Extending the Overlap Graph for Gene Assembly in Ciliates

Robert Brijder and Hendrik Jan Hoogeboom

Leiden Institute of Advanced Computer Science, Universiteit Leiden,
Niels Bohrweg 1, 2333 CA Leiden, The Netherlands,
rbrijder@liacs.nl

Abstract. Gene assembly is an intricate biological process that has been studied formally and modeled through string and graph rewriting systems. Recently, a restriction of the general (intramolecular) model, called simple gene assembly, has been introduced. This restriction has subsequently been defined as a string rewriting system. We show that by extending the notion of overlap graph it is possible to define a graph rewriting system for two of the three types of rules that make up simple gene assembly. It turns out that this graph rewriting system is less involved than its corresponding string rewriting system. Finally, we give characterizations of the ‘power’ of both types of graph rewriting rules. Because of the equivalence of these string and graph rewriting systems, the given characterizations can be carried over to the string rewriting system.

1 Introduction

Gene assembly is a highly involved process occurring in one-cellular organisms called ciliates. Ciliates have two both functionally and physically different nuclei called the micronucleus and the macronucleus. Gene assembly occurs during sexual reproduction of ciliates, and transforms a micronucleus into a macronucleus. This process is highly parallel and involves a lot of splicing and recombination operations – this is true for the stichotrichs group of ciliates in particular. During gene assembly, each gene is transformed from its micronuclear form to its macronuclear form.

Gene assembly has been extensively studied formally, see [1]. The process has been modeled as either a string or a graph rewriting system [2, 3]. Both systems are ‘almost equivalent’, and we refer to these as the general model. In [4] a restriction of this general model has been proposed. While this model is less powerful than the general model, it is powerful enough to allow each known gene [5] in its micronuclear form to be transformed into its macronuclear form. Moreover this model is less involved and therefore called the simple model. The simple model was first defined using signed permutations [4], and later proved...
equivalent to a string rewriting system [6]. The graph rewriting system of the
general model is based on overlap graphs. This system is an abstraction from
the string rewriting system in the sense that certain local properties within the
strings are lost in the overlap graph. Therefore overlap graphs are not suited for
the simple gene assembly model. In this paper we show that by naturally ex-
tending the notion of overlap graph we can partially define simple gene assembly
as a graph rewriting system. These extended overlap graphs form an abstraction
of the string model, and is some way easier to deal with. This is illustrated by
characterizing the power of two of the three types of recombination operations
that make up simple gene assembly. While this characterization is based on ex-
tended overlap graphs, due to its equivalence, it can be carried over to the string
rewriting system for simple gene assembly.

2 Background: Gene Assembly in Ciliates

In this section we very briefly describe the process of gene assembly. For a de-
tailed account of this process we refer to [1]. Gene assembly occurs in a group
of one-cellular organisms called ciliates. A characterizing property of ciliates is
that they have two both functionally and physically different nuclei called the
micronucleus (MIC) and the macronucleus (MAC). Each gene occurs both in the
MIC and in the MAC, however they occur in very different forms in the MIC
and the MAC. The MIC form of a gene consists of a number of DNA segments
$M_1, \ldots, M_\kappa$, called MDSs, which occur in some fixed permutation on a chromo-
some. The MDSs are separated by non-coding DNA segments. Moreover, each
MDS can occur inverted, i.e. rotated 180 degrees. For example, the gene in MIC
form encoding for the actin protein in ciliate sterkiella nova is given in Figure 1
(see [7, 5]). Notice that $M_2$ occurs inverted.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{mic_structure.png}
\caption{The structure of the MIC gene encoding for the actin protein in sterkiella nova.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{mac_structure.png}
\caption{The structure of a MAC gene consisting of $\kappa$ MDSs.}
\end{figure}
In the MAC form of the gene, the MDSs occur as a sequence \( M_1, \ldots, M_\kappa \) where each two consecutive MDSs overlap, see Figure 2. These shaded area’s represent the overlapping segments and are called **pointers**. Moreover, there are two sequences denoted by \( b \) and \( e \), which occur on \( M_1 \) and \( M_\kappa \) respectively, that indicate the beginning and ending of the gene. The sequences \( b \) and \( e \) are called **markers**. The process of gene assembly transforms the MIC into the MAC, thereby transforming each gene in the MIC form to the MAC form. Hence, for each gene the MDSs are ‘sorted’ and put in the right orientation (i.e., they do not occur inverted). This links gene assembly to the well-known theory of sorting by reversal [8].

