{4} &= 2, \quad \text{R } 1 \to \texttt{C}\\ \frac{2}{4} &= 0, \quad \text{R } 2 \to \texttt{G}\end{aligned}$$

◀

Both methods need q iterations and reconstruct the q-gram from the first position to the last. Each iteration takes a starting value r and determines the corresponding character of the q-gram while updating r for the next iteration. The starting value for the first iteration is the rank of the q-gram. In iteration i in the unranking method (a), for $1 \leq i \leq q$, the starting value r is divided by σ^{q-i}. The integer result c of the division determines the decoded character as described by r_Σ of the used ranking function. The remainder r' of the division serves as starting value for the next iteration.

$$\text{(a) } \frac{r}{\sigma^{q-i}} = c, \text{ R } r'$$

In unranking method (b), the starting value r is always divided by σ. Here, the remainder c of the integer division decodes the corresponding character and the integer result r' is the starting value for the next iteration.

$$\text{(b) } \frac{r}{\sigma} = r', \text{ R } c$$

1.1.2 Choice of q

In principle, we could use any $q \in [1, \min\{m, n\}]$ to compare two strings of lengths m and n. In practice, some choices are more reasonable than others.

For example, for $q = 1$, we merely add the differences of the single letter counts. If m and n are large, there are many (even very differently looking) strings with the same letter counts, which would all have distance zero from each other.

If on the other hand $q = m \leq n$, and $m \approx n$, the distance d_q between the strings is always close to $n - m$, which does not give us a good resolution either. Also, the q-gram profile of both strings is extremely sparse in these cases. We thus ask for a value of q such that each q-gram occurs about once (or $c \geq 1$ times) on average.

Assuming a uniform random distribution of letters in a given string $x \in \Sigma^m$, the probability that a fixed q-gram z starts at a fixed position $i \in [1, m - q + 1]$ is $1/\sigma^q$, where $\sigma = |\Sigma|$. The expected number of occurrences of z in x is thus $(m - q + 1)/\sigma^q$. The condition

that this number equals c leads to the condition $(m+1)/c = q/c + \sigma^q$, or approximately $m/c = \sigma^q$, since $1/c$ and q/c are comparatively small. Thus setting

$$q = \left\lfloor \frac{\log(m) - \log(c)}{\log(\sigma)} \right\rfloor$$

ensures an expected occurrence count $\geq c$ of each q-gram.

1.1.3 Efficiency

Obviously, for $z \in \Sigma^q$, the ranking function $r(z)$ can be computed in $O(q)$ time. Both the falling and the rising ranking functions have the advantage that when a window of length q is shifted along a text, the rank of the q-gram can be updated in $O(1)$ time:

Assume that $t = aub$ with $a \in \Sigma$, $u \in \Sigma^{q-1}$, and $b \in \Sigma$. The first q-gram is au, whereas ub is the updated one. Now we can define an update system for each of the ranking systems above. Let $j = r(au)$. For the falling ranking function we compute:

$$\text{(a) } r(ub) = (j \bmod \sigma^{q-1}) \cdot \sigma + r_\Sigma(b),$$

and for the rising ranking function:

$$\text{(b) } r(zb) = \left\lfloor \frac{j}{\sigma} \right\rfloor + f(b), \text{ where } f(b) = r_\Sigma(b) \cdot \sigma^{q-1}.$$

If we take a more detailed look at both systems now, we can see that the second way is possibly more efficient (as it avoids the modulo operation and only uses integer division) if f is implemented as a lookup table $f : \Sigma \to \mathbb{N}_0$. If $\sigma = 2^k$ for some $k \in \mathbb{N}$, multiplications and divisions can be implemented by bit shifting operators, e.g. in the second system (b), $r(ub) = (j \texttt{ >> } k) + f(b)$.

It is easy to see that the q-gram corresponding to a ranking value can be obtained in $O(q)$ time for both unranking methods.

Now, to compute $d_q(x, y)$ for $x \in \Sigma^m$ and $y \in \Sigma^n$,

1. initialize integer array $p_q[0 \ldots \sigma^q - 1]$ with zeros,
2. for each $i \in [1, m-q+1]$, increment $p_q[r(x[i \ldots i+q-1])]$,
3. for each $i \in [1, n-q+1]$, decrement $p_q[r(y[i \ldots i+q-1])]$,
4. initialize $d := 0$,
5. for each $j \in [0, \sigma^q - 1]$, increment d by $|p_q[j]|$.

This procedure obviously takes $O(\sigma^q + m + n)$ time. If $m \leq n$ and q is as suggested above, this is $O(n)$ time overall. The space complexity is $O(\sigma^q)$ to store the array p_q.

If q is comparatively large, it does not make sense to maintain a full array. Instead, we maintain a data structure that contains only the nonzero entries of p_q. If $q = O(\log(m+n))$, we can still achieve $O(m+n)$ time using hashing.

1.1.4 Relation to the Edit Distance

While the q-gram distance d_q has its own applications in the comparison of sequences that have undergone block movements, it also has a weak connection to the unit cost edit distance d. Recall that we can have a high edit distance even though $d_q(x, y) = 0$. On the other hand, a single edit operation changes up to q q-grams. Generally, one can show the following relationship.

Theorem 1.2 Let d denote the standard unit cost edit distance. Then

$$\frac{d_q(x, y)}{2q} \leq d(x, y).$$

Proof. Each edit operation locally affects up to q q-grams. Each changed q-gram can cause a change of up to 2 in d_q, as the occurrence count of one q-gram decreases, the other increases. Therefore each edit operation can lead to an increase of $2q$ of the q-gram distance in the worst case (e.g. consider `AAAAAAAAAAA` vs. `AABAABAABAA` with edit distance 3, $q = 3$, and, as can be verified, $d_3 = 18$). □

How this relationship can be used productively in order to speed up the computation of the edit distance will be discussed in Section 1.3.

1.2 The Maximal Matches Distance

Another notion of distance that admits large block movements is the maximal matches distance. It is based on the idea that we can cover a sequence x with substrings of another sequence (and vice versa) that are interspersed with single characters.

Partitioning a sequence. Consider a sequence $x = z_0 b_1 z_1 b_2 \dots z_{\ell-1} b_\ell z_\ell$, with $|x| = m$, such that each z_i is a (possibly empty) string (for $0 \leq i \leq \ell$) and each b_i is a single character (for $1 \leq i \leq \ell$). The tuple $P = (z_0, \langle b_1 \rangle, z_1, \langle b_2 \rangle, \dots, z_{\ell-1}, \langle b_\ell \rangle, z_\ell)$ is a **partition** of x with respect to another sequence y if each z_i is also a substring of y. The **size** of the partition P, denoted by $|P|$, is the number of its separating single characters, that is, $|P| = \ell$. Note that, if x is a substring of y, there exists the shortest partition $P_0 = (x)$, of size 0. In the other extreme, the longest partition $P_m = (\varepsilon, \langle x[1] \rangle, \varepsilon, \langle x[2] \rangle, \dots, \varepsilon, \langle x[m] \rangle, \varepsilon)$ of size m always exists, even if $y = \varepsilon$.

Example 1.4 Let $x =$ `CTAATGCT` and $y =$ `ATCTA`. The following sequences are partitions of x with respect to y: $P = (\texttt{CTA}, \langle \texttt{A} \rangle, \texttt{T}, \langle \texttt{G} \rangle, \texttt{CT})$ with $|P| = 2$, $P' = (\texttt{CTA}, \langle \texttt{A} \rangle, \texttt{T}, \langle \texttt{G} \rangle, \texttt{C}, \langle \texttt{T} \rangle, \varepsilon)$ with $|P'| = 3$ and $P'' = (\texttt{CT}, \langle \texttt{A} \rangle, \texttt{AT}, \langle \texttt{G} \rangle, \texttt{CT})$ with $|P''| = 2$. ◀

Definition 1.4 The **maximal matches distance** to x from y is defined as

$$\delta(x \| y) := \min\{|P| : P \text{ is a partition of } x \text{ with respect to } y\}.$$

1.2.1 Efficient Computation

The next question is how to find a minimum size partition of x with respect to y among all the partitions. Fortunately, this turns out to be easy: A *greedy strategy* does the job. We start covering x at the first position by a match of maximal length k such that $z_0 := x[1 \dots k]$ is a substring of y, but $z_0 b_1 = x[1 \dots k+1]$ is not a substring of y. We apply the same strategy to the remainder of x, $x[k+2 \dots m]$.

The **left-to-right partition** $P_{\text{LR}}(x, y) = (z_0, \langle b_1 \rangle, z_1, \langle b_2 \rangle, \dots, z_{\ell-1}, \langle b_\ell \rangle, z_\ell)$ of x with respect to y is the partition defined by the condition that for all $i \in [1, \ell]$, $z_{i-1} b_i$ is not a substring of y.

Similarly, the **right-to-left partition** $P_{\text{RL}}(x, y) = (z_0, \langle b_1 \rangle, z_1, \dots, \langle b_{\ell-1} \rangle, z_{\ell-1}, \langle b_\ell \rangle, z_\ell)$ of x with respect to y is the partition defined by the condition that for all $i \in [1, \ell]$, $b_i z_i$ is not a substring of y.

Theorem 1.3 The left-to-right partition is a partition of minimal length, i.e.,

$$|P_{\text{LR}}(x, y)| = \min\{|P| : P \text{ is a partition of } x \text{ with respect to } y\} = \delta(x \| y).$$

The same is true for the right-to-left partition, which means:

$$|P_{\text{RL}}(x, y)| = |P_{\text{LR}}(x, y)| = \delta(x \| y).$$

Proof. If x is a substring of y, then the optimal partition is the left-to-right partition and has size zero. So assume that x is not a substring of y and any partition has size ≥ 1.

First, we prove the following (obvious) statement: If an optimal partition for a string x' (with respect to y) has size ℓ, and if $x = px'$ contains x' as a suffix, then an optimal partition for x cannot have size smaller than ℓ. If it did, we could restrict that smaller partition to the suffix x', and would thus obtain a smaller partition for x'.

Now we show that the above statement proves the theorem: A partition $P = (z_0, \langle b_1 \rangle, \dots)$ decomposes $x = z_0 b_1 x'$, where z_0 is a substring of y, b_1 is the first separating single character, and x' is the remaining suffix of x. The size of such a partition is at least one plus the size of an optimal partition of x'. Since the left-to-right partition induces the shortest suffix x', by the above statement, its optimal partition is never larger than all partitions of longer suffixes.

The statement for the right-to-left partition follows since it is equal to $\overleftarrow{P_{\text{LR}}(\overleftarrow{x}, \overleftarrow{y})}$. □

It is important to discuss how (and how fast) we can compute the size of a left-to-right partition. In fact, because the longest common substring z that starts at a given position i in x and occurs somewhere in y can easily be found in $O(|z|)$ time using the suffix tree of y, we obtain the following result.

Theorem 1.4 The maximal matches distance $\delta(x \| y)$ to a sequence x from another sequence y can be computed in $O(|x| + |y|)$ time.

Proof. Given the suffix tree of y, $T(y)$, starting at the root follow the letters of x until no continuation is possible. This is by definition the first sequence z_0 of the left-to-right partition. The next character of x is then the first single character b_1. Now continue recursively with the remaining suffix of x, again starting at the root of $T(y)$, and repeat until x is exhausted. Clearly, the resulting partition is the left-to-right partition of x with respect to y. The procedure takes $O(|y|)$ time to construct $T(y)$ and then $O(|x|)$ time for the suffix tree traversals whose total length is bounded by $|x|$. □

1.2.2 Relation to the Edit Distance

Like the q-gram distance, also the maximal matches distance can be used to compute a lower bound of the edit distance.

Theorem 1.5 Let $d(x, y)$ be the unit cost edit distance between sequences x and y. Then $d(x\|y) \leq d(x, y)$.

Proof. Consider a minimum-cost edit sequence e transforming x into y. Each run of copy operations in e corresponds to a substring of x that is exactly covered by a substring of y and can thus be used as part of a partition. It follows that there exists a partition of x with respect to y whose size is bounded by the number of non-copy operations in e; this number is by definition equal to the edit distance. □

Again, this bound permits us to speed up the computation of the edit distance, as will be shown in Section 1.3.

1.2.3 Maximal Matches Metric

A final observation is that δ is obviously not symmetric: $\delta(x\|y) = 0$ is equivalent to x being a substring of y. Therefore the maximal matches distance is not a metric and the name *distance* is misleading. But it is possible to use δ for designing an appropriate symmetric function that satisfies the triangular inequality and is a metric.

Theorem 1.6 Given sequences x and y from Σ^*, let the function $d_{||}(x, y)$ be defined as $d_{||}(x, y) = \log\big(\delta(x\|y) + 1\big) + \log\big(\delta(y\|x) + 1\big)$. Then $d_{||}(x, y)$ is a metric on Σ^*.

Proof. Symmetry and identity of indiscernibles are easily checked; we now check the triangular inequality. Consider a third sequence $z \in \Sigma^*$. The number of substrings of z needed to cover x is $\delta(x\|z) + 1$. We further need $\delta(z\|y) + 1$ substrings of y to cover z. This means that we can cover x with at most $(\delta(x\|z) + 1) \cdot (\delta(z\|y) + 1)$ substrings of y, i.e.,

$$\delta(x\|y) + 1 \leq (\delta(x\|z) + 1) \cdot (\delta(z\|y) + 1)$$

and (by the same argumentation)

$$\delta(y\|x) + 1 \leq (\delta(y\|z) + 1) \cdot (\delta(z\|x) + 1).$$

Taking the logarithm of the product of these two inequalities, we obtain

$$\begin{aligned}&\log(\delta(x\|y)+1)+\log(\delta(y\|x)+1)\\ &\quad\leq\ \log(\delta(x\|z)+1)+\log(\delta(z\|y)+1)+\log(\delta(y\|z)+1)+\log(\delta(z\|x)+1),\end{aligned}$$

which is the triangular inequality $d_{||}(x,y) \leq d_{||}(x,z) + d_{||}(z,y)$. □

1.3 Filtration for Edit Distance

Coming back to the edit distance, while – given the fact that the search space (consisting of all pairwise alignments of x and y) is of exponential size – the quadratic time dynamic programming solution can be considered very efficient, it can also be considered quite slow in practice when the sequences are long. Moreover, the quadratic *space* requirements (unless a space-saving technique is used that we will discuss in Chapter 6) can cause even larger problems.

Now, remember that in many applications the goal of sequence comparison is not the construction of an accurate alignment. Instead, for example in a database search context, one is mostly interested in identifying sequences similar to a given query. More formally:

Problem 1.2 (Database Search Problem) Given a database $X = \{x_1, x_2, \ldots, x_k\}$ of k sequences $x_i \in \Sigma^*$, a query sequence $y \in \Sigma^*$ and a distance threshold $t \geq 0$, find all sequences $x \in X$ such that $d(x,y) \leq t$.

Clearly, a straightforward way to solve this problem is to compare y to each sequence $x \in X$, one after the other. Whenever in such a comparison the distance $d(x,y)$ is smaller or equal to the threshold t, then the sequence x is reported, otherwise it is discarded. This strategy clearly takes $O(k \cdot mn)$ time, where m is an upper bound for the database sequence length and $n = |y|$.

However, since usually t is small and many sequences in X will be quite dissimilar from y, it may be possible to quickly screen the database and separate interesting *candidates* from irrelevant sequences, faster than computing the edit distance for all of them. If such a screening procedure guarantees that all relevant sequences $x \in X$ with $d(x,y) \leq t$ are among the remaining candidates, it is called a **filter**.

Generally, we thus have a two-step procedure. First, in a fast **filtration step**, candidates are identified that have a chance to be hits. Then, in a **verification step** these candidates are checked with the (usually slower) exact method whether they really qualify as hits. Obviously, filtration makes sense only if the procedure employed in the filtration step has a lower time complexity than the procedure used in the verification step.

Indeed, if one has multiple filters, one can first apply all of them, and then employ the verification only to those elements $x \in X$ that pass all the filters.

In the previous sections we have seen two fast sequence comparison methods that can be used as filters for the unit cost edit distance. Both of them require only time proportional to the sum of the lengths of the two sequences ("linear time") and provide lower bounds for the edit distance.

Filtration with the q-gram distance. The property outlined in Theorem 1.2 can be used as a filter criterion for the edit distance: For a given query sequence y and edit distance threshold t, and a given value of q, a database sequence $x \in X$ can be discarded whenever $d_q(x, y)/(2q) > t$. In case of a global similarity of the sequences and the differences spread out evenly, this filter will work quite well. If the difference between the two sequences, however, is by block movements, then the edit distance is usually very high, although the q-gram distance may still be low and produce a false positive candidate when used as a filter.

Filtration with the maximal matches distance. As shown in Theorem 1.5, the maximal matches distance also gives us a bound for the unit cost edit distance. In fact, since the edit distance is symmetric, the filtration can even be used twice, once with $d(x\|y)$ and once with $d(y\|x)$. This results in the following filter: For a given query sequence y and edit distance threshold t, a database sequence $x \in X$ can be discarded whenever $\delta(x\|y) > t$ or $\delta(y\|x) > t$.

Note that the distinguishing feature of a filter is that it never discards a hit (has no false negatives). On the other hand, one can also develop **heuristics** that approximate the edit distance and are efficiently computable. Good heuristics (like BLAST) will be close to the true result with high probability; hence the error made by using them is small most of the time. There is no guarantee, though, that a true hit will not be missed.

2 Practical Aspects of de Bruijn Graphs

A note on terminology: In Section 1.1 we were speaking of q-grams when referring to short (sub)strings of a certain length q. This notation has originally been introduced in the computer science literature and is there in use until today. An alternative, equivalent notion that one finds in most of the biological[1] and bioinformatics literature, is that of a ***k*-mer**. Here k refers to the (sub)string length. To be more consistent with the literature, in this chapter we will adopt that notation.

2.1 Definition and Basic Properties

Remember the following definition, where $s_{k,i} := s[i \dots i+k-1]$ denotes the k-mer starting at index i, $1 \leq i \leq |s| - k + 1$:

Definition 2.1 The **de Bruijn subgraph** $\mathrm{DBG}(s, k) = (V, E)$ for a given sequence s and a positive integer $k \leq |s|$ is a directed multigraph whose vertices V correspond to the distinct k-mers of s, $s_{k,1}, \dots, s_{k,|s|-k+1}$, and whose edges are derived from the $(k+1)$-mers of s as follows: Let a $(k+1)$-mer of s be denoted as $s[i \dots i+k] = aub$, where a and b are symbols and $|u| = k-1$, then a directed edge is contained in E, linking vertex au to vertex ub. The value k is called the **dimension** of $\mathrm{DBG}(s, k)$.

Note that if the same $(k+1)$-mer occurs multiple times in s, say m times, we have m parallel edges occurring in the multigraph $\mathrm{DBG}(s, k)$.

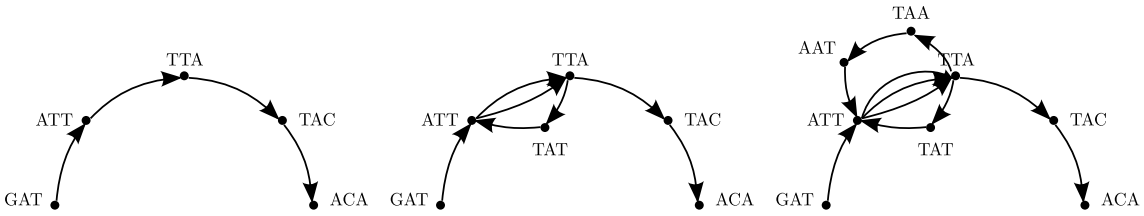
2.2 Words with the same $(k+1)$-mer Profile

Motivated by the fact that the q-gram distance does not satisfy the identity property of a metric (see Section 1.1) because there can be distinct sequences with the same q-gram profile, we want to determine whether for a given sequence s and integer k there are other sequences that are distinct but have the same $(k+1)$-mer profile as s. And, if there are, how many?

