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Complexity Measures for Gene Assembly

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Abstract

The process of gene assembly in ciliates is a fascinating example of programmed DNA manipulations in living cells. Macronuclear genes are split into coding blocks (called MDSs), shuffled and separated by non-coding sequences to form micronuclear genes. Assembling the coding blocks from micronuclear genes to form functional macronuclear genes is facilitated by an impressive in-vivo implementation of the linked list data structure of computer science. Complexity measures for genes may be defined in many ways, including the number of MDSs, the number of loci, etc. We take a different approach in this paper and propose four complexity measures for genes in ciliates, based on the 'effort' required to assemble the gene. We consider: (a) the types of operations used in the assembly, (b) the number of operations used in the assembly, (c) the length of the molecular folds involved, and (d) the length of the shortest possible parallel assembly for that gene.

"One of the oldest forms of life on Earth has been revealed as a natural born computer programmer."
BBC, September 10, 2001.

Keywords: Gene assembly, complexity measures, weights of operations

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1 Introduction

Ciliates are very old eukaryotic unicellular organisms that, through evolution, have developed an unusual way of organizing their genome. Each cell has two types of functionally different nuclei - the *macronucleus* is the somatic nucleus, while the *micronucleus* is the germline nucleus. Depending on the species each type of nuclei may be present in many copies in each cell.

The macronuclear genes are very short molecules, e.g., ranging in the S.nova organisms between 200bp and 3700bp, with an average of 2200 bp in length, see [22], [19], [3], [4]. As a matter of fact, these are the shortest DNA molecules known in Nature, see [20]! On the other hand, micronuclear genome is organized on very long chromosomes (about 120 chromosomes, each with about 10^7 bp in S.nova, see [19]), with coding sequences occupying as little as 2 - 5% of the genome, see, e.g., [3]. During the process of sexual reproduction, ciliates destroy the old macronuclei and transform a micronucleus into a new macronucleus. Ciliates thus have to identify precisely the genetic material and splice it out from the chromosomes. The complexity of the process is profoundly magnified by the fundamentally different organization of the micronuclear and the macronuclear genomes. This process of converting micronuclear genes to their macronuclear form, called *gene assembly*, is especially involved in a family of ciliates called *Stichotrichs* – we concentrate in this paper on this family.

The macronuclear gene is a contiguous DNA sequence, which is placed on its own chromosome, that (with few exceptions only) is not shared with other genes. The same gene in the micronucleus is broken into pieces called *MDSs (macronuclear destined sequences)* that are separated by noncoding blocks called *IESs (internally eliminated sequences)*. Moreover, the order of MDSs may be permuted (with respect to their order in the macronuclear gene), and some of the MDSs may be inverted. Here is where the challenge of gene assembly lies: ciliates have to identify correctly more than 100 000 MDSs in their genome, see [20], assemble them together in the macronuclear (orthodox) order, and eliminate all IESs. We refer to [12], [19], [23] for more details on ciliates and gene assembly.

A hint on how ciliates achieve gene assembly is given by the structure of MDSs. It turns out that ciliates organize their genomic data as *linked lists* in the style used in computer science, see [19]. A short sequence at the end of each MDS is repeated at the beginning of the MDS that should follow it in the orthodox order, thus (in the terminology of computer science) serving as a pointer in a linked list. It is currently believed that ciliates splice together the consecutive MDSs on the common pointers to assemble the gene. The models for gene assembly in Stichotrichs, such as, e.g., [16], [17] and [8], [21], agree on this generic mechanism.

We consider in this paper the *intramolecular* model of [8], [21]. The model is based on three molecular operations: ld, hi, and dlad. In each of these operations, the molecule folds on itself so that two or more pointers get aligned and through

recombination two or more MDSs get combined into a bigger composite MDS. The process continues until all MDSs have been assembled.

First operation: ld**.** In the operation *(loop, direct repeat)-excision*, or ld for short, a pair of pointers flanking an IES guides the excision of this IES as a circular molecule, as illustrated in Fig. 1. The DNA molecule folds on itself so that the two pointers can get aligned, after which the IES is excised through recombination. As a result, two MDSs get joined and form a bigger composite MDS. It is crucial to note that the excised molecule is an IES (closed into a circular form) and so it does not contain any coding blocks – therefore it is not required to participate anymore in the gene assembly process.

Figure 1: Illustration of the ld-rule.

Second operation: hi**.** The operation *(hairpin, inverted repeat)-excision/reinsertion*, or hi for short, is applicable to a molecule containing a pair of pointers where one pointer is the inversion of the other. This is illustrated in Fig. 2. The molecule folds on itself forming a hairpin so that the two copies of the pointer can get aligned with the same polarity, thus facilitating the recombination. Through recombination, the sequence between the two occurrences of the pointer is inverted. One may also note that as a result of applying hi, two MDSs are joined together into a bigger composite MDS, while two IESs are joined together into a bigger noncoding block (a bigger composite IES).

Figure 2: Illustration of the hi-rule.

Third operation: dlad**.** The operation *(double loop,alternating direct repeat)-excision/reinsertion*, or dlad for short applies to a DNA molecule containing two pairs of pointers where the segments delimited by the pairs of pointers overlap with each other. This is illustrated in Fig. 3. The molecule folds into two loops so that the two copies of the first pointer align with each other in one loop, and the two copies of the second pointer align with each other in the other loop. Thus, the molecule is in position for two recombinations. As a result of this double recombination, two sequences are translocated; several MDSs are joined together into bigger composite MDSs(see [6] for details).

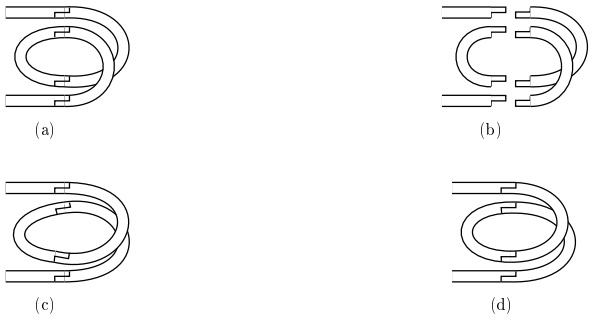


Figure 3: Illustration of the dlad-rule.

2 Definitions

We give in this section some basic notions concerning permutations, strings, and graphs.

For a finite alphabet $\Sigma = \{a_1, \ldots, a_n\}$, we denote by Σ^* the free monoid generated by Σ and call any element of Σ^* a *string*. Let $\overline{\Sigma} = \{\overline{a}_1, \ldots, \overline{a}_n\}$, where $\Sigma \cap \overline{\Sigma} = \emptyset$. For $p, q \in \Sigma \cup \overline{\Sigma}$, we say that p, q have the same *signature* if either $p, q \in \Sigma$, or $p, q \in \overline{\Sigma}$ and we say that they have *different signatures* otherwise. For $p \in \Sigma$, we say that p is an *unsigned letter*, while for $p \in \overline{\Sigma}$, we say that p is a *signed letter*.

Let $\Sigma^{\maltese} = (\Sigma \cup \overline{\Sigma})^*$. For any $u \in \Sigma^{\maltese}$, $u = x_1 \ldots x_k$, with $x_i \in \Sigma \cup \overline{\Sigma}$, for all $1 \leq i \leq k$, we set $\|u\| = \|x_1\| \ldots \|x_k\|$, where $\|a\| = \|\overline{a}\| = a$, for all $a \in \Sigma$. Also, $\overline{u} = \overline{x}_k \ldots \overline{x}_1$, where $\overline{\overline{a}} = a$, for all $a \in \Sigma$.

We say that $u \in \Sigma^{\maltese}$ is a *signed double occurrence string* if for any $p \in \Sigma$, u has either 0, or 2 occurrences from the set $\{p, \overline{p}\}$. In case u has two occurrences from the set $\{p, \overline{p}\}$, we say that p is a *positive letter* in u if the two occurrences have different signatures, and we say that p is a *negative letter* in u if the two occurrences have the same signature. We say that letters p and q, $p \neq q$, *overlap* in u if $u = u_1 p u_2 q u_3 p u_4 q u_5$, for some $u_i \in \Sigma^{\maltese}$, $1 \leq i \leq 5$.