It is postulated that there are three types of recombination operations that cut-and-paste the DNA to transform the gene from the MIC form to the MAC form. These operations are defined on pointers, so one can abstract from the notion of MDSs by simply considering the MIC gene as a sequence of pointers and markers, see Figure 3 corresponding to the gene in MIC form of Figure 1. The pointers are numbered according to the MDS they represent: the pointer on the left (right, resp.) of MDS \( M_i \) is denoted by \( i \) \((i + 1, \text{resp.)} \). Pointers or markers that appear inverted are indicated by a bar: hence pointers 2 and 3 corresponding to MDS \( M_2 \) appear inverted and are therefore denoted by \( \overline{2} \) and \( \overline{3} \) respectively. In the general model the markers are irrelevant, so in that case only the sequence of pointers is used.

![Fig. 3. Sequence of pointers and markers representing the gene in MIC form.](image)

### 3 Legal Strings with Markers

For an arbitrary finite alphabet \( A \), we let \( \bar{A} = \{ \overline{a} \mid a \in A \} \) with \( A \cap \bar{A} = \emptyset \). We use the ‘bar operator’ to move from \( A \) to \( \bar{A} \) and back from \( \bar{A} \) to \( A \). Hence, for \( p \in A \cup \bar{A} \), \( \overline{p} = p \). For a string \( u = x_1x_2 \cdots x_n \) with \( x_i \in A \), the **inverse** of \( u \) is the string \( \overline{u} = \overline{x_nx_{n-1}} \cdots \overline{x}_1 \). We denote the empty string by \( \lambda \).

We fix \( \kappa \geq 2 \), and define the alphabet \( \Delta = \{ 2, 3, \ldots, \kappa \} \) and the alphabet \( \Pi = \Delta \cup \Delta \). The elements of \( \Pi \) are called **pointers**. For \( p \in \Pi \), we define \( \|p\| \) to be \( p \) if \( p \in \Delta \), and \( \overline{p} \) if \( p \in \bar{\Delta} \), i.e., \( \|p\| \) is the ‘unbarred’ variant of \( p \). A **legal string** is a string \( u \in \Pi^* \) such that for each \( p \in \Pi \) that occurs in \( u \), \( u \) contains exactly two occurrences from \( \{ p, \overline{p} \} \).

Let \( M = \{ b, e \} \) with \( \Delta \cap \{ b, e \} = \emptyset \). The elements of \( M \) are called **markers**. We let \( \Xi = \Delta \cup \{ b, e \} \), and let \( \Psi = \Xi \cup \bar{\Xi} \). We define the morphism \( \text{rm} : \Psi^* \to \Pi^* \) as follows: \( \text{rm}(a) = a \), for all \( a \in \Pi \), and \( \text{rm}(m) = \lambda \), for all \( m \in M \cup \bar{M} \). We say that a string \( u \in \Psi^* \) is an **extended legal string** if \( \text{rm}(u) \) is a legal string and \( u \) has one occurrence from \( \{ b, \overline{b} \} \) and one occurrence from \( \{ e, \overline{e} \} \). We fix \( m \not\in \Psi \) and define for each \( q \in M \cup \bar{M} \), \( \|q\| = m \).
An extended legal string represents the sequence of pointers and markers of a gene in MIC form. Hence, the extended legal string corresponding to Figure 3 is 3445675679c32b289. The legal string corresponding to this figure is 344567567932289 (without the markers). Legal strings are considered in the general model since markers are irrelevant there.

