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Persistent and Permanent Point of Views of Two Stages DNA Splicing Languages

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Abstract

Yusof-Goode (Y-G) splicing system was formulated by Yusof in 2012 to present the existence relation between formal language theory and molecular biology in a convenient approach. In terms of biology, the recombinant deoxyribonucleic acid (DNA) molecules can often be split with the existence of actual restriction enzymes. For this property of the recombinant DNA strands is called persistent. Therefore, determining the persistency of the hybrid templets of DNA strands, after acting restriction enzymes on the initial DNA strands, by providing mathematical proof is considered as a new contribution in the areas of DNA molecular. In this work, the persistent and permanent aspects of two stages splicing languages are investigated and discussed via Y-G approach. This investigation focuses on number of cutting sites in initial strings as well as sequences factors of splicing rules. Accordingly, the persistency and permanency of the above splicing languages with respect to two initial strings (with two cutting sites) and two rulesare presented as lemmas, theorems, and corollaries. Nevertheless, an example is provided, which shows the real meaning of theorem in terms of biology.

Keywords: Permanent, persistent, two stages Y-G splicing languages.

Introduction

Deoxyribonucleic acid or DNA is a longchain of nucleotides made up of repeating components. The components of each nucleotide arenitrogen involving four nucleobases such as adenine (*A*), guanine (*G*), cytosine (*C*) and thymine (*T*), and a monosaccaharide sugar as well as a phosphate group. The four nucleobases of DNA is classified into two families of purine and pyrimidine. In a DNA, for adenine and guanine is called purine, while for cytosine and thymine is called pyrimidine. Since DNA exists in the form of double-stranded DNA (dsDNA), thusthe two single strands are joined together by hydrogen bonding between nucleotides bases. There are two hydrogen bonding between adenine and thymineand three hydrogen bonding between guanine and cytosine, and vice-versa. In dsDNA, the two strandsare complementaryto each and run into two opposite directions. Due to this property of dsDNA, the lower single strand can be determined by its upper single strand and vice-versa[1]. However, a restriction enzyme (RE) is an enzyme that finds specific sequence of the nitrogenous bases usually 4-6 base pairs in length in the strand of DNA and carves it from phospodiester bond between adjacent bases, resulting in a molecule with staggered or blunt ends[2]. Then, the DNA fragments are re-joined with their complementary staggered ends or blunt ends by ligase to produce new DNA strands. Naturally, the vast majority of RE have been isolated from bacteria, where they accomplish a host-defense function for the cell.

Y-G splicing system is the latest model of splicing system, which was introduced byYusof[3]. This splicing system studies the splitting and ligating characteristics of DNA under the effect of appropriate restriction enzyme and a ligase in a translucent approach. The notion of splicing system was first proposed by Head under a framework of formal language theory[4]. Furthermore, the splicing language, which is generated by splicing system, was discussed. Besides, persistent splicing system was defined and itsrelation with strictly locally testable languagewas proven. Later, Gatterdamdefined the notion of permanent and proved that permanent is a proper subset of persistent[5]. Although the permanent splicing system is proper subset of persistent, the splicing languages generate by these two splicing systems are equal[6]. Karimiproved that if there are two persistent (or permanent) splicing systems, the union and intersection of these two splicing systems are not persistent or permanent[7]. Some sufficient conditions for persistency of splicing system were provided[8]. The concepts of crossing preserved and self-closed were introduced and theirrelations with a persistent splicing system were presented[9]. Besides, the concept of extended crossing preserved was introduced and the relation between self-closed, extended crossing preserved and permanent splicing system was given. Common crossing splicing system was defined and its relationwitha self-closed and persistent splicing system was investigated[10]. Some sufficient conditions for splicing system to be permanent were provided[11]. An analysis on four different models of splicing systems namely, Head, Paun, Goode-Pixton and Y-G as well as splicing languages produce by them is investigated[12]. The persistent and permanent points of views of some non-semi simple splicing system were discussed using Y-G approach[3]. In this research, the persistent and permanent points of views of two stages splicing languages based on crossing sites and contexts of splicing rules and cutting sites of initial strings are investigated and presentedaslemmas, theorems and corollaries.

Methodology

Preliminaries: In this section, the definitions of Y-G splicing system, persistent, permanent and crossing disjoint are given, wherethese concepts are the key pointsin this research. Since this study is based on Y-G approach, this splicing system is defined as below.

Definition 1: Yusof-Goode Splicing system[3]: If $r \in R$, where $r = (a, x, b : c, x, d)$ and $s_1 = \alpha axb\beta$ and $s_2 = \gamma cxd\delta$ are elements of I, then splicing s_1 and s_2 using r produce the initial string I together with $\alpha axd\delta$ and $\gamma cxb\beta$, presented in either order where $\alpha, \beta, \gamma, \delta, a, b, c$ and $d \in A^*$ are free monoid generated by A with the concatenation operation and 1 as the identity element.□

Biologically, the new formed hybrid DNA strands are not usually persistent and permanent. Therefore, to investigate the persistency and permanency of two stages recombinant DNA molecules, these two important properties of splicing system are defined in the following.