For two signed double occurrence strings, we say that v is a *substring* of u, denoted $v \leq u$, $u = u_1 v u_2$, for some strings u_1, u_2. We say that the signed double

(a) (b)

Figure 4: (a) The square C_4; (b) the diamond D_4.

occurrence string u is *elementary* if u has no substring v with v a signed double occurrence string.

A *permutation* π over alphabet Σ is a bijection $\pi : \Sigma \to \Sigma$. Fixing the order relation $(a_1, a_2, \ldots, a_m)$ over Σ, we often denote π as the string $\pi(a_1) \ldots \pi(a_m) \in \Sigma^*$. A *signed permutation* over Σ is a string $\psi \in \Sigma^{\maltese}$, where $\|\psi\|$ is a permutation over Σ.

A *signed graph* is a triple $G = (V, E, \phi)$, where V is a finite set of *vertices*, $E \subseteq V \times V$ is the set of (undirected) *edges*, with the property that $(x, y) \in E$ if and only if $(y, x) \in E$, and $\phi : V \to \{+, -\}$ is the *signature function*. We say that vertex $p \in V$ is positive if $\phi(p) = +$ and it is *negative* otherwise. For all $p \in V$, we denote by $N_G(p)$ the neighborhood of p in G, i.e., $N_G(p) = \{q \in V \mid (p, q) \in E\}$. For $V' \subseteq V$, the subgraph of G *induced* by V' is $G_V = (V', E', \phi')$, where $E' = \{(p, q) \in E \mid p, q \in V'\}$ and $\phi' : V' \to \{+, -\}$, $\phi'(p) = \phi(p)$, for all $p \in V'$.

For all $p \in V$ we denote by $G - p$ the graph induced by the set of vertices $V \setminus \{p\}$. We also denote by $\mathsf{loc}_p(G)$ the *local complement* of G at p: $\mathsf{loc}_p(G) = (V, E', \phi')$, where $(x, y) \in E'$ if and only if $(x, y) \notin E$, for all $x, y \in N_G(p)$, and $(x, y) \in E'$ if and only if $(x, y) \in E$ otherwise. Also, $\phi'(x) = +$ if and only if $\phi(x) = -$, for all $x \in N_G(p)$, and $\phi'(x) = \phi(x)$, otherwise.

We denote by C_4 and D_4 the graphs shown in Fig. 4.

With any signed double occurrence string u over alphabet Σ, we associate a signed graph $G_u = (V_u, E_u, \phi_u)$ as follows: $V_u = \{p \in \Sigma \mid p \text{ or } \overline{p} \text{ occurs in } u\}$, $E_u = \{(p, q) \mid p \text{ and } q \text{ overlap in } u\}$, and $\phi_u(p) = +$ if and only if p is a positive letter in u. The graph G_u is called the *overlap graph* of u.

For $k \geq 2$ we will use throughout the paper the alphabets $\Sigma_k = \{1, \ldots, k\}$ and $\Delta_k = \{2, \ldots, k\}$.

3 Three models for gene assembly

The intramolecular model for gene assembly, [8], [21], has been formalized on several levels of abstraction. The structures of genes can be represented as: signed permutations, MDS descriptors, signed double occurrence strings, or signed overlap graphs. Consequently, the process of gene assembly can be formalized through processing of strings, or through processing of graphs. As it turns out, all these

levels of abstraction are equivalent as far as the modeling of gene assembly is concerned, see [6] for a detailed discussion on model forming. Nevertheless, different levels of abstraction prove more suitable (more elegant or technically simpler) for different research topics.

In this paper we consider issues dealing with formalization of the gene assembly on the level of string permutation, signed double occurrence strings, and signed graphs. We present briefly these three abstraction levels referring to [6] for more details.

For any gene γ having k MDSs, $k \geq 1$, we may associate a signed permutation in the following way: associate to the MDS M_i letter i, $1 \leq i \leq k$, and to its inversion $\overline{M}_i$ the signed letter $\bar{i}$. Thus the signed permutation associated to the MDS sequence $M_3\overline{M}_1M_2$ is simply the signed permutation $3\bar{1}2$.

We may also associate a signed double occurrence string with any gene (more generally, to any sequence of MDSs), simply by writing its sequence of pointers. Thus, given a sequence of k MDSs, we associate with each MDS M_i, $2 \leq i \leq k-1$, the string consisting of its incoming and outgoing pointers: $i(i+1)$. With $\overline{M}_i$ we associate string $(\overline{i+1})\bar{i}$. The first and the last MDS are special because they contain only one pointer each, and moreover we mark the beginning of the first MDS by the beginning marker, and the end of the last MDS by the end marker. In our coding of these MDSs, we ignore the beginning and the end markers – thus, with M_1 we associate string 2 and with $\overline{M}_1$ string $\bar{2}$. Similarly, with M_k we associate string k and with $\overline{M}_k$ string $\bar{k}$, Consequently, with the MDS sequence $M_3\overline{M}_1M_2$ we associate string $3\bar{2}23$. Also, with the MDS sequence $M_2\bar{M}_4M_1M_3$ we associate string $23\bar{4}234$.

On a higher level of abstraction, we may associate a graph with a sequence of MDS in the following way. If u_γ is the string associated with gene γ, then G_γ is the signed overlap graph of u_γ, as defined in Section 2. Thus, the graph associated with the MDS sequence $M_3\overline{M}_1M_2$ consists of positive vertex 2, adjacent to negative vertex 3.

The molecular operation ld, hi, dlad are modeled by string rewriting rules as follows (we will use the notation ld, hi, dlad also for the string rules, but this should not lead to confusion).

Let u be a signed double occurrence string over alphabet Δ_k.

1. For all $p \in \Delta_k \cup \overline{\Delta}_k$, ld_p is defined as follows:

$$\mathsf{ld}_p(uppv) = uv,$$

   where $u, v \in \Delta_k^{\maltese}$.

2. For all $p \in \Delta_k \cup \overline{\Delta}_k$, hi_p is defined as follows:

$$\mathsf{hi}_p(upv\bar{p}w) = u\bar{v}w,$$

   where $u, v \in \Delta_k^{\maltese}$.

3. For all $p \in \Delta_k \cup \overline{\Delta}_k$, $\mathsf{dlad}_{p,q}$ is defined as follows:

$$\mathsf{dlad}_{p,q}(u_1 p u_2 q u_3 p u_4 q u_5) = u_1 u_4 u_3 u_2 u_5,$$

where $u_i \in \Delta_k^{\maltese}$, for all $1 \leq i \leq 5$.

We say that a composition ϕ of ld, hi, and dlad operations is a *reduction strategy* for string u if $\phi(u) = \Lambda$.

Example 1. *Let* $u = 3\bar{5}26547367\bar{2}\bar{4}88$*, then* ld_8 *is applicable to* u*:* $\mathsf{ld}_8(u) = 3\bar{5}2654736\bar{7}2\bar{4}$*. Also,* hi_4 *and* $\mathsf{dlad}_{5,6}$ *are applicable to* u*:* $\mathsf{hi}_4(u) = 3\bar{5}265\bar{2}\bar{7}\bar{6}\bar{3}\bar{7}88$*, and* $\mathsf{dlad}_{3,2}(u) = 67654\bar{6}\bar{5}\bar{4}88$.

The corresponding operations for signed graphs are defined as follows (again, also for the graph rules we will use the same notation ld, hi, and dlad). Let $G = (V, E)$ be a signed graph.

1. For all $p \in V$, ld_p is applicable to G if and only if p is an isolated negative vertex in G. When applicable, $\mathsf{ld}_p(G) = G - p$.

2. For all $p \in V$, $\mathsf{hi}_p\, 4$ is applicable to G if and only if p is an positive vertex in G. When applicable, $\mathsf{hi}_p(G) = \mathsf{loc}_p(G) - p$.