The domain of a string \( u \in \Psi^* \) is \( \text{dom}(u) = \{ \| p \| \mid p \text{ occurs in } u \} \). Note that \( m \in \text{dom}(v) \) for each extended legal string \( v \). Let \( q \in \text{dom}(u) \) and let \( q_1 \) and \( q_2 \) be the two occurrences of \( u \) with \( \| q_1 \| = \| q_2 \| = q \). Then \( q \) is positive in \( u \) if exactly one of \( q_1 \) and \( q_2 \) is in \( \Xi \) (the other is therefore in \( \Xi^c \)). Otherwise, \( q \) is negative in \( u \).

Example 1. String \( u = 24b4e2 \) is an extended legal string since \( \text{rm}(u) = 2442 \) is a legal string. The domain of \( u \) is \( \text{dom}(u) = \{ m, 2, 4 \} \). Now, \( m \) and 2 are positive in \( u \), and 4 is negative in \( u \).

Let \( u = x_1x_2 \cdots x_n \) be an (extended) legal string with \( x_i \in \Xi \) for \( 1 \leq i \leq n \), and let \( p \in \text{dom}(u) \). The \( p \)-interval of \( u \) is the substring \( x_ix_{i+1} \cdots x_j \) where \( i \) and \( j \) with \( i < j \) are such that \( \| x_i \| = \| x_j \| = p \).

Next we consider graphs. A signed graph is a graph \( G = (V, E, \sigma) \), where \( V \) is a finite set of vertices, \( E \subseteq \{ \{ x, y \} \mid x, y \in V, x \neq y \} \) is a set of (undirected) edges, and \( \sigma : V \rightarrow \{ +, - \} \) is a signing, and for a vertex \( v \in V \), \( \sigma(v) \) is the sign of \( v \). We say that \( v \) is negative in \( G \) if \( \sigma(v) = - \), and \( v \) is positive in \( G \) if \( \sigma(v) = + \). A signed directed graph is a graph \( G = (V, E, \sigma) \), where the set of edges are directed \( E \subseteq V \times V \). For \( e = (v_1, v_2) \in E \), we call \( v_1 \) and \( v_2 \) endpoints of \( e \). Also, \( e \) is an edge from \( v_1 \) to \( v_2 \).

4 Simple and General String Pointer Rules

Gene Assembly has been modeled using three types of string rewriting rules on legal strings. These types of rules correspond to the types of recombination operations that perform gene assembly. We will recall the string rewriting rules now— together they form the string pointer reduction system, see [2, 1]. The string pointer reduction system consists of three types of reduction rules operating on legal strings. For all \( p, q \in \Pi \) with \( \| p \| \neq \| q \| \):

- the string negative rule for \( p \) is defined by \( \text{snr}_p(u_1pu_2) = u_1u_2 \);
- the string positive rule for \( p \) is defined by \( \text{spr}_p(u_1pu_2pu_3) = u_1u_2u_3 \);
- the string double rule for \( p, q \) is defined by \( \text{sd}_p,q(u_1pu_2pu_3pu_4pu_5) = u_1u_4u_3u_2u_5 \),

where \( u_1, u_2, \ldots, u_5 \) are arbitrary (possibly empty) strings over \( \Pi \).

We now recall a restriction to the above defined model. The motivation for this restricted model is that it is less involved but still general enough to allow for the successful assembling of all known experimental obtained micronuclear genes [5]. The restricted model, called simple gene assembly, was originally defined on signed permutations, see [4, 9]. The model can also be defined as string rewriting
rules (in an equivalent way) as done for the general model above. This model is defined and proven equivalent in [6], and we recall it here. It turns out that it is necessary to use extended legal strings adding symbols $b$ and $e$ to legal strings.

The simple string pointer reduction system consists of three types of reduction rules operating on extended legal strings. For all $p, q \in \Pi$ with $\|p\| \neq \|q\|$: 

- the string negative rule for $p$ is defined by $\text{snr}_p(u_1pu_2) = u_1u_2$ as before,
- the simple string positive rule for $p$ is defined by $\text{sspr}_p(u_1pu_2\bar{p}u_3) = u_1\bar{u}_2u_3$, where $|u_2| = 1$, and
- the simple string double rule for $p, q$ is defined by $\text{ssdr}_{p,q}(u_1pu_2u_3pu_4) = u_1u_2u_3$,

where $u_1, u_2,$ and $u_3$ are arbitrary (possibly empty) strings over $\Psi$. Note that the string negative rule is not changed, and that the simple version of the string positive rule requires $|u_2| = 1$, while the simple version of the string double rule requires $u_2 = u_4 = \lambda$ (in the string double rule definition).

