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Abstract. We introduce a new bio-inspired operation called a *site-directed deletion* motivated from site-directed mutagenesis performed by enzymatic activity of DNA polymerase: Given two strings x and y, a site-directed deletion partially deletes a substring of x guided by the string y that specifies which part of a substring can be deleted. We study a few decision problems with respect to the new operation and examine the closure properties of the (iterated) site-directed deletion operations. We, then, define a site-directed deletion-closed (and -free) language L and investigate its decidability properties when L is regular or context-free.

Keywords: Bio-inspired operations · Site-directed deletion · Closure properties · Decidability · Automata theory

1 Introduction

A deletion operation is one of the well-established operations [4, 5, 10, 12, 14]. We focus on a variant of deletion, *site-directed deletion mutagenesis*, which can be performed *in vitro*, whose goal is to create a deletion on a given gene sequence under enzymatic activities [19]. The activity under DNA polymerase requires a single-stranded DNA segment (called a template) and primers designated to form a partially double-stranded region with the template. In particular, primers complementarily bind to a given template excluding a region to be deleted and extend to the end of the template forming a double-stranded DNA segment.

From the formal language viewpoint, Head et al. [11] and Kari and Kopecki [15] considered a splicing system inspired by DNA recombination under enzymatic activities. Kari and Thierrin [16] modeled a contextual deletion that occurs in a context sensitive manner: It deletes a string v from a string u only if certain contexts are presented in a given set $\mathcal{C}$. Furthermore, Daley and McQuillan [3], and Enaganti et al. [6, 7] studied operations inspired by the action of DNA polymerase enzyme that plays a major role in DNA recombination. Recently, Cho et al. [2] studied the site-directed insertion operation[6] that inserts a string y at a specific position of x according to the matching outfix of x.

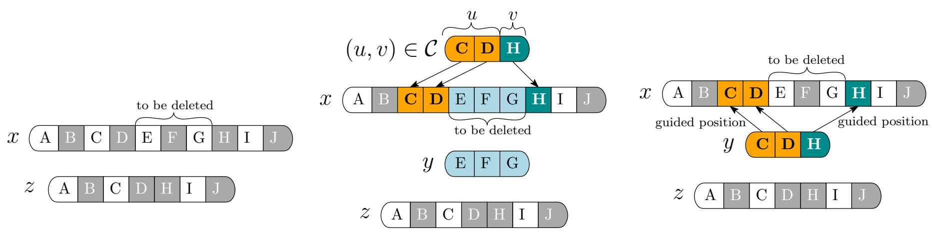
We define a new operation called *site-directed deletion* that *partially* deletes a substring from a given string x based on a guide string y that specifies which part of a substring can be deleted. In other words from a biological viewpoint, x is a template and y is a guide primer with information of the region to be deleted. We say that a site-directed deletion occurs on x if a site-directed deletion of x and y partially deletes a substring from x guided by y. This is analogous to the contextual deletion operation studied by Kari and Thierrin [16]. A contextual deletion of a string y from x occurs only if certain contexts exist in a given set $\mathcal{C}$ of contexts. Roughly speaking, contextual deletion specifies y—a string to be deleted—according to a set $\mathcal{C}$ whereas site-directed deletion deletes a substring from x according to y. Fig. 1 illustrates the difference among ordinary, contextual and site-directed deletions.

A site-directed deletion successfully generates a desired DNA sequence *in vitro* if a primer is designed to complementarily interact with a template which can result in the desired DNA sequence. From the formal language viewpoint, we consider a question of deciding whether or not the template and primer interact each other as desired. We show linear time algorithms to determine whether or not the site-directed deletion ($\mathbb{SDD}$) of two strings x and y is not empty and whether or not the site-directed deletion of x and y results in a given string z. We show that regular languages are closed under site-directed deletion, whereas context-free languages are not, and we give a context-free language L which is not closed under iterated site-directed deletion. Finally, we consider decidability for *sdd*-closedness and *sdd*-freeness on a language L.

2 Preliminaries

Let Σ be a finite alphabet of characters and Σ^* be the set of all strings over Σ. The symbol $\emptyset$ denotes the empty language and the symbol λ denotes the empty string. Given a string x, $|x|$ denotes the length of x and x^R denotes the reversal of x. A string $x = x[1]x[2] \cdots x[n] \in \Sigma^n$ is a finite sequence of n symbols. For $1 \leq i \leq j \leq n$, we denote $x[i : j] = x[i]x[i+1] \cdots x[j]$. Given a string $x = uv$, where $u, v \in \Sigma^*$, we say that u is a *prefix* of x and v is a *suffix* of x. Given a string $y = uwv$, where $u, w, v \in \Sigma^*$, we say that (u, v) is an outfix of y. If $u \neq \lambda$ and $v \neq \lambda$, then we say that (u, v) is a *non-trivial outfix* of y. Sometimes (in particular, when talking about the site-directed deletion operation) we call an outfix (u, v) simply a string uv (when it is known from the context what

[6] Site-directed insertion has earlier been considered under the name *outfix-guided insertion* [2].