3. For all $p, q \in V$, $\mathsf{dlad}_{p,q}$ is applicable to G if and only if p and q are adjacent negative vertices in G. When applicable, $\mathsf{dlad}_{p,q}(G) = (V \setminus \{p, q\}, E')$, where E' is obtained from E by complementing the edges that join vertices in $N_G(p)$ with vertices in $N_G(q)$. This means that $(x, y) \in (E' \setminus E) \cup (E \setminus E')$ if and only if

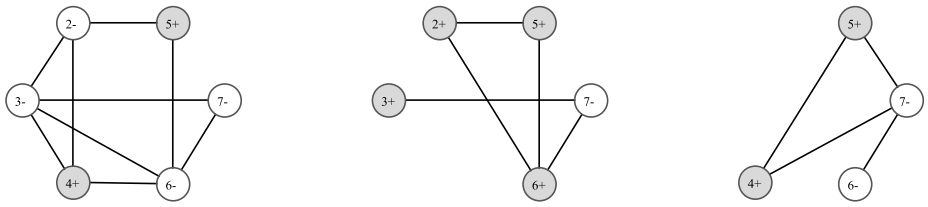
$$\begin{array}{l} x \in N_G(p) \setminus N_G(q) \text{ and } y \in N_G(q), \text{ or} \\ x \in N_G(q) \cup N_G(q) \text{ and } y \in (N_G(p) \setminus N_G(q)) \cup (N_G(q) \setminus N_G(p)), \text{ or} \\ x \in N_G(q) \setminus N_G(p) \text{ and } y \in N_G(p). \end{array}$$

We say that a composition ψ of ld, hi, and dlad operations is a *reduction strategy* for graph G if $\psi(G) = \emptyset$.

Example 2. *The signed overlap graph associated to string* u *in Example 1 is depicted in Fig. 5*

Without risk of confusion, for both the string rules and for the graph rules we set $\mathsf{Ld} = \{\mathsf{ld}_p \mid p \geq 2\}$, $\mathsf{Hi} = \{\mathsf{hi}_p \mid p \geq 2\}$, and $\mathsf{Dlad} = \{\mathsf{dlad}_{p,q} \mid p, q \geq 2, p \neq q\}$.

Note that the process of gene assembly, and all its formalizations, as sorting permutations, reducing strings, or reducing graphs, are non-deterministic. We illustrate this in the following example.



(a) (b) (c)

Figure 5: (a) The graph G_u in Example 2; (b) $\mathsf{hi}_4(G_u)$; (c) $\mathsf{dlad}_{2,3}(G_u)$

Example 3. *Consider a double occurrence string* $u = 562324573467$. *There are at least two reduction strategies for* u*:* $\phi_1 = \mathsf{ld}_7 \circ \mathsf{ld}_4 \circ \mathsf{dlad}_{5,6} \circ \mathsf{dlad}_{2,3}$ *and* $\phi_2 = \mathsf{ld}_5 \circ \mathsf{ld}_6 \circ \mathsf{dlad}_{2,3} \circ \mathsf{dlad}_{4,7}$. *Indeed,*

$$\phi_1(u) = (\mathsf{ld}_7 \circ \mathsf{ld}_4 \circ \mathsf{dlad}_{5,6})(56457467) = (\mathsf{ld}_7 \circ \mathsf{ld}_4)(7447) = \mathsf{ld}_7(77) = \Lambda,$$

$$\phi_2(u) = (\mathsf{ld}_5 \circ \mathsf{ld}_6 \circ \mathsf{dlad}_{2,3})(56232635) = (\mathsf{ld}_5 \circ \mathsf{ld}_6)(5665) = \mathsf{ld}_5(55) = \Lambda.$$

Note that the two strategies have the same number of ld *operations, albeit applied to different pointers.*

The following result, adapted from [6] provides an invariant for all sorting strategies of a given string.

Theorem 1 ([6])**.** *Let* u *be a signed double occurrence string and* ϕ_1, ϕ_2 *two reduction strategies for* u*. Then* ϕ_1 *and in* ϕ_2 *contain the same number of* ld *operations.*

4 First complexity measure: the minimal subset of operations sufficient for gene assembly

We introduce in this section our first measure of gene complexity in terms of the smallest set of (types of) operations that are capable to assemble a given gene. Our formalism in this section will be that of signed double occurrence strings. Note that a similar presentation may also be done in terms of signed graphs, see [5].

The concept of (gene) complexity here is the following. For a given string x, consider reduction strategies φ for x, and take the set $S_\varphi \subseteq \{\mathsf{Ld}, \mathsf{Hi}, \mathsf{Dlad}\}$ of those types of operations that are used in φ. We say that S_φ is a *reduction set* for x.

Example 4. *Note that a string may have several reduction sets. For instance, if* $u = 2\bar{3}\bar{2}434$*, then* $\varphi_1(u) = \mathsf{dlad}_{3,4} \circ \mathsf{hi}_2$ *is a reduction strategy for* u*:* $\varphi_1 = \mathsf{dlad}_{3,4}(3434) = \Lambda$. *Thus,* $\{\mathsf{Hi}, \mathsf{Dlad}\}$ *is a reduction set for* u*. However,* $\{\mathsf{Hi}\}$ *is also a reduction set for* u*. Indeed,* $\varphi_2 = \mathsf{hi}_2 \circ \mathsf{hi}_4 \circ \mathsf{hi}_3$ *is a reduction strategy for* u*:* $\varphi_2(u) = (\mathsf{hi}_2 \circ \mathsf{hi}_4)(2\bar{4}24) = \mathsf{hi}_2(2\bar{2}) = \Lambda$.

We say that a set $S \subseteq \{\mathsf{Ld}, \mathsf{Hi}, \mathsf{Dlad}\}$ is a *minimal reduction set* for X, if for any $T \subseteq S$, where T is a reduction set for X, we have $T = S$.

As we will observe at the end of this section, a string X has a unique minimal reduction set. Anticipating this result, the following notion of complexity is well defined.

Definition 1. *The complexity $\mathcal{C}_1(X)$ of a signed double occurrence string X is a minimal reduction set of X.*

To prove the result announced above, we need to consider for every $S \subseteq \{\mathsf{Ld}, \mathsf{Hi}, \mathsf{Dlad}\}$, what are the strings with S as a reduction set. The first complete characterization was given in [5] in the case of realistic strings. The results were then extended to signed double occurrence strings in [1]; the characterizations in [1] are based in part on a notion of break point graphs. For simplicity, we only consider here the case of elementary strings and the approach in [5].

Theorem 2 ([5])**.** *Let u be an elementary string.*

(i) $\{\mathsf{Ld}\}$ *is a reduction set for u if and only if u contains neither overlap, nor signed letters.*

(ii) $\{\mathsf{Ld}, \mathsf{Hi}\}$ *is a reduction set for u if and only if $|u| \leq 2$ or u contains at least one positive pointer.*

(iii) $\{\mathsf{Ld}, \mathsf{Dlad}\}$ *is a reduction set for u if and only if u contains no signed letters.*

(iv) $\{\mathsf{Ld}, \mathsf{Hi}, \mathsf{Dlad}\}$ *is a reduction set for any signed double occurrence string.*

We omit in this paper the characterizations of the strings with $\{\mathsf{Hi}\}$, $\{\mathsf{Dlad}\}$, or $\{\mathsf{Hi}, \mathsf{Dlad}\}$ as reduction sets. Such characterization have been given in [5].

Example 5. *The R_1 gene of S.nova is described by the MDS sequence $M_1M_2M_3M_4M_5M_6$ and its associated string is 2233445566.*

(a) $\{\mathsf{Ld}\}$ *as a minimal reduction set for the whole string.*

(b) $\{\mathsf{Dlad}\}$ *is a minimal reduction set for string 2323.*

(c) $\{\mathsf{Hi}\}$ *is a minimal reduction set for string $23\bar{2}3$.*

(d) String $2\bar{3}\bar{2}3$ has two reduction strategies: $\mathsf{ld}_3 \circ \mathsf{hi}_2$ and $\mathsf{ld}_2 \circ \mathsf{hi}_3$. Thus, $\{\mathsf{Ld}, \mathsf{Hi}\}$ *is a minimal reduction set for it.*

(e) The α-TP gene of S.nova is described by the MDS sequence $M_1M_3M_5M_9$ $M_{11}M_2M_4M_6M_8M_{10}M_{12}M_{13}M_{14}$. Its associated string 2345691011122345 $67891011121313141 4$ has $\{\mathsf{Ld}, \mathsf{Dlad}\}$ *as a minimal reduction set.*

(f) The actin I gene of S.nova is described by the MDS sequence $M_3M_4M_6M_5M_7M_9\overline{M}_2M_1M_8$. *Its associated string* $3445675678\bar{9}\bar{3}\bar{2}289$ *has* $\{\mathsf{Ld}, \mathsf{Hi}, \mathsf{Dlad}\}$ *as a minimal reduction set.*

We can now prove the following result.