For $k = 0$, the problem is trivial. Indeed, if a sequence t that is distinct from s corresponds to a permutation of the symbols of s, then s and t clearly share the same 1-mer profile. For $k \geq 1$, an elegant answer can be found with the help of the de Bruijn subgraph $\mathrm{DBG}(s, k)$.

[1] The true origin is probably in biochemistry, where notions like *monomer* or *polymer* refer to molecular chains of certain lengths.

Clearly, by construction DBG(s, k) forms an Eulerian path p_s, represented by its sequence of vertices $s_{k,1}, s_{k,2}, \ldots, s_{k,|s|-k+1}$. Consequently DBG$(s, k)$ is *connected* and *balanced* and might contain even more than one Eulerian path. In fact, there is a one-to-one correspondence between Eulerian paths in DBG(s, k) and sequences sharing the same $(k+1)$-mer profile. In other words, a sequence t that is distinct but has the same $(k+1)$-mer profile as s exists if and only if DBG(s, k) has another Eulerian path p_t corresponding to the sequence of $(k+1)$-mers of t. Since p_t uses the same edges as p_s in a different order, the path p_t can only exist if DBG(s, k) contains a cycle. Note, however, that the presence of a cycle is not a sufficient condition. Indeed, it is possible that DBG(s, k) contains one or more cycles, but still admits only one Eulerian path. Some examples are given in Figures 2.1 and 2.2.



$x =$ `GATTACA` and $k = 3$

(a) Since this graph contains no cycle, it has only one Eulerian path. There can be no other word sharing the same 4-mer profile.

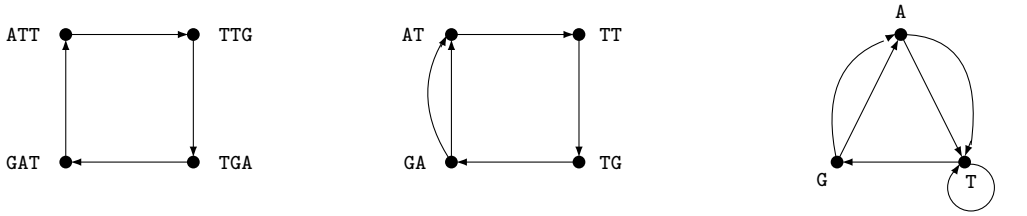
$x =$ `GATTATTACA` and $k = 3$

(b) Here the graph contains two cycles, but still only one Eulerian path. There is no other word sharing the same 4-mer profile.

$x =$ `GATTATTAATTACA` and $k = 3$

(c) This graph contains several cycles and two distinct Eulerian paths. There is exactly one other word with the same 4-mer profile, `GATTAATTATTACA`.

Figure 2.1: Examples of de Bruijn subgraphs for distinct sequences and $k = 3$.



$x =$ `GATTGAT` and $k = 3$

(a) This graph has an Eulerian cycle starting at any of its four vertices. Therefore there are three other sequences sharing the same 4-mer profile: `ATTGATT`, `TTGATTG` and `TGATTGA`.

$x =$ `GATTGAT` and $k = 2$

(b) This graph has two cycles, but only one Eulerian path. There is no other word sharing the same 3-mer profile.

$x =$ `GATTGAT` and $k = 1$

(c) This graph contains cycles and two distinct Eulerian paths. The other word sharing the same 2-mer profile is `GATGATT`.

Figure 2.2: De Bruijn subgraphs for the same sequence but with k varying from 3 to 1.

We summarize the observations above in the following theorem.

Theorem 2.1 Given a sequence s and an integer $k \geq 1$, the number of distinct sequences

that have the same $(k+1)$-mer profile as s equals the number of distinct Eulerian paths in $\mathrm{DBG}(s,k)$. If $\mathrm{DBG}(s,k)$ is acyclic, it has a single Eulerian path, and no sequence t exists that is distinct but has the same $(k+1)$-mer profile as s.

One can write a computer program that constructs $\mathrm{DBG}(s,k)$, identifies the initial and final vertices (if they exist), and systematically enumerates all Eulerian paths in $\mathrm{DBG}(s,k)$ using backtracking.

2.3 Variants of de Bruijn Graphs

Although, strictly speaking, we consider most of the time de Bruijn subgraphs (for a given sequence), often they are just called *de Bruijn graphs*. In fact, there are more variants of de Bruijn graphs, and often they are also not named very carefully. Here we will discuss them and introduce a precise nomenclature.

The original **de Buijn graph** of dimension k over a finite alphabet Σ of size σ is the graph that contains one vertex for each k-mer (and therefore σ^k vertices) and a directed edge for each overlap of length $k-1$ (and therefore σ^{k+1} edges).

The **de Bruijn subgraph** for a given sequence s of dimension k is the one that we introduced in Definition 2.1. It has several interesting properties (see Section 2.2) and is used in various contexts in bioinformatics like genome assembly and pangenomics.

In fact, in most bioinformatics applications where DNA sequence reads come from a sequencing machine, it is not clear from which strand of the DNA double helix a read originates. Therefore one prefers to identify a k-mer z with its reverse complement $\overleftarrow{z^{-1}}$ and represent them by the same vertex in the graph. The result is then called **bidirected de Bruijn subgraph**. The remaining definitions remain unchanged.

Finally, in a (bidirected) de Bruijn subgraph one often finds stretches of vertices that have only one successor, the next k-mer in the originating sequence(s). Such a non-branching path can be collapsed into a into single vertex (representing a k'-mer for some $k' \geq k$), called **unitig**, saving space and often computation time. The resulting graph is then called **compacted (bidirected) de Bruijn subgraph**.

An additional variant comes into play when the input sequences are partitioned into several groups, for example representing different genomes, and these groups shall also be distinguished in the de Bruijn graph. Then one assigns a **color** (usually a single bit indicating presence or absence) to each group. A k-mer inherits this color, so that each k-mer gets assigned a **color set**, indicating in which groups it appears and in which not. This graph is then called **colored (compacted) (bidirected) de Bruijn subgraph**. Colored de Bruijn subgraphs form a very powerful data structure, but their memory-efficient implementation is not trivial. In fact, the main challenge is no longer the storage of the k-mers (whose number is limited by σ^k, see above), but the storage of the colors, which may occupy several thousand bits per k-mer in case of large pangenomes.

2.4 Implementation of de Bruijn Graphs: Sets of k-mers

Here we consider only the basic variant of de Bruijn subgraphs. With a little bit of effort all results can also be generalized to the other graph types.

A simple observation allows to save considerable space: Assume we have a very fast method to test if a certain k-mer is represented in de Bruijn graph $G = \mathrm{DBG}(s, k)$ or not. Then one does not need to store the edges explicitly, for the following reason. When traversing from a vertex representing k-mer au to an adjacent vertex representing k-mer ub ($a, b \in \Sigma$, $u \in \Sigma^{k-1}$), instead of following the edge $au \to ub$ explicitly (if it exists), we can instead just test if ub is represented as a vertex in G or not. If the test is successful, we know that the edge exists and therefore can be traversed. If the test is unsuccessful, then the edge does not exist and can not be traversed. Similarly, if we want to find all possible successors of the vertex representing k-mer au, we perform σ tests, one for each possible last character c of the new, overlapping k-mer uc. Especially for small alphabets like DNA, this is not very much work.

Therefore, in (DNA-) bioinformatics applications, a data structure is very popular that represents only a set S of k-mers and offers a very quick presence/absence test, instead of storing a complete graph data structure: the **Bloom filter**.

The idea is to use one large bit array B of length m, say, as a hash table with all bits initially set to *false* (`F`), and then have a small number of h different hash functions $f_1, f_2, \ldots, f_h$ that map k-mers (respectively, their ranks) to integers in the range $[0, m-1]$. In order to store a k-mer (respectively its rank r), the hash functions are computed and the corresponding bits in B are set:

$$B[f_1(r)] = B[f_2(r)] = \ldots = B[f_h(r)] = \mathtt{T}.$$

This is repeated for each k-mer.

As always, the hash functions should be independent and uniformly distributed. A very simple one is of the form $f_i(r) = (r^{i+1} \texttt{>>} k) \mod m$, where $\texttt{>>}k$ denotes k-fold bitwise right-shift. There exist, however, much more advanced hash functions in the literature.

In order to test whether a k-mer with rank r has been stored in a Bloom filter B, we perform the same procedure: We evaluate all hash functions in parallel, and if all of them point to `T` entries in the hash table, then it is very likely that the element r has been stored in B:

$$\text{probably-contains}(B, r) = B[f_1(r)] \wedge B[f_2(r)] \wedge \cdots \wedge B[f_h(r)],$$

where $\wedge$ denotes logical AND.

By the combination of several distinct hash functions, a k-mer z (with rank $r = \mathrm{rank}(z)$) will be reported as a false positive, although it has not been stored in the Bloom filter, only if all hash functions are involved in collisions, i.e., if for each of the h hash values $f_i(r)$, $1 \le i \le h$, some other k-mer $z' \neq z$ produces with some hash function $f_{i'}$ the same hash value ($f_{i'}(\mathrm{rank}(z')) = f_i(r)$) and therefore sets B at this position to `T`. While this is quite unlikely in practice as long as the hash table is only moderately filled, it can not be

completely avoided. Therefore the above function is called "probably-contains" and not "contains". There are some techniques to handle false positives in certain applications manually, so that they do not distort the result and the Bloom filter becomes exact. Details are omitted here.

Other techniques to handle k-mer sets in practice. Here comes a list of techniques that can be used to reduce memory requirements or speed up storage and retrieval of k-mer sets: **minimizers**, **spectrum-preserving string sets**, **super-k-mers**, **hyper-k-mers**, **multiminimizers**. Details go beyond the purpose of this lecture.

It may, however, be emphasized again that the methods mentioned here reach their limits when heterogeneous datasets with a large number of individual k-mer sets are stored, corresponding to colored de Bruijn graphs with many different colors. Finding good solutions for such cases is still an active area of research.

Software. Two powerful implementations of compacted colored de Bruijn graphs are `bifrost` (Holley and Melsted, 2020) and `ggcat` (Cracco and Tomescu, 2023).

3 Approximate String Matching

In the "Sequence Analysis 1" class, we have already discussed a variant of the Universal Alignment Algorithm that can be used to find a *best* (highest score) alignment of a (short) pattern x within a (long) text y: semi-global alignment. Often, we are interested in *all* matches with at least a certain score. A simple modification makes this possible: We just need to visit all predecessors of the final cell v_E and check whether the corresponding solutions fulfill the given criterion. (In the case of semi-global alignment, these cells correspond to the last row of the alignment matrix.)

In this section, we consider the cost-based formulation and therefore alignment matrix D. We assume that a sensible cost function cost : $\mathcal{A} \to \mathbb{R}_0^+$ for alignment columns is given where $\text{cost}(\binom{a}{a}) = 0$, $\text{cost}(\binom{a}{b}) > 0$ for $a \neq b$, and all indels have the same cost $\gamma > 0$. Then we consider the following problem variant:

Definition 3.1 Given a pattern $x \in \Sigma^m$, a text $y \in \Sigma^n$, a sensible cost function and a threshold $k \geq 0$, then the **approximate string matching problem** consists of finding all substrings y' of y, such that $d(x, y') \leq k$, where $d(\cdot, \cdot)$ denotes the edit distance with respect to the given cost function.

In fact, it suffices to discover the ending positions j of the substrings y' because we can always reconstruct the whole substring and its starting position by backtracing. These ending positions are also called ***k*-approximate matches** of x in y.

3.1 Sellers' Algorithm

For a change in style, here we present an explicit formulation of an algorithm that solves the problem not as a recurrence, but in pseudo-code notation. Algorithm 1 is known as **Sellers' algorithm** (Sellers, 1980). Of course, it corresponds to the Universal Alignment Algorithm variant discussed in the "Sequence Analysis 1" class for semi-global alignment, except that we do not look at the final vertex v_E to find the *best* match, but look at all the vertices in the last row to identify *all* matches with $D(m, j) \leq k$ for $0 \leq j \leq n$.

Looking closely at Algorithm 1, we see that each iteration $j = 1, \ldots, n$ transforms the previous column $C_{j-1} := D(\cdot, j-1)$ of the edit matrix into the current column $C_j := D(\cdot, j)$, based on character $y[j]$. Starting with C_0, Sellers' algorithm effectively consists of repeatedly computing C_j from C_{j-1} and the next text character $y[j]$, for each $j = 1, \ldots, n$.

Some remarks about Sellers' algorithm:

Algorithm 1: Sellers' algorithm solving the approximate string matching problem

input : pattern $x \in \Sigma^m$, text $y \in \Sigma^n$, threshold $k \in \mathbb{N}_0$, sensible cost function with indel cost $\gamma > 0$, defining an edit distance $d(\cdot, \cdot)$

output: ending positions j such that $d(x, y') \leq k$, where $y' := y[j' \dots j]$ for some $j' \leq j$

```
//initialize the 0-th column of the alignment matrix
for i ← 0, ..., m do
    C_0(i) ← i · γ
end
//proceed column-by-column
for j ← 1, ..., n do
    C_j(0) ← 0
    for i ← 1, ..., m do
        C_j(i) ← min{C_{j-1}(i − 1) + cost(x[i], y[j]), C_{j-1}(i) + γ, C_j(i − 1) + γ}
    end
    if C_j(m) ≤ k then
        report (j, C_j(m))    //report match ending at column j and its cost
    end
end
```

1. Since we only report end positions and costs, there is no need to store back pointers and the entire alignment matrix D in Algorithm 1. The current and the previous column suffice. This decreases the space requirement to $O(m)$, where m is the (short) pattern length. We still need to store the text, which is $O(n)$, however this need not necessarily be in memory.

2. If we have a very good match with cost $< k$ at position j, the neighboring positions will also match with cost $\leq k$. To avoid this redundancy, it makes sense to post-process the output and only look for **runs of local minima**. Without formally defining it, if $k = 3$ and the respective costs at positions $j - 2, \dots, j + 4$ are $(3, 2, 1, 1, 2, 2, 3)$, the original algorithm will report all of these positions. In most applications, however, we are only interested in the locally minimal value 1, which occurs as a run of length 2. So it would make sense to report the run interval and the minimum cost as $([j, j + 1], 1)$.

3. Note that at no time in the algorithm we need to know the exact value of $C_j(m)$ as long as we can be sure that it exceeds k and therefore will not be reported.

3.2 Ukkonen's Cutoff Algorithm

The last of the above three observations leads to a considerable improvement of Sellers' algorithm: If we know that in column j all values after a certain index i_j^* exceed k, we do not need to compute them (e.g. we could assume that they are all equal to ∞ or $k + 1$). The following definition captures this formally.

Definition 3.2 The **last essential index** i^* (for threshold k) of a column C is defined as $i^*(C) := \max\{i \mid C(i) \leq k\}$.

Thus the last essential index i_j^* of column C_j is $i_j^* := i^*(C_j) = \max\{i \mid C_j(i) \leq k\}$. From the definition it is clear that we cannot miss any k-approximate matches if we do not compute the cells below the last essential index.

To set this in operation, the last essential index i_0^* of the 0-th column is easily found, because the scores increase monotonically from 0 to $m \cdot \gamma$. The other columns, however, are not necessarily monotone! Therefore, as we move column-by-column through the matrix, we have to make sure that we can efficiently find the last essential index of the next column, given the previous column. Consider the effects of the C_{j-1}-entries below the last essential index on the next column C_j. Since $C_{j-1}(i) > k$ for all $i > i_{j-1}^*$ and costs are non-negative, these cells provide candidate values $> k$ for $C_j(i)$ for $i > i_{j-1}^* + 1$ which therefore do not need to be considered during the minimization. The only chance that such $C_j(i)$ remain bounded by k is vertically, i.e., if $C_j(i) = C_j(i-1) + \gamma$. As soon as $C_j(i) > k$ for some $i > i_{j-1}^* + 1$, we know that the last essential index i_j^* of C_j occurs before that i. Algorithm 2 shows the details.

Example 3.1 Let $x =$ `AABB`, $y =$ `BABAABAABBABAA` and $k = 1$. The following table shows which values are computed by Algorithm 2 considering unit cost. The last essential index in each column is encircled. The k-approximate matches are underlined in the last row.

x \ y	ϵ	B	A	B	A	A	B	A	A	B	B	A	B	A	A
ϵ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A	①	①	0	1	0	0	1	0	0	1	1	0	1	0	0
A		2	①	1	①	0	1	1	0	1	2	1	1	①	0
B				①	2	①	0	1	①	0	1	2	①	2	①
B					2		①	①	2	①	⓪	①	2	2	
							I	II		III	IV	V			

Five exemplary alignments are shown below, one for each of the underlined positions.

I	II	III	IV	V
x: A A B B	x: A A B B	x: A A B B	x: A A B B	x: A A B B -
y: A A B -	y: A A B A	y: A A B -	y: A A B B	y: A A B B A

◀

Sometimes, because it was first published by Ukkonen (1985), this algorithm is referred to as **Ukkonen's cutoff algorithm**, although it is more common to use this name if the cost function is standard unit cost. In that case, even further optimizations are possible, but we do not discuss the details here.

Algorithm 2: Ukkonen's cutoff algorithm solving the approximate string matching problem

input : pattern $x \in \Sigma^m$, text $y \in \Sigma^n$, threshold $k \in \mathbb{N}_0$, sensible cost function with indel cost $\gamma > 0$, defining an edit distance $d(\cdot, \cdot)$
output: ending positions j such that $d(x, y') \leq k$, where $y' := y[j' \dots j]$ for some $j' \leq j$

```
//initialize the 0-th column of the alignment matrix
i*_0 ← min{m, ⌊k/γ⌋}
for i ← 0, ..., i*_0 do
    C_0(i) ← i · γ
end
//proceed column-by-column
for j ← 1, ..., n do
    C_j(0) ← 0
    i*_j ← 0
    i ← 1
    while i ≤ i*_{j−1} do
        C_j(i) ← min{C_{j−1}(i − 1) + cost(x[i], y[j]), C_{j−1}(i) + γ, C_j(i − 1) + γ}
        if C_j(i) ≤ k then i*_j ← i end
        i ← i + 1
    end
    if i ≤ m then
        C_j(i) ← min{C_{j−1}(i − 1) + cost(x[i], y[j]), C_j(i − 1) + γ}
        if C_j(i) ≤ k then i*_j ← i end
        i ← i + 1
        while i ≤ m and C_j(i − 1) + γ ≤ k do
            C_j(i) ← C_j(i − 1) + γ
            if C_j(i) ≤ k then i*_j ← i end
            i ← i + 1
        end
    end
    if i*_j = m and C_j(m) ≤ k then
        report (j, C_j(m))    //report match ending at column j and its cost
    end
end
```

3.3 Search Schemes and Bidirectional Indices

Assume we want to do approximate string matching with a text index, as a generalization of exact string matching that can be very efficiently solved with suffix trees, suffix arrays or the Burrows-Wheeler Transform (BWT), as we have seen. In principle one can use these data structures also for approximate matching, if the solution space of pattern alignments with all candidate substrings of the text is enumerated by backtracking. However, if this is done in a straight left-to-right (as with suffix trees) or right-to-left (as with the BWT) organization, it will be very inefficient already for small error threshold k. A better way is the use of *search schemes* in combination with more flexible *bidirectional indices* that we will study in this section.

The general idea is that the pattern x is divided into several non-overlapping parts $x_1, x_2, \ldots, x_p$. The **search scheme** (Kucherov et al., 2016) then formally consists of multiple searches, where a search is represented by the triple $S = (\pi, L, U)$. The permutation π of $\{1, 2, \ldots, p\}$ specifies the order in which the parts of the pattern are matched. L and U are arrays of size p such that $L[i]$ and $U[i]$ denote lower and upper bounds of the number of errors after the first i parts are matched (in the order specified by π).