**Example 2.** Let $u = 524453626b3e$ be an extended legal string. Then within the simple string pointer reduction system only $\text{snr}_4$ and $\text{sspr}_6$ are applicable to $u$. We have $\text{sspr}_6(u) = 52445326b3e$. Within the string pointer reduction system also $\text{spr}_5$ and $\text{spr}_2$ are applicable to $u$. We will use $u$ (in addition to a extended legal string $v$, which is defined later) as a running example.

A composition $\varphi = \rho_n \cdots \rho_2 \rho_1$ of string pointer rules $\rho_i$ is a reduction of (extended) legal string $u$, if $\varphi$ is applicable to (i.e., defined on) $u$. A reduction $\varphi$ of legal string $u$ is successful if $\varphi(u) = \lambda$, and a reduction $\varphi$ of extended legal string $u$ is successful if $\varphi(u) \in \{be, eb, \bar{e}b, b\bar{e}\}$. A successful reduction corresponds to the transformation using recombination operations of a gene in MIC form to MAC form. It turns out that not every extended legal string has a successful reduction using only simple rules – take e.g. $2342b3e$.

**Example 3.** In our running example, $\varphi = \text{sspr}_3 \text{sspr}_2 \text{sspr}_5 \text{snr}_4 \text{sspr}_6$ is a successful reduction of $u$, since $\varphi(u) = b\bar{e}$. All rules in $\varphi$ are simple.

## 5 Extended Overlap Graph

The general string pointer reduction system has been made more abstract by replacing legal strings by so-called overlap graphs, and replacing string rewriting rules by graph rewriting rules. The obtained model is called the graph pointer reduction system. Unfortunately, this model is not fully equivalent to the string pointer reduction system since the string negative rule is not faithfully simulated. Also, overlap graphs are not suited for a graph model for simple gene assembly. We propose an extension to overlap graphs that allows one to faithfully model the string negative rule and the simple string positive rule using graphs and graph rewriting rules. First we recall the definition of overlap graph.
**Definition 4.** The overlap graph for (extended) legal string $u$ is the signed graph $(V, E, \sigma)$, where $V = \text{dom}(u)$ and for all $p, q \in \text{dom}(u)$, $\{p, q\} \in E$ iff $q \in \text{dom}(p')$ and $p \in \text{dom}(q')$ where $p'$ ($q'$, resp.) is the $p$-interval ($q$-interval) of $u$. Finally, for $p \in \text{dom}(u)$, $\sigma(p) = +$ iff $p$ is positive in $u$.

![Diagram](image)

**Fig. 4.** The overlap graph of $u$ from Example 5.

**Example 5.** Consider again extended legal string $u = 5\overline{2}4_{45}6\overline{2}6b\overline{3}e$. Then the overlap graph $G_u$ of $u$ is given in Figure 4.

We say that $p, q \in \text{dom}(u)$ overlap if there is an edge between $p$ and $q$ in the overlap graph of $u$. We now define the extended overlap graph.

**Definition 6.** The extended overlap graph for (extended) legal string $u$ is the signed directed graph $(V, E, \sigma)$, denoted by $G_u$, where $V = \text{dom}(u)$ and for all $p, q \in \text{dom}(u)$, there is an edge $(q, p)$ iff $q$ or $\overline{q}$ occurs in the $p$-interval of $u$. Finally, for $p \in \text{dom}(u)$, $\sigma(p) = +$ iff $p$ is positive in $u$.

Notice first that between any two (different) vertices $p$ and $q$ we can have the following possibilities:

1. There is no edge between them. This corresponds to $u = u_1pu_2pu_3qu_4qu_5$ or $u = u_1qu_2qu_3pu_4pu_5$ for some (possibly empty) strings $u_1, \ldots, u_5$ and possibly inversions of the occurrences of $p$ and $q$ in $u$.