(a) Ordinary deletion on x

(b) Contextual deletion of x and y with $\mathcal{C}$

(c) Site-directed deletion on x and y

Fig. 1: Examples of ordinary deletion, contextual deletion and site-directed deletion. (a) An ordinary deletion of x results in a string z removing a substring EFG. The resulting string z is one of many possible deletion outputs. (b) A contextual deletion of x and y with a set $\mathcal{C} \subseteq \Sigma^* \times \Sigma$ of contexts results in a string z if there exists an infix uyv of x, where $(u, v) \in \mathcal{C}, uv = CDH$ and $y = EFG$. (c) A site-directed deletion of two strings x and y—in other words, a template and a primer, respectively—results in a string z if $y = CDH$ is the catenation of an outfix (CD, H) of a substring $CDEFGH$ of x. The string z is the only possible site-directed deletion output when the guide string is CDH.

the components u and v are). Given a language L, we use $\overline{L}$ to denote the complement of L; $\overline{L} = \Sigma^* \setminus L$.

A nondeterministic finite automaton (NFA) is a tuple $A = (\Sigma, Q, \delta, q_0, F)$, where Σ is an input alphabet, Q is a finite set of states, $\delta \colon Q \times \Sigma \to 2^Q$ is a multivalued transition function, $q_0 \in Q$ is the initial state and $F \subseteq Q$ is a set of final states. In the usual way, δ is extended as a function $Q \times \Sigma^* \to 2^Q$ and the language recognized by A is $L(A) = \{w \in \Sigma^* \mid \delta(q_0, w) \cap F \neq \emptyset\}$. The automaton A is a deterministic finite automaton (DFA) if δ is a single valued partial function. A sequence $q_0, q_1, \ldots, q_n$ of states denotes a *path*. A string w is accepted by A if there is a labeled path from q_0 to $q_n \in F$, and we call this path an *accepting path*.

We now introduce a new operation called a *site-directed deletion* that formalizes an enzymatic reaction called the site-directed deletion mutagenesis.

Definition 1. *The site-directed deletion from a string x by y is*

$$x \overset{sdd}{\leftarrow} y = \{x_1 uvx_2 \mid x = x_1 uwvx_2, y = uv, u \neq \lambda \text{ and } v \neq \lambda\}.$$

We say that y is a deletion guide *of x if $x \overset{sdd}{\leftarrow} y \neq \emptyset$.*

We use the notation $x \overset{[y]}{\Rightarrow} z$ to indicate that z is obtained by $x \overset{sdd}{\leftarrow} y$. Note that a string x is obtained from itself by selecting the outfix to be x itself, in other words $x \overset{[x]}{\Rightarrow} x$, when $|x| \geq 2$. The site-directed deletion operation can be extended to languages as follows:

$$L_1 \overset{sdd}{\leftarrow} L_2 = \bigcup_{w_i \in L_i, i=1,2} w_1 \overset{sdd}{\leftarrow} w_2.$$

We define the *iterated site-directed deletion* on a language, which is an iterated version of the site-directed deletion operation.

Definition 2. *Site-directed deletion* $\mathbb{SDD}$ *of* L *is inductively defined as*

$$\mathbb{SDD}^{(0)}(L) = L, \text{ and } \mathbb{SDD}^{(i+1)}(L) = \mathbb{SDD}^{(i)}(L) \overset{sdd}{\leftarrow} \mathbb{SDD}^{(i)}(L), i \geq 0.$$

The iterated site-directed deletion $\mathbb{SDD}^*$ *of* L *is*

$$\mathbb{SDD}^*(L) = \bigcup_{i=0}^{\infty} \mathbb{SDD}^{(i)}(L).$$

3 Decidability of Site-Directed Deletion on Strings

We first consider the question of determining whether or not a string y can be a deletion guide of a string x; in other words, is $x \overset{sdd}{\leftarrow} y \neq \emptyset$? The site-directed deletion mutagenesis deliberately creates mutant DNA sequences using designed DNA sequences—a source DNA sequence to be partially deleted, a DNA sequence that contains the target mutant region and designed primers that indicate a starting point of synthesis. Given two strings x and y, a DNA sequence that contains a target mutant region is a deletion-guide y of a source DNA sequence x. Note that site-directed deletion cannot create a mutant DNA sequence if a DNA sequence containing a mutant region does not interact with a source DNA sequence. Thus, it is important to check whether or not designed DNA sequences can interact each other. We present a linear time algorithm that determines whether or not $x \overset{sdd}{\leftarrow} y \neq \emptyset$.