Theorem 3. *Let* $u \neq \Lambda$ *be an elementary string and* S_1, S_2 *two minimal reduction sets for* u. *Then* $S_1 = S_2$.

Proof. Assume that there is an elementary string $u \neq \Lambda$ with two different minimal reduction sets S_1, S_2. Clearly, $S_1 \not\subseteq S_2$ and $S_2 \not\subseteq S_1$. We then have the following cases:

(i) $S_1 = \{\mathsf{Ld}\}$, $S_2 = \{\mathsf{Hi}\}$;
(ii) $S_1 = \{\mathsf{Ld}\}$, $S_2 = \{\mathsf{Dlad}\}$;
(iii) $S_1 = \{\mathsf{Ld}\}$, $S_2 = \{\mathsf{Hi}, \mathsf{Dlad}\}$;
(iv) $S_1 = \{\mathsf{Ld}\,\mathsf{Hi}\}$, $S_2 = \{\mathsf{Dlad}\}$;
(v) $S_1 = \{\mathsf{Ld}, \mathsf{Hi}\}$, $S_2 = \{\mathsf{Ld}, \mathsf{Dlad}\}$;
(vi) $S_1 = \{\mathsf{Ld}, \mathsf{Hi}\}$, $S_2 = \{\mathsf{Hi}, \mathsf{Dlad}\}$;
(vii) $S_1 = \{\mathsf{Ld}, \mathsf{Dlad}\}$, $S_2 = \{\mathsf{Hi}\}$;
(viii) $S_1 = \{\mathsf{Ld}, \mathsf{Dlad}\}$, $S_2 = \{\mathsf{Hi.Dlad}\}$;
(ix) $S_1 = \{\mathsf{Hi}\}$, $S_2 = \{\mathsf{Dlad}\}$.

In all cases except (ii) and (vi) we have that one of the reduction sets contains Hi, while the other does not. Consequently, according to one reduction set, u should have at least one signed letter, while according to the other reduction set, u should have none. Thus, $u = \Lambda$, a contradiction.

In Cases (ii) and (vi) u has two reduction strategies: one containing at least one Ld- operation, another containing none. This is a contradiction by Theorem 1. □

Corollary 4. *The complexity measure* $\mathcal{C}_1$ *is well defined.*

5 Second complexity measure: weights associated with the assembly operations

The concept of our second measure of complexity is straightforward: a gene is more "complex" than another if it requires more "effort" to be assembled. The simplest way to measure the "effort" required to assemble a given gene is through counting the number of operations required in the reduction.

Definition 2. *Let* u *be a signed double occurrence string and* φ *a reduction strategy for* u. *We denote by* $\mathcal{C}_2^{(1)}(\varphi)$ *the number of* ld, hi, *and* dlad *operations in* φ. *Then the complexity* $\mathcal{C}_2^{(1)}(u)$ *is defined as:*

$$\mathcal{C}_2^{(1)}(u) = min\{\mathcal{C}_2^{(1)}(\varphi) \mid \varphi \text{ is a reduction strategy for } u\}.$$

Example 6. *Consider* $u = 2\bar{3}\bar{2}434$ *and reduction strategies* φ_1, φ_2 *for* u *as given in Example 4. Then* $\mathcal{C}_2^{(1)}(\varphi_1) = 2$, *while* $\mathcal{C}_2^{(1)}(\varphi_2) = 3$. *It is easy to see that* $\mathcal{C}_2^{(1)}(u) = 2$.

Clearly, to find the complexity $\mathcal{C}_2^{(1)}(u)$ for a given string u, one needs to find the length of a reduction strategy φ for u using maximum number of dlad operations. Indeed, note that ld and hi operations reduce the length of the string by two, while dlad operations reduce the length of the string by four.

Finding the complexity $\mathcal{C}_2^{(1)}(u)$ is easy if $\mathcal{C}_1(u) \neq \{\mathsf{Hi}, \mathsf{Dlad}\}$. Indeed, based on Theorem 1, it is easy to see that in this case, for any two reduction strategies φ and ψ for u, we have $\mathcal{C}_2^{(1)}(\varphi) = \mathcal{C}_2^{(1)}(\psi)$. It is currently unknown how to compute $\mathcal{C}_2^{(1)}(u)$ if $\mathcal{C}_1(u) = \{\mathsf{Hi}, \mathsf{Dlad}\}$.

Considering the molecular model of the dlad operations, with a double fold and two simultaneous recombination, it may sometimes be undesirable to maximize the number of dlad operations as done when computing $\mathcal{C}_2^{(1)}(u)$. A different idea is to associate *weights* with each of ld, hi and dlad and consequently to any reduction strategy. Associating weights to the operations may be done in at least two ways: either by introducing a (fixed) weight for each type of operation, or through variable weights depending on the type of operation and the string to which the operation applies. We illustrate both ideas in the following.

Definition 3. *For any operation $f \in \mathsf{Ld} \cup \mathsf{Hi} \cup \mathsf{Dlad}$, we define $\mathcal{C}_2^{(2)}(f)$ as follows: $\mathcal{C}_2^{(2)}(f) = c_1$, if $f \in \mathsf{Ld}$; $\mathcal{C}_2^{(2)}(f) = c_2$, if $f \in \mathsf{Hi}$; and $\mathcal{C}_2^{(2)}(f) = c_3$, if $f \in \mathsf{Dlad}$, where $c_1, c_2, c_3 \geq 0$. Then for a composition $\varphi = f_k \circ \cdots \circ f_1$, $f_i \in \mathsf{Ld} \cup \mathsf{Hi} \cup \mathsf{Dlad}$, we let $\mathcal{C}_2^{(2)}(\varphi) = \sum_{i=1}^{k} \mathcal{C}_2^{(2)}(f_i)$.*

For a signed double occurrence string u, the complexity $\mathcal{C}_2^{(2)}(u)$ is defined as

$$\mathcal{C}_2^{(2)}(u) = min\{\mathcal{C}_2^{(2)}(\varphi) \mid \varphi \text{ is a reduction strategy for } u\}.$$

Note that if we define $\mathcal{C}_2^{(2)}(f) = 1$, for any $f \in \mathsf{Ld} \cup \mathsf{Hi} \cup \mathsf{Dlad}$, then $\mathcal{C}_2^{(2)} = \mathcal{C}_2^{(1)}$: we only count the number of operations in each strategy.

Example 7. *Let $u = 2\bar{3}\bar{2}434$ and let φ_1, φ_2 be reduction strategies for u as in Examples 4 and 6. Let assign the weights as follows: $\mathcal{C}_2^{(2)}(f) = 0$ for $f \in \mathsf{Ld}$, $\mathcal{C}_2^{(2)}(f) = 1$ for $f \in \mathsf{Hi}$, $\mathcal{C}_2^{(2)}(f) = 3$ for $f \in \mathsf{Dlad}$. Then $\mathcal{C}_2^{(2)}(\varphi_1) = 4$ and $\mathcal{C}_2^{(2)}(\varphi_2) = 3$.*

A more refined measure of complexity may be introduced depending on the length of the strings "manipulated" by each operation: the length of the string inverted by hi_p, and the length of the strings translocated by $\mathsf{dlad}_{p,q}$. In the case of ld_p, the excised string is always the same, pp and so, for simplicity, we may set the complexity of ld equal to zero. Formally this is defined as follows.

Definition 4. *Let u be a signed double occurrence string.*

(i) For any operation ld_p applicable to u, we let $\mathcal{C}_2^{(3)}(\mathsf{ld}_p, u) = 0$.

(ii) For any operation hi_p applicable to u, we let $\mathcal{C}_2^{(3)}(\mathsf{hi}_p, u) = |u_2|$, where $u = u_1 p u_2 \overline{p} u_3$, for some strings u_1, u_2, u_3.