Pigeonhole search scheme. A simple and very natural search scheme is the following: For error threshold k, split the pattern x in $p = k + 1$ parts $x_1, x_2, \ldots, x_{k+1}$, usually of roughly the same length. Clearly, if the whole pattern occurs in the text with at most k errors, then at least one of the parts must match exactly in the corresponding text region. Therefore, the search scheme consists of $k + 1$ searches $S_1, S_2, \ldots, S_{k+1}$ where search S_i first matches pattern part x_i exactly, followed by a backtracking search to the left $(x_{i-1}, x_{i-2}, \ldots, x_1)$ with not more than k errors, and finally a backtracking search to the right $(x_{i+1}, x_{i+2}, \ldots, x_{k+1})$, with additional errors, until a total of k is reached (or the pattern is exhausted).

Formally, for $k = 4$ the pigeonhole principle search scheme $\mathcal{S} = (S_1, \ldots, S_5)$ looks as follows:

$$\begin{aligned} S_1 &= (12345, 00000, 04444) \\ S_2 &= (21345, 00000, 04444) \\ S_3 &= (32145, 00000, 04444) \\ S_4 &= (43215, 00000, 04444) \\ S_5 &= (54321, 00000, 04444) \end{aligned}$$

Note that there may be redundant results that need to be filtered in a postprocessing step, for example if more than one pattern part occurs in one match with no errors.

MinU search scheme. Another example is the MinU search scheme (Renders et al., 2024). Its definition for $k = 4$ is as follows:

$$
\begin{aligned}
S_1 &= (12345, 00222, 02244) \\
S_2 &= (23145, 00000, 01244) \\
S_3 &= (32145, 01111, 01244) \\
S_4 &= (45321, 00003, 01444) \\
S_5 &= (54321, 01114, 01444)
\end{aligned}
$$

It is easy to see that MinU is more efficient, since the ranges $(L[i], U[i])$ grow slower than for the pigeonhole scheme. The main question is, though, whether it is lossless, i.e., no approximate match with up to k errors will be missed. This can be proven by showing that all possible *error configurations* $(e_1, e_2, \ldots, e_p)$ are covered, where e_i is the number of errors in part x_i and $e_1 + e_2 + \ldots + e_p \leq k$. This has been shown for the MinU scheme, although we will not go into details here.

Bidirectional indices. In order to combine search schemes with a text index, it is necessary that the search can be organized more flexibly than just left-to-right or just right-to-left. To this end, bidirectional text indices are very useful. They allow for extending a partial match either to the left or to the right, just as the search scheme requires. Several bidirectional indices have been developed over time, starting with **affix trees** (Stoye, 1995, 2000; Maaß, 2003) and **affix arrays** (Strothmann, 2007), followed by the **bidirectional BWT** (Lam et al., 2009), the **bidirectional wavelet index** (Schnattinger et al., 2012), the **br-index** (Arakawa et al., 2022) and finally the **bidirectional move index** (Depuydt et al., 2025). Details go beyond the scope of these lecture notes.

Software. A very efficient implementation of approximate string matching via search schemes and bidirectional indices is `Columba` (Renders et al., 2025). `Columba` implements the MinU search scheme for up to $k = 13$ errors, uses the b-move (bidirectional move) index, and filters redundant results; both of the type when the same match is found multiple times because of the search scheme and when several overlapping matches are found around a match with less than the maximum allowed number of errors, where better only a *run* of consecutive matches should be reported.

4 Biologically Inspired Alignment Scores

The unit cost edit distance simply counts certain differences between strings. There is no inherent biological meaning behind this scheme. If we want to compare DNA or protein sequences, for example, biologically more meaningful distances should be used.

The following is called the **transversion/transition cost** on the DNA alphabet:

	A	G	C	T
A	**0**	1	2	2
G	1	**0**	2	2
C	2	2	**0**	1
T	2	2	1	**0**

Bases A and G are called **purines**, and bases C and T are called **pyrimidines**. The transversion/transition cost function reflects the biological fact that a purine/purine and a pyrimidine/pyrimidine replacement is more likely to occur than a purine/pyrimidine replacement. Often, an insertion/deletion cost of 3 is used with the above costs to take into account that a deletion or an insertion of a base is even more seldom. These costs define the **transition/transversion distance** between two DNA sequences.

To compare protein sequences and define a cost function on the amino acid alphabet, we may count how many DNA bases must change minimally in a codon to transform one amino acid into another.

A more sophisticated cost function for replacement of amino acids was calculated by W. Taylor according to the method described in Taylor and Jones (1993) and is shown in Table 4.1. (To completely define a distance function, we would also have to define the costs for insertions and deletions.)

Note, however, that in a distance model a match has always zero cost, $\text{cost}(\binom{a}{a}) = 0$ for all $a \in \Sigma$. This restricts flexibility a little bit because any match is scored in the same way.

4.1 Sensible Similarity Scores

The concept of a metric is useful because it embodies the essential properties of our intuition about distances. For example, if the triangle inequality is violated, we could find a shorter way from x to y via a detour over z, which is not intuitive. However, distances also have disadvantages, as we have seen in the discussion of local alignment, where distance functions are not useful. Similarity scores, instead, where matches are

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	**0**	14	7	9	20	9	8	7	12	13	17	11	14	24	7	6	4	32	23	11
R	14	**0**	11	15	26	11	14	17	11	18	19	7	16	25	13	12	13	25	24	18
N	7	11	**0**	6	23	5	6	10	7	16	19	7	16	25	10	7	7	30	23	15
D	9	15	6	**0**	25	6	3	9	11	19	22	10	19	29	11	11	10	34	27	17
C	20	26	23	25	**0**	26	25	21	25	22	26	25	26	26	22	20	21	34	22	21
Q	9	11	5	6	26	**0**	5	12	7	17	20	8	16	27	10	11	10	32	26	16
E	8	14	6	3	25	5	**0**	9	10	17	21	10	17	28	11	10	9	34	26	16
G	7	17	10	9	21	12	9	**0**	15	17	21	13	18	27	10	9	9	33	26	15
H	12	11	7	11	25	7	10	15	**0**	17	19	10	17	24	13	11	11	30	21	16
I	13	18	16	19	22	17	17	17	17	**0**	9	17	8	17	16	14	12	31	18	4
L	17	19	19	22	26	20	21	21	19	9	**0**	19	7	14	20	19	17	27	18	10
K	11	7	7	10	25	8	10	13	10	17	19	**0**	15	26	11	10	10	29	25	16
M	14	16	16	19	26	16	17	18	17	8	7	15	**0**	18	17	16	13	29	20	8
F	24	25	25	29	26	27	28	27	24	17	14	26	18	**0**	27	24	23	24	8	19
P	7	13	10	11	22	10	11	10	13	16	20	11	17	27	**0**	9	8	32	26	14
S	6	12	7	11	20	11	10	9	11	14	19	10	16	24	9	**0**	5	29	22	13
T	4	13	7	10	21	10	9	9	11	12	17	10	13	23	8	5	**0**	31	22	10
W	32	25	30	34	34	32	34	33	30	31	27	29	29	24	32	29	31	**0**	25	32
Y	23	24	23	27	22	26	26	26	21	18	18	25	20	8	26	22	22	25	**0**	20
V	11	18	15	17	21	16	16	15	16	4	10	16	8	19	14	13	10	32	20	**0**

Table 4.1: Amino acid replacement costs as suggested by W. Taylor.

scored positively, while mismatches and indels receive negative scores, provide a useful alternative. Here we consider a **general similarity score** that should satisfy certain intuitive properties.

Definition 4.1 A **sensible score function** for columns in a pairwise alignment is a function $\text{score} : \mathcal{A}(\Sigma) \to \mathbb{R}$ such that

$$\begin{aligned}
&\text{score}(\tbinom{a}{a}) \geq 0 \geq \text{score}(\tbinom{a}{-}) = \text{score}(\tbinom{-}{a}) && \text{for all } a \in \Sigma \\
&\text{score}(\tbinom{a}{a}) \geq \text{score}(\tbinom{a}{c}) = \text{score}(\tbinom{c}{a}) && \text{for all } a, c \in \Sigma \\
&\text{score}(\tbinom{a}{c}) \geq \text{score}(\tbinom{a}{-}) + \text{score}(\tbinom{-}{c})) && \text{for all } a, c \in \Sigma
\end{aligned}$$

The first and second conditions impose symmetry and state that the similarity between a and itself is always higher than between a and anything else. In particular, insertions and deletions never score positively. The third condition states that a direct substitution is preferable to a deletion followed by an insertion.

In general, it is a good idea to verify that a score function in sensible, like in the case of log-odds score matrices introduced in the next section. In other cases, one might deviate from this property for good reason, see Section 4.3.

4.2 Log-Odds Score Matrices

Now it is time to discuss how we can define similarity scores for biological sequences from an evolutionary point of view; we focus on proteins and define a similarity function on the alphabet Σ of 20 amino acids.

The basic observation is that over evolutionary time-scales, in functional proteins two similar amino acids are replaced more frequently by each other than dissimilar amino

acids. The twist is now to *define* similarity by observing how often one amino acid is replaced by another one in a given amount of time.

Let $\pi = (\pi_a)_{a\in\Sigma}$ be the **frequency vector**, which means that $\pi_a \geq 0$ for all $a \in \Sigma$ and $\sum_{a\in\Sigma} \pi_a = 1$, of the naturally occurring amino acids. If we look at two random positions in two randomly selected proteins, the probability that we see the ordered pair (a, b) is then $\pi_a \cdot \pi_b$. The entries of π are also called **background frequencies** of the amino acids.

Now assume that we can track the fate of individual amino acids over a **fixed evolutionary time period** t. Let $M^t(a, b)$ be the observed frequency table of homologous amino acid pairs where we observed a at the beginning of the period and b at the end of the period, such that $\sum_{a,b} M^t(a, b) = 1$. Usually, we count substitutions and identities twice, i.e., once in each direction. This ensures the symmetry of $M^t : M^t(a, b) = M^t(b, a)$. The reason is that we can in fact not track amino acids during evolution; we can only observe the state of two present-day sequences and do not know their most recent common ancestor. The fields of **molecular evolution** and **phylogenetics** examine more of the resulting implications.

We can find the background frequencies as the marginals of M^t: $\pi_a = \sum_{b\in\Sigma} M^t(a, b)$ and $\pi_b = \sum_{a\in\Sigma} M^t(a, b)$ because of symmetry.

There are two reasons why an entry $M^t(a, b)$ can be relatively large. The first reason is the one we are interested in: because a and b are similar. The second reason is that simply a or b could be frequent amino acids. To remove the second effect, we consider the so-called **likelihood ratio** $M^t(a, b)/(\pi_a \cdot \pi_b)$, which relates the probability of the pair (a, b) in an evolutionary model to its probability in a random model. We declare that a and b are similar if this ratio exceeds 1 and that they are dissimilar if this ratio is below 1. To obtain an additive function, we take the logarithm and define the **log-odds score matrix** with respect to time period t by

$$\text{score}^t(a, b) := \log\left(\frac{M^t(a, b)}{\pi_a \cdot \pi_b}\right).$$

The time parameter t should be chosen in such a way that the score matrix is optimal for the problem at hand: If we want to compare two sequences whose most recent common ancestor existed, say, 530 million years ago, then we should ideally use a score matrix constructed from sequence pairs with distance $t \approx 1060$ million years.

Some remarks:

- For convenience, scores are often scaled by a constant factor and then rounded to integers. The score unit is called a **bit** if the score is obtained by a $\log_2(\cdot)$ operation. When multiplied by a factor of three, say, we get scores in **third-bits**.

- Since we cannot easily observe how DNA or proteins change over millions of years, constructing a score matrix is somewhat difficult. Since the pioneering work of Margaret Dayhoff and colleagues in the 1970s, Markov processes are used to model molecular evolution. Methods that allow to integrate information from sequences of different divergence times in a consistent fashion have been developed more recently.

- Important score matrix families for proteins are the **PAM(t)** (Dayhoff et al., 1978) and the **BLOSUM(s)** (Henikoff and Henikoff, 1992) matrices. Here t is a divergence time parameter and s is a clustering similarity parameter inversely correlated to t. (The inventors of BLOSUM have chosen to index their matrices in a different way.) The BLOSUM matrices are the most widely used ones for protein sequence comparison.

4.3 Non-symmetric score matrices

We usually demand that similarity functions and hence score matrices are symmetric. In some cases, however, there are good reasons to choose a non-symmetric similarity function. Often then, the unsymmetry does not stem from an unsymmetry of M^t (for reasons explained above), but from different background frequencies. We mention an example.

Assume that we have a particular protein fragment (the "query") that forms a transmembrane helix. The amino acid composition in membrane regions differs strongly from that of the "average" protein: It is hydrophobically biased. Assume that we want to look for similar fragments in a large database of peptide sequences (of unknown or "average" type). It follows that the matrix M^t of pair frequencies should be one derived from an evolutionary process acting on transmembrane helices and that two different types of background frequencies should be used: The query background frequencies τ are those of the transmembrane model, different from the background frequencies π of the database. It follows that we should use

$$\text{score}^t(a,b) := \log\left(\frac{M^t(a,b)}{\tau_a \cdot \pi_b}\right),$$

or a rounded multiple thereof, so that now $\text{score}(a,b) \neq \text{score}(b,a)$ in general. Table 4.2 shows the SLIM161 matrix for transmembrane helices (Müller et al., 2001). The 161 is the time parameter t referring to the expected number of evolutionary events per 100 positions.

4.4 Position-Specific Scores

The match/mismatch score in all our alignment algorithms depends on a (sensible) similarity function score as defined in Definition 4.1, often given by a log-odds score matrix. However, this does not need to be the case. The algorithm does not change in any way if indel and match/mismatch scores depend also on the *position* in the sequences. Therefore we can replace $\text{score}(x[i], y[j])$ by $\text{score}(i,j)$ and worry later about where to obtain reasonable score values.

A useful application of this observation is as follows: Local alignment is often used in large-scale database searches to find remote homologs of a given protein. Transmembrane (TM) proteins have an unusual sequence composition (which has a large influence on the score matrix entries, as pointed out in Section 4.2). According to Müller et al. (2001), one can increase the sensitivity of the homology search by using a bipartite scoring scheme

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	**5**	-8	-5	-10	3	-7	-11	0	-5	1	1	-10	1	1	-5	2	1	-2	-3	2
R	-3	**10**	-4	-10	-2	-3	-11	-4	-4	-2	-1	-3	-1	-2	-7	-4	-3	-2	-3	-3
N	-1	-5	**8**	-2	0	-2	-7	-2	2	-1	-1	-6	0	1	-5	2	0	-2	2	-2
D	-3	-9	1	**9**	-4	-2	1	-2	-2	-2	-2	-6	-2	-1	-5	-3	-3	-3	-2	-2
C	2	-8	-5	-11	**11**	-7	-12	-3	-8	0	1	-12	1	3	-11	2	0	-1	0	1
Q	-1	-2	0	-3	0	**7**	-4	-2	0	0	0	-4	2	2	-4	0	-1	4	1	0
E	-3	-7	-2	3	-2	-1	**7**	-3	-1	-2	-2	-8	-1	0	-4	-1	-3	-1	-1	-2
G	1	-7	-4	-7	0	-5	-10	**7**	-7	-1	0	-8	0	1	-5	1	-1	-3	-2	-1
H	-1	-5	3	-5	-2	-1	-5	-4	**10**	-1	-1	-8	-1	2	-7	-1	-2	1	5	-2
I	-1	-9	-7	-11	-1	-7	-12	-4	-7	**6**	3	-11	4	2	-6	-3	-1	-2	-3	4
L	-1	-8	-6	-10	1	-7	-12	-4	-7	4	**5**	-11	4	3	-7	-3	-1	-1	-2	2
K	-1	2	-1	-4	-2	0	-7	-1	-3	0	-1	**6**	0	0	-1	-1	-1	-1	1	-2
M	-1	-7	-6	-10	1	-5	-11	-3	-7	4	4	-11	**7**	3	-7	-2	0	-1	-2	2
F	-1	-9	-5	-10	2	-6	-11	-3	-4	1	2	-11	2	**8**	-7	-2	-2	3	4	0
P	-3	-9	-7	-9	-7	-8	-10	-4	-9	-2	-3	-8	-3	-2	**11**	-3	-3	-3	-4	-2
S	2	-8	-1	-8	4	-5	-9	0	-4	0	0	-9	0	1	-5	**6**	1	-2	-1	0
T	2	-7	-3	-8	2	-6	-10	-2	-5	2	2	-9	3	2	-4	2	**4**	-4	-2	2
W	-3	-7	-6	-10	0	-2	-11	-5	-3	-1	0	-10	0	4	-6	-4	-5	**15**	2	-2
Y	-4	-8	-2	-9	1	-5	-10	-5	0	-2	-1	-8	-1	6	-7	-3	-3	2	**11**	-2
V	1	-9	-7	-10	1	-7	-11	-4	-7	5	3	-12	3	2	-6	-2	0	-2	-3	**5**

Table 4.2: The non-symmetric SLIM 161 score matrix. Scores are given in third-bits, i.e., as $\text{score}(a,b) = \text{round}\big(3 \cdot \log_2(M(a,b)/(\tau_a \pi_b))\big)$.

with a (non-symmetric) transmembrane helix specific scoring matrix (such as SLIM) for the TM helices and a general-purpose scoring matrix (such as BLOSUM) for the remaining regions of the query protein; see Figure 4.1. As a consequence, the score of aligning $x[i]$ with $y[j]$ does not only depend on the amino acids $x[i]$ and $y[j]$ themselves, but also on the position i within the query sequence x.

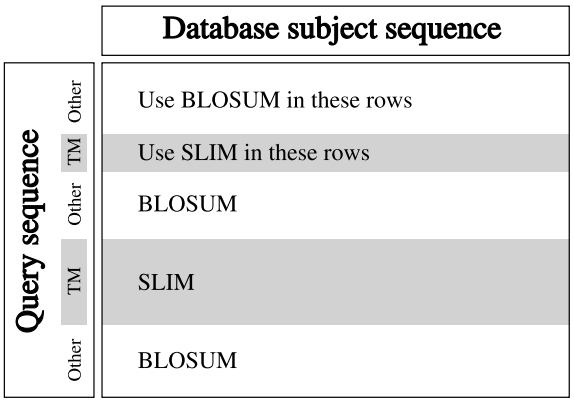


Figure 4.1: Bipartite scoring scheme for detection of homologous transmembrane proteins due to Müller et al. (2001). The figure represents the Smith-Waterman alignment matrix and indicates which scoring matrix is used for which query positions (rows): In transmembrane helices (TM), the transmembrane-specific scoring matrix SLIM is used, elsewhere the general-purpose matrix BLOSUM is used.

5 RNA Secondary Structure Prediction

5.1 Introduction

RNA is in many ways similar to DNA, with a few important differences:

- Thymine (T) is replaced by uracil (U), so the RNA alphabet is $\Sigma_{\text{RNA}} = \{\mathtt{A}, \mathtt{C}, \mathtt{G}, \mathtt{U}\}$.
- The additional base pair $\{\mathtt{G}, \mathtt{U}\}$ can be formed.
- RNA is less stable than DNA and more easily degraded.
- RNA mostly occurs as a single-stranded molecule that forms secondary structures by base pairing.
- While DNA is mainly the carrier of genetic information, RNA has a wide variety of tasks in the cell, e.g.
  - it acts as messenger (mRNA), transporting the transcribed genetic information,
  - it has regulatory functions,
  - it has structural tasks, e.g. in forming a part of the ribosome (rRNA),
  - it takes an active role in cellular processes, e.g. in the translation of mRNA to proteins, by transferring amino acids (tRNA).