Theorem 1. *Given two strings* x *and* y*, we can determine whether or not* $x \overset{sdd}{\leftarrow} y \neq \emptyset$ *in* $O(n)$ *time, where* $|x| = n, |y| = m$ *and* $m \leq n$*.*

Proof. Our goal is to determine whether or not there exist two substrings u, v of x such that $y = uv$. In other words, we look for a pentuple (g, h, i, j, k) that satisfies the following conditions:

1. $y[1 : g] = x[j{-}g{+}1 : j]$
2. $y[m{-}h{+}1 : m] = x[k : k{+}h{-}1]$
3. $j - g + i \leq k + h - m + i$
4. $i \leq g$
5. $m - i \leq h$

Suppose there exists a pentuple (g, h, i, j, k) that satisfies all five conditions. Also suppose that there exists g' such that $y[1 : g'] = x[j-g'+1 : j]$ and $g' < g$. If $i \le g'$, then $i \le g$ and (g, h, i, j, k) also satisfies the five conditions. Therefore, for each index in x, we need to find the longest prefix of y that ends at j and the longest suffix of y that starts from k. We check the required conditions using a modified Aho-Corasick algorithm [1].

Using all prefixes of y and y^R as two dictionaries, we construct two Aho-Corasick automata $\mathcal{A}_P$ and $\mathcal{A}_S$ where the output function from a state directs to the longest matching pattern. Since we use prefixes of a string as a dictionary, it is straightforward that these modified automata can be built in $O(m)$ time using $O(m)$ space. The result is two lists of pairs (j, g), (k, h) of indices from $\mathcal{A}_P$ and $\mathcal{A}_S$, where the first element indicates the ending (starting, respectively) index and the second elements indicates the length of the longest matched pattern.

In condition 3, the inequality indicates that $m \le (g - j) + (k + h)$. From conditions 4 and 5, we know that $m \le g+h$. On the other hand, if two conditions $m \le (g - j) + (k + h)$ and $m \le g + h$ are satisfied, then we can find $i = g$ that satisfies conditions 3, 4 and 5.

We store j and k using g and h as indices respectively. Moreover, to check the condition $m \le (g - j) + (k + h)$, we only need to keep the smallest j and the largest k for same g and h, respectively. Constructing such two arrays $j[g]$ and $k[h]$ requires $O(n)$ time and $O(m)$ space. Note that we have $k[h] > k[h+1]$, since an occurrence of $y[m-h+2 : m]$ that starts at index k guarantees an occurrence of $y[m-h+1 : m]$ that starts at index $k+1$. Then we start from $g = 1$ and $h = m$, and check if $j[g] \le k[h] + 1$. If the condition is satisfied, we know that $m \le g+h$ and $m \le g - j[g] + k[h] + h$. If the condition is not satisfied, we increase g and decrease h by 1 continuously and check the condition. The runtime to compare arrays is $O(m)$, and the total runtime becomes $O(n)$. □

We next consider a problem of determining whether or not $z \in x \overset{sdd}{\leftarrow} y$ for given strings x, y and z. The biological implication of $z \in x \overset{sdd}{\leftarrow} y$ is that a site-directed deletion mutagenesis by PCR (Polymerase Chain Reaction) successfully generates a desired DNA sequence, which is denoted by z. We show that the problem can be solved in linear time.

Theorem 2. *Given three strings $x, y, z \in \Sigma^*$, we can determine whether or not $z \in x \overset{sdd}{\leftarrow} y$ in $O(n)$ time, where $n = |x|$ and $|x| \ge |z| \ge |y| \ge 2$.*

Proof. Suppose that there exist three strings $x[1 : n], y[1 : m]$ and $z[1 : l]$ such that $z \in x \overset{sdd}{\leftarrow} y$, where $n, m, l \ge 2$. Then there are decompositions of strings $x = x_1uwvx_2, y = uv$ and $z = x_1uvx_2$, where $x_1, x_2, w \in \Sigma^*$ and $u, v \ne \lambda$. We decide whether or not $z \in x \overset{sdd}{\leftarrow} y$ as follows:

1. **Scanning x and z for finding common prefix x_1u and suffix vx_2:** We scan both ends of x and z until a mismatch occurs—in other words, we find the longest matching prefix and suffix between $x[1 : n]$ and $z[1 : l]$. Let $z[1 : i] = x[1 : i]$ be the longest matching prefix, and $z[l-j+1 :$

$m] = x[n-j+1 : n]$ be the longest matching suffix, where $i, j > 1$. When $i+1 = l-j+1$, we show that the catenation of the longest matching prefix and suffix $z[1 : i] \cdot z[l-j+1 : l]$ equals to $z = x_1uvx_2$.