(iii)For any operation $\mathsf{dlad}_{p,q}$ *applicable to* u*, we let* $\mathcal{C}_2^{(3)}(\mathsf{dlad}_{p,q}, u) = |u_2|+|u_4|$*, where* $u = u_1pu_2qu_3pu_4qu_5$*, for some strings* u_1, u_2, u_3, u_4, u_5*.*

For a reduction strategy $\varphi = f_k \circ \cdots \circ f_1$ *for* u*,* $f_i \in \mathsf{Ld} \cup \mathsf{Hi} \cup \mathsf{Dlad}$*, we let* $\mathcal{C}_2^{(3)}(\varphi, u) = \sum_{i=1}^{k} \mathcal{C}_2^{(3)}(f_i, (f_{i-1} \circ \cdots f_1)(u))$*. Then we define the complexity* $\mathcal{C}_2^{(3)}(u)$ *by*

$$\mathcal{C}_2^{(3)}(u) = min\{\mathcal{C}_2^{(3)}(\varphi, u) \mid \varphi \textit{ is a reduction strategy for } u\}.$$

Example 8. *Let* $u = 3445675678\bar{9}\bar{3}\bar{2}289$ *be the string associated with the gene actin I in S.nova. Then* $\varphi_1 = \mathsf{ld}_6 \circ \mathsf{dlad}_{7,5} \circ \mathsf{ld}_4 \circ \mathsf{hi}_2 \circ \mathsf{hi}_8 \circ \mathsf{hi}_9 \circ \mathsf{hi}_3$ *is a reduction strategy for* u*:*

$$\begin{aligned}
u_1 &= \mathsf{hi}_3(u) = \bar{9}\bar{8}\bar{7}\bar{6}\bar{5}\bar{7}\bar{6}\bar{5}\bar{4}\bar{4}\bar{2}289,\\
u_2 &= \mathsf{hi}_9(u_1) = \bar{8}\bar{2}2445675678,\\
u_3 &= \mathsf{hi}_8(u_2) = \bar{7}\bar{6}\bar{5}\bar{7}\bar{6}\bar{5}\bar{4}\bar{4}\bar{2}2,\\
u_4 &= \mathsf{hi}_2(u_3) = \bar{7}\bar{6}\bar{5}\bar{7}\bar{6}\bar{5}\bar{4}\bar{4},\\
u_5 &= \mathsf{ld}_4(u_4) = \bar{7}\bar{6}\bar{5}\bar{7}\bar{6}\bar{5},\\
u_6 &= \mathsf{dlad}_{7,5}(u_5) = \bar{6}\bar{6},\\
u_7 &= \mathsf{ld}_6(u_6) = \Lambda.
\end{aligned}$$

Hence $\mathcal{C}_2^{(3)}(\varphi_1, u) = \mathcal{C}_2^{(3)}(\mathsf{hi}_3, u)+\mathcal{C}_2^{(3)}(\mathsf{hi}_9, u_1)+\mathcal{C}_2^{(3)}(\mathsf{hi}_8, u_2)+\mathcal{C}_2^{(3)}(\mathsf{hi}_2, u_3)+\mathcal{C}_2^{(3)}(\mathsf{ld}_4, u_4)+\mathcal{C}_2^{(3)}(\mathsf{dlad}_{7,5}, u_5)+\mathcal{C}_2^{(3)}(\mathsf{ld}_6, u_6) = 10+12+10+0+0+2+0 = 34$.

Note that $\varphi_2 = \mathsf{hi}_3 \circ \mathsf{hi}_2 \circ \mathsf{dlad}_{8,9} \circ \mathsf{ld}_7 \circ \mathsf{dlad}_{5,6} \circ \mathsf{ld}_4$ *is also a reduction strategy for* u*:*

$$\begin{aligned}
v_1 &= \mathsf{ld}_4(u) = 3567567893\bar{2}\bar{2}289,\\
v_2 &= \mathsf{dlad}_{5,6}(v_1) = 3778932\bar{2}289,\\
v_3 &= \mathsf{ld}_7(v_2) = 389\bar{3}\bar{2}289,\\
v_4 &= \mathsf{dlad}_{8,9}(v_3) = 3\bar{3}\bar{2}2,\\
v_5 &= \mathsf{hi}_2(v_4) = 3\bar{3},\\
v_6 &= \mathsf{hi}_3(v_5) = \Lambda.
\end{aligned}$$

Thus $\mathcal{C}_2^{(3)}(\varphi_2, u) = \mathcal{C}_2^{(3)}(\mathsf{ld}_4, u)+\mathcal{C}_2^{(3)}(\mathsf{dlad}_{5,6}, v_1)+\mathcal{C}_2^{(3)}(\mathsf{ld}_7, v_2)+\mathcal{C}_2^{(3)}(\mathsf{dlad}_{8,9}, v_3) + \mathcal{C}_2^{(3)}(\mathsf{hi}_2, v_4) + \mathcal{C}_2^{(3)}(\mathsf{hi}_3, v_5) = 0$.

A natural question here is: what are the strings with the minimal $\mathcal{C}_2^{(3)}(u)$ complexity? We discuss this issue in the next section, where we consider the simple operations for gene assembly.

6 Third complexity measure: simple operations

As discussed above, one way to introduce a complexity measure for gene assembly is by considering the length of the molecular folds involved in every step of the assembly. We consider in this section simple versions of ld, hi, and dlad where the operations can only be applied on the shortest possible folds. It is known that Ld∪Hi∪Dlad is a complete model, in the sense that any gene (alternatively: signed permutation, string, or graph) may be assembled in this model, see [7]. It turns out that the simple operations are not complete: there are certain patterns that cannot be assembled through simple operations. Remarkably though, all known micronuclear gene sequences, see [2], can indeed be assembled through simple operations.

The molecular model for simple ld, hi, and dlad was introduced in [11]. Due to lack of space, we only give here a short intuitive presentation, followed by its formalization as rewriting rules for signed permutations. For formalizations on the level of MDS descriptors and signed double occurrence strings we refer to [11].

As observed in Section 3, ld must always be simple – the excised sequences may never contain coding blocks for the assembly to succeed. In simple hi, one only inverts sequences containing *at most one MDS*. Similarly, in simple dlad, the two sequences that are translocated may contain altogether *at most one MDS*. We refer to [11] for details.

As noted in Section 3, when working with signed permutations, we ignore the ld operation and model gene assembly as a process of sorting a signed permutation rather than as a process of pointer elimination. Simple hi and dlad are modeled through the following operations for signed permutations.

1. For each $p \geq 1$, sh_p is defined as follows:

$$\mathsf{sh}_p(xp\dots(p+i)(\overline{p+k})\dots(\overline{p+i+1})y) = xp\dots(p+i)(p+i+1)\dots(p+k)y,$$
$$\mathsf{sh}_p(x(\overline{p+i})\dots\overline{p}(p+i+1)\dots(p+k)y) = xp\dots(p+i)(p+i+1)\dots(p+k)y,$$
$$\mathsf{sh}_p(x(p+i+1)\dots(p+k)(\overline{p+i})\dots\overline{p}) = x(\overline{p+k})\dots(\overline{p+i+1})(\overline{p+i})\dots\overline{p}y,$$
$$\mathsf{sh}_p(x(\overline{p+k})\dots(\overline{p+i+1})p\dots(p+i)y) = x(\overline{p+k})\dots(\overline{p+i+1})(\overline{p+i})\dots\overline{p}y,$$

   where $k > i \geq 0$ and x, y, z are signed strings over Σ_n. Let $\mathsf{Sh} = \{\mathsf{sh}_i \mid 1 \leq i \leq n\}$.