Persistent[4]: Let $S = (A, I, R)$ be a splicing system. Then S is persistent if for each pair of strings $ucxdv$ and $pexfq$, in A^* with (c, x, d) and (e, x, f) patterns of the same hands: if y is a sub segment of ucx (respectively xfq) that is crossing of a site in $ucxdv$ (respectively $pexfq$) then this same sub segment y of $ucxfq$ contains an occurrence of a crossing of a site in $ucxfq$.□

In the next, the definition of permanent is stated.

Permanent[5]: Let $S = (A, I, R)$ be a splicing system. Then S is permanent if for each pair of strings $ucxdv$ and $pexfq$, in A^* with (c, x, d) and (e, x, f) patterns of the same hands: if y is a sub segment of ucx (respectively xfq) that is crossing of a site in $ucxdv$ (respectively $pexfq$) then this same sub segment y of $ucxfq$ is an occurrence of a crossing of a site in $ucxfq$.

When the restriction enzymes are chosen from any supplier, for example, New England BioLabs (NEB), their behaviors as well as the splicing sites are not always the same. In terms of DNA recombination, the splicing sites of restriction enzymes play an important role on the number of generating DNA molecules at stage one and stage two as well as their persistent and permanent properties. Thus, the concept of crossing disjoint (disjoint splicing sites) splicing system using is defined below.

Crossing Disjoint[13]: A splicing system $S = (A, I, B, C)$ is crossing disjoint if there do not exist patterns (a, x, b) in B and (c, x, d) in C with same crossing x.

Since Y-G splicing system is being used in this research, the above definition can be defined as: A Y-G splicing system $S = (A, I, R)$ is crossing disjoint if there does not exist pattern $(a, x, b : a, x, b)$ in R with same crossing x .□

Results and Discussions

This section discusses on persistent as well as permanent points of views of two stages DNA splicing languages. Somelemmas, theorems, and corollaries regarding to characteristics of two stages splicing languages with respect to number of cutting sites of initial strings as well ascrossing sites and sequences of splicing rules are provided in terms of persistent and permanent points of views. In biological perspective, the provided mathematical theorems predict whether the new forming DNA fragments after accomplishing the recombination process at two stages (stage one and stage two) will be split by acting of existence RE or not. It means that if the recombinant DNA strands are persistent, then they can be cut by the existence of RE. However, if the recombinant DNA molecules are non-persistent, then they cannot be cut by the existence of same RE. In other words, the recombination process is stopped. To understand the concept of two stages splicing languages,its definition is first presented below.

Two Stages Splicing Languages: Let $S = (A, I, R)$ is a splicing system. Furthermore, let $L = L(S)$ is the set of stage one splicing languages produced by splicing system S and $L' = L'(S)$ is the set of stage two splicing languages produced by S that consists of $L = L(S)$ and all splicing languages that can be resulted by splicing L. Then, the union of stage one and stage two splicing languages are called two stages splicing languages.□

Lemma 1 discusses on the persistency of two stages DNA splicing languages with respect to two initial strings (with two cutting sites) and two rules, where first rule is used on first initial string, and second rule is used on second initial string.

Lemma 1: If the crossing sites of the rules in a Y-G splicing system be disjoint and non-palindromic so that the first rule cuts the first initial string (with two cutting sites) and the second rule cuts the second initial string (with two cutting sites) from two specific places, respectively, then the set of two stages splicing languages, which is produced by Y-G splicing system, is persistent.

Proof: Suppose that $S=(A,I,R)$ be a Y-G splicing system. Thus the rules $r_1,r_2\in R$ are presented in the forms of $r_1=(a_1,a_1a_2,a_2:a_1,a_1a_2,a_2)$ and $r_2=(b_1,b_1b_2,b_2:b_1,b_1b_2,b_2)$, respectively where a_1 is not complementary with a_2, b_1 is not complementary with b_2 and vice- versa, $a_1,a_2,b_1,b_2\in A^*$ .Since the patterns have disjoint crossings, therefore the fragments of initial strings after cutting by the existing splicing rules cannot join together. To show the two stages splicing languages are persistent, the splicing languages at two stages need to be obtained. Let s_1 and s_2 be two initial strings in A^* which are presented the forms $s_1=\alpha a_1a_1a_2a_2a_1a_1a_2a_2\beta$ and $s_2=\gamma b_1b_1b_2b_2b_1b_1b_2b_2\delta$, respectively such that string s_1 can be cut by r_1 and string s_2 can be cut by r_2 from two specific sequences, and by splicing the following splicing languages can be generated at stage one.

$\alpha a_1(a_1a_2a_2a_1)^k a_1a_2a_2\beta$, $\gamma b_1(b_1b_2b_2b_1)^k b_1b_2b_2\delta$,
where $k\in\mathbb{N}$.

Now the splicing languages of stage two is obtained. By applying the rules r_1 and r_2 on the resulted splicing languages $\alpha a_1(a_1a_2a_2a_1)^k a_1a_2a_2\beta$ and $\gamma b_1(b_1b_2b_2b_1)^k b_1b_2b_2\delta$, respectively the following two long DNA splicing languages will be produced at stage two.