2. **Identifying search-range of z with respect to y**: For the case $i > l-j+1$, which implies that a suffix of $z[1 : i]$ is a prefix of $z[l-j+1 : l]$, we check an occurrence of y within $z[l-(j+m)+2 : i+m-1]$. We call the range from $l-(j+m)+2$ to $i+m-1$ a *search-range* on z. The search-range is calculated from the assumption that the longest prefix $z[1 : i]$ contains u as an infix, and the longest suffix $z[l-j+1 : l]$ contains v as an infix, where the catenation of u and v becomes y for $|u|, |v| \geq 1$. We consider a case where y matches with $z[l-j-m+2 : i+m-1]$. Suppose $y = z[l-j-m+2 : l-j+1]$. Then, $z[l-j+1 : l] = x[n-j+1 : n]$ denotes vx_2 and there exists a corresponding prefix $x[1 : l-j]$, which denotes x_1u, and we show that $z \in x \overset{sdd}{\leftarrow} y$.
3. **Finding y from search-range of z**: We can prove for all the other cases when y appears within the search-range using a similar argument. We check the existence of y within the search-range in $O(m)$ time using the KMP algorithm [17]. Thus, the total runtime is $O(n)$.

□

4 Closure Properties and Decidability on Languages

4.1 Closure Properties of Site-Directed Deletion on Languages

We show that regular languages are closed under the site-directed deletion, whereas context-free languages are not. Context-free languages are closed under site-directed deletion with regular languages. We give a context-free language L such that $\mathbb{SDD}^*(L)$ is not context-free.

Theorem 3. *If L_1 and L_2 are regular, then $L_1 \overset{sdd}{\leftarrow} L_2$ is regular.*

Proof. Let L_1 be recognized by an NFA $A = (Q_A, \Sigma, \delta, q_0, F_A)$ and L_2 be recognized by an NFA $B = (Q_B, \Sigma, \gamma, p_0, F_B)$. Let $\widetilde{Q_A} = \{\widetilde{q} \mid q \in Q_A\}$ such that Q_A is disjoint with $\widetilde{Q_A}$. We construct an NFA $C = (Q_C, \Sigma, \omega, s, F_C)$ recognizing all strings of $L_1 \overset{sdd}{\leftarrow} L_2$, where $Q_C = Q_A \times (\{\clubsuit, \heartsuit\} \cup Q_B) \cup \widetilde{Q_A} \times Q_B$, $s = (q_0, \clubsuit)$ and $F = \{(q, \heartsuit) \mid q \in F_A\} \cup \{(\widetilde{q}, p) \mid q \in F_A, p \in F_B\}$. Let α be an arbitrary symbol of Σ. We set the following transitions of ω:

(i) for $q \in Q_A, \omega((q, \clubsuit), \alpha) = \{(q', \clubsuit \mid q' \in \delta(q, \alpha)\} \cup \{(q', p') \mid q' \in \delta(q, \alpha) \text{ and } p' \in \gamma(p_0, \alpha)\}$,

(ii) for $q \in Q_A$ and $p \in Q_B$, $\omega((q, p), \alpha) \in \{(q', p') \mid q' \in \delta(q, \alpha) \text{ and } p' \in \gamma(p, \alpha)\} \cup \{(\widetilde{q''}, p') \mid q' \in \delta(q, \alpha), q'' \in \delta(q', \lambda), p' \in \gamma(p, \alpha) \text{ and } \widetilde{q} \in \widetilde{Q_A}\}$,

(iii) for $\widetilde{q} \in \widetilde{Q_A}$ and $p \in Q_B$, $\omega((\widetilde{q}, p), \alpha) \in \{(\widetilde{q'}, p') \mid q' \in \delta(q, \alpha) \text{ and } p' \in \gamma(p, \alpha)\} \cup Z_q$, where

$$Z_q = \begin{cases} \{(q', \heartsuit) \mid q' \in \delta(q, \alpha)\} & \text{if } p \in F_B, \\ \emptyset & \text{if } p \notin F_B, \end{cases}$$

(iv) for $q \in Q_A$, $\omega((q, \heartsuit), \alpha) = \{(q', \heartsuit) \mid q' \in \delta(q, \alpha)\}$.