As with proteins, the three-dimensional structure of an RNA molecule is crucial in determining its function. Since 3D-structure is hard to determine, an intermediate structural level, the **secondary structure**, is frequently considered. The secondary structure of an RNA molecule simply determines the intra-molecular basepairs. As with DNA, the Watson-Crick pairs $\{\mathtt{A}, \mathtt{U}\}$ and $\{\mathtt{C}, \mathtt{G}\}$ stabilize the molecule, but also the "wobble pair" $\{\mathtt{G}, \mathtt{U}\}$ adds structural stability. We now define this formally.

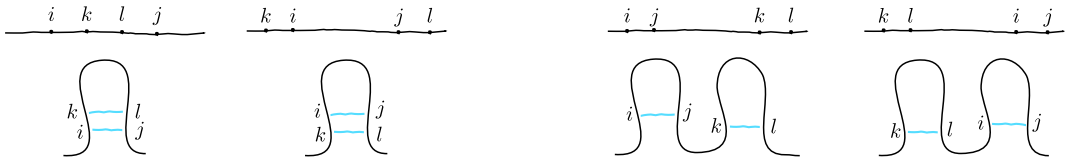
Let $s \in \{\mathtt{A}, \mathtt{C}, \mathtt{G}, \mathtt{U}\}^n$ be an RNA sequence of length n.

Definition 5.1 An ***s*-basepair** is a set of two different numbers $\{i, j\} \subset \{1, \ldots, n\}$ such that $\{s[i], s[j]\} \in \{\{\mathtt{A}, \mathtt{U}\}, \{\mathtt{C}, \mathtt{G}\}, \{\mathtt{G}, \mathtt{U}\}\}$. Basepairs must bridge at least $\delta \geq 0$ positions (e.g. $\delta = 3$); to account for this, we have the additional constraint $|i - j| > \delta$ for a basepair $\{i, j\}$.

Definition 5.2 A **simple structure** on s is a set $S = \{b_1, b_2, \ldots, b_N\}$ of s-basepairs with $N \geq 0$ and the following properties:

- Either $N \leq 1$, or

- if $N \geq 2$ and $\{i, j\} \in S$ and $\{k, l\} \in S$ are two different basepairs such that $i < j$ and $k < l$, then $i < k < l < j$ or $k < i < j < l$ (nested basepairs) or $i < j < k < l$ or $k < l < i < j$ (separated basepairs).



In other words, basepairs in a simple structure may not cross.

The restriction that basepairs may not cross is actually unrealistic, but it helps to keep several computational problems regarding RNA analysis of manageable complexity, as we shall see shortly.

With the above definition of a simple structure, we cover the following structural elements of RNA molecules: **single-stranded RNA**, **stem** or **stacking region**, **hairpin loop**, **bulge loop**, **interior loop**, **junction** or **multi-loop**; see Figure 5.1.

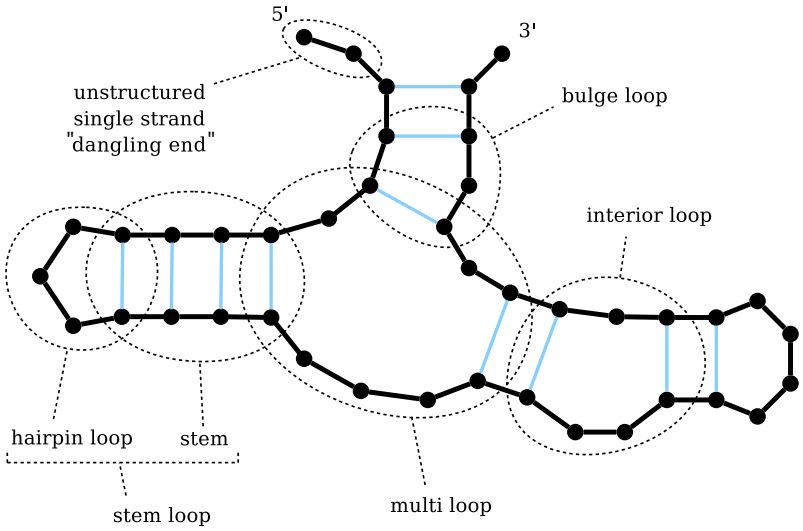


Figure 5.1: RNA secondary structure elements covered by Definition 5.2.

Several more complex RNA interactions are *not* covered, e.g. **pseudoknot**, **kissing hairpins**, **hairpin-bulge contact**. This is not such a severe restriction, because these elements seem to be comparatively rare and can be considered separately at a later stage.

We point out that all of the above structural elements can be rigorously defined in terms of sets of basepairs. For our purposes, an intuitive visual understanding of these elements is sufficient. There exist different ways to represent a simple structure in the computer. A popular way is the **dot-bracket** notation, where dots represent unpaired bases and corresponding parentheses represent paired bases:

```
5′ AACGCUCCCAUAGAUUACCGUACAAAGUAGGGCGC 3′
   ..(((.((..((...)).(.(...).)..)))))).
```

Another popular way to visualize a structure is by means of a **mountain plot**: Starting with the dot-bracket representation, replace dots by `-` and brackets `()` by `/\`, and use a second dimension. Another alternative is a **circle plot**; see Figure 5.2.

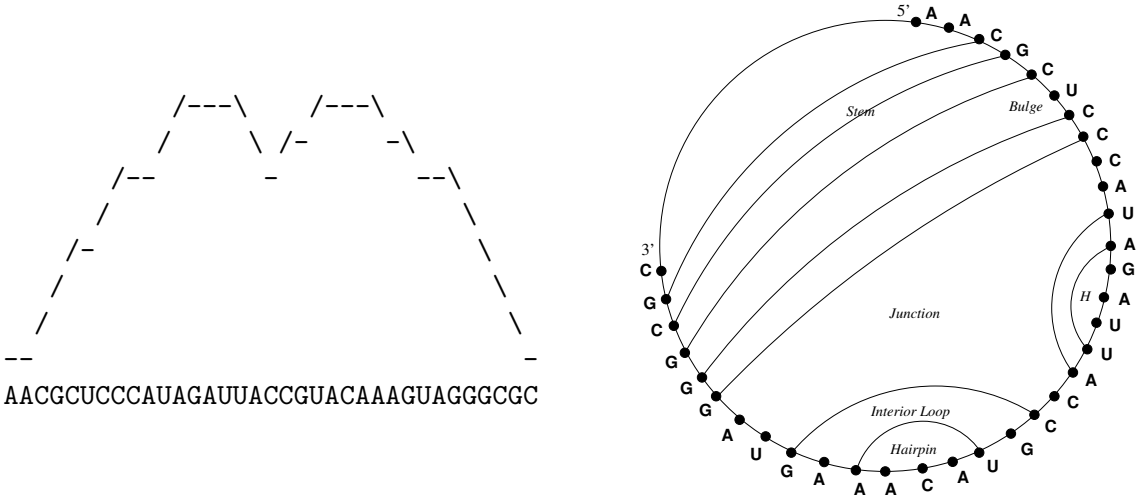


Figure 5.2: Mountain plot (left) and circle plot (right) of an RNA secondary structure.

5.2 The Optimization Problem

Over time, an RNA molecule may assume several distinct structures. The more energetically stable a structure is, the more likely the molecule will assume this structure. To make this precise, we need to define an energy model for all secondary structure elements. This can become quite complicated (the problem of predicting RNA structures could fill an entire course), so here we only consider a drastically simplified version of the problem: We assign a score (that can be interpreted as "stability" measure or negative free energy) to each basepair, depending on its type. We can simply count basepairs by assigning $+1$ to each pair, or we may take their relative stability into account by assigning $\text{score}(\{\texttt{A},\texttt{U}\}) := 2$, $\text{score}(\{\texttt{C},\texttt{G}\}) := 3$, $\text{score}(\{\texttt{G},\texttt{U}\}) := 1$, for example. On a sequence s of length n, the score of a basepair $b = \{i, j\}$ is then defined as $\text{score}(b) := \text{score}(\{s[i], s[j]\})$. The computational problem becomes the following one.

Problem 5.1 (Simple RNA Secondary Structure Prediction Problem) Let an RNA sequence s of length n be given, determine a simple structure S^* on s such that

$$\text{score}(S^*) = \max\{\text{score}(S) \mid S \text{ is a simple structure on } s\},$$

where $\text{score}(S) := \sum_{b \in S} \text{score}(b)$ is the sum of the base pair scores in the structure S.

Generally, the number of valid simple structures for a molecule of length n is exponential in n, so the most stable structure cannot be found in reasonable time by enumerating all possible structures. After a short digression to context free grammars, we present an efficient algorithm to solve the problem.

5.3 Context-Free Grammars

From the above discussion of the dot-bracket notation, we may say that a (simple secondary) structure is

0. either the empty structure,
1. or an unpaired base, followed by a structure,
2. or a structure, followed by an unpaired base,
3. or an outer basepair (with a sequence constraint), enclosing a structure (with a minimum length constraint),
4. or two consecutive structures.

We recall the notion of a **context-free grammar** here to show that simple secondary structures allow a straightforward recursive description.

Definition 5.3 A **context-free grammar** (CFG) is a quadruple $G = (N, T, P, S)$, where

- N is a finite set of **non-terminal symbols**,
- T is a finite set of **terminal symbols**, $N \cap T = \emptyset$,
- P is a finite subset of $N \times (N \cup T)^*$, the **productions**, which are rules for transforming a non-terminal symbol into a (possibly empty) sequence of terminal and non-terminal symbols, and
- $S \in N$ is the **start symbol**.

Ignoring basepair sequence and distance constraints, in the RNA structure setting, the terminal symbols are $T = \{\texttt{(}, \texttt{.}, \texttt{)}\}$ and $N = \{S\}$, where S is also the start symbol and represents a secondary structure. The productions are formalized versions of the above rules, i.e.,

0. $S \to \varepsilon$, where ε denotes the empty sequence,
1. $S \to \texttt{.}S$ 
2. $S \to S\texttt{.}$ 
3. $S \to \texttt{(}S\texttt{)}$ 
4. $S \to SS$

Note that this context-free recursive description is only possible because we imposed the constraint that basepairs do not cross.

Also note that this grammar is ambiguous: There are generally many ways to produce a given dot-bracket string from the start symbol S. For the Nussinov Algorithm in the next section, this ambiguity is not a problem. However, for other tasks, e.g. counting the number of possible structures, we need to consider a grammar that produces each structure in a unique way. We can argue as follows: Either the structure is empty, or it contains at least one base. In each nonempty RNA structure, look at the last base. Either

it is unpaired and preceded by a (shorter) prefix structure, or it is paired with some base at a previous position. We obtain the following non-ambiguous grammar:

$$S \to \varepsilon \mid S. \mid S(S)$$

where the vertical bar (|) denotes "or", i.e., it separates alternative right-hand sides of the production.

5.4 The Nussinov Algorithm

To solve Problem 5.1, we now give a dynamic programming algorithm based on the structural description of simple secondary structures from the previous section. This algorithm is due to Nussinov and Jacobson (1980). We also take into account the distance and sequence constraints for basepairs.

In the previous section, we have seen how to build longer secondary structures from shorter ones that can be arbitrary substrings of the longer ones. As before, let $s \in \{\mathtt{A}, \mathtt{C}, \mathtt{G}, \mathtt{U}\}^n$ be an RNA sequence of length n. For $i \leq j$, we define $M(i,j)$ as the solution to Problem 5.1 (i.e., maximally attainable score) for the string $s[i \dots j]$.

Since it is easy to find an optimal secondary structure of short sequences, let us start with those.

- If $j - i \leq \delta$ (in particular if $i = j$, and to avoid complications also if $i > j$), then $M(i,j) = 0$, since no basepair is possible due to the length or distance constraints (case 0).
- If $j - i > \delta$, then we can decompose the optimal structure according to one of the four cases. In each case, the resulting substructures must also be the optimal ones. Thus

$$M(i,j) = \max \begin{cases} M(i+1,j) & \text{(case 1: } S \to .S\text{)} \\ M(i,j-1) & \text{(case 2: } S \to S.\text{)} \\ M(i+1,j-1) + \mu(i,j) & \text{(case 3: } S \to (S)\text{)} \\ \max_{i+1<k<j}\{M(i,k-1) + M(k,j)\} & \text{(all cases 4: } S \to SS\text{)} \end{cases}$$

  where $\mu(i,j) := \text{score}(\{s[i], s[j]\}) > 0$ if $\{s[i], s[j]\}$ is a valid basepair, and $\mu(i,j) := -\infty$ otherwise, excluding the possibility of any forbidden basepair $\{i,j\}$.

This recurrence states: To find an optimal structure for $s[i \dots j]$, consider all possible decompositions according to the grammar and their respective scores, and evaluate which score for $s[i \dots j]$ would result from each decomposition. Then pick the best one.

This is possible because the composing elements of the optimal structure are themselves optimal. The proof is by contradiction: If they were not optimal and there existed better sub-structures, we could build a better overall structure from them, which would contradict its optimality.

The above recurrence has still to be developed into an algorithm. In order to evaluate $M(i,j)$, we need access to all entries $M(x,y)$ for which $[x,y]$ is a (strictly shorter) subinterval of $[i,j]$. This suggests to compute matrix M in order of increasing $j - i$ values.

The initialization for $j - i \leq \delta$ is easily done. Then we proceed for increasing $d = j - i$, as in Algorithm 3.

Algorithm 3: Nussinov-Algorithm

input : RNA sequence $s = s[1 \dots n]$; a scoring function for basepairs; minimum number δ of unpaired bases in a hairpin loop

output: maximal score $M(i, j)$ for a simple secondary structure of each substring $s[i \dots j]$; traceback indicators $T(i, j)$ indicating which case leads to the optimum

for $i \leftarrow 2, \dots, n$ **do**
  $M(i, i-1) \leftarrow 0$
  $T(i, i-1) \leftarrow$ "init" *// ε (case 0)*
end
for $d \leftarrow 0, \dots, \delta$ **do**
  for $i \leftarrow 1, \dots, n - d$ **do**
    $M(i, i+d) \leftarrow 0$
    $T(i, i+d) \leftarrow$ "unpaired" *// .S and/or S. (case 1 or 2)*
  end
end
for $d \leftarrow \delta + 1, \dots, n - 1$ **do**
  for $i \leftarrow 1, \dots, n - d$ **do**
    compute $M(i, i+d)$ according to the recurrence
    store the maximizing case in $T(i, i+d)$ *// (cases 1–3; or 4 with max. k)*
  end
end
report score $M(1, n)$
start traceback with $T(1, n)$

To find an optimal secondary structure (in addition to the optimal score), we also store traceback indicators in a second matrix T that shows which of the production rules (and which value of k for the $S \to SS$ rule) was used at each step. By following the traceback pointers backwards, we can build the dot-bracket representation of the secondary structure. For this, we start in cell $(1, n)$ and look at the recursion of the algorithm: When we follow a $S \to SS$ production traceback, the process branches recursively into the two substructures, reconstructing each substructure independently. Diagonals mean $S \to (S)$ and horizontal or vertical movement means $S \to S. \mid .S$. Note that when reaching the δ-diagonals only horizontal and vertical movement is allowed. Finally, when we end in the lowest diagonal (below the $i = j$ diagonal) we have $S \to \varepsilon$.

Analysis. The memory requirement for the whole procedure is obviously $O(n^2)$ and its time complexity is $O(n^3)$.

There exists a faster algorithm using the *Four-Russians* speedup yielding an $O(\frac{n^3}{\log n})$ time algorithm (Frid and Gusfield, 2009), but this goes far beyond the scope of these lecture notes.

Example 5.1 The Nussinov matrix for the RNA sequence `GACUCGAGUU` is shown in Figure 5.3. We use $\delta = 1$ and the scoring scheme: score(`G`, `C`) = 3, score(`A`, `U`) = 2 and score(`G`, `U`) = 1.

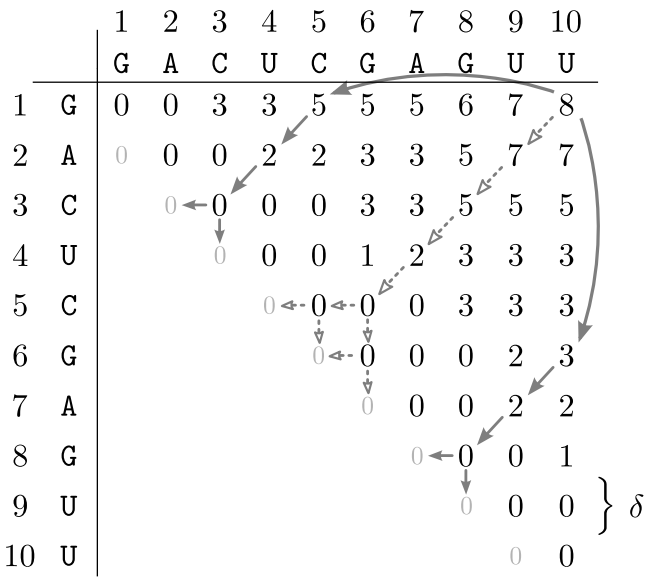


Figure 5.3: The Nussinov matrix M for the RNA sequence `GACUCGAGUU`.

There exist two distinct variants (indicated in solid and dashed traces) in which the given RNA sequence can optimally fold under this basepair scoring scheme. The resulting dot-bracket notations are `((.))((.))` and `((((..))))` for the solid and dashed variant, respectively. ◀

When reaching the δ-diagonals we could move vertically or horizontally which is possible in the ambiguous grammar. However, since in a hairpin loop a sequence of $S \to .S \mid S.$ will eventually end with $S \to \varepsilon$ no matter whether the `.` is put left or right, this would lead to identical dot-bracket notations. Hence, we could also choose to allow *only* horizontal or *only* vertical movement within the initialized 0-diagonals as to simplify the backtracing of all optimal dot-bracket notations (only 1 backtrace instead of 4 for the dashed variant).

6 Pairwise Sequence Alignment in Linear Space

6.1 The Forward-Backward Technique

The following problem frequently arises as a sub-problem of more advanced alignment methods, that is why we discuss it beforehand in detail:

Problem 6.1 (Advanced Alignment Problem) Given two sequences x and y of lengths m and n, respectively, find their optimal global alignment under the condition that the alignment path passes through a given cell (i_0, j_0) of the alignment matrix.

This is not a new problem at all! In fact, we can decompose it into two standard global alignment problems: Aligning the prefixes $x[1 \dots i_0]$ and $y[1 \dots j_0]$, and aligning the suffixes $x[i_0 + 1 \dots m]$ and $y[j_0 + 1 \dots n]$. By combining the results of these two sub-problems in an appropriate way, we obtain the minimally attainable **total cost** of all alignment paths that go through (i_0, j_0).

Total cost matrix. In fact, it is even possible to solve this problem for *every* point (i, j) *simultaneously*. The solution is called the **forward-backward technique**. In addition to the usual alignment matrix with alignment costs $D(i, j)$ (which record the minimally attainable cost for a prefix alignment), we additionally define the **reverse alignment matrix**: We exchange initial and final cell and reverse the direction of all computations. Effectively, we are thus globally aligning the reversed sequences. Hence, the associated alignment matrix $D^{\text{rev}} = D^{\text{rev}}(i, j)$ records the best possible costs for the suffix alignments.

Definition 6.1 Given two sequences x and y of lengths m and n, respectively, their **total cost matrix** is defined by

$$T(i,j) \quad = \quad \min \left\{ \text{cost}(A +\!\!+ B) \;\middle|\; \begin{array}{l} A \text{ is an alignment of the prefixes} \\ \quad x[1 \dots i] \text{ and } y[1 \dots j] \\ B \text{ is an alignment of the suffixes} \\ \quad x[i+1 \dots m] \text{ and } y[j+1 \dots n] \end{array} \right\}$$

where "++" denotes concatenation of alignments.