$\alpha a_1(a_1a_2a_2a_1)^n a_1a_2a_2\beta$, $\gamma b_1(b_1b_2b_2b_1)^n b_1b_2b_2\delta$, where $n=k+i,\ i=0,\ 1,\ 2,\dots,k$

To show that the above splicing languages are persistent the patterns with same crossing should be considered. Therefore, According to definition of persistent, if a_1a_2 bea sub segment of $\alpha a_1a_1a_2$ and b_1b_2 be a sub segment of $\gamma b_1b_1b_2$. These a_1a_2 and b_1b_2 is a crossing of $\alpha a_1a_1a_2a_2a_1a_1a_2a_2\beta$ and $\gamma b_1b_1b_2b_2b_1b_1b_2b_2\delta$, respectively. These same sub segments a_1a_2 and b_1b_2, respectively contain an occurrence of the crossing of a site in the resulted DNA splicing languages $\alpha a_1(a_1a_2a_2a_1)^k a_1a_2a_2\beta$ and $\gamma b_1(b_1b_2b_2b_1)^k b_1b_2b_2\delta$, respectively as well the rest of the above yielding splicing languages. Thus, the two stages splicing languages are persistent.

In the following lemma, the persistency of two stages DNA splicing languages is proven according to two initial string (with two cutting sites) and two rules, where first rule is applied on first initial string and both rules are used on second string.

Lemma 2: If the crossing sites of the rules in a Y-G splicing system be disjoint and non-palindromic so that the first rule cuts the first initial string (with two cutting sites) and both splicing rules cut the second initial string (with two cutting sites) from two specific places, respectively, then the set of two stages splicing languages, which is produced by Y-G splicing system, is persistent.

Proof: Suppose that $S=(A,I,R)$ be a Y-G splicing system. Thus the rules $r_1,r_2\in R$ are presented the forms $r_1=(a_1,a_1a_2,a_2:a_1,a_1a_2,a_2)$ and $r_2=(b_1,b_1b_2,b_2:b_1,b_1b_2,b_2)$, where a_1 is not complementary with a_2, b_1 is not complementary with b_2 and vice- versa, and $a_1,a_2,b_1,b_2\in A^*$ .Since the patterns have disjoint crossings, thus the fragments of initial strings after cutting by the existing splicing rules cannot re-join together. To show the two stages splicing languages are persistent; the splicing languages at two stages need to be obtained. Let s_1 and s_2 be two initial strings in A^* which are presented in the forms $s_1=\alpha a_1a_1a_2a_2a_1a_1a_2a_2\beta$ and $s_2=\gamma a_1a_1a_2a_2b_1b_1b_2b_2\delta$ such that string s_1 can be cut by r_1 and string s_2 can be cut by both rules r_1 and r_2 from two specific places. Splicing them using above rules the following splicing languages will be resulted at stage one.

$\alpha a_1(a_1a_2a_2a_1)^k a_1a_2a_2\beta$, $\alpha a_1(a_1a_2a_2a_1)^k a_1a_2a_2b_1b_1b_2b_2\delta$,
$\gamma a_1(a_1a_2a_2a_1)^k a_1a_2a_2\beta$, $\gamma a_1(a_1a_2a_2a_1)^k a_1a_2a_2b_1b_1b_2b_2\delta$,
where $k\in\mathbb{N}$.

When splicing operation takes place among the resulted DNA splicing languages, the following DNA splicing languages will be generated at stage two.

$\alpha a_1(a_1a_2a_2a_1)^n a_1a_2a_2\beta$, $\alpha a_1(a_1a_2a_2a_1)^n a_1a_2a_2b_1b_1b_2b_2\delta$,
$\gamma a_1(a_1a_2a_2a_1)^n a_1a_2a_2\beta$, $\gamma a_1(a_1a_2a_2a_1)^n a_1a_2a_2b_1b_1b_2b_2\delta$,
where $n=k+i,\ i=0,\ 1,\ 2,\dots,k$.

To prove the above sets of splicing languages are persistent, the patterns with same crossing site need to be considered. If a_1a_2 be a sub segment of $\alpha a_1a_1a_2$, that is crossing of a site in $\alpha a_1a_1a_2a_2a_1a_1a_2a_2\beta$. This same sub segment a_1a_2 contains an occurrence of crossing of a site in the yielding string $\alpha a_1(a_1a_2a_2a_1)^k a_1a_2a_2b_1b_1b_2b_2\delta$. Hence, the two stages splicing languages are persistent.

In the next lemma, the persistency of two stages DNA splicing languages is presented with respect to a Y-G splicing system consisting of two initial strings (with two cutting sites) and two rules where both rules are used on first initial string and second rule is used on second initial string.

Lemma 3: If the crossing sites of the rules in a Y-G splicing system be disjoint and non-palindromic so that both splicing rules cut the first initial string (with two cutting sites) and the second rule cuts the second initial string (with two cutting sites) from two specific places, respectively, then the set of two stages splicing languages, which is produced by Y-G splicing system, is persistent.□

Proof: Suppose that $S=(A,I,R)$ be a Y-G splicing system. Thus the rules $r_1,r_2 \in R$ are presented the forms $r_1=(a_1,a_1a_2,a_2:a_1,a_1a_2,a_2)$ and $r_2=(b_1,b_1b_2,b_2:b_1,b_1b_2,b_2)$, where a_1 is not complementary with a_2, b_1 is not complementary with b_2 and vice- versa, $a_1,a_2,b_1,b_2 \in A^*$. Since the patterns have disjoint crossings, thus the fragments of initial strings after cutting by the existing splicing rules cannot recombine together. To show the two stages splicing languages are persistent, the splicing languages at two stages need to be obtained. Assume s_1 and s_2 be two initial strings in $I \in A^*$ have a form as $s_1=\alpha a_1a_1a_2a_2b_1b_1b_2b_2\beta$ and $s_2=\gamma b_1b_1b_2b_2b_1b_1b_2b_2\delta$ such that string s_2 can be cut by r_2 and string s_1 can be cut by both rules r_1 and r_2 from two specific places and by splicing the following splicing languages will be the generating splicing languages at stage two.