All transitions not listed above are undefined. We show that $L(A) \overset{sdd}{\leftarrow} L(B) \subseteq L(C)$. Let $x_1uwvx_2 \in L(A)$ and $uv \in L(B)$, where $x_1, x_2, w \in \Sigma^*$ and $u, v \in \Sigma^+$. The construction is based on the idea that C uses the states $(q, \clubsuit)$ of C to process the prefix x_1, the states (q, p) to process the following substring u, the states $(\widetilde{q}, p)$ to process the substring v and the states $(q, \heartsuit)$ to process the following substring x_2. Let $\mathcal{P}_A(x)$ be an accepting path $q_0, \ldots, q_{x_1}, \ldots, q_u, \ldots, q_w, \ldots, q_u, \ldots, q_{x_2}$ of $x \in L(A)$ and $\mathcal{P}_B(y)$ be an accepting path $p_0, \ldots, p_u, \ldots, p_v$ of $y \in L(B)$. From the initial state $(q_0, \clubsuit)$ of C the transition rule (i) allows C to simulate the path $q_0, \ldots, q_{x_1}$ of $\mathcal{P}_A(x_1uwvx_2)$ reaching a state $(q_{x_1}, \clubsuit)$. When the first symbol α of u appears, the transition rule (ii) allows C to simulate both paths $q_{x_1}, \ldots, q_u$ of $\mathcal{P}_A$ and $p_0, \ldots, p_u$ of $\mathcal{P}_B$. Note that the transition (ii) allows C to nondeterministically enter the state $(\widetilde{q_w}, p_u)$ which shows that C simulates the path $q_u, \ldots, q_w$ of $\mathcal{P}_A$ reading w. Then, the transition (iii) allows C to simulate both paths $q_w, \ldots, q_v$ of $\mathcal{P}_A$ and $p_u, \ldots, p_v$ of $\mathcal{P}_B$, and C enter the state $(\widetilde{q_v}, p_v)$. Note that C enter the state $(q_v, \heartsuit)$ if $p_v \in F_B$. The transition (iv) allows C to simulate the remaining path $q_v, \ldots, q_{x_2}$ of $\mathcal{P}_A$, and simulation ends in an accepting state $(q_{x_2}, \heartsuit)$, where $q_{x_2} \in F_A$.

We now show that if C recognizes a string x_1uvx_2, then A recognizes a string x_1uwvx_2 and B recognizes a string uv, in other words; $L(C) \subseteq L(A) \overset{sdd}{\leftarrow} L(B)$. We decompose an accepting path $(q_0, \clubsuit), \ldots, (q_{x_2}, \heartsuit)$ for a string $z = x_1uvx_2 \in L(C)$ into four paths $\mathcal{P}_1, \mathcal{P}_2, \mathcal{P}_3$ and $\mathcal{P}_4$ such that

$$\underbrace{(q_0, \clubsuit), \ldots, (q_{x_1}, \clubsuit)}_{\mathcal{P}_1}, \underbrace{(q_{x_1}, p_s), \ldots, (q_u, p_u)}_{\mathcal{P}_2},$$

$$\underbrace{(\widetilde{q_w}, p_u), \ldots, (\widetilde{q_v}, p_v)}_{\mathcal{P}_3}, \text{ and } \underbrace{(q_v, \heartsuit), \ldots, (q_{x_2}, \heartsuit)}_{\mathcal{P}_4}.$$

Path $\mathcal{P}_1$ shows that there is a path $q_0, \ldots, q_{x_2}$ in $\mathcal{P}_A$ that recognizes a string x_1. Path $\mathcal{P}_2$ shows that there exist paths $q_{x_1}, \ldots, q_u$ of $\mathcal{P}_A$ and $p_0, \ldots, p_u$ of $\mathcal{P}_B$ that recognize a string u, and path $\mathcal{P}_3$ shows that there exist paths $\widetilde{q_u}, \ldots, \widetilde{q_v}$ of $\mathcal{P}_A$ and $p_u, \ldots, p_v$ of $\mathcal{P}_B$ that recognize a string v. Note that based on the transitions (ii), C nondeterministically enters a state $(\widetilde{q}, p_u)$ from (q_u, p_u), then, C simulates a path $q, \ldots, q_w$ of $\mathcal{P}_A$ reaching $(\widetilde{q_w}, p_u)$. Path $\mathcal{P}_4$ shows that C only simulates a path $q_v, \ldots, q_{x_2}$ of $\mathcal{P}_A$, and the simulation ends on the final state $(q_{x_2}, \heartsuit)$. It implies that if C accepts a string x_1uvx_2, then there exist accepting paths on A and B that recognize strings x_1uwvx_2 and uv. □

Theorem 4. *There exist context-free languages L_1 and L_2 such that $L_1 \overset{sdd}{\leftarrow} L_2$ is not context-free.*

Proof. Consider two context-free languages $L_1 = \{a^n\$b^n\$\%c^m\# \mid n, m \geq 1\}$ and $L_2 = \{\$b^n\$c^n\# \mid n \geq 1\}$. Then, the language $(L_1 \overset{sdd}{\leftarrow} L_2) \cap (a^*\$b^*\$c^*\#) = \{a^n\$b^n\$c^n\# \mid n \geq 1\}$ is not context-free. □

The proof of Theorem 5 is omitted due to the limitation on the number of pages.