2. There are exactly two edges between them, which are in opposite direction. This corresponds to the case where $p$ and $q$ overlap in $u$.

3. There is exactly one edge between them. If there is an edge from $p$ to $q$, then this corresponds to the case where $u = u_1qu_2pu_3pu_4qu_5$ for some (possibly empty) strings $u_1, \ldots, u_5$ and possibly inversions of the occurrences of $p$ and $q$ in $u$.

As usual, we represent two directed edges in opposite direction (corresponding to case number two above) by one undirected edge. In the remaining we will use this notation and consider the extended overlap graph as having two sets of edges: undirected edges and directed edges. In general, we will call graphs with a special vertex $m$ and having both undirected edges and directed edges simple marked graphs.
Example 7. Consider again extended legal string $u = 5244536263\overline{e}$. Then the extended overlap graph $G_u$ of $u$ is given in Figure 5. Also, the extended overlap graph of $v = \overline{4234}e3\overline{b}$ is given in Figure 6.

The undirected graph obtained by removing the directed edges is denoted by $[G_u]$. This is the ‘classical’ overlap graph of $u$, cf. Figures 4 and Figure 5. On the other hand, the directed graph obtained by removing the undirected edges is denoted by $[[G_u]]$. This graph represents the proper nesting of the $p$-intervals in the legal string.

6 Simple Graph Rules

We will now define two types of rules for simple marked graphs $\gamma$. Each of these rules transform simple marked graph of a certain form into another simple marked graph. We will subsequently show that in case $\gamma$ is the extended overlap of a legal strings, then these rules faithfully simulate the effect of the snr and sspr rules on the underlying legal string.

Definition 8. Let $\gamma$ be a simple marked graph. Let $p$ be any vertex of $\gamma$ not equal to $m$.

- The graph negative rule for $p$, denoted by $\text{gnr}_p$, is applicable to $\gamma$ if $p$ is negative, there is no undirected edge $e$ with $p$ as an endpoint, and there is no directed edge from a vertex to $p$ in $\gamma$. The result is the simple marked graph $\text{gnr}_p(\gamma)$ obtained from $\gamma$ by removing vertex $p$ and removing all edges connected to $p$. The set of all graph negative rules is denoted by Gnr.

- The simple graph positive rule for $p$, denoted by $\text{sgpr}_p$, is applicable if $p$ is positive, there is exactly one undirected edge $e$ with $p$ as an endpoint, and there is no directed edge from a vertex to $p$ in $\gamma$. The result is the simple
marked graph \(\text{sgpr}_p(\gamma)\) obtained from \(\gamma\) by removing vertex \(p\), removing all edges connected to \(p\), and flipping the sign of the other vertex \(q\) of \(e\) (i.e. changing the sign of \(q\) to + if it is − and to − if it is +). The set of all simple graph positive rules is denoted by \(\text{sGpr}\).

These rules are called simple graph pointer rules.

Remark 9. The \(\text{sgpr}\) rule is much simpler than the \(\text{gpr}\) for ‘classical’ overlap graphs. One does not need to compute the ‘local complement’ of the set of adjacent vertices. Obviously, this is because the simple rule allows only a single pointer in the \(p\)-interval.

Example 10. Rules \(\text{gnr}_4\) and \(\text{sgpr}_6\) are the only applicable rules on the simple marked graph \(\gamma = \mathcal{G}_u\) of Figure 5. Simple marked graph \(\text{sgpr}_6(\gamma)\) is depicted in Figure 7.

Similar as for strings, a composition \(\varphi = \rho_n \cdots \rho_2 \rho_1\) of graph pointer rules \(\rho_i\) is a reduction of simple marked graph \(\gamma\), if \(\varphi\) is applicable to (i.e., defined on) \(\gamma\). A reduction \(\varphi\) of \(\gamma\) is successful if \(\varphi(\gamma)\) is the graph having only vertex \(m\) where \(m\) is negative. For \(S \subseteq \{\text{Gnr}, \text{sGpr}\}\), we say that \(\gamma\) is successful in \(S\) if there is a successful reduction of \(\gamma\) using only graph pointer rules from \(S\).