$\gamma b_1(b_1b_2b_2b_1)^k b_1b_2b_2\delta$, $\gamma b_1(b_1b_2b_2b_1)^k b_1b_2b_2\beta$,
$\alpha a_1a_1a_2a_2b_1(b_1b_2b_2b_1)^k b_1b_2b_2\delta$,
$\alpha a_1a_1a_2a_2b_1(b_1b_2b_2b_1)^k b_1b_2b_2\beta$,
where $k \in \mathbb{N}$.

Since the resulted DNA splicing languages can be cut by the rules r_1 and r_2, thus by adding the rules on the generated splicing languages of stage one the following DNA splicing languages will be obtained at stage two.

$\gamma b_1(b_1b_2b_2b_1)^n b_1b_2b_2\delta$, $\gamma b_1(b_1b_2b_2b_1)^n b_1b_2b_2\beta$,
$\alpha a_1a_1a_2a_2b_1(b_1b_2b_2b_1)^n b_1b_2b_2\delta$,
$\alpha a_1a_1a_2a_2b_1(b_1b_2b_2b_1)^n b_1b_2b_2\beta$,
where $n=k+i,\ i=0,\ 1,\ 2,\ldots,k$.

The proof for persistency of above sets of splicing languages follows **Lemma 2**.

In **Lemma 4**, the persistency of two stages DNA splicing languages is discussed with respect to a Y-G splicing system consisting of two initial strings (with two cutting sites) and two rules where both rules are used on each of the initial string.

Lemma 4: If the crossing sites of the rules in a Y-G splicing system be disjoint and non-palindromic so that both splicing rules cut both of the initial strings (with two cutting sites) from two specific places, respectively, thenthe set of two stages splicing languages, that is produced by Y-G splicing system, is persistent.□

Proof: Suppose that $S=(A,I,R)$ be a Y-G splicing system. Thus the rules $r_1,r_2 \in R$ are presented the forms $r_1=(a_1,a_1a_2,a_2:a_1,a_1a_2,a_2)$ and $r_2=(b_1,b_1b_2,b_2:b_1,b_1b_2,b_2)$, respectivelywhere a_1 is not complementary with a_2, b_1 is not complementary with b_2 and vice- versa, $a_1,a_2,b_1,b_2 \in A^*$ .Since the patterns have disjoint crossings, therefore, the fragments of initial strings after cutting by the existing splicing rules will not re-join together. To show the two stages splicing languages are persistent, the splicing languages at two stages need to be obtained. Suppose that s_1 and s_2 be two initial strings in $I \in A^*$ have a form as $s_1=\alpha a_1a_1a_2a_2b_1b_1b_2b_2\beta$ and $s_2=\gamma a_1a_1a_2a_2b_1b_1b_2b_2\delta$ such that strings s_1 and s_2 can be cut by both rules r_1 and r_2 from two specific sequences and by splicing them the following splicing languages will be generated at stage one namely, $\alpha a_1a_1a_2a_2b_1b_1b_2b_2\delta$, $\gamma a_1a_1a_2a_2b_1b_1b_2b_2\beta$

However, by applying both rules r_1 and r_2 on the resulted DNA splicing languages of first stage, no distinct DNA splicing languages will be generated at stage two.

Now it is shown that the above set of splicing languages is persistent. According to definition of persistent if a_1a_2 and b_1b_2 be sub segments of $\alpha a_1a_1a_2$ (respectively $b_1b_2b_2\delta$), these are crossings of the sites in $\alpha a_1a_1a_2a_2b_1b_1b_2b_2\beta$ (respectively $\gamma a_1a_1a_2a_2b_1b_1b_2b_2\delta$). These same sub segments a_1a_2 and b_1b_2 contain an occurrence of crossing of a site in the yielding string $\alpha a_1a_1a_2a_2b_1b_1b_2b_2\delta$ and $\gamma a_1a_1a_2a_2b_1b_1b_2b_2\beta$. Hence, the two stages splicing languages are persistent.

In the following theorem, the persistency of two stages DNA splicing languages according to Y-G splicing system consisting two initial strings (with two cutting sites) and two rules with disjoint non-palindromic crossing sites is investigated.

Theorem 1: The set of two stages splicing languages, which is produced by Y-G splicing system consisting two initial strings (with two cutting sites) and two rules with disjoint crossing sites and non-palindromic sequences, is persistent.

Proof: Suppose that $S=(A,I,R)$ be a Y-G splicing system. Thus, the rules $r_1,r_2 \in R$ are presented in the forms $r_1=(a_1,a_1a_2,a_2:a_1,a_1a_2,a_2)$ and $r_2=(b_1,b_1b_2,b_2:b_1,b_1b_2,b_2)$, respectivelywhere a_1 is not complementary with a_2, b_1 is not complementary with b_2 and vice- versa, $a_1,a_2,b_1,b_2 \in A^*$. Since the patterns have disjoint crossings, therefore the fragments of initial strings after cutting by the existing splicing rules cannot recombine together. According to the number of cutting sites of initial strings four cases happen.