Theorem 5. *Given a context-free language L_1 and a regular language L_2, $L_1 \overset{sdd}{\leftarrow} L_2$ and $L_2 \overset{sdd}{\leftarrow} L_1$ are context-free.*

Theorem 6. *There exists a context-free language L such that $\mathbb{SDD}^*(L)$ is not context-free.*

Proof. Let $\Sigma = \{a, b, c, \$, \%, \#\}$ and define $L = L_1 \cup L_2$, where $L_1 = \{\#a^n\$b^n\$\%c^m\# \mid n, m \geq 1\}$ and $L_2 = \{\$b^n\$c^n\# \mid n \geq 1\}$. Denote $M_1 = \#a^+\$b^+\$\%c^+\#$, $M_1' = \#a^+\$b^+\$c^+\#$ and $M_2 = \$b^+\$c^+\#$ (and thus $L_1 \subseteq M_1$ and $L_2 \subseteq M_2$). We observe the following properties:

(i) It is not possible to use a string $y \in M_1 \cup M_1'$ as a deletion guide for a string $x \in M_2$ (because strings of M_2 contain only one occurrence of $\#$).
(ii) Using a string in $M_1 \cup M_1'$ as a deletion guide for a string in $M_1 \cup M_1'$ can be done only in a trivial deletion step (that produces the original deletion guide).
(iii) Using a string in M_2 as a deletion guide for a string in M_2 can be done only in a trivial deletion step.
(iv) Using a string in M_2 as a deletion guide for strings of $M_1 \cup M_1'$ always produces a string in M_1'.

We explain observation (iv) below. The first three observations follow directly from the definition of site-directed deletion.

Consider $z \in x \overset{sdd}{\leftarrow} y$ where $x \in M_1 \cup M_1'$ and $y \in M_2$. The end-marker $\#$ of y must be matched with the last symbol of x and the two occurrences of \$ in y must be matched with the two occurrences of \$ in the string x. This means that z must be the result of deleting from $x \in M_1$ the symbol % and zero or more symbols c and, if $x \in M_1'$, z must be the result of deleting from x zero or more symbols c. Not all c's can be deleted because the string $y \in M_2$ contains at least one c. Thus, in both cases $z \in M_1'$.

Since trivial deletion steps produce only the original deletion guide, observations (i)–(iv) imply that

$$L \cup \mathbb{SDD}^{(1)}(L) = L_1 \cup L_2 \cup (L_1 \overset{sdd}{\leftarrow} L_2),$$

and in particular,

$$L \cup \mathbb{SDD}^{(1)}(L) = L_1 \cup L_2 \cup \{\#a^n\$b^n\$c^n\# \mid n \geq 1\}. \tag{1}$$

The last equality is verified as in the proof of Theorem 4. Note that our current construction is a modification of the construction used in the proof of Theorem 4.

Now $L_1 \overset{sdd}{\leftarrow} L_2 \subseteq M_1'$ and none of the deletion steps (i)–(iv) produces new strings of M_2 (the steps (ii) and (iii) must use a trivial deletion step). This means that using induction on k and the observations (i)–(iv), we get that

$$\bigcup_{i=0}^{k+1} \mathbb{SDD}^{(i)}(L) = L_1 \cup L_2 \cup (\mathbb{SDD}^{(k)}(L) \overset{sdd}{\leftarrow} L_2),$$

and, thus, $\mathbb{SDD}^*(L)$ consists of L and strings obtained from L by iterated deletions that all use a string in L_2 as a guide.

When using a string $y \in L_2$ as a deletion guide for a string $x \in \{\#a^n\$b^n\$c^n\# \mid n \geq 1\}$, the only possibility is to match y with a suffix of x, because the string x has the same number of b's and c's and the string y has the same property. When y is matched with a suffix of x, the result of the deletion is just x. This means that iterating the deletions does not produce anything new and $\mathbb{SDD}^*(L) = L \cup \mathbb{SDD}^{(1)}(L)$ and from equation (1) we get that $\mathbb{SDD}^*(L) \cap \#a^+\$b^+\$c^+\# = \{\#a^n\$b^n\$c^n\# \mid n \geq 1\}$ which is not context-free. $\square$

Closure of regular languages under iterated site-directed deletion remains open.

4.2 Decidability of *sdd*-closed and *sdd*-free Languages

A language L is *sdd-closed* if $L \overset{sdd}{\leftarrow} L \subseteq L$, which implies that L does not generate a string that is not in L under the site-directed deletion operation. A language L is *sdd-free* if $x \overset{sdd}{\leftarrow} y = \emptyset$, where $x, y \in L$ and $x \neq y$. Note that a string in L is obtained only from itself by site-directed deletion if L is *sdd*-free.

We show that it is decidable whether or not a given language L is *sdd*-closed when L is regular and is undecidable when L is context-free.