2. For each p, $2 \leq p \leq n-1$, sd_p is defined as follows:

$$\mathsf{sd}_p(x\,p\dots(p+i)\,y\,(p-1)\,(p+i+1)\,z) = xy(p-1)p\dots(p+i)(p+i+1)z,$$
$$\mathsf{sd}_p(x\,(p-1)(p+i+1)yp\dots(p+i)z) = x(p-1)p\dots(p+i)(p+i+1)yz,$$

where $i \geq 0$ and x, y, z are signed strings over Σ_n. We also define $\mathsf{sd}_{\overline{p}}$ as follows:

$$\mathsf{sd}_{\overline{p}}(x(\overline{p+i+1})(\overline{p-1})y(\overline{p+i})\ldots\overline{p}z) = x\,(\overline{p+i+1})(\overline{p+i})\ldots\overline{p}(\overline{p-1})yz,$$
$$\mathsf{sd}_{\overline{p}}(x(\overline{p+i})\ldots\overline{p}y(\overline{p+i+1})(\overline{p-1})z) = xy(\overline{p+i+1})(\overline{p+i})\ldots\overline{p}(\overline{p-1})z,$$

where $i \geq 0$ and x, y, z are signed strings over Σ_n. Let $\mathsf{Sd} = \{\mathsf{sd}_i, \mathsf{sd}_{\overline{i}} \mid 1 \leq i \leq n\}$.

We say that a signed permutation π over a set of integers $\{i, i+1, \ldots, i+l\}$ is *sortable* if there are operations $\phi_1, \ldots, \phi_k \in \mathsf{Sh} \cup \mathsf{Sd}$ such that $(\phi_k \circ \ldots \circ \phi_1)(\pi)$ is a sorted permutation. We say that π is *blocked* if neither an sh operation, nor an sd operation is applicable to π and π is not sorted.

Let $\phi = \phi_k \circ \ldots \circ \phi_1$, $\phi_i \in \mathsf{Sh} \cup \mathsf{Sd}$, for all $1 \leq i \leq k$. We say that ϕ is a *strategy* for π if $\phi(\pi)$ is either sorted or blocked. In the former case we say that ϕ is a *sorting strategy*, while in the latter case we say that ϕ is a *unsuccessful strategy* for π.

Example 9. *Let* $\pi = 2\,4\,3\,\overline{1}$ *be a signed permutation. Then* $(\mathsf{sh}_1 \circ \mathsf{sd}_3)(\pi) = \mathsf{sh}_1(2\,3\,4\,\overline{1}) = \overline{4}\,\overline{3}\,\overline{2}\,\overline{1}$*, a sorted permutation.*

One may introduce "elementary" versions of sh and sd, where only one letter is rewritten in every step, rather than strings as in sh and sd. We introduce them in the following.

3. For each $p \geq 1$, eh_p is defined as follows:

$$\mathsf{eh}_p(x\,p(\overline{p+1})\,y) = x\,p\,(p+1)\,y, \qquad \mathsf{eh}_p(x\,(\overline{p+1})\,p\,y) = x\,(\overline{p+1})\,\overline{p}\,y,$$
$$\mathsf{eh}_p(x\,\overline{p}\,(p+1)\,y) = x\,p\,(p+1)\,y, \qquad \mathsf{eh}_p(x\,(p+1)\,\overline{p}\,y) = x\,(\overline{p+1})\,\overline{p}\,y,$$

where x, y are signed strings over Σ_n. Let$\mathsf{Eh} = \{\mathsf{sh}_p \mid 1 \leq p \leq n\}$.

4. For each $p \geq 1$, $2 \leq p \leq n-1$, ed_p is defined as follows:

$$\mathsf{ed}_p(x\,p\,y\,(p-1)\,(p+1)\,z) = x\,y\,(p-1)\,p\,(p+1)\,z,$$
$$\mathsf{ed}_p(x\,(p-1)\,(p+1)\,y\,p\,z) = x\,(p-1)\,p\,(p+1)\,y\,z,$$
$$\mathsf{ed}_p(x\,(\overline{p+1})\,(\overline{p-1})\,y\,\overline{p}\,z) = x\,(\overline{p+1})\,\overline{p}\,(\overline{p-1})\,y\,z,$$
$$\mathsf{ed}_p(x\,\overline{p}\,y\,(\overline{p+1})\,(\overline{p-1})\,z) = x\,y\,(\overline{p+1})\,\overline{p}\,(\overline{p-1})\,z,$$

where x, y, z are signed strings over Σ_n. Let $\mathsf{Ed} = \{\mathsf{sd}_p \mid 1 \leq p \leq n\}$.

Example 10. *(a) Let* $\pi = 3\,\overline{4}\,\overline{5}\,6\,\overline{1}\,2$*. Then* $(\mathsf{eh}_1 \circ \mathsf{eh}_6 \circ \mathsf{eh}_4 \circ \mathsf{eh}_3)(\pi) = 3\,4\,5\,6\,1\,2$ *is a sorted permutation.*
(b) Let $\pi' = 3\,4\,5\,6\,\overline{1}\,\overline{2}$*. Then* π' *is not* $\mathsf{Eh} \cup \mathsf{Ed}$*-sortable. Indeed, no* eh *or* ed *operation is applicable to* π'*.*

Lemma 5. *For any signed permutation π, if* eh_p*(*ed_p*, resp.) is applicable to π, for some p, then* sh_p*(*sd_p*, resp.) is also applicable to π and* $\mathsf{eh}_p(\pi) = \mathsf{sh}_p(\pi)$ *(*$\mathsf{ed}_p(\pi) = \mathsf{sd}_p(\pi)$*, resp.)*

Note that Lemma 5 does not hold in the reverse direction: if $\pi = 1\,4\,2\,3$, then $\mathsf{sd}_2(\pi) = 1\,2\,3\,4$, while ed_2 is not applicable to π.

As illustrated by the next example, it turns out that the $\mathsf{Eh} \cup \mathsf{Ed}$-model is *non-deterministic*.

Example 11. *Let $\pi = 1\,3\,5\,2\,4$. Note that π has both sorting and non-sorting strategies in the elementary model. Indeed, $(\mathsf{ed}_2 \circ \mathsf{ed}_4)(\pi) = 1\,2\,3\,4\,5$, a sorted permutation. On the other hand, $\pi' = \mathsf{ed}_3(\pi) = 1\,5\,2\,3\,4$ is not sorted and no* eh *or* ed *operation is applicable to π'.*

Due to nondeterminism, deciding whether a given permutation is Eh-, Ed-, or $\mathsf{Eh} \cup \mathsf{Ed}$-sortable is difficult. A complete answer may be found in [10], based on an involved notion of dependency graph.

The simple model however is different. A permutation may indeed have several different strategies, but they are either all sorting, or all non-sorting. Moreover, [13] also defines a notion of *structure of a permutation* and notes that the results obtained after applying these strategies, though different, *have the same structure*. In this way, deciding whether or not a given permutation π is Sh-, Sd-, or $\mathsf{Sh} \cup \mathsf{Sd}$-sortable is easy: simply apply operations from the desired set in an arbitrary order; if the final blocked permutation is sorted, then the answer is 'yes', otherwise the answer is 'no': there are no sorting strategies for π.

Example 12. *(a) The permutation $\pi_1 = 4\overline{6}71\overline{2}35$ has several sorting strategies. Here are some of them:*

$$\pi_1^{(1)} = \mathsf{sd}_5 \circ \mathsf{sh}_6 \circ \mathsf{sh}_1(\pi_1) = 4567123,$$
$$\pi_1^{(2)} = \mathsf{sd}_5 \circ \mathsf{sh}_6 \circ \mathsf{sh}_2(\pi_1) = 4567123,$$
$$\pi_1^{(3)} = \mathsf{sd}_4 \circ \mathsf{sh}_6 \circ \mathsf{sh}_2(\pi_1) = 6712345,$$
$$\pi_1^{(4)} = \mathsf{sh}_6 \circ \mathsf{sh}_1 \circ \mathsf{sd}_4(\pi_1) = 6712345.$$

(b) The permutation $\pi_2 = 13685724$ has several unsuccessful strategies. Here are some of them:

$$\pi_2^{(1)} = \mathsf{sd}_2 \circ \mathsf{sd}_7(\pi_2) = 12367854,$$
$$\pi_2^{(2)} = \mathsf{sd}_2 \circ \mathsf{sd}_6(\pi_2) = 12385674,$$
$$\pi_2^{(3)} = \mathsf{sd}_3 \circ \mathsf{sd}_7(\pi_2) = 18567234,$$
$$\pi_2^{(4)} = \mathsf{sd}_3 \circ \mathsf{sd}_6(\pi_2) = 18567234.$$

7 Fourth complexity measure: parallelism

The previous three measures of complexity all deal with *sequential* compositions of operations leading to the assembly of a given gene. We introduce in this section a fourth measure of complexity dealing with more general *parallel assemblies* of genes.