Note that $T(0, 0) = T(m, n)$ is the optimal global alignment cost $d(x, y)$ (since all paths, in particular the optimal ones, pass through $(0, 0)$ and (m, n)), and so $T(i, j) \geq d(x, y)$ for all (i, j).

The computation of the total cost matrix is particularly simple for *additive alignment costs*. Here

$$T(i,j) = D(i,j) + D^{\text{rev}}(i,j).$$

Clearly matrices D, D^{rev}, and thus T can be computed in $O(mn)$ time.

For *affine gap costs* the computation of the total cost matrix is slightly more difficult: Here, the cost of a concatenated alignment is not always the sum of the costs of the individual alignments because gaps might be merged, so that the gap initiation penalty that is imposed twice in the two separate alignments must be counted only once in the concatenated alignment. However, since the efficient computation of affine gap costs with Gotoh's algorithm uses the two history matrices V and H, and we know that by definition the costs stored in these matrices refer to those alignments that end with gaps, the total cost matrix for affine gap costs can also be computed in quadratic time if the history matrices V and H and their reverse counterparts V^{rev} and H^{rev} are given:

$$T(i,j) \quad = \quad \min \left\{ \begin{array}{l} D(i,j) + D^{\text{rev}}(i,j) \\ V(i,j) + V^{\text{rev}}(i,j) - d + e \\ H(i,j) + H^{\text{rev}}(i,j) - d + e \end{array} \right\}$$

where d is the gap open cost and e is the gap extension cost.

Additional cost matrix. For every (i,j), we can now obtain the **additional cost** $C(i,j)$ for passing through (i,j), compared to staying on an optimal alignment path.

Definition 6.2 Given two sequences x and y of lengths m and n, respectively, their **additional cost matrix** is defined by

$$C(i,j) \quad = \quad T(i,j) - d(x,y)$$

where $d(x,y)$ is the edit distance of sequences x and y.

By definition, if (i,j) is on the path of an optimal alignment, this value is zero, otherwise it is positive; see Figure 6.1.

Again, for any additive alignment cost, where $\text{cost}(A +\!\!+ B) = \text{cost}(A) + \text{cost}(B)$, we have that $C(i,j) = T(i,j) - d(x,y) = D(i,j) + D^{\text{rev}}(i,j) - d(x,y)$. Since T and $d(x,y)$ can be computed in $O(mn)$ time, the additional cost matrix C can be computed in $O(mn)$ time, too.

Example 6.1 Consider the sequences $x = \texttt{CT}$ and $y = \texttt{AGT}$. For unit cost, the edit matrix D and reverse edit matrix D^{rev}, which is drawn in reverse direction, are:

D:

	ϵ	A	G	T
ϵ	0	1	2	3
C	1	1	2	3
T	2	2	2	2

D^{rev}:

	A	G	T	ϵ
C	2	1	1	2
T	2	1	0	1
ϵ	3	2	1	0

This results in the following additional cost matrix:

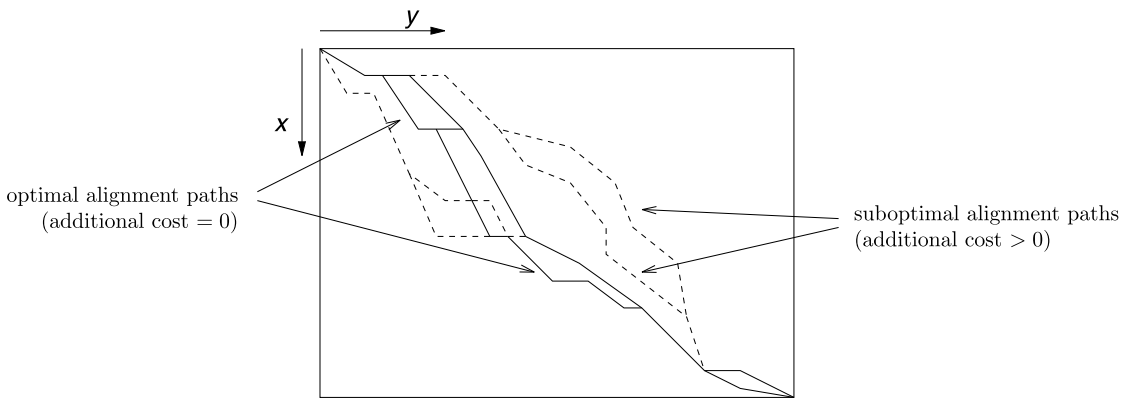


Figure 6.1: An additional cost matrix. Entry $C(i, j)$ contains the additional cost of the best possible alignment that passes through vertex (i, j) of the alignment graph.

C:

	ϵ	A	G	T
ϵ	0	0	1	3
C	1	0	0	2
T	3	2	1	0

We see that the paths of the two optimal alignments $A_1^{\text{opt}} = \begin{pmatrix} \texttt{C-T} \\ \texttt{AGT} \end{pmatrix}$ and $A_2^{\text{opt}} = \begin{pmatrix} \texttt{-CT} \\ \texttt{AGT} \end{pmatrix}$ correspond to the two paths of 0-entries in C. ◀

Applications. The forward-backward technique is used, for example, in the following problems:

- linear-space alignment (Hirschberg technique, see Section 6.2),
- discovering regions of slightly sub-optimal alignments (those cells where the score gap, resp. additional cost, remains below a small tolerance parameter),
- computing regions for the Carrillo-Lipman heuristic for multiple sequence alignment (see Section 8.2),
- finding cut-points for divide-and-conquer multiple alignment (see Section 10.1).

6.2 Hirschberg's Algorithm

We have seen that the *optimal alignment cost* of two sequences x and y of lengths m and n, respectively, as well as an *optimal alignment* can be computed in $O(mn)$ time and $O(mn)$ space.

Even though the $O(mn)$ time required to compute an optimal global or local alignment is sometimes frowned upon, it is not the main bottleneck in sequence analysis. The main bottleneck so far is the $O(mn)$ space requirement. Think of a matrix of traceback-pointers

for two bacterial genomes of 5 million nucleotides each. That matrix contains $25 \cdot 10^{12}$ entries and would thus need 25 Terabytes of memory. Fortunately, there is a solution!

We have already seen that the score by itself can easily be computed in $O(m+n)$ space, since we only need to store the sequences and two rows or two columns of the edit matrix at a time. However, if we also want to output an optimal alignment, the whole alignment matrix (or the backtracing matrix) is required for the backtracing phase.

In this section we present an algorithm that allows to compute an optimal global alignment in *linear* space. It was first presented by Hirschberg (1975). Our presentation is for the simple case of homogeneous linear gap costs $g(\ell) = \ell \cdot \gamma$, where γ is the cost for a single gap character. However, it can be generalized to affine gap costs with some additional complications.

The algorithm works in a **divide-and-conquer** manner, where in each recursion the alignment matrix $D = (D(i,j))_{1 \le i \le m, 1 \le j \le n}$ is divided into an upper half and a lower half at its middle row $m' = \lceil m/2 \rceil$. Hereby, the cut positions are chosen so that an optimal alignment of the two obtained prefixes, concatenated with an optimal alignment of the two obtained suffixes, yields an optimal alignment of the original sequences.

To find the cut positions for the division of the sequences, the "forward-backward" technique is used. The upper half is computed in a forward manner, as usual, and the lower one in a backward manner. At index m' both halves intersect, which makes it possible to compute the additional costs $C(m', j)$ for this row m'. An optimal alignment passes row m' at some column n' if and only if $C(m', n') = 0$.

The procedure is called recursively, once for the upper left "quarter", and once for the lower right "quarter" of the alignment matrix. The recursion is continued until only one row remains, in which case the problem can easily be solved directly. See Figure 6.2 for an illustration.

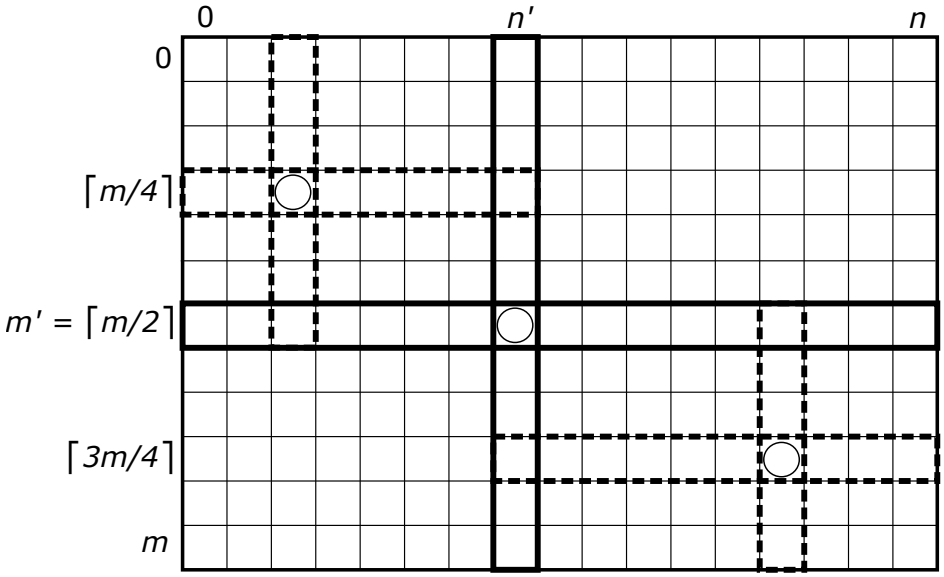


Figure 6.2: Illustration of linear-space alignment.

Complexity analysis. The space complexity is in $O(m+n)$ since all matrix computations can be performed row-wise using memory for two adjacent rows at most, and the final alignment has at most $m + n$ columns.

A central question is, how much do we have to "pay" in terms of running time to obtain the linear space complexity? Fortunately, the answer is: Only approximately a constant factor of 2. In the original (quadratic-space) algorithm, each cell of the edit matrix S is computed once. In the linear-space version, some cells are computed several times during the recursions.

The amount of cells computed in the first pass is mn, in the second pass it is only half of the matrix, in the third pass it is a quarter of the matrix and so on, for a total of $mn \cdot (1 + 1/2 + 1/4 + \dots) \leq 2mn$ cells (geometric series). Thus, the asymptotic time complexity is still $O(mn)$.

Example 6.2 Given strings $x =$ `CACG` and $y =$ `GAG`, Figure 6.3 shows how alignment in linear space works. Note that just the black numbers need to be calculated. The grey ones are only for comparison to Section 6.1 to provide an easier comprehension.

The recursion ends in four base cases resulting in the overall alignment $\binom{\texttt{CACG}}{\texttt{GA-G}}$. Note that if a cut position n' is found (indicated by a circle in additional cost matrix C), the sequences are cut in two *after* this position, such that the left side of t' contains the circled character and the right side does not. ◀

Affine and general gap costs. Combining the Hirschberg technique with affine gap costs is conceptually straightforward, while the devil is in the details. This was first presented by Myers and Miller (1988).

General gap costs cannot be handled in linear space. We cannot even compute just the alignment score in linear space since we always need to refer back to *all* cells to the left and above of the current one.

Local alignment. We have described how to compute an optimal *global* alignment in linear space. What about *local* alignments? Clearly, we have to use a scoring scheme instead of a cost function, but that modification is an easy exercise. For the main adaptation, it is important to note that a local alignment is in fact a global alignment of the two best-aligning substrings of x and y. So, once we know the start- and endpoints of these substrings, we can use the above global alignment procedure.

It is easy to find the endpoints: They are given by the entry with the highest score in the (forward) local alignment matrix.

To find the start points, we can use the reversal technique: They are just the endpoints of the optimal local alignment of the reversed strings. This technique is simple, but may become problematic if there are several equally high-scoring local alignments, when the start- and end-points have to be matched correctly. Details are left to the reader.

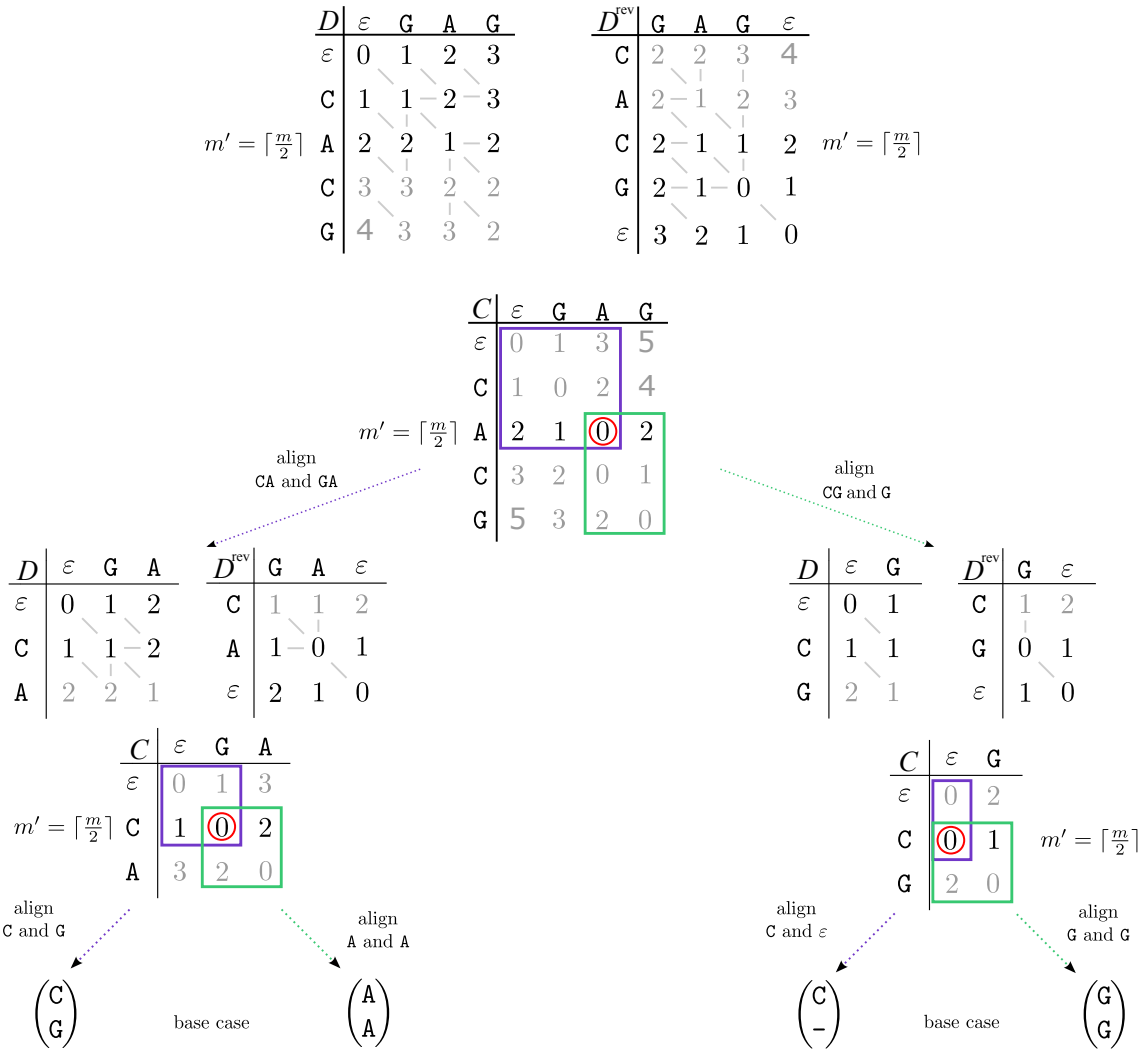


Figure 6.3: Complete example of linear-space alignment.

Suboptimal alignments. In practice, we are often interested in several, say K good alignments instead of a single optimal one. Can this also be done in linear space? Essentially, we need to remember the K highest entries in the edit matrix, and once we have found an optimal alignment we store a list or hash table to remember its path and subsequently take care that the diagonal edges of this path cannot be used anymore when we re-compute the next (now optimal) alignment. This requires additional space proportional to the total lengths of all discovered alignments, which is linear for a constant number of alignments.

A more satisfying way to define and compute suboptimal local alignments is presented in the next chapter.

7 Suboptimal Local Alignments

Often it is desirable not only to find one or all *optimal* alignments of two sequences, but also to find **suboptimal alignment**s, i.e., alignments that score (slightly) below the optimal alignment score. This is especially relevant in a local alignment context where several similar regions might exist and be of biological interest, while the Smith-Waterman algorithm reports only a single (optimal) one.

A simple way to find suboptimal local alignments could be to report first the alignment(s) (obtained by backtracing) corresponding to the highest score in the local alignment graph, then the alignment(s) corresponding to the second highest score, and so on. This approach has two disadvantages, though:

1. Redundancy: Many of the local alignments reported by this procedure will be largely overlapping, differing only by the precise placement of single gaps, single mismatches or the exact end characters of the alignments. An illustration is given in Figure 7.1.

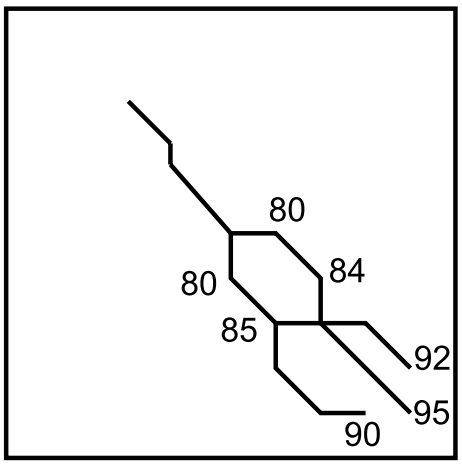


Figure 7.1: Illustration of the redundancy effect. Numbers indicate the score at positions next to them. Altogether there are five (sub-) optimal alignments, which share large parts of their structures.

2. Shadowing effect: Some high scoring alignments will be invisible in this procedure if an even higher scoring alignment is very close by, for example if the two alignments cross each other in the edit graph, where always the higher scoring prefix will be chosen. An illustration is given in Figure 7.2.

In order to circumvent these disadvantages, Waterman and Eggert (1987) gave a more sensible definition of suboptimal local alignments.

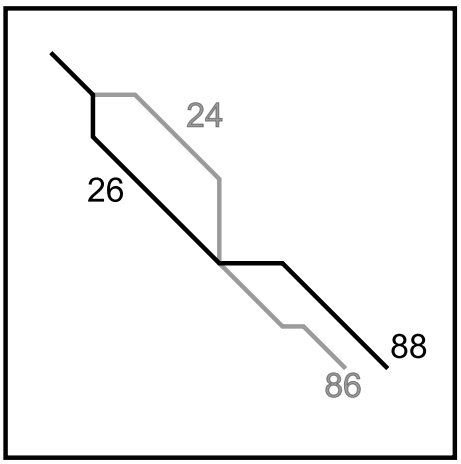


Figure 7.2: Illustration of the shadowing effect. Numbers indicate the score at positions next to them. The black prefix overshadows the grey, i.e., alignments with a black prefix will always be preferred, although alignments with a grey prefix are still very good.

Definition 7.1 An alignment is a **non-overlapping suboptimal local alignment** if it has no match or mismatch in common with any higher-scoring (sub)optimal local alignment. (Two alignments A and A' have a match or mismatch in common (they overlap) if there is at least one pair of positions (i, j) such that $x[i]$ is aligned with $y[j]$ both in A and in A'.)

Algorithmically, non-overlapping alignments can be computed as follows: First the Smith-Waterman algorithm is run as usual, returning an optimal local alignment. Then, the matrix S is computed again, but any match or mismatch from the reported optimal alignment is forbidden, i.e., in the alignment matrix the corresponding diagonal steps are ignored in the maximization. In terms of computing the alignment matrix, in the maximizations for these cells only two cases, namely insertion and deletion, are allowed.