Theorem 7. *Given a regular language L, it is decidable in polynomial time whether or not L is sdd-closed.*

Theorem 8. *For a given context-free language L, determining whether or not L is sdd-closed is undecidable.*

Proof. Let $((u_1, \ldots, u_n), (v_1, \ldots, v_n))$ be an instance of the *Post's Correspondence Problem* (PCP) [18], where $u_i, v_i \in \Sigma^*$ and $1 \leq i \leq n$. A solution for the instance is a sequence of integers $(i_1, \ldots, i_k)$, $i_j \in \{1, \ldots, n\}$, $j = 1, \ldots, k$ such that $u_{i_1} \cdots u_{i_k} = v_{i_1} \cdots v_{i_k}$. It is well known that the PCP is undecidable [18]. Let $\Sigma' = \Sigma \cup \{\#, \%, ¢\}$.

We consider a context-free language $L = L_1 \cup L_2$, where

$$L_1 = \{¢\$i_1 i_2 \cdots i_r \# u_{i_r}^R u_{i_{r-1}}^R \cdots u_{i_1}^R \#\%\# v_{j_1} v_{j_2} \cdots v_{j_s} \# j_s j_{s-1} \cdots j_1 \$¢\} \text{ and}$$

$$L_2 = \{\$i_1 i_2 \cdots i_r \# w \#\# w^R \# i_r i_{r-1} \cdots i_1 \$\}$$

for $w \in \Sigma^*, 1 \leq r, s,$ and $1 \leq i_r, j_s \leq n$. Then, L has two types of string

$$w_1 = ¢\$i_1 i_2 \cdots i_r \# u_{i_r}^R u_{i_{r-1}}^R \cdots u_{i_1}^R \#\%\# v_{i_1} v_{i_2} \cdots v_{i_r} \# i_r i_{r-1} \cdots i_1 \$¢ \in L_1, \text{ and}$$

$$w_2 = \$i_1 i_2 \cdots i_r \# u_{i_r}^R u_{i_{r-1}}^R \cdots u_{i_1}^R \#\# u_{i_1} u_{i_2} \cdots u_{i_r} \# i_r i_{r-1} \cdots i_1 \$ \in L_2.$$

Here, $w = u_{i_r}^R u_{i_{r-1}}^R \cdots u_{i_1}^R$ and it follows $w^R = u_{i_1} u_{i_2} \cdots u_{i_{r-1}}$. We claim that the PCP has a solution if and only if L is not *sdd*-closed, and we prove both implications of the claim.

- "If the PCP has a solution, then L is not *sdd*-closed": Suppose that $(i_1, \ldots, i_r)$ is a solution for the PCP, which implies $u_{i_1} u_{i_2} \cdots u_{i_r} = v_{i_1} v_{i_2} \cdots v_{i_r}$. We decompose $w_1 = x_1 u w v x_2$ and $w_2 = uv$, where $x_1, x_2 = ¢, w = \%$, and

$$u = \$ i_1 i_2 \cdots i_r \# u_{i_r}^R u_{i_{r-1}}^R \cdots u_{i_1}^R \#, v = \# u_{i_1} u_{i_2} \cdots u_{i_{r-1}} \# i_r i_{r-1} \cdots i_1 \$.$$

Then, the claim holds that L is not *sdd*-closed since

$$w_1 \overset{sdd}{\leftarrow} w_2 = ¢\$ i_1 i_2 \cdots i_r \# w \# \# w^R \# i_r i_{r-1} \cdots i_1 \$ ¢,$$

which is not in L—there is no string contains all occurrences of symbols $¢, \$$ and $\#$ without the symbol $\%$.
- "If the PCP has no solution, then L is *sdd*-closed": We consider two strings

$$w_1 = ¢\$ i_1 i_2 \cdots i_r \# u_{i_r}^R u_{i_{r-1}}^R \cdots u_{i_1}^R \# \% \# v_{j_1} v_{j_2} \cdots v_{j_s} \# j_s j_{s-1} \cdots j_1 \$ ¢ \in L_1 \text{ and}$$

$$w_2 = \$ i_1 i_2 \cdots i_r \# u_{i_r}^R u_{i_{r-1}}^R \cdots u_{i_1}^R \# \# u_{i_1} u_{i_2} \cdots u_{i_r} \# i_r i_{r-1} \cdots i_1 \$ \in L_2.$$

We consider the following four possible cases of $L \overset{sdd}{\leftarrow} L$, and show that there is a contradiction of the assumption.

(i) $L_1 \overset{sdd}{\leftarrow} L_1$: A string $w_1 \in L_1$ itself is a deletion guide of w_1, which is obtained by $w_1 \overset{[w_1]}{\Rightarrow} w_1$, and the claim holds that $L \overset{sdd}{\leftarrow} L \subseteq L$.