Case 1: r_1 is used on s_1 and r_2 is used on s_2.
Case 2: r_1 is used on s_1 and both r_1 and r_2 are used on s_2
Case 3: both r_1 and r_2 are used on s_1 and r_2 is used on s_2.
Case 4: both r_1 and r_2 are used on s_1 and s_2.

Since the cases of **Theorem 1**have compatibility with the lemmas that have been proven above, therefore this theorem can be proven by combining the proofs of **Lemma 1**, **Lemma 2**, **Lemma 3** and **Lemma 4**, respectively.

The following corollary, which is resulted from **Theorem 1**,indicates that the set of two stages splicing languages with the above conditions is also permanent.

Corollary 1: The set of two stages splicing languages, that is produced by Y-G splicing system consisting two initial strings (with two cutting sites) and two rules with disjoint crossing sites and non-palindromic sequences, is permanent.

Lemma 5 Presentsthe persistency of two stages DNA splicing languages at the existence of two initial strings (with two cutting sites) and two palindromic rules, where the first rule is used on first initial string and second rule on second initial string.

Lemma 5: If the crossing sites of the rules in a Y-G splicing system be disjoint and palindromic so that the first rule cuts the first initial string (with two cutting sites) and the second rule cuts the second initial string (with two cutting sites) from two specific places, respectively, thenthe set of two stages splicing languages, that is produced by Y-G splicing system, is persistent.□

Proof: Assume $S=(A,I,R)$ be a Y-G splicing system that consists of two initial strings $s_1,s_2 \in I$ and two rules $r_1,r_2 \in R$. Thus, the rules $r_1,r_2 \in R$ are presented in the forms of $r_1=(a_1,a_1a_2,a_2:a_1,a_1a_2,a_2)$ and $r_2=(b_1,b_1b_2,b_2:b_1,b_1b_2,b_2)$, respectively where a_1 is complementary with a_2, b_1 is complementary with b_2 and vice- versa, $a_1,a_2,b_1,b_2 \in A^*$. To prove the persistency of the generating splicing languages at two stages with respect to recognition sites of initial strings four cases need be considered. Suppose s_1 and s_2 are two initial strings in I such that the string s_1 can be cut by rule r_1 and string s_2 can be cut by rule r_2 from two specific sequences. Let $s_1=\alpha a_1a_1a_2a_2a_1a_1a_2a_2\beta$ and $s_2=\gamma b_1b_1b_2b_2b_1b_1b_2b_2\delta$ be two initial strings in $I \in A^*$, and α', β', γ' and δ' are complemented of α, β, γ and δ, respectively. Since the crossings of rules are disjoint, splicing s_1 itself and s_2 itself the following six DNA splicing languages will be produced at stage one.

$\alpha a_1(a_1a_2a_2a_1)^k a_1a_2a_2\beta$, $\alpha a_1(a_1a_2a_2a_1)^k a_1a_2a_2\alpha'$,
$\beta' a_1(a_1a_2a_2a_1)^k a_1a_2a_2\beta$,
$\gamma b_1(b_1b_2b_2b_1)^k b_1b_2b_2\delta$, $\gamma b_1(b_1b_2b_2b_1)^k b_1b_2b_2\gamma'$,
$\delta' b_1(b_1b_2b_2b_1)^k b_1b_2b_2\delta$,
where $k \in \mathbb{N}$.

To get the resulted DNA splicing languages of stage two, the rules r_1 and r_2 are added on the splicing languages of stage one, the generated DNA splicing languages at stage two are as below.

$\alpha a_1(a_1a_2a_2a_1)^n a_1a_2a_2\beta$, $\alpha a_1(a_1a_2a_2a_1)^n a_1a_2a_2\alpha'$,
$\beta' a_1(a_1a_2a_2a_1)^n a_1a_2a_2\beta$,
$\gamma b_1(b_1b_2b_2b_1)^n b_1b_2b_2\delta$, $\gamma b_1(b_1b_2b_2b_1)^n b_1b_2b_2\gamma'$,
$\delta' b_1(b_1b_2b_2b_1)^n b_1b_2b_2\delta$,
where $n=k+i,\ i=0,\ 1,\ 2,...,k$.

To prove that the families of two stages splicing languages are persistent, the pattern with same crossing should be considered. According to definition of persistent by taking a_1a_2 as sub segment of $\alpha a_1a_1a_2$, that is crossing of $\alpha a_1a_1a_2a_2a_1a_1a_2a_2\beta$. Thus, this sub segment a_1a_2 also contains an occurrence of the crossing of a site in the resulted DNA splicing language $\alpha a_1(a_1a_2a_2a_1)^k a_1a_2a_2\alpha'$ and rest of other above splicing languages that have a pattern of $(a_1,a_1a_2,a_2:a_1,a_1a_2,a_2)$. Also, the same method the persistency of the above splicing languages, which have a pattern of $(b_1,b_1b_2,b_2:b_1,b_1b_2,b_2)$, is proven.Consequently, the set of two stages splicing languages is persistent.

In **Lemma 6**, the persistent aspect of two stages DNA splicing languages is discussed at the existence of two initial strings (with two cutting sites) and two rules, so that first rule is applied on first initial string and both of splicing rules are used on second initial string.