This could be done by re-computing the whole matrix, but it is easy to see that it suffices to compute only that part of the matrix from the starting point of the first alignment to the bottom-right of the matrix. Moreover, whenever in a particular row or column the entries in the re-computation do not change compared to the entries in the original matrix, the computation along this row or column can be stopped. Waterman and Eggert suggest to alternate the computation of one row and one column at a time, each one until the new value coincides with the old value.

This procedure can be repeated for the third-best non-overlapping local alignment, and so on.

If we assume the usual type of similarity function with expected negative score of a random symbol pair so that large parts of the alignment matrix are filled with zeros, then the part of the matrix that needs to be re-computed is not much larger than the alignment itself.

Then, to compute the K best non-overlapping suboptimal local alignments, the algorithm takes an expected time of $O(mn + \sum_{k=1}^{K} L_k^2)$, where L_k is the length of the k-th reported alignment. In the worst case this is $O(mnK)$, but typically much less in practice.

Huang and Miller (1991) showed that the Waterman-Eggert algorithm can also be implemented for affine gap costs and in linear space.

8 Exact Algorithms for Sum-of-Pairs Multiple Sequence Alignment

Although sum-of-pairs (SP) multiple sequence alignment is NP-complete, it is sometimes desirable to compute optimal solutions for a moderate number of sequences; either as a subprocedure of other methods, or for comparison and benchmarking of heuristic alignment algorithms.

Therefore here we will come back to the original model of sum-of-pairs multiple alignment and look at it a bit more closely. We first formally describe the multi-dimensional generalization of the pairwise dynamic programming algorithm. Then we show how to reduce the search space considerably according to an idea of Carrillo and Lipman.

In this section we give the formulation for distance minimization. Naturally, equivalent algorithms exist for score maximization.

8.1 The Exact Algorithm Revisited

The problem to be solved is the following. Given k sequences $s_1, s_2, \ldots, s_k$ of lengths $n_1, n_2, \ldots, n_k$, respectively, the goal is to find a multiple alignment A such that

$$\text{cost}_{\text{SP}}(A) := \sum_{1 \le p \le q \le k} \text{cost}_2(\pi_{p,q}(A))$$

is minimum among all multiple alignments of $s_1, s_2, \ldots, s_k$.

8.1.1 The Basic Algorithm

We have already seen that the SP multiple alignment problem can be solved by applying the Universal Alignment Algorithm from Chapter 5 of the "Sequence Analysis 1" lecture to a k-dimensional alignment matrix, one dimension for each of the input sequences:

For each cell v of the alignment matrix, compute in an appropriate order the dissimilarity value

$$D(v) = \min_{u \in \text{pred}(v)} \big\{ D(u) + \text{cost}(u \to v) \big\}. \tag{8.1}$$

More explicitly, this can be written as follows:

$$D(0,0,\ldots,0) = 0$$

$$D(\overbrace{i_1, i_2, \ldots, i_k}^{v}) = \min_{\substack{\Delta_1,\ldots,\Delta_k \in \{0,1\} \\ \Delta_1+\ldots+\Delta_k \neq 0}} \left\{ D(\overbrace{i_1-\Delta_1, i_2-\Delta_2, \ldots, i_k-\Delta_k}^{\text{predecessor } u}) + D_{\text{SP}} \overbrace{\begin{pmatrix} \Delta_1 s_1[i_1] \\ \Delta_2 s_2[i_2] \\ \ldots \\ \Delta_k s_k[i_k] \end{pmatrix}}^{\text{alignm. col.}} \right\}$$

where for a character $c \in \Sigma$ the notation Δc denotes $\Delta c = c$ if $\Delta = 1$ and $\Delta c = -$ if $\Delta = 0$.

Space and time complexity. The space complexity $O(n^k)$ is clearly given by the size of the multidimensional alignment matrix, where $n \geq n_1, n_2, \ldots, n_k$.

The time complexity $O(n^k \cdot 2^k \cdot k^2)$ is also easy to see in this notation: For each of the $O(n^k)$ cells, all $2^k - 1$ predecessors have to be evaluated, given by the 2^k possible settings of the vector $(\Delta_1, \ldots, \Delta_k)$ of binary variables Δ_i, without the all-zero vector where $\Delta_1 + \ldots + \Delta_k = 0$. In addition, the sum-of-pairs cost of the resulting alignment column has to be computed, which requires additional $O(k^2)$ time per predecessor.

8.1.2 Variations of the Basic Algorithm

Memory reduction. From the pairwise alignment, we remember that it is possible to reduce the space for optimal alignment computation from quadratic to linear using Hirschberg's divide-and-conquer approach (Section 6.2).

In principle, the same idea can also be followed for multiple alignment, but the effect is much less dramatic. The space reduction is by one dimension, from $O(n^k)$ to $O(n^{k-1})$. Wile for $k = 3$ this has quite some effect, the space savings become less and less impressive as k grows. For example, for $k = 12$ sequences the space consumption using this technique is still $O(n^{11})$.

Figure 8.1 sketches on the left side the first division step for the pairwise case, and on the right side the corresponding step in the multiple (here 3-dimensional) case.

Free end gaps. In the same spirit as in semi-global pairwise alignment, it is sometimes desired to penalize gaps at the beginning and at the end of a multiple alignment less than internal gaps (or not at all, known as *free end-gap alignment*), in order to avoid that shorter sequences are "spread" over longer ones, just to get a slightly higher score that is not counter-penalized by gap costs, because these have to be imposed anyway to account for the difference in sequence length.

This can be done in multiple alignment as in the pairwise case. It is just a different initialization of the base cases, i.e. the outer edges of the k-dimensional alignment matrix.

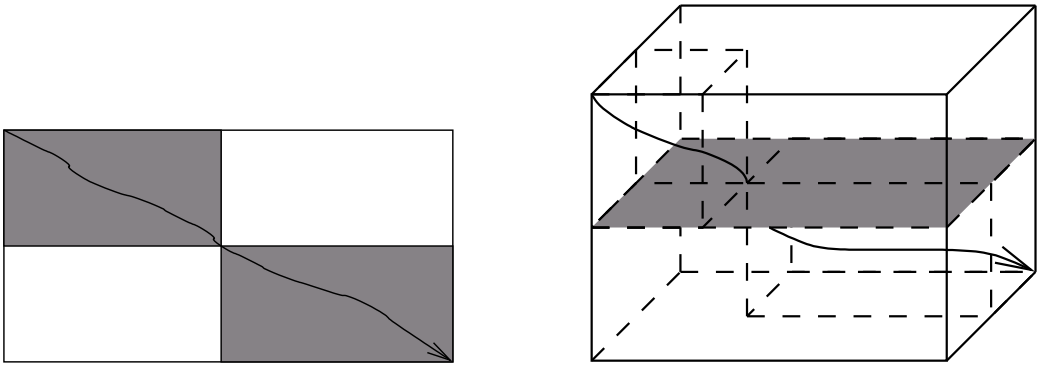


Figure 8.1: Divide-and-conquer space reduction technique for multiple alignment.

Affine gap costs. Affine gap costs can be defined in a straightforward manner as a simple generalization of the pairwise case, called **natural gap costs**: The cost of a multiple alignment is the sum of all costs of the pairwise projections, where the cost of a pairwise projection is computed with affine gap costs. In principle it is possible to perform the computation of such alignments similarly as it is done for pairwise alignments. However, the number of "history matrices" (V and H in the pairwise case) grows exponentially with the number of sequences, so that very much space is needed.

That is why Altschul (1989) suggested to use an approximation of the exact case, so-called **quasi-natural gap costs**. The idea is to look back by only one position in the edit graph and decide from the previous column in the alignment if a new gap is started in the column to be computed or not. The choices, compared to the "correct" choices in natural gap costs, are given in the following table whose first line depicts the different possible scenarios of pairwise alignment projections where an asterisk (`*`) refers to a character, a dash (`-`) refers to a blank, and a question mark (`?`) refers to either a character or a blank. The entries correspond to the cost (d is gap open cost, e is gap extension cost), where "no" means no new gap is opened (substitution cost):

	`?*` `?*`	`?-` `?-`	`**` `*-`	`**` `--`	`-*` `*-`	`-*` `--`
natural	no	0	d	e	d	d/e
quasi-natural	no	0	d	e	d	d

The last case in natural gap costs cannot be decided if only one previous column is given. It would be d for `*---*` `*----` and e for `***--*` `*-----` .

With this heuristic, the overall computation becomes slower by a factor of 2^k, yielding a time complexity for the complete multiple alignment computation of $O(n^k 2^{2k} k^2)$.

The space requirements do not change in the worst case, they remain $O(n^k)$.

8.2 Carrillo and Lipman's Search Space Reduction

Carrillo and Lipman (1988) suggested an effective way to prune the search space for multiple alignments. Their algorithm is still exponential in the worst case, but in many cases the time of alignment computation is considerably reduced compared to the full dynamic programming algorithm from Section 8.1, making it applicable in practice for data sets of moderate size (7–10 protein sequences of length 300–400).

Let sequences $s_1, s_2, \ldots, s_k$ be given. The algorithm of Carrillo and Lipman is a branch-and-bound running time heuristic, based on a simple observation.

Idea. We have seen that the pairwise projections of an optimal multiple alignment are not necessarily the optimal pairwise alignments (making the problem hard). However, intuitively, they can also not be particularly bad alignments: If all pairwise projections were bad, the whole multiple alignment would also have a bad sum-of-pairs score.

If we can quantify this idea, we can restrict the search space to those vertices that are part of close-to-optimal pairwise alignments for each pairwise projection.

Bounding pairwise Alignment costs. We assume that we already know some (sub-optimal) multiple alignment A^{heur} of the given sequences with cost $\text{cost}_{SP}(A^{\text{heur}}) \geq \text{cost}_{SP}(A^{\text{opt}})$, for example computed by a progressive alignment method or by the center-star heuristic (Section 9.3).

Remember the definition of the sum-of-pairs multiple alignment cost:

$$\text{cost}_{\text{SP}}(A) = \sum_{p<q} \text{cost}_2(\pi_{\{p,q\}}(A)).$$

We now pick a particular index pair (x, y), $x < y$, and remove it from the sum. In the remaining pairs, we use the fact that the pairwise projections of the optimal multiple alignment cannot have a lower cost than the optimal corresponding pairwise alignments: $\text{cost}_2(\pi_{\{p,q\}}(A^{\text{opt}})) \geq d(s_p, s_q)$. Thus

$$\begin{aligned}
\text{cost}_{\text{SP}}(A^{\text{heur}}) &\geq \text{cost}_{SP}(A^{\text{opt}}) \\
&= \sum_{p<q} \text{cost}_2(\pi_{\{p,q\}}(A^{\text{opt}})) \\
&= \text{cost}_2(\pi_{\{x,y\}}(A^{\text{opt}})) + \sum_{\substack{p<q \\ (p,q)\neq(x,y)}} \text{cost}_2(\pi_{\{p,q\}}(A^{\text{opt}})) \\
&\geq \text{cost}_2(\pi_{\{x,y\}}(A^{\text{opt}})) + \sum_{\substack{p<q \\ (p,q)\neq(x,y)}} d(s_p, s_q)
\end{aligned}$$

for any pair (x, y), $1 \leq x < y \leq k$.

This implies the following upper bound for the cost of the projection of an optimal multiple alignment on rows x and y:

$$\text{cost}_2(\pi_{\{x,y\}}(A^{\text{opt}})) \;\leq\; \text{cost}_{\text{SP}}(A^{\text{heur}}) - \sum_{\substack{p<q \\ (p,q)\neq(x,y)}} d(s_p, s_q) \;=:\; U_{(x,y)}.$$

Note that in order to compute the upper bound $U_{(x,y)}$, only pairwise optimal alignments and a heuristic multiple alignment need to be calculated. This can be done efficiently.

Pruning the alignment matrix. We now keep only those cells $(i_1, \ldots, i_k)$ of the alignment matrix that satisfy the following condition for *all* pairs (x, y), $1 \leq x < y \leq k$:

> The best pairwise alignment of s_x and s_y through cell (i_x, i_y) has cost $\leq U_{(x,y)}$.

How do we know the cost of the best pairwise alignment through any given cell (i_x, i_y)? This question was answered in Section 6.1: We use the forward-backward techniqe. In other words, for every pair (x, y), $x < y$, we compute the total cost matrix $T_{(x,y)}$ such that $T_{(x,y)}(i_x, i_y)$ contains the cost of the best alignment of s_x and s_y that passes through the cell (i_x, i_y).

We can define the set of cells in the multi-dimensional alignment matrix that satisfy the constraint imposed by the pair (x, y) as follows:

$$B_{(x,y)} := \{(i_1, \ldots, i_k) : T_{(x,y)}(i_x, i_y) \leq U_{(x,y)}\}$$

From the above considerations it is clear that the projection of an SP-optimal multiple alignment on sequences s_x and s_y can not have a cost higher than $U_{(x,y)}$, implying that regions (i, j) with values $T_{(x,y)}(i, j) > U_{(x,y)}$ cannot contain such a projection.

Thus an optimal multiple alignment passes only through cells that are in $B_{(x,y)}$ for *all pairs* (x, y), i.e., in

$$B := \bigcap_{x<y} B_{(x,y)}.$$

This means: Instead of computing the distances in all $O(n^k)$ cells in the alignment matrix, we need to compute the distances of only $|B|$ cells. The whole procedure is conceptually illustrated in Figure 8.2.

The size of B depends on many things, e.g. on

- the quality of the heuristic alignment with cost $\text{cost}_{\text{SP}}(A^{\text{heur}})$ that serves as an upper bound of the optimal alignment cost. The closer $\text{cost}_{\text{SP}}(A^{\text{heur}})$ is to the true optimal value, the smaller is B;
- the difference between the optimal pairwise alignment costs and the costs of the pairwise projections of the optimal multiple alignment.

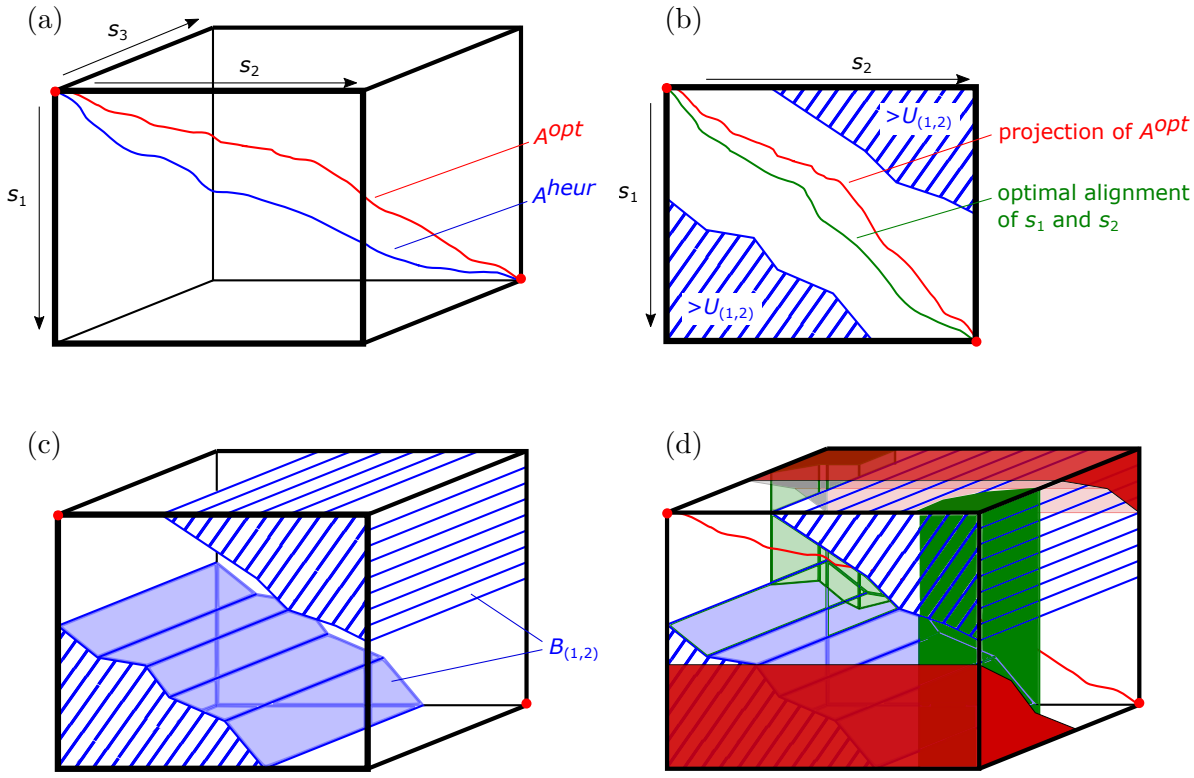


Figure 8.2: Illustration of the Carrillo-Lipman heuristic for three sequences s_1, s_2 and s_3. (a) In order to compute the (initially unknown) optimal multiple alignment A^{opt}, first a suboptimal multiple alignment A^{heur} is required. (b) For each pair of sequences, here (s_1, s_2), from the cost of A^{heur} and of the optimal pairwise alignments, forbidden regions ($> U_{(1,2)}$) are identified that can not contain the projection of A^{opt}. (c) The forbidden regions are then back-projected into the multidimensional space, yielding $B_{(1,2)}$. (d) When this is done for all pairs (s_1, s_2) (blue), (s_1, s_3) (red) and (s_2, s_3) (green), ideally only a small region remains that needs to be fully searched for the optimal alignment A^{opt}.

Generally, if all k sequences are similar and the gap costs are small, then a good heuristic alignment (maybe even an optimal one) is easy to find, so B may contain almost no additional vertices besides the true optimal multiple alignment path. Thus, for a medium number of similar sequences, the simple Carrillo-Lipman heuristic may already perform very well (because the relevant regions become very small) while for even fewer but less closely related sequences even the best known heuristics will not finish within weeks of computation. Sequence similarity plays a much higher role than sequence length or the number of sequences.

Some implementations of the Carrillo-Lipman bound attempt to reduce the size of B further by "cheating": Remember that we need a heuristic alignment to obtain an upper bound $\text{cost}_{SP}(A^{\text{heur}}) =: \delta$ on the optimal cost. If we have reason to suspect that we have found a bad alignment, and believe that a better one exists, we may replace δ by a smaller value, which could even be chosen differently for each pair (x, y): $\delta - \epsilon_{(x,y)}$. Of course, since we cannot be sure that such a better alignment exists, we may reduce the size of B

too much and in fact exclude the optimal multiple alignment from B. In practice, however, this kind of cheating works quite well if done carefully, and further reduces the running time.

Algorithm. We give a few remarks about implementing the above idea. We emphasize that the set of relevant matrix cells B is conceptual and does not need to be constructed explicitly. Instead, it is sufficient to precompute all $T_{(x,y)}$ matrices, the optimal pairwise alignment scores $d(s_p, s_q)$ for all (p, q), $1 \leq p < q \leq k$, and a heuristic alignment A^{heur} to obtain δ.

It is advantageous not to implement the "pull-type" recurrence (8.1), but instead to use a "push-type" algorithm as explained in the following.

Imagine that every cell has a value of ∞ at the beginning of the algorithm, although this information is not stored explicitly. Instead, a list (e.g. a queue or priority queue) of "active" cells is maintained, that initially contains only $(0, 0, \ldots, 0)$.

The algorithm then consists of repeatedly "visiting" a cell from the queue until the final cell $(n_1, n_2, \ldots, n_k)$ is reached. When a cell is visited, it already contains the correct alignment distance.

Visiting a cell v consists of the following steps.