Example 11. In our running example, \(\varphi = \text{sgpr}_3 \text{sgpr}_2 \text{sgpr}_5 \text{gnr}_4 \text{sgpr}_6\) is a successful reduction of \(\mathcal{G}_u\).

We now show that these two types of rules faithfully simulate the string negative rule and the simple string positive rule.

Lemma 12. Let \(u\) be a legal string and let \(p \in \Pi\). Then \(\text{snr}_p\) is applicable to \(u\) iff \(\text{gnr}_{\|p\|}\) is applicable to \(\mathcal{G}_u\). In this case, \(\mathcal{G}_{\text{snr}_p(u)} = \text{gnr}_{\|p\|}(\mathcal{G}_u)\).

Proof. We have \(\text{snr}_p\) is applicable to \(u\) iff \(u = u_1 u_2\) for some strings \(u_1\) and \(u_2\) iff \(\|p\|\) is negative in \(u\) and the \(\|p\|\)-interval is empty iff \(\|p\|\) is negative in \(\mathcal{G}_u\) and there is no undirected edge with \(\|p\|\) as endpoint and there is no directed edge to \(\|p\|\) iff \(\text{gnr}_{\|p\|}\) is applicable to \(\mathcal{G}_u\).

In this case, \(\mathcal{G}_{\text{snr}_p(u)}\) is obtained from \(\mathcal{G}_u\) by removing vertex \(\|p\|\) and the edges connected to \(\|p\|\), hence \(\mathcal{G}_{\text{snr}_p(u)}\) is equal to \(\text{gnr}_{\|p\|}(\mathcal{G}_u)\).
The previous lemma is illustrated as a commutative diagram in Figure 8. The next lemma shows that a similar diagram can be made for the simple string positive rule.

**Lemma 13.** Let $u$ be a legal string and let $p \in \Pi$. Then $\text{sspr}_p$ is applicable to $u$ iff $\text{sgpr}_{\parallel p\parallel}$ is applicable to $G_u$. In this case, $G_{\text{sspr}_p(u)} = \text{sgpr}_{\parallel p\parallel}(G_u)$.

**Proof.** We have $\text{sspr}_p$ is applicable to $u$ iff $u = u_1pu_2\bar{p}u_3$ for some strings $u_1$, $u_2$, and $u_3$ with $|u_2| = 1$ iff $\parallel p\parallel$ is positive in $u$ (or equivalently in $G_u$) and there is exactly one undirected edge $e$ with $\parallel p\parallel$ as endpoint and there is no directed edge with $\parallel p\parallel$ as endpoint iff $\text{sgpr}_{\parallel p\parallel}$ is applicable to $G_u$.

In this case, $G_{\text{sspr}_p(u)}$ is obtained from $G_u$ by removing vertex $\parallel p\parallel$, removing all edges connected to $\parallel p\parallel$, and flipping the sign of the other vertex of $e$. Hence $G_{\text{sspr}_p(u)}$ is equal to $\text{gnr}_{\parallel p\parallel}(G_u)$. \(\square\)

**Example 14.** In our running example, one can easily verify that the extended overlap graph of $\text{sspr}_6(u) = 5244532\bar{b}3\bar{c}$ is equal to graph $\text{sgpr}_6(G_u)$ given in Figure 7.

One may be wondering at this point why we have not defined the simple graph double rule. To this aim, consider extended legal string $w = b234234\bar{c}$. Note that $\text{ssdr}_{2,3}$ and $\text{ssdr}_{3,4}$ are applicable to $w$, but $\text{ssdr}_{2,4}$ is not applicable to $w$. However, this information is lost in $G_w$ – applying the isomorphism that interchanges vertices 2 and 3 in $G_w$ obtains us $G_w$ again, see Figure 9. Thus, given only $G_w$ it is impossible to deduce applicability of the simple graph double rule.

To successfully define a simple graph double rule, one needs to retain information on which pointers are next to each other, and therefore different concepts are required. However, this concept would require that the linear representation...
of the pointers in an extended legal string is retained. Hence, string representations are more natural compared to graph representations.