(ii) $L_2 \overset{sdd}{\leftarrow} L_2$: Similarly, a string $w_2 \in L_2$ is obtained by $w_2 \overset{[w_2]}{\Rightarrow} w_2$, and it implies that $L \overset{sdd}{\leftarrow} L \subseteq L$.

(iii) $L_2 \overset{sdd}{\leftarrow} L_1$: A string $w_1 \in L_1$ begins and ends with the symbol $¢$, which does not occur in $w_2 \in L_2$, and it also implies that $L \overset{sdd}{\leftarrow} L \subseteq L$.

(iv) $L_1 \overset{sdd}{\leftarrow} L_2$: Suppose that $w_2 \in L_2$ is a deletion guide of $w_1 \in L_1$ such that $w_1 = x_1 u w v x_2$ and $w_2 = uv$, where $u, n \neq \lambda$. Since w_1 begins and ends with $¢$, and w_2 does not contain occurrences of $¢$, in the decomposition of w_1, the first symbol $¢$ must be in x_1 and the last symbol $¢$ must be in x_2. Similarly, in the decomposition of w_1, $\%$ must be in w since w_2 does not contain $\%$. As an outfix (u, v) of a substring of w_1 should be $w_2 = uv$ when w_2 is a deletion-guide of w_1, it follows that

$$u = \$ i_1 i_2 \cdots i_r \# u_{i_r}^R u_{i_{r-1}}^R \cdots u_{i_1}^R \# \text{ and } v = \# v_{j_1} v_{j_2} \cdots v_{j_s} \# j_s j_{s-1} \cdots j_1 \$,$$

where $r = s, i_r = j_r$ and $v_{j_1} v_{j_2} \cdots v_{j_s} = u_{i_1} u_{i_2} \cdots u_{i_{r-1}} = (u_{i_r}^R u_{i_{r-1}}^R \cdots u_{i_1}^R)^R$. Thus, $(i_1, \ldots, i_r)$ is a solution for the PCP—a contradiction.

Therefore, it is undecidable whether or not L is *sdd*-closed since the PCP is undecidable. □

We also show that it is decidable whether or not L is *sdd*-free in polynomial time when L is regular, and it is undecidable when L is context-free. The insertion of y into x in an arbitrary place in x is defined as the set of strings $x \overset{ins}{\leftarrow} y = \{x_1 y x_2 \mid x = x_1 x_2 \text{ and } x_1, x_2 \in \Sigma^*\}$ [9, 13]. We use the closure property of regular languages under the insertion operation.

Lemma 1. *Consider $L \subseteq \Sigma^*$. Then L is sdd-free if and only if*

$$L \cap [\Sigma^+(L \overset{ins}{\leftarrow} \Sigma^*)\Sigma^* \cup \Sigma^*(L \overset{ins}{\leftarrow} \Sigma^+)\Sigma^* \cup \Sigma^*(L \overset{ins}{\leftarrow} \Sigma^*)\Sigma^+] = \emptyset.$$

When L is regular the language on the left-side of the equation of Lemma 1 is regular. Theorem 9 is proved using Lemma 1.

Theorem 9. *Given an NFA A, we can decide in polynomial time whether or not $L(A)$ is sdd-free.*

Theorem 10. *It is undecidable whether or not a context-free language L is sdd-free.*

We now consider the question of determining whether or not *sdd*-closed and *sdd*-free languages preserve their properties. We establish the following results which characterize *sdd*-closed and *sdd*-free languages with respect to basic operations such as intersection, union, catenation and complement. Notice that the basic operations are widely used in molecular biology, biochemistry and pharmacology, for instance, a complement under enzymatic activities inhibits an enzymatic activation that causes a disease or inflammation [8].

Theorem 11. *sdd-closed languages are closed under intersection and complement but not closed under union and catenation.*

Theorem 12. *sdd-free languages are closed under intersection but not closed under union, compliment and catenation.*

5 Conclusions

From a site-directed deletion mutagenesis under enzymatic activities, we have introduced the site-directed deletion operation. We have designed linear time algorithms for determining whether or not $x \overset{sdd}{\leftarrow} y = \emptyset$ and $z \in x \overset{sdd}{\leftarrow} y$. We have shown that $L_1 \overset{sdd}{\leftarrow} L_2$ is regular when L_1 and L_2 are regular, however, $L_1 \overset{sdd}{\leftarrow} L_2$ may not be context-free when L_1 and L_2 are context-free. Given a context-free language L_1 and a regular language L_2, both $L_1 \overset{sdd}{\leftarrow} L_2$ and $L_2 \overset{sdd}{\leftarrow} L_1$ are context-free. In addition, we have established that it is decidable whether or not a regular language L is *sdd*-closed and *sdd*-free in polynomial time, and it is undecidable whether or not a context-free language is *sdd*-closed and *sdd*-free.

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