Lemma 6: If the crossing sites of the rules in a Y-G splicing system be disjoint and palindromic so that the first rule cuts the first initial string (with two cutting sites) and both splicing rules cut the second initial string (with two cutting sites) from two specific places, respectively, then the set of two stages splicing languages, that is produced by Y-G splicing system, is persistent.□

Proof: Assume $S=(A,I,R)$ be a Y-G splicing system that consists of two initial strings $s_1,s_2\in I$ and two rules $r_1,r_2\in R$. Thus, the rules $r_1,r_2\in R$ are presented in the forms of $r_1=(a_1,a_1a_2,a_2:a_1,a_1a_2,a_2)$ and $r_2=(b_1,b_1b_2,b_2:b_1,b_1b_2,b_2)$, respectively where a_1 is complementary with a_2, b_1 is complementary with b_2 and vice- versa, $a_1,a_2,b_1,b_2\in A^*$. Therefore, for the persistency of the generating splicing languages at two stages with respect to recognition sites of initial strings four cases need be considered. Suppose s_1 and s_2 are two initial strings in I that have two cutting sites and the string s_1 can be cut by r_1 and string s_2 can cut by both rules $r_1,r_2\in R$ from two specific places. Assume $s_1=\alpha a_1a_1a_2a_2a_1a_1a_2a_2\beta$ and $s_2=\gamma a_1a_1a_2a_2b_1b_1b_2b_2\delta$ are two initial strings in $I\in A^*$, and α', β', γ' and δ' are complemented of α, β, γ and δ, respectively. Applying the rules on initial strings and splicing the fragments of them with their complementary ends, the following thirteen DNA splicing languages will be generated at stage one namely,

$\alpha a_1(a_1a_2a_2a_1)^k a_1a_2a_2\beta$,

$\alpha a_1(a_1a_2a_2a_1)^k a_1a_2a_2\alpha'$,

$\beta' a_1(a_1a_2a_2a_1)^k a_1a_2a_2\beta$, $\alpha a_1(a_1a_2a_2a_1)^k a_1a_2a_2b_1b_1b_2b_2\delta$,

$\alpha a_1(a_1a_2a_2a_1)^k a_1a_2a_2\gamma'$, $\beta' a_1(a_1a_2a_2a_1)^k a_1a_2a_2b_1b_1b_2b_2\delta$,

$\gamma a_1(a_1a_2a_2a_1)^k a_1a_2a_2\beta$, $\gamma a_1(a_1a_2a_2b_1\cup b_1b_2b_2a_1)^k a_1a_2a_2\gamma'$,

$\delta' b_1(b_1b_2b_2a_1\cup a_1a_2a_2b_1)^k b_1b_2b_2\delta$,

$\gamma a_1a_1a_2a_2b_1(b_1b_2b_2a_1\cup a_1a_2a_2b_1)^k b_1b_2b_2\delta$,

$\alpha a_1(a_1a_2a_2b_1\cup b_1b_2b_2a_1)^k a_1a_2a_2\gamma'$,

$\gamma a_1(a_1a_2a_2a_1)^k a_1a_2a_2b_1b_1b_2b_2\delta$,

$\gamma a_1(a_1a_2a_2b_1\cup b_1b_2b_2a_1)^k a_1a_2a_2\beta$,

where $k\in\mathbb{N}$.

To generate the splicing languages of stage two, the resulted DNA splicing languages are spliced using rules r_1 and r_2. When splicing occurs the following DNA splicing languages will be formed as listed below.

$\alpha a_1(a_1a_2a_2a_1)^n a_1a_2a_2\beta$, $\alpha a_1(a_1a_2a_2a_1)^n a_1a_2a_2\alpha'$,

$\beta' a_1(a_1a_2a_2a_1)^n a_1a_2a_2\beta$, $\alpha a_1(a_1a_2a_2a_1)^n a_1a_2a_2b_1b_1b_2b_2\delta$,

$\alpha a_1(a_1a_2a_2a_1)^n a_1a_2a_2\gamma'$, $\beta' a_1(a_1a_2a_2a_1)^n a_1a_2a_2b_1b_1b_2b_2\delta$,

$\gamma a_1(a_1a_2a_2a_1)^n a_1a_2a_2\beta$, $\gamma a_1(a_1a_2a_2b_1\cup b_1b_2b_2a_1)^n a_1a_2a_2\gamma'$,

$\delta' b_1(b_1b_2b_2a_1\cup a_1a_2a_2b_1)^n b_1b_2b_2\delta$,

$\gamma a_1a_1a_2a_2b_1(b_1b_2b_2a_1\cup a_1a_2a_2b_1)^n b_1b_2b_2\delta$,

$\alpha a_1(a_1a_2a_2b_1\cup b_1b_2b_2a_1)^n a_1a_2a_2\gamma'$,

$\gamma a_1(a_1a_2a_2a_1)^n a_1a_2a_2b_1b_1b_2b_2\delta$,

$\gamma a_1(a_1a_2a_2b_1\cup b_1b_2b_2a_1)^n a_1a_2a_2\beta$,

where $n=k+i,\ i=0,\ 1,\ 2,...,k$.