The next lemma shows that simple marked graphs that are extended overlap graphs are quite restricted in form. We will use this restriction in the next section.

**Lemma 15.** Let \( u \) be a legal string. Then \( \langle G_u \rangle \) is acyclic and transitively closed.

**Proof.** There is a (directed) edge from \( p \) to \( q \) in \( \langle G_u \rangle \) iff the \( p \)-interval is completely contained in the \( q \)-interval of \( u \). A nesting relation of intervals is acyclic and transitive. \( \square \)

**Remark 16.** We have seen that \( G_u \) is the overlap graph of \( u \). Not every graph is an overlap graph – a characterization of which graphs are overlap graphs are shown in [10]. Hence, both \( \langle G_u \rangle \) and \( G_u \) are restricted in form compared to graphs in general. \( \square \)

### 7 Characterizing Successfulness

In this section we characterize successfulness of simple marked graphs in \( S \subseteq \{ \text{Gnr}, \text{sGpr} \} \). First we consider the case \( S = \{ \text{Gnr} \} \).

**Remark 17.** In the general (not simple) model, which has different graph pointer rules and is based on overlap graphs, successfulness in \( S \) has been characterized for those \( S \) which includes the graph negative rules (note that these rules are different from the graph negative rules defined here) – the cases where \( S \) does not contain the graph negative rules remain open. \( \square \)

**Theorem 18.** Let \( \gamma \) be a simple marked graph. Then \( \gamma \) is successful in \( \{ \text{Gnr} \} \) iff each vertex of \( \gamma \) is negative, \( \gamma \) has no undirected edges, and \( \gamma \) is acyclic.

**Proof.** Since \( \langle \gamma \rangle = \gamma \) is acyclic, there is a linear ordering \((p_1, p_2, \ldots, p_n)\) of the vertices of \( \gamma \) such that if there is an edge from \( p_i \) to \( p_j \), then \( i < j \). The result now follows by the definition of \( \text{gnr} \). In this case, linear ordering \((p_1, p_2, \ldots, p_n)\) corresponds to a successful reduction \( \varphi = \text{gnr}_{p_{n-1}} \cdots \text{gnr}_{p_2} \text{gnr}_{p_1} \) of \( \gamma \). \( \square \)

Using Lemma 15, more can be said if \( \gamma = G_u \) for some legal string \( u \).

**Corollary 19.** Let \( \gamma = G_u \) for some legal string \( u \). Then \( \gamma \) is successful in \( \{ \text{Gnr} \} \) iff each vertex of \( \gamma \) is negative and \( \gamma \) has no undirected edges. In this case, \( \gamma \) is the transitive closure of a forest, where edges in the forest are directed from children to their parents.

Next we turn to the case \( S = \{ \text{sGpr} \} \).

**Theorem 20.** Let \( \gamma \) be a simple marked graph. Then \( \gamma \) is successful in \( \{ \text{sGpr} \} \) iff the following conditions hold:

1. \( \langle \gamma \rangle \) is a (undirected) tree,
2. for each vertex \( v \) of \( [\gamma] \), the degree of \( v \) is even iff \( v \) is negative in \( \gamma \), and

3. the graph obtained by replacing each undirected edge in \( \gamma \) by a directed edge from the child to the parent in tree \([\gamma]\) with root \( m \) is acyclic.

**Proof.** Proof sketch. It can be verified that each of both statements hold iff there is a linear ordering \((p_1, p_2, \ldots, p_n)\) of the vertices of \( \gamma \) such that \( p_n = m \), and for each \( p_i \) with \( i \in \{1, \ldots, n\} \) the following holds:

1. the number of undirected edges from vertices \( p_j \) with \( j < i \) to \( p_i \) is even iff \( p_i \) is positive in \( \gamma \),

2. if \( i < n \), then there is exactly one undirected edge between \( p_i \) and another vertex \( p_j \) with \( j > i \), and

3. there is no directed edge from a vertex \( p_j \) to \( p_i \) with \( j > i \).