A systematic study of parallelism for gene assembly has been initiated in [15]. We only consider in this paper a graph-based presentation of parallelism, although a string-based study is also possible, see [15].

Intuitively, a set of operations can be applied in parallel to a gene pattern if only if each operation's applicability is independent of the other's. In other words, a number of operations can be applied in parallel to a gene pattern if they can be (sequentially) applied in any order to that gene pattern. Note that this is consistent with how parallelism and concurrency are defined in computer science.

E.g., the C_2 gene of S.nova described by the MDS sequence $M_1M_2M_3M_4$ requires three Ld operations. The three Lds can be applied independently of each other and so, they can be applied in parallel. Also, the micronuclear gene R_1 of S.nova described by the MDS sequence $M_1M_2M_3M_4M_5M_6$, requires five Ld operations, and all of them can be applied at once. Consequently, its parallel complexity is one, the same as gene C_2.

Parallelism can be defined in terms of signed graphs as follows.

Definition 5 ([15])**.** *Let $S \subseteq \mathsf{Ld} \cup \mathsf{Hi} \cup \mathsf{Dlad}$ be a set of k rules and let $G = (V, E, \sigma)$ be a signed graph. We say that the rules in S can be applied in parallel to G if for any ordering $\varphi_1, \varphi_2, \ldots, \varphi_k$ of S, the composition $\varphi_k \circ \cdots \circ \varphi_1$ is applicable to G.*

The following result provides a simple criterium for two rules to be applicable in parallel.

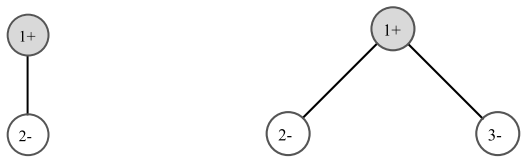
Lemma 6 ([15])**.** *Let $G = (V, E, \sigma)$ be a signed graph and let $\varphi, \psi \in \mathsf{Ld} \cup \mathsf{Hi} \cup \mathsf{Dlad}$ be two rules applicable to G with $\mathsf{dom}(\varphi) \cap \mathsf{dom}(\psi) = \emptyset$.*

(i) If $\varphi \in \mathsf{Ld}$, then φ and ψ can be applied in parallel to G.

(ii) If $\varphi = \mathsf{hi}_p$ with $p \in V$, then φ and ψ can be applied in parallel to G if and only if $N_G(p) \cap \mathsf{dom}(\psi) = \emptyset$.

(iii) If $\varphi, \psi \in \mathsf{Dlad}$, then φ and ψ can applied in parallel to G if and only if the subgraph of G induced by $\mathsf{dom}(\varphi) \cup \mathsf{dom}(\psi)$ is not isomorphic to either C_4 or D_4.

According to the definition, if a set of rules is applicable in parallel to a signed graph, then any composition of these rules is applicable to that graph. This definition does not require that the result of applying different compositions of rules must be the same. However, it can be proved that this is indeed the case.



(a) (b)

Figure 6: (a) A graph with parallel complexity two; (b) A graph with parallel complexity three.

Lemma 7 ([15])**.** *If $\varphi, \psi \in \mathsf{Ld} \cup \mathsf{Hi} \cup \mathsf{Dlad}$ are applicable in parallel to the signed graph G, then $\varphi(\psi(G)) = \psi(\varphi(G))$.*

The general case follows now easily from Lemma 7.

Theorem 8 ([15])**.** *Let G be a signed graph and let $S \subseteq \mathsf{Ld} \cup \mathsf{Hi} \cup \mathsf{Dlad}$ be a set of rules applicable in parallel to G. Then for any two compositions φ, φ' of the rules in S, $\varphi(G) = \varphi'(G)$.*

Based on Theorem 8 we can now define the notion of parallel complexity.

Definition 6. *Let G be a signed graph. If $S \subseteq \mathsf{Ld} \cup \mathsf{Hi} \cup \mathsf{Dlad}$ is a set of rules applicable in parallel to G, then we say that S is applicable to G and we denote by S(G) the graph obtained as a result of applying to G any sequential composition of the rules in S.*

If $S_1, S_2, \ldots, S_k \subseteq \mathsf{Ld} \cup \mathsf{Hi} \cup \mathsf{Dlad}$ are disjoint sets of rules, $S_i \cap S_j = \emptyset$, for $i \neq j$, we say that $S_k \circ \ldots \circ S_1$ is applicable to G if S_i is applicable to $(S_{i-1} \circ \ldots \circ S_1)(G)$, for all $1 \leq i \leq k$. If $(S_k \circ \ldots \circ S_1)(G) = \emptyset$, then we say that $S_k \circ \ldots \circ S_1$ is a parallel reduction strategy for G. We say that the parallel complexity of $S = S_k \circ \ldots \circ S_1$ is $\mathcal{C}_4(S) = k$.

We define the parallel complexity $\mathcal{C}_4(G)$ of G as follows:

$$\mathcal{C}_4(G) = \min\{\mathcal{C}_4(S) \mid \ S \text{ is a parallel reduction strategy for } G\}.$$

Example 13. *(a) Any discrete graph can be reduced in one parallel step.*

(b) The smallest graph with parallel complexity two is shown in Fig. 6(a).

(c) The smallest graph with parallel complexity three is shown in Fig. 6(b).

Example 14. *Let G be the signed overlap graph associated with actin I gene in S. nova, illustrated in Fig. 7. There are only 6 different maximal parallel strategies to reduce G:*

$S_1 = \{\mathsf{ld}_7, \mathsf{hi}_3\} \circ \{\mathsf{hi}_2, \mathsf{ld}_4, \mathsf{dlad}_{5,6}, \mathsf{dlad}_{8,9}\}$;
$S_2 = \{\mathsf{ld}_6, \mathsf{hi}_8, \mathsf{hi}_9\} \circ \{\mathsf{hi}_2, \mathsf{hi}_3, \mathsf{ld}_4, \mathsf{dlad}_{5,7}\}$;
$S_3 = \{\mathsf{ld}_6, \mathsf{hi}_3\} \circ \{\mathsf{hi}_2, \mathsf{ld}_4, \mathsf{dlad}_{5,7}, \mathsf{dlad}_{8,9}\}$;
$S_4 = \{\mathsf{ld}_7, \mathsf{hi}_8, \mathsf{hi}_9\} \circ \{\mathsf{hi}_2, \mathsf{hi}_3, \mathsf{ld}_4, \mathsf{dlad}_{5,6}\}$;
$S_5 = \{\mathsf{ld}_5, \mathsf{hi}_3\} \circ \{\mathsf{hi}_2, \mathsf{ld}_4, \mathsf{dlad}_{6,7}, \mathsf{dlad}_{8,9}\}$;
$S_6 = \{\mathsf{ld}_5, \mathsf{hi}_8, \mathsf{hi}_9\} \circ \{\mathsf{hi}_2, \mathsf{hi}_3, \mathsf{ld}_4, \mathsf{dlad}_{6,7}\}$.

Note that there are 3060 sequential strategies to reduce this graph (and assemble the gene), see [6] – the reason for this difference is that many sequential strategies coincide modulo commutation of some rules. Those rules may be applied in parallel.

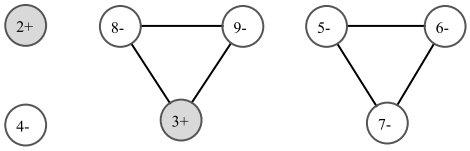


Figure 7: The signed overlap graph associated with string $3\,4\,4\,5\,6\,7\,5\,6\,7\,8\,9\,\overline{3}\,\overline{2}\,2\,8\,9$, both representing the structure of the micronuclear gene *actin I* in S.nova.

The following problem seems to be difficult: check whether or not a given set of rules can be applied in parallel to a given signed graph. In the next theorem we give a simple criterium in the case when at most two dlad rules are to be applied. Giving a general answer, for an arbitrary number of dlad rules, remains an open problem.