To prove that the families of the above two stages splicing languages are persistent, the pattern with same crossing should be considered. According to definition of persistent by taking $y=a_1a_2$ as a sub segment of $\alpha a_1a_1a_2$ (respectively $a_1a_2a_2b_1b_1b_2b_2\delta$), that is crossing of $\alpha a_1a_1a_2a_2a_1a_1a_2a_2\beta$. Thus, this $y=a_1a_2$ also contains an occurrence of the crossing of a site in $\alpha a_1(a_1a_2a_2a_1)^k a_1a_2a_2b_1b_1b_2b_2\delta$, as well crossing of the rest of the splicing languages, which have a pattern of $(a_1,a_1a_2,a_2:a_1,a_1a_2,a_2)$. By using the same approach the persistency of two stages splicing languages, which have a pattern of $(b_1,b_1b_2,b_2:b_1,b_1b_2,b_2)$, is proven. Consequently, the above two stages splicing languages are persistent.

In the next lemma, the persistent point of view of two stages DNA splicing languages is presented at the existence of two initial strings and two palindromic rules, where both of splicing rules are applied on first initial strings and second on second initial string.

Lemma 7: If the crossing sites of the rules in a Y-G splicing system be disjoint and palindromic so that both splicing rules cut the first initial string (with two cutting sites) and the second rule cuts the second initial string (with two cutting sites) from two specific places, respectively, then the set of two stages splicing languages, which is produced by Y-G splicing system, is persistent.

Proof: Assume $S=(A,I,R)$ be a Y-G splicing system that consists the two initial strings $s_1,s_2\in I$ and two rules $r_1,r_2\in R$.

Thus, the rules $r_1, r_2 \in R$ are presented in the forms of $r_1 = (a_1, a_1a_2, a_2 : a_1, a_1a_2, a_2)$ and $r_2 = (b_1, b_1b_2, b_2 : b_1, b_1b_2, b_2)$, respectively where a_1 is complementary with a_2, b_1 is complementary with b_2 and vice- versa, $a_1, a_2, b_1, b_2 \in A^*$. Therefore, for the persistency of the generating splicing languages at two stages with respect to recognition sites of initial strings four cases need be considered. Suppose s_1 and s_2 are two initial strings in I that have two cutting sites and the string s_1 can be cut by both rules r_1 and r_2 and string s_2 can cut by rule r_2 from two specific places. Assume $s_1 = \alpha a_1a_1a_2a_2b_1b_1b_2b_2\beta$ and $s_2 = \gamma b_1b_1b_2b_2b_1b_1b_2b_2\delta$ be two initial strings. in $I \in A^*$, and α', β', γ' and δ' are complemented of α, β, γ and δ, respectively. Applying the rules on initial strings and splicing the fragments of them with their complementary ends, the following thirteen DNA splicing languages will be generated at stage one namely,

$\alpha a_1 (a_1a_2a_2b_1 \cup b_1b_2b_2a_1)^k a_1a_2a_2\alpha'$,
$\beta' b_1 (b_1b_2b_2a_1 \cup a_1a_2a_2b_1)^k b_1b_2b_2\beta$, $\gamma b_1 (b_1b_2b_2b_1)^k b_1b_2b_2\delta$,
$\gamma b_1 (b_1b_2b_2b_1)^k b_1b_2b_2\gamma'$, $\delta' b_1 (b_1b_2b_2b_1)^k b_1b_2b_2\delta$,
$\gamma b_1 (b_1b_2b_2b_1)^k b_1b_2b_2\beta$, $\gamma b_1 (b_1b_2b_2b_1)^k b_1b_2b_2a_1a_1a_2a_2\alpha'$,
$\delta' b_1 (b_1b_2b_2b_1)^k b_1b_2b_2\beta$, $\delta' b_1 (b_1b_2b_2b_1)^k b_1b_2b_2a_1a_1a_2a_2\alpha'$,
$\alpha a_1a_1a_2a_2b_1 (b_1b_2b_2b_1)^k b_1b_2b_2\beta$,
$\beta' b_1 (b_1b_2b_2a_1 \cup a_1a_2a_2b_1)^k b_1b_2b_2\delta$,
$\beta' b_1 (b_1b_2b_2a_1 \cup a_1a_2a_2b_1)^k b_1b_2b_2\gamma'$,
$\beta' b_1 (b_1b_2b_2a_1 \cup a_1a_2a_2b_1)^k b_1b_2b_2a_1a_1a_2a_2\alpha'$,
where $k \in \mathbb{N}$.

Since the resulted DNA splicing languages contains the cleavage patterns of splicing rules, if splicing takes place among them the only change that will arise in the DNA splicing languages of stage two is the value of $k \in \mathbb{N}$, and the repeating sequences will have the power n, where $n = k + i$, $i = 0, 1, 2, ..., k$.

To prove that the families of the above two stages splicing languages are persistent, the pattern with same crossing should be considered. By taking b_1b_2 as a sub segment of $\gamma b_1b_1b_2$, that is crossing of $\gamma b_1b_1b_2b_2b_1b_1b_2b_2\delta$. Thus, this b_1b_2 also contains an occurrence of the crossing of a site in $\gamma b_1 (b_1b_2b_2b_1)^k b_1b_2b_2\beta$, as well crossing of the rest of the splicing languages that have a pattern of $(b_1, b_1b_2, b_2 : b_1, b_1b_2, b_2)$. By using the same approach the persistency of two stages splicing languages, which have a pattern of $(a_1, a_1a_2, a_2 : a_1, a_1a_2, a_2)$ is proven. Consequently, the above two stages splicing languages are persistent.