1. We look at the $2^k - 1$ successors w of v in the alignment matrix and check for each of them if it belongs to the set B. This is the case if w is already in the queue, or if the current distance value plus edge cost $v \to w$ satisfies the Carrillo-Lipman bound for every index pair (x, y). In the former case, the distance (and backpointer) information of w is updated if the new distance value coming from v is lower than the current one. In the latter case w is "activated" with the appropriate distance and backpointer information and added to the queue.
2. We remove v from the queue. If we want to create an alignment in the end, we need to store v's information somewhere else to reconstruct the traceback information. If we only want the distance value, v can now be completely discarded.
3. We pick the next cell x to visit. The algorithm works correctly if x is
   - a vertex of minimum distance; then the algorithm is essentially **Dijkstra's algorithm** for single-source shortest paths in the (reduced) alignment graph, or
   - any vertex that has no active predecessors, so we can be sure that we do not need to update x's distance information after visiting x.

   Since often more than one vertex is available for visiting, a good target-driven choice may speed up the algorithm.

Implementations. Implementations of the Carrillo-Lipman heuristic are the programs `MSA` (Gupta et al., 1995) and `QALIGN` (Sammeth et al., 2003b).

9 An Approximation Algorithm for Sum-of-Pairs Multiple Sequence Alignment

9.1 Digression: NP-completeness

It was stated before that sum-of-pairs multiple alignment is NP-complete with respect to the number of sequences k, without further explaining what that means. This section shall now give a brief introduction to the field of NP-completeness, about which every good bioinformatician should know. The contents in this section are based on the book of Cormen et al. (2001).

Speaking in an abstract way, a **problem** Q is a binary relation on a set I of **problem instances** and a set S of **problem solutions** (see Figure 9.1). A problem instance can point to no, one, or several problem solutions, and a problem solution can be one of no, one, or several problem instances.

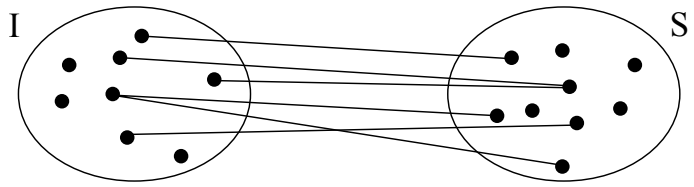


Figure 9.1: Binary relations between problem instances I and problem solutions S.

Example 9.1 A simple example of a problem is integer addition. Then the problem instances could be "$5 + 3$", "$4 + 4$" or "$1 + 4$" pointing to the problem solutions "8", "8" and "5", respectively. ◀

An **optimization problem (OP)** or **search problem** addresses the minimization of a cost or maximization of a score function. For example, finding the minimum-cost alignment of two or more sequences is an optimization problem.

A **decision problem (DP)** is a "yes/no question" with just two problem solutions, $S = \{\mathtt{T}, \mathtt{F}\}$ (*true*/*false*). The question whether for two given sequences there exists an alignment with a cost lower than a certain threshold t is a decision problem.

The discussion about NP-completeness mainly considers decision problems, but the results influence also optimization problems: Searching for a bound, every optimization problem can be stated as a series of decision problems. If a decision problem is difficult, the corresponding optimization problem is usually difficult as well.

Complexity classes. The set of all decision problems for which algorithms exist that have the same asymptotic behavior (e.g. running time) are contained in one complexity class. The class **P** contains all problems for which a deterministic algorithm exists that can *solve* in polynomial time a certain problem instance, i.e., it can find, for a given problem instance, in polynomial time whether there exists a configuration of input variables such that the problem has solution `T` or not. The class **NP** contains all problems for which a deterministic algorithm exists that can *verify* in polynomial time whether a given certificate (configuration of input variables) is able to solve an actual problem instance, i.e., it can tell whether the certificate indeed yields solution `T` or not. In short **P = efficiently solvable** and **NP = efficiently verifiable**. It follows directly that $\mathrm{P} \subseteq \mathrm{NP}$. The question is still open whether $\mathrm{P} \stackrel{?}{=} \mathrm{NP}$.

Remark. The given definition of NP above is not the historically original one. The original definition describes NP as the class that contains all problems for which a nondeterministic algorithm exists that can solve in polynomial time a certain problem instance. However, as this definition is less intuitive, the verification-based definition is usually preferred.

Reducibility. A problem Q is **reducible** to a problem Q' if every instance of Q can be formulated as an instance of Q' so that the solution S of problem Q follows from the solution S' of problem Q'. If Q can be reduced to Q' in a simple way, it is not harder to solve Q than to solve Q'. Q is **polynomial-time reducible** to Q' (shortly: $Q \leq_p Q'$) if the reduction takes only polynomial time. In particular, $Q \leq_p Q'$ implies $Q' \in P \Rightarrow Q \in P$.

The idea of reducibility is illustrated in Figure 9.2.

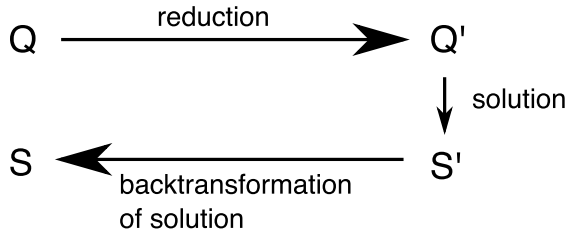


Figure 9.2: Illustration of the concept of reducing a problem Q to another problem Q' and backtransforming the solution S' of Q' to a solution S of Q.

Example 9.2 As an example, consider the **Consecutive Ones Problem** (C1P) that asks whether the columns of an $n \times m$ binary matrix M can be permuted, such that the resulting matrix M' has at most one run of ones in each row. In order to solve C1P, one can resort to a well-known problem in graph theory, the **Traveling Salesperson Problem** (TSP), that asks for a shortest Hamiltonian cycle in a weighted graph.

The reduction C1P $\leq_p$ TSP works as follows (see Figure 9.3): Given the binary matrix M, assume without loss of generality that it has one column of zeros only, and no two columns of M are identical. Now, define the weighted complete graph $G(M)$ that has one vertex per column of M, where the weight of an edge (v, w) is defined as the Hamming distance

between the two columns represented by the two vertices v and w. Then it is easy to see that the C1P is fulfilled for M if and only if $G(M)$ contains a TSP tour of length $2n$, and otherwise not. Moreover, the construction of $G(M)$ can be done in polynomial time and a solution of the TSP can easily be backtransformed into a solution of the C1P. Therefore, given an algorithm that solves TSP efficiently, we would also have an algorithm solving C1P efficiently. ◀

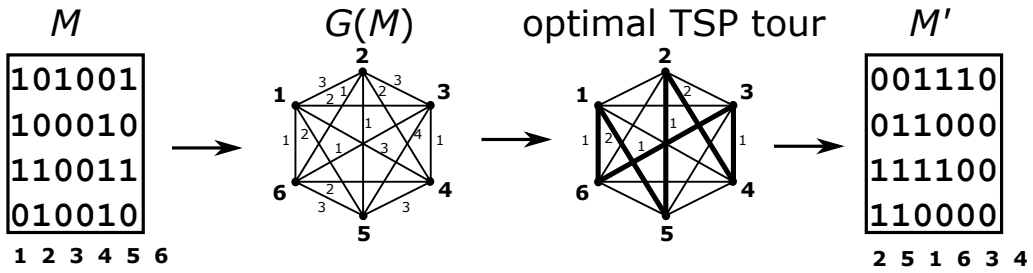


Figure 9.3: Solving the Consecutive Ones Problem for binary matrix M with $n = 4$ rows via the Traveling Salesperson Problem. The optimal TSP tour has weight $8 = 2n$, therefore M fulfills the Consecutive Ones Property, as shown in matrix M'.

NP-hardness, NP-completeness. A problem Q' is **NP-hard** if $Q \leq_p Q'$ for every $Q \in$ NP. A problem Q' is in the class **NP-complete (NPC)** if it is NP-hard and $Q' \in$ NP. The following property holds for the class NPC: If some NP-complete problem is solvable in polynomial time, then P = NP. If some problem in NP is *not* solvable in polynomial time, then no single NP-complete problem is solvable in polynomial time.

For solving the question whether P $\stackrel{?}{=}$ NP, NP-complete problems are intensely studied. The most common assumption is that P $\neq$ NP. An alternative possibility is that P = NP (see Figure 9.4 for schematic views of these two alternatives).

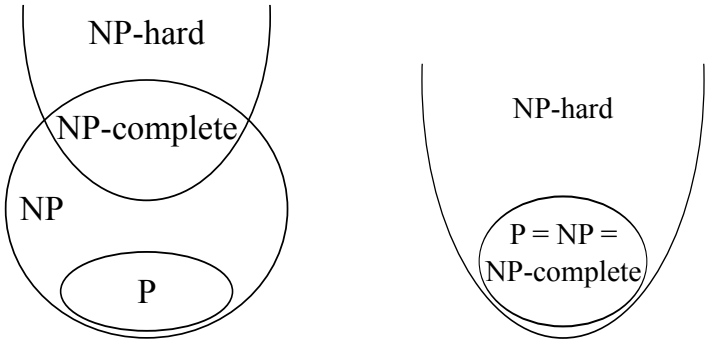


Figure 9.4: Left: P $\neq$ NP. Right: P = NP.

The satisfiability problem SAT. It is not intuitively clear that any NP-complete problems exist because the conditions are quite strong and the implications enormous. Nevertheless, they do exist, and here we summarize a bit of that story.

The first known example of an NP-complete problem was the **satisfiability problem (SAT)**. It is stated as follows: Given a boolean formula of length n, is it satisfiable, i.e., is there a configuration of variables so that the evaluation of the formula yields `T`?

Example 9.3 Given the formula[1] $(a \vee b) \wedge (\neg a \vee b) \wedge (\neg b \vee c)$, is there a configuration of variables so that the formula is true? Indeed, there are two certificates that satisfy the given formula, first: $a = b = c = \texttt{T}$ and second: $a = \texttt{F}$ and $b = c = \texttt{T}$. ◀

To show that SAT is NP-complete, we prove two properties:

1. We show that SAT belongs to the class NP. This is the case, because given a certificate (assignment of truth values to the variables in a boolean formula), it is easy to decide in polynomial time whether the evaluation of the formula with these variable values indeed yields `T` or not.
2. We show that any problem Q belonging to the class NP can be reduced to SAT ($Q \leq_p$ SAT) in polynomial time. This can be seen as follows:
   - $Q \in$ NP implies that a verification algorithm $A(x, y)$ exists for Q that tells in polynomial time for any problem instance $x \in I$ and certificate $y \in S$ whether y is a valid solution of x (i.e., $A(x, y) = \texttt{T}$), or not (i.e., $A(x, y) = \texttt{F}$).
   - Let $f(A, x)$ be a reduction function that partially evaluates the verification algorithm A according to its first argument, yielding the function $C_x(y) = f(A(x, y), x)$, which only depends on $y \in S$. Then we have:
     - Encoded as a boolean function, C_x is satisfiable ($C_x(y) = \texttt{T}$) if and only if y is a solution to x, otherwise $C_x(y) = \texttt{F}$.
     - It can be shown that for any $Q \in$ NP the corresponding boolean function $C_x(y)$ can be constructed in polynomial time (via CIRCUIT-SAT, see Cormen et al. (2001, Chapter 36.6)), therefore if SAT can be solved in polynomial time, also Q can be solved in polynomial time, i.e. $Q \leq_p$ SAT.

Therefore SAT is an NP-complete problem.

Extension of the class NPC. For showing that some problem Q is in NPC, it is sufficient to show that $Q \in$ NP and to reduce a known NP-complete problem Q' (e.g. $Q' =$ SAT) to Q:

1. Show that $Q \in$ NP.
2. Choose a known NP-complete problem Q'.
3. Describe an algorithm that calculates in polynomial time a function f that projects any instance of Q' to an instance of Q.
4. Show that for f holds: $x \in Q'$ if and only if $f(x) \in Q$ for all $x \in \{\texttt{F}, \texttt{T}\}^*$.

[1] The symbols in the formula denote the following: $\vee$ = OR, $\wedge$ = AND, $\neg$ = NOT.

Consequences of NP completeness. While the proof of NP-completeness of a problem implies that it is quite unlikely to find a deterministic polynomial-time algorithm to solve the problem to optimality in general, there are various ways to respond to such a result if one wants to solve the problem in feasible time:

1. Small problem instances might be solvable anyway. See Section 8.1.
2. Using adequate techniques to efficiently prune the search space, even larger instances may be solvable in reasonable time (**running time heuristics**). See Section 8.2.
3. The problem may not be hard for certain subclasses, so polynomial-time algorithms may exist for them, called FPT (**Fixed Parameter Tractability**) algorithms.
4. In an optimization problem, it may be sufficient to approximate the optimal solution to a certain degree. If closeness to the optimal solution can be guaranteed, one speaks of an **approximation algorithm**. See Section 9.2.
5. By no longer confining oneself to any optimality guarantee, one may get very fast heuristic algorithms that produce good but not always optimal or guaranteed close-to-optimal solutions (**correctness heuristics**). See Sections 10.1 and 10.2.

9.2 Approximation Algorithms

As mentioned in Item 4 above, in some situations it may be sufficient to approximate the optimal solution of a problem to a certain degree. The following definition states more precisely, what that means.

Definition 9.1 For $c \geq 1$, an algorithm for a cost *minimization problem* is called a ***c*-approximation** if it is guaranteed that its solution has cost at most c times the optimal solution. For a *maximization problem*, an algorithm is called a ***c*-approximation** if it is guaranteed that its solution has score at least $1/c$ times the optimal solution.

9.3 The Center-Star Approximation

There exists a series of results on the approximability of the sum-of-pairs multiple sequence alignment problem.

A simple algorithm, the so-called **center star method** (Gusfield, 1991, 1993), is a 2-approximation for the sum-of-pairs multiple alignment problem if the underlying weighted edit distance satisfies the triangle inequality.

The algorithm is the following. First, for each sequence s_p, $1 \leq p \leq k$, its overall distance d_p to the other sequences is computed, i.e. the sum of the pairwise optimal alignment costs:

$$d_p = \sum_{1 \leq q \leq k} d(s_p, s_q).$$

Let c, $1 \leq c \leq k$, be an index such that d_c minimizes this overall distance. The sequence s_c is then called **center sequence**.

Secondly, a multiple alignment A_c is constructed from all pairwise optimal alignments $A_{\{c,q\}}$ where the center sequence is involved, i.e., all the optimal alignments of s_c and the other s_p, $p \neq c$, are combined into one multiple alignment A_c, such that $\pi_{\{c,q\}}(A_c) = A_{\{c,q\}}$.

The claim is that this alignment is a 2-approximation for the optimal sum-of-pairs multiple alignment cost of the sequences $s_1, s_2, \ldots, s_k$, i.e.,

$$\text{cost}_{\text{SP}}(A_c) \leq 2 \cdot \text{cost}_{\text{SP}}(A^{\text{opt}}).$$

This can be seen as follows:

$$\begin{aligned}
\text{cost}_{\text{SP}}(A_c) &= \sum_{1 \leq p < q \leq k} \text{cost}_2(\pi_{\{p,q\}}(A_c)) \text{ //definition} \\
&= \frac{1}{2} \sum_{1 \leq p \leq k, 1 \leq q \leq k} \text{cost}_2(\pi_{\{p,q\}}(A_c)) \text{ //since } \text{cost}_2(\pi_{\{p,p\}}) = 0 \\
&\leq \frac{1}{2} \sum_{1 \leq p \leq k, 1 \leq q \leq k} \left(\text{cost}_2(\pi_{\{p,c\}}(A_c)) + \text{cost}_2(\pi_{\{c,q\}}(A_c))\right) \text{ //triangle inequality} \\
&= \frac{1}{2} \sum_{1 \leq p \leq k, 1 \leq q \leq k} (d(s_p, s_c) + d(s_c, s_q)) \text{ //alignments to } s_c \text{ are optimal} \\
&= k \cdot \sum_{1 \leq q \leq k} d(s_c, s_q) \text{ //star property} \\
&\leq \sum_{1 \leq p \leq k, 1 \leq q \leq k} d(s_p, s_q) \text{ //minimal choice of } s_c \\
&\leq \sum_{1 \leq p \leq k, 1 \leq q \leq k} \text{cost}_2(\pi_{\{p,q\}}(A^{\text{opt}})) \\
&= 2 \cdot \sum_{1 \leq p < q \leq k} \text{cost}_2(\pi_{\{p,q\}}(A^{\text{opt}})) \\
&= 2 \cdot \text{cost}_{\text{SP}}(A^{\text{opt}})
\end{aligned}$$

Hence A_c is a 2-approximation of the optimal alignment A^{opt}.

Time and space complexity. The running time of the algorithm can be estimated as follows, assuming all k sequences have the same length n:

In the first step, $\binom{k}{2}$ pairwise alignments are computed, requiring overall $O(k^2n^2)$ time. In the second step, the $k-1$ alignments are combined into one multiple alignment which can be done in time proportional to the size of the constructed alignment, i.e. $O(k^2n)$. This yields an overall running time of $O(k^2n^2)$.

The space complexity is $O(n+k)$ for the linear-space distance computation and to store k computed values d_p. Additionally, $O(k^2n)$ space is used to store k pairwise alignments and the intermediate/final multiple alignment. Hence, the overall space complexity is $O(k^2n)$.

This idea can be generalized to a $2 - \frac{\kappa}{k}$-approximation if instead of pairwise alignments, optimal multiple alignments of κ sequences are computed, see (Bafna et al., 1997).

10 Heuristics for Multiple Sequence Alignment

10.1 Divide-and-Conquer Alignment

In this section we present a heuristic for sum-of-pairs multiple sequence alignment that no longer gives any guarantee about the resulting alignment cost, although in practice it is often very good. Therefore, the method is a correctness heuristic. While in the worst case the running time is still exponential in the number of sequences, the advantages are a polynomial space usage and that on average the practical running time is much faster than the exhaustive search, while the result is very often still optimal or close to optimal.

Idea. The basic idea of the heuristic is to cut all sequences at suitable cut points into left and right parts, and then to solve the two such generated alignment problems for the left parts and the right parts separately. This is done either by recursion or, if the sequences are short enough, by an exact algorithm. For a graphical sketch of the procedure, see Figure 10.1.

Finding cut positions. The main question about this procedure is how to choose good cut positions, since this choice is obviously critical for the quality of the final alignment. The ideal case is easy to formulate:

Definition 10.1 A family of cut positions $(c_1, c_2, \ldots, c_k)$ of the sequences $s_1, s_2, \ldots, s_k$ of lengths $n_1, n_2, \ldots, n_k$, respectively, is called an **optimal cut** if and only if there exists an alignment A of the prefixes $(s_1[1 \ldots c_1], s_2[1 \ldots c_2], \ldots, s_k[1 \ldots c_k])$ and an alignment B of the suffixes $(s_1[c_1 + 1 \ldots n_1], s_2[c_2 + 1 \ldots n_2], \ldots, s_k[c_k + 1 \ldots n_k])$ such that the concatenation $A +\!\!+ B$ is an optimal alignment of $s_1, s_2, \ldots, s_k$.

There are many optimal cut positions, e.g. the trivial ones $(0, 0, \ldots, 0)$ and $(n_1, n_2, \ldots, n_k)$. In fact:

Lemma 10.1 For any cut position $\hat{c}_1 \in \{0, \ldots, n_1\}$ of the first sequence s_1, there exist cut positions $c_2, \ldots, c_k$ of the remaining sequences $s_2, \ldots, s_k$ such that $(\hat{c}_1, c_2, \ldots, c_k)$ is an optimal cut of the sequences $s_1, s_2, \ldots, s_k$.