In this case, linear ordering \((p_1, p_2, \ldots, p_n)\) corresponds to a successful reduction \( \varphi = \text{sgpr}_{p_{n-1}} \cdots \text{sgpr}_{p_2} \text{sgpr}_{p_1} \) of \( \gamma \).

**Example 21.** Consider again extended legal string \( u \) of Example 7 with its extended overlap graph \( \mathcal{G}_u \) given in Figure 5. Then by Theorem 20, \( \mathcal{G}_u \) is *not* successful in \( \{sGpr\} \), since condition 1 is violated – \([\gamma]\) is not a tree as it has two connected components.

Reconsider now extended legal string \( v \) of Example 7 with its extended overlap graph \( \mathcal{G}_v \) given in Figure 6. By Theorem 20, \( \mathcal{G}_v \) is successful in \( \{sGpr\} \). According to the proof of Theorem 20, \((2, 4, 3, m)\) is a linear ordering of the vertices corresponding to a successful (graph) reduction \( \varphi = \text{sgpr}_3 \text{sgpr}_2 \text{sgpr}_1 \) of \( \mathcal{G}_v \). By Lemma 13, this in turn corresponds to a successful (string) reduction \( \varphi' \) of \( v \) – one can verify that we can take \( \varphi' = \text{sspr}_3 \text{sspr}_2 \). Moreover, by the proof of Theorem 20, linear ordering \((4, 2, 3, m)\) does not correspond to a successful reduction of \( \mathcal{G}_v \) (or of \( v \)).

Finally, we consider the case \( S = \{Gnr, sGpr\} \).

**Theorem 22.** Let \( \gamma \) be a simple marked graph. Then \( \gamma \) is successful in \( \{Gnr, sGpr\} \) iff all of the conditions of Theorem 20 hold, except that in condition 1) \([\gamma]\) is a forest instead of a tree, and in condition 3) for each tree in the forest we can identify a root, where \( m \) is one such root, such that the graph obtained by replacing each undirected edge \( e \) in \( \gamma \) by a directed edge from the child to the parent in the tree to which \( e \) belongs, is acyclic.

**Proof.** Proof sketch. It can be verified that each of both statements hold iff there is an ordering \((p_1, p_2, \ldots, p_n)\) of the vertices of \( \gamma \) such that for each \( p_i \) with \( i \in \{1, \ldots, n\} \), condition 1) holds and either conditions 2) and 3) hold in the proof of Theorem 20 or there is no edge (directed or not) between a vertex \( p_j \) to \( p_i \) with \( j > i \).

Again, in this case, linear ordering \((p_1, p_2, \ldots, p_n)\) corresponds to a successful reduction \( \varphi \) of \( \gamma \) where the vertices corresponding to roots in forest \([\gamma]\) (except \( m \)) are used in \( \text{gnr} \) rules, while the other vertices are used in \( \text{sgpr} \) rules. \(\Box\)
Example 23. Consider again extended legal string $u$ of Example 7 with its extended overlap graph $G_u$ given in Figure 5. By Theorem 22, $G_u$ is successful in $\{G_{nr}, sG_{pr}\}$. By the proof of Theorem 22, $(6, 4, 5, 2, 3, m)$, $(4, 6, 5, 2, 3, m)$, and $(4, 5, 6, 2, 3, m)$ are the linear orderings of the vertices that correspond to successful reductions of $G_u$ in $\{G_{nr}, sG_{pr}\}$. Moreover, in each case vertex 4 corresponds to the $\text{gnr}_4$ rule while the other pointers correspond to $\text{sgpr}$ rules.

8 Discussion

We have shown that we can partially model simple gene assembly based on a natural extension of the well-known concept of overlap graph. The model is partial in the sense that the simple double string rule does not have graph rule counterpart. Within this partial model we characterize which micronuclear genes can be successfully assembled using 1) only graph negative rules, 2) only simple graph positive rules, and 3) both of these types of rules. These results carry over to the corresponding simple string pointer rules.

What remains is a graph rule counterpart of the simple double string rule. However such a counterpart would require different concepts since the overlap graph or any natural extension does not capture the requirement that pointers $p$ and $q$ (in the rule) are next to each other in the string.

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