Theorem 9 ([15])**.** *Let G be a signed graph and $S \subseteq \mathsf{Ld} \cup \mathsf{Hi} \cup \mathsf{Dlad}$ a set of rules containing at most two* dlad*'s. Let P be the union of domains of rules in S with $P^+ = \{p \in P \mid \sigma(p) = +\}$, and $P^- = P \setminus P^+$. Then the rules in S can be applied in parallel to G if and only if the following conditions are satisfied:*

(i) The subgraph induced by P^+ is discrete. Moreover, there is no edge between vertices in P^+ and vertices in P^-.

(ii) The subgraph induced by P^- does not contain induced squares C_4 or diamonds D_4.

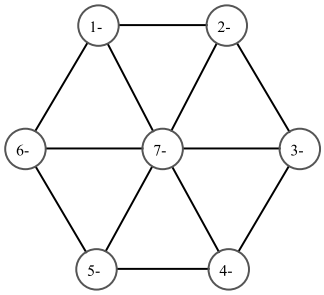


Figure 8: A negative graph with parallel complexity three.

Two conjectures were given in [15] regarding the parallel complexity of graphs. We proposed that any negative graph may be reduced in at most two parallel steps and that any graph may be reduced in at most four parallel steps. Revisiting these conjectures and based on a newly available gene assembly simulator, see [18], we give in the following counterexamples to both these conjectures. It is currently unknown if the parallel complexity of arbitrary graphs is bounded. Several classes of graphs are shown to have bounded parallel complexity in [9].

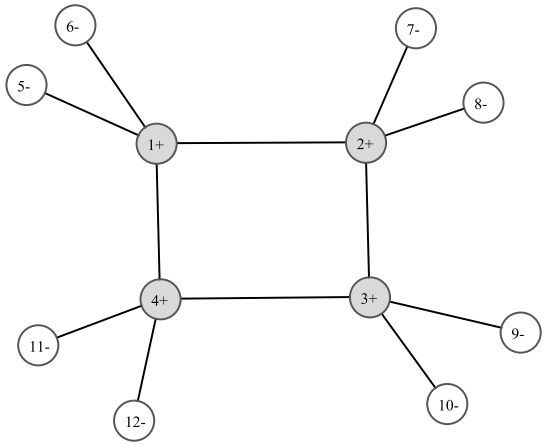


Figure 9: A graph with parallel complexity five.

Example 15. *(a) The negative graph G_3 depicted in Fig. 8 has parallel complexity three. (As a matter of fact, an automated search shows that this is a smallest such graph in terms of number of vertices.) Indeed, one three-step parallel strategy for G_3 is $\{\mathsf{ld}_6\} \circ \{\mathsf{dlad}_{5,7}\} \circ \{\mathsf{dlad}_{1,2}, \mathsf{dlad}_{3,4}\}$. Some straightforward analysis shows that no two-step or one-step parallel strategy for G_3 exists.*

(b) The graph G_5 depicted in Fig. 9 has parallel complexity 5. One 5-step parallel reduction for G_5 is $\{\mathsf{ld}_8, \mathsf{ld}_{12}\} \circ \{\mathsf{ld}_6, \mathsf{ld}_{10}, \mathsf{hi}_7, \mathsf{hi}_{11}\} \circ \{\mathsf{hi}_5, \mathsf{hi}_9\} \circ \{\mathsf{hi}_2, \mathsf{hi}_4\} \circ \{\mathsf{hi}_1, \mathsf{hi}_3\}$.

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References

[1] Brijder, R., Hoogeboom, H.J., Rozenberg, G., Reducibility of gene patterns in cliates using the breakpoint graph, to appear in *Theoret. Comput. Sci* (2006)

[2] Cavalcanti, A., Clarke, T.H., Landweber, L., MDS_IES_DB: a database of macronuclear and micronuclear genes in spirotrichous ciliates. *Nucleic Acids Research* **33** (2005) 396–398.

[3] Chang, W.J., Bryson, P.D., Liang, H., Shin, M.K., Landweber, L., The evolutionary origin of a complex scrambled gene. *Proceedings of the National Academy of Sciences of the US* **102**(42) (2005) 15149–15154

[4] Chang, W.J., Kuo, S., Landweber, L., A new scrambled gene in the ciliate *Uroleptus*. *Gene* (2006), to appear

[5] Ehrenfeucht, A., Harju, T., Petre, I., and Rozenberg, G. (2002) Characterizing the micronuclear gene patterns in ciliates. *Theory of Comput. Syst.* **35** pp 501–519

[6] Ehrenfeucht, A., Harju, T., Petre, I., Prescott, D. M., and Rozenberg, G. (2004) Computation in Living Cells: Gene Assembly in Ciliates, Springer

[7] Ehrenfeucht, A., Petre, I., Prescott, D. M., and Rozenberg, G., Universal and simple operations for gene assembly in ciliates. In: V. Mitrana and C. Martin-Vide (eds.) *Words, Sequences, Languages: Where Computer Science, Biology and Linguistics Meet*, Kluwer Academic, Dortrecht, (2001) pp. 329–342

[8] Ehrenfeucht, A., Prescott, D. M., and Rozenberg, G., Computational aspects of gene (un)scrambling in ciliates. In: L. F. Landweber, E. Winfree (eds.) *Evolution as Computation*, Springer, Berlin, Heidelberg, New York (2001) pp. 216–256

[9] Harju, T., Li, C., and Petre, I., Results on parallel reductions of signed overlap graphs, manuscript (2006)

[10] Harju, T., Petre, I., Rogojin, V., and Rozenberg, G., Simple operations for gene assembly. In: A. Carbone, N. A. Pierce (eds.) *DNA Computing: 11th International Workshop on DNA Computing*, Lecture Notes in Comput. Sci. **3892** (2006), 96 – 111.

[11] Harju, T., Petre, I., and Rozenberg, G., Modelling simple operations for gene assembly. In: J.Chen, N.Jonoska, G.Rozenberg (Eds.) *Nanotechnology: Science and Computation* (2006) 361–376

[12] Jahn, C. L., and Klobutcher, L. A., Genome remodeilng in ciliated protozoa. *Ann. Rev. Microbiol.* **56** (2000), 489–520.

[13] Langille, M., Petre, I. (2006) Simple gene assembly is deterministic. *Fundamenta Informaticae* IOS Press

[14] Harju, T., Petre, I., Rogojin, V., and Rozenberg, G. (2006), Simple operations for gene assembly, In: Proceedings of the 11th International Meeting on DNA-based computers DNA11 *Lecture Notes in Computer Science* (2006) Springer

[15] Harju, T., Li, C., Petre, I., and Rozenberg, G., Parallelism in gene assemby, In: Proceedings of the 10th International Meeting on DNA-based computers DNA 10, Milan, Italy, *Lecture Notes in Computer Science* **3384** (2005) 140–150

[16] Landweber, L. F., and Kari, L., The evolution of cellular computing: Nature's solution to a computational problem. In: *Proceedings of the 4th DIMACS Meeting on DNA-Based Computers*, Philadelphia, PA (1998) pp. 3–15

[17] Landweber, L. F., and Kari, L., Universal molecular computation in ciliates. In: L. F. Landweber and E. Winfree (eds.) *Evolution as Computation*, Springer, Berlin Heidelberg New York (2002)

[18] Petre, I., Skogman, S. (2006) Gene assembly simulator. `http://combio.abo.fi/simulator/simulator.php`

[19] Prescott, D. M., The DNA of ciliated protozoa. *Microbiol. Rev.* **58**(2) (1994) 233–267

[20] Prescott, D. M., DNA manipulations in ciliates. In: W.Brauer, H.Ehrig, J.Jarhumäki, A.Salomaa (eds.) *Formal and Natural Computing: essays dedicated to Grzegorz Rozenberg*, LNCS 2300, Springer (2002) 394–417

[21] Prescott, D. M., Ehrenfeucht, A., and Rozenberg, G., Molecular operations for DNA processing in hypotrichous ciliates. *Europ. J. Protistology* **37** (2001) 241–260

[22] Swanton, M.T., Heumann, J.M., Prescott, D.M., Gene-sized DNA molecules of the macronuclei in three species of hypotrichs: size distribution and absence of nicks. Chromosoma **77** (1980) 217–227

[23] Yao, M.C., Fuller, P., Xi, X., Programmed DNA Deletion As an RNA-Guided System of Genome Defense, *Science* 300 (2003) 1581–1584

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