Proof. Assume that A^{opt} is an optimal alignment. Choose one of the points between the characters in the first row of A^{opt} that correspond to the characters $s_1[\hat{c}_1]$ and $s_1[\hat{c}_1 + 1]$. The vertical "cut" at this point through the optimal alignment defines the cut positions

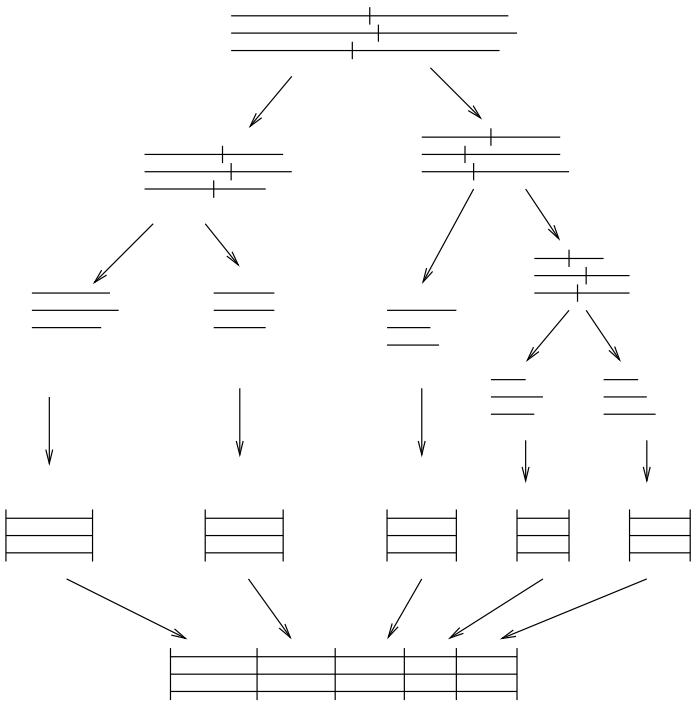


Figure 10.1: Overview of divide-and-conquer multiple sequence alignment.

$c_2, \ldots, c_k$ that, together with $\hat{c}_1$, are optimal by definition. See Figure 10.2 for an illustration. □

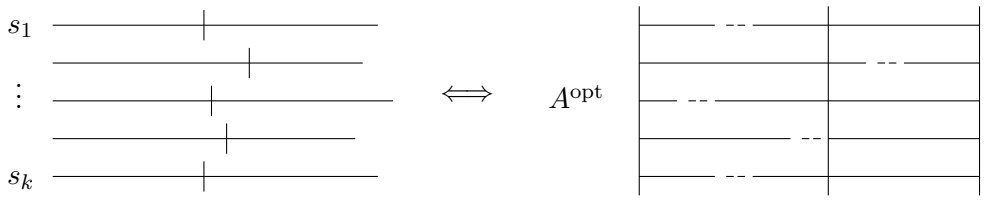


Figure 10.2: Connection between unaligned sequences $s_1, \ldots, s_k$ (left) and a multiple alignment A of them (right). If A is an optimal alignment A^{opt}, any vertical cut in the alignment corresponds to an optimal cut of the sequences.

The lemma tells us that a first cut position $\hat{c}_1$ of s_1 can be chosen arbitrarily (for symmetry reasons and to arrive at an efficient divide-and-conquer procedure, we will use $\hat{c}_1 = \lceil n_1/2 \rceil$), and then there always exist cut positions of the other sequences that produce an optimal cut.

The NP-completeness of the SP-alignment problem implies, however, that it is unlikely that there exists a polynomial time algorithm for finding optimal cuts of this type. Hence a heuristic is used to find good, though not always optimal cut positions.

This heuristic is based on pairwise sequence comparisons whose results are stored in an *additional cost matrix* that contains for each pair of cut positions (c_p, c_q) the penalty

(additional cost) imposed by cutting the two sequences at these points, instead of cutting them at points compatible with an optimal pairwise alignment (see Definition 6.2). The efficient computation of additional cost matrices was discussed in Section 6.1.

The idea of using additional cost matrices to quantify the quality of a family of cut positions is that the closer a potential cut point is to an optimal pairwise alignment path, the smaller will be the contribution of this pair to the overall sum-of-pairs multiple alignment cost. Since we are dealing with sum-of-pairs alignment, we define the additional cost of a family of cuts along the same rationale.

Definition 10.2 The **multiple additional cost** of a cut $(c_1, c_2, \ldots, c_k)$ is defined as

$$C(c_1, c_2, .., c_k) := \sum_{1 \le p < q \le k} C_{(p,q)}(c_p, c_q),$$

where $C_{(p,q)}$ is the additional cost matrix for sequence pair (s_p, s_q), $p < q$.

Although maybe unintuitive, even a family of cut positions with zero multiple additional cost, i.e. one whose implied pairwise cuts are all compatible with the corresponding pairwise optimal alignments, is not necessarily optimal (see Example 10.1). The converse is also not true: Neither has an optimal cut always multiple additional cost zero, nor is a cut with smallest possible additional cost necessarily optimal.

Example 10.1 Let $s_1 =$ `CT`, $s_2 =$ `AGT`, and $s_3 =$ `G`. Using unit cost edit distance, the three additional cost matrices are the following:

$C_{(1,2)}$:

	ϵ	A	G	T
ϵ	0	0	1	3
C	1	0	0	2
T	3	2	1	0

$C_{(1,3)}$:

	ϵ	G
ϵ	0	1
C	0	0
T	1	0

$C_{(2,3)}$:

	ϵ	G
ϵ	0	2
A	0	1
G	1	0
T	2	0

There are two cuts $(1, 1, 0)$ and $(1, 2, 1)$, both of which yield a multiple additional cost $C(1, 1, 0) = C(1, 2, 1) = 0$. Nevertheless, the implied multiple alignments are not necessarily optimal as can be seen for the cut $(1, 1, 0)$:

$$\begin{array}{c|c} \texttt{C} & \texttt{T} \\ \texttt{A} & \texttt{GT} \\ \varepsilon & \texttt{G} \end{array} \quad \rightarrow \quad \begin{pmatrix} \texttt{C} \\ \texttt{A} \\ \texttt{-} \end{pmatrix} +\!\!+ \begin{pmatrix} \texttt{-} & \texttt{T} \\ \texttt{G} & \texttt{T} \\ \texttt{G} & \texttt{-} \end{pmatrix} = \begin{pmatrix} \texttt{C} & \texttt{-} & \texttt{T} \\ \texttt{A} & \texttt{G} & \texttt{T} \\ \texttt{-} & \texttt{G} & \texttt{-} \end{pmatrix} \quad \text{(SP-cost 7, not optimal).}$$

But they might be optimal, as for the cut $(1, 2, 1)$:

$$\begin{array}{c|c} \texttt{C} & \texttt{T} \\ \texttt{AG} & \texttt{T} \\ \texttt{G} & \varepsilon \end{array} \quad \rightarrow \quad \begin{pmatrix} \texttt{-} & \texttt{C} \\ \texttt{A} & \texttt{G} \\ \texttt{-} & \texttt{G} \end{pmatrix} +\!\!+ \begin{pmatrix} \texttt{T} \\ \texttt{T} \\ \texttt{-} \end{pmatrix} = \begin{pmatrix} \texttt{-} & \texttt{C} & \texttt{T} \\ \texttt{A} & \texttt{G} & \texttt{T} \\ \texttt{-} & \texttt{G} & \texttt{-} \end{pmatrix} \quad \text{(SP-cost 6, optimal).}$$

◀

Nevertheless, it is a good heuristic to choose cuts minimizing the multiple additional cost.

DCA Algorithm. The divide-and-conquer alignment algorithm first chooses an arbitrary cut position $\hat{c}_1$ for the first sequence (usually the sequence is cut in half for symmetry reasons) and then searches for the remaining cut positions $c_2, \ldots, c_k$ such that the cut $(\hat{c}_1, c_2, \ldots, c_k)$ minimizes $C(\hat{c}_1, c_2, \ldots, c_k)$.

Then the sequences are cut in this way, and the procedure is repeated recursively until the maximal sequence length drops below a threshold value L, when an exact multiple sequence alignment algorithm MSA (e.g. using Carrillo-Lipman bounds) is applied.

The pseudocode for this procedure is given in Algorithm 4 where $+\!\!+$ is the operator concatenating two alignments.

Algorithm 4: DCA$(s_1, s_2, \ldots, s_k; L)$

let $n_p \leftarrow |s_p|$ for all p, $1 \leq p \leq k$
if $\max\{n_1, n_2, \ldots, n_k\} \leq L$ **then**
    return MSA$(s_1, s_2, \ldots, s_k)$
else
    $\hat{c}_1 \leftarrow \lceil n_1/2 \rceil$
    compute $(c_2, \ldots, c_k)$ such that $C(\hat{c}_1, c_2, \ldots, c_k)$ is minimum
    return DCA$(s_1[1 \ldots \hat{c}_1], s_2[1 \ldots c_2], \ldots, s_k[1 \ldots c_k]; L)$
        $+\!\!+$ DCA$(s_1[\hat{c}_1 + 1 \ldots n_1], s_2[c_2 + 1 \ldots n_2], \ldots, s_k[c_k + 1 \ldots n_k]; L)$
end

The search space in the computation of cut positions is sketched in Figure 10.3. Since the cut position of the first sequence s_1 is fixed to $\hat{c}_1$ but the others can vary over their whole sequence length, the search space has size $O(n_2 \cdot n_3 \cdots n_k)$. This implies that the time for computing a cut (i.e., finding the minimum in this search space) by exhaustive search takes $O(k^2 n^2 + n^{k-1})$ time and uses $O(k^2 n^2)$ space.

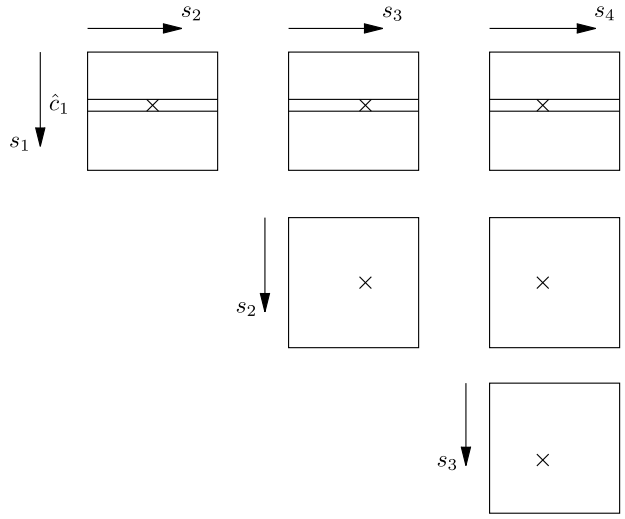


Figure 10.3: Calculation of cut positions minimizing the multiple additional cost.

Overall complexity. Let L be the length bound below which the exact MSA algorithm is applied. Assuming that the maximum sequence length n is of the form $n = L \cdot 2^D$ and all cuts are symmetric (i.e., $n_{(d)} = n/2^d$ for all $d = 0, \ldots, D-1$), the whole divide-and-conquer alignment procedure has the overall time complexity

$$
\begin{aligned}
&\left(\sum_{d=0}^{D-1} 2^d \left(k^2 n_{(d)}^2 + n_{(d)}^{k-1}\right)\right) + \frac{n}{L}(2^k L^k k^2) \\
&= \left(\sum_{d=0}^{D-1} 2^d \left(k^2 \frac{n^2}{2^{2d}} + \frac{n^{k-1}}{2^{d(k-1)}}\right)\right) + 2^k n L^{k-1} k^2 \\
&= \left(\sum_{d=0}^{D-1} \left(k^2 \frac{n^2}{2^d} + \frac{n^{k-1}}{2^{d(k-2)}}\right)\right) + 2^k n L^{k-1} k^2 \\
&\leq \left(\sum_{d=0}^{D-1} \left(k^2 \frac{n^2}{2^d} + \frac{n^{k-1}}{2^d}\right)\right) + 2^k n L^{k-1} k^2 && \text{(since } k \geq 3\text{)} \\
&= \left(k^2 n^2 + n^{k-1}\right) \left(\sum_{d=0}^{D-1} \frac{1}{2^d}\right) + 2^k n L^{k-1} k^2 \\
&\leq \left(k^2 n^2 + n^{k-1}\right) \cdot 2 + 2^k n L^{k-1} k^2 && \text{(geometric series)} \\
&\in O(k^2 n^2 + n^{k-1} + 2^k n L^{k-1} k^2)
\end{aligned}
$$

and space complexity

$$
\max_{0 \leq d < D} \left\{ k n_{(d)} + \binom{k-1}{2} n_{(d)}^2 \right\} + L^k \quad \in \quad O(k^2 n^2 + L^k).
$$

Compared to the standard dynamic programming for multiple alignment, the time is reduced only by one dimension, so it is not clear if the whole method is really an advantage. However:

- The space usage for the whole divide-and-conquer alignment procedure can be seen as polynomial since the space is mainly required for the $\binom{k}{2}$ additional cost matrices. The exponential term in the space complexity to compute the exact multiple alignment for sequences shorter than L can be neglected because L is small and can be chosen freely.

- Additional cost matrices have a very nice structure, since the low values that are of interest here are often close to the main diagonal, and the values increase rapidly when one leaves this diagonal. Based on this observation, several branch-and-bound heuristics have been developed to speed up the search for good cut positions, for more details see (Stoye, 1998).

An implementation of the divide-and-conquer alignment algorithm, plus further documentation, can be found at `https://bibiserv.cebitec.uni-bielefeld.de/dca`.

10.2 Segment-Based Alignment

The global multiple alignment methods mentioned so far, like the progressive methods or the simultaneous method DCA, have properties that are not necessarily desirable in all applications.

In particular, the alignment depends on many parameters, like the cost function or substitution matrix, gap penalties, and a scoring function (e.g. sum of pairs) for the multiple alignment. Apart from the problem that these parameters need to be chosen in advance, the global methods have the well-known weakness that local similarities may be aligned in a wrong way if they are not significant in a global context.

For example, the following alignment shows a potential misalignment that may be caused by the choice of the affine gap penalties:

$$A = \begin{pmatrix} \mathtt{A} & \mathtt{F} & \mathtt{A} & \mathtt{T} & \mathtt{C} & \mathtt{A} & \mathtt{T} & \mathtt{C} & \mathtt{A} \\ \mathtt{A} & \mathtt{C} & \mathtt{A} & \mathtt{T} & - & - & - & - & \mathtt{A} \end{pmatrix}.$$

If instead of individual positions, whole units of local similarities are compared and used to build a global multiple alignment, the result will depend much less on the particular alignment parameters, and instead reflect more the local similarities in the data, like in the following example:

$$A' = \begin{pmatrix} \mathtt{A} & \mathtt{F} & \mathtt{A} & \mathtt{T} & \mathtt{C} & \mathtt{A} & \mathtt{T} & \mathtt{C} & \mathtt{A} \\ \mathtt{A} & - & - & - & \mathtt{C} & \mathtt{A} & \mathtt{T} & - & \mathtt{A} \end{pmatrix}.$$

This is the idea of segment-based sequence alignment.

10.2.1 Segment Identification

The first step in a segment-based alignment approach is to identify the segments. There are different possibilities to obtain segments.

The possibly simplest strategy, followed by the program `TWOALIGN` (Abdeddaïm, 1997) is to use *(suboptimal) local alignments* like they are computed by the Smith-Waterman or Waterman-Eggert algorithms. A disadvantage of this approach is that the local alignment computation also requires the usual alignment parameters like score function and gap penalties.

An alternative approach is to use gap-free local alignments. In this case, the segments correspond to *diagonal segments* of the alignment matrix. Since gap-free alignments can be computed faster than gapped alignments, more time can be spent on the computation of their score. The approach followed in the `DIALIGN` method (Section 10.2.3) is to use a statistical objective function that assigns the score $P_D(l_D, s_D)$ to a diagonal D of length l_D with s_D matches.

This score is the probability that a random segment of length l_D has at least s_D matches:

$$P_D(l_D, s_D) = \sum_{i=s_D}^{l_D} \binom{l_D}{i} p^i \, (1-p)^{l_D - i},$$

where $p = 0.25$ for nucleotides and $p = 0.05$ for amino acids. The *weight* w_D of a diagonal D is then defined as the negative logarithm of its score,

$$w_D = -\ln(P_D).$$

(In more statistical terms, the score would be called the p-value of the diagonal, and the weight would be called the score.)

The time to compute all diagonals is $O(n^3)$, but this can be reduced to $O(n^2)$ if the maximal length of a diagonal segment is bounded by a constant.

10.2.2 Segment Selection and Assembly

After a set of segments is identified, it is not necessarily possible to assemble them into a global alignment, since they might be *inconsistent*, i.e., the inclusion of some of the segments does not allow the inclusion of other segments into the alignment. In the case of two sequences, this is the case if and only if two segments "cross" as the two segments "ON" and "ANA" shown in Figure 10.4.

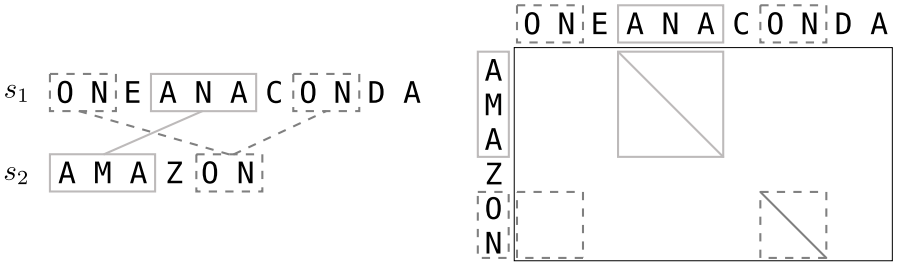


Figure 10.4: Inconsistent pairwise alignment segments.

More generally, a set of segments of multiple sequences $s_1, s_2, \ldots, s_k$ is **inconsistent** if there exists no multiple alignment of these sequences that induces all these segments. Several methods exist to select from a set of segments a subset of consistent segments.

For example, the methods `TWOALIGN` and `DIALIGN` employ a greedy algorithm that considers one maximum scoring pairwise local alignment after the other (in order of decreasing alignment weight) and, if it is consistent with the previously assembled segments, adds it to the solution. Alternative methods are based on *chaining*, that we have seen in the context of whole genome alignment.

Given a set of consistent segments, in general there will still remain unaligned characters that are not contained in any of the consistent segments. In order to arrive at a global multiple alignment, it is desirable to also add these characters to the alignment. Different strategies can be followed.

The `DIALIGN` method simply fills the remaining regions with gaps, letting those characters unaligned that are not contained in any segment. Another possibility is to re-align the regions between the segments. This is not so easy, though, because the region "between the segments" is not clearly defined.

In any case, a global alignment is to be computed that respects the segments as fixed anchors, while for the remaining characters freedom is still given, as long as the resulting alignment is consistent with the segments. It has been suggested to align the sequences progressively (Myers, 1996) or simultaneously (Sammeth et al., 2003a).

10.2.3 DIALIGN

`DIALIGN` (Morgenstern et al., 1996; Morgenstern, 1999) is a practical implementation of segment-based alignment, enhanced in several ways.

By taking into account the information from various sequences during the selection of consistent segments, `DIALIGN` uses an optimized objective function in order to enhance the score of diagonals with a weak, but consistent signal over many sequences. The **overlap weight** of a diagonal D is defined as

$$olw(D) = w_D + \sum_{E \in \mathcal{D}} \tilde{w}(D, E),$$

where $\mathcal{D}$ is the set of all diagonals and $\tilde{w}(D_1, D_2) := w_{D_3}$ is the weight of the (implied) overlap D_3 of the two diagonals D_1 and D_2.

For coding DNA sequences it is possible that the program automatically translates the codons into amino acids and then scores the diagonal with the amino acid scoring scheme.

In its second version, `DIALIGN 2.0`, the statistical score for diagonals was changed in order to upweight longer diagonals. Also, the algorithm for consistency checking and greedy addition of diagonals into the growing multiple alignment has been improved several times.

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