In the last lemma, the persistency of two stages DNA splicing languages is proven using Y-G approach with respect to two initial string and two palindromic rules, where both of splicing rules are applied on both of initial strings.

Lemma 8: If the crossing sites of the rules in a Y-G splicing system be disjoint and palindromic so that both splicing rules cut both of the initial strings (with two cutting sites) from two specific places, respectively, then the set of two stages splicing languages, that is produced by Y-G splicing system, is persistent.□

Proof: Assume $S = (A, I, R)$ be a Y-G splicing system that consists of two initial strings $s_1, s_2 \in I$ and two rules $r_1, r_2 \in R$. Thus, the rules $r_1, r_2 \in R$ are presented in the forms of $r_1 = (a_1, a_1a_2, a_2 : a_1, a_1a_2, a_2)$ and $r_2 = (b_1, b_1b_2, b_2 : b_1, b_1b_2, b_2)$, respectively where a_1 is complementary with a_2, b_1 is complementary with b_2 and vice- versa, $a_1, a_2, b_1, b_2 \in A^*$. Therefore, for the persistency of the generating splicing languages at two stages with respect to recognition sites of initial strings four cases need be considered. Suppose s_1 and s_2 are two initial strings in I such that the strings s_1 and s_2 can be cut by both rules r_1 and r_2 from two specific sequences. Let $s_1 = \alpha a_1a_1a_2a_2b_1b_1b_2b_2\beta$ and $s_2 = \gamma a_1a_1a_2a_2b_1b_1b_2b_2\delta$ be two initial strings in $I \in A^*$, and α', β', γ' and δ' are complemented of α, β, γ and δ, respectively.Apply the rules r_1 and r_2 on the initial strings s_1 and s_2, the following ten DNA splicing languages will be produced at stage one.

$\alpha a_1 (a_1a_2a_2b_1 \cup b_1b_2b_2a_1)^k a_1a_2a_2\alpha'$,
$\beta' b_1 (b_1b_2b_2a_1 \cup a_1a_2a_2b_1)^k b_1b_2b_2\beta$,
$\gamma a_1 (a_1a_2a_2b_1 \cup b_1b_2b_2a_1)^k a_1a_2a_2\gamma'$,
$\delta' b_1 (b_1b_2b_2a_1 \cup a_1a_2a_2b_1)^k b_1b_2b_2\delta$,
$\alpha a_1 (a_1a_2a_2b_1 \cup b_1b_2b_2a_1)^k a_1a_2a_2\gamma'$,
$\beta' b_1 (b_1b_2b_2a_1 \cup a_1a_2a_2b_1)^k b_1b_2b_2\delta$,
$\gamma a_1a_1a_2a_2b_1 (b_1b_2b_2a_1 \cup a_1a_2a_2b_1)^k b_1b_2b_2\delta$,
$\alpha a_1a_1a_2a_2b_1 (b_1b_2b_2a_1 \cup a_1a_2a_2b_1)^k b_1b_2b_2\delta$,
$\alpha a_1a_1a_2a_2b_1 (b_1b_2b_2a_1 \cup a_1a_2a_2b_1)^k b_1b_2b_2\beta$,
$\gamma a_1a_1a_2a_2b_1 (b_1b_2b_2a_1 \cup a_1a_2a_2b_1)^k b_1b_2b_2\beta$,
where $k \in \mathbb{N}$.

Since the yielded DNA splicing languages have the sites two split by the rules r_1 and r_2, therefore splicing them via existence rules, produce the following splicing languages at stage two as listed below.

$$\alpha a_1 \left(a_1 a_2 a_2 b_1 \cup b_1 b_2 b_2 a_1\right)^n a_1 a_2 a_2 \alpha',$$
$$\beta' b_1 \left(b_1 b_2 b_2 a_1 \cup a_1 a_2 a_2 b_1\right)^n b_1 b_2 b_2 \beta,$$
$$\gamma a_1 \left(a_1 a_2 a_2 b_1 \cup b_1 b_2 b_2 a_1\right)^n a_1 a_2 a_2 \gamma',$$
$$\delta' b_1 \left(b_1 b_2 b_2 a_1 \cup a_1 a_2 a_2 b_1\right)^n b_1 b_2 b_2 \delta,$$
$$\alpha a_1 \left(a_1 a_2 a_2 b_1 \cup b_1 b_2 b_2 a_1\right)^n a_1 a_2 a_2 \gamma',$$
$$\beta' b_1 \left(b_1 b_2 b_2 a_1 \cup a_1 a_2 a_2 b_1\right)^n b_1 b_2 b_2 \delta,$$
$$\gamma a_1 a_1 a_2 a_2 b_1 \left(b_1 b_2 b_2 a_1 \cup a_1 a_2 a_2 b_1\right)^n b_1 b_2 b_2 \delta,$$
$$\alpha a_1 a_1 a_2 a_2 b_1 \left(b_1 b_2 b_2 a_1 \cup a_1 a_2 a_2 b_1\right)^n b_1 b_2 b_2 \delta,$$
$$\alpha a_1 a_1 a_2 a_2 b_1 \left(b_1 b_2 b_2 a_1 \cup a_1 a_2 a_2 b_1\right)^n b_1 b_2 b_2 \beta,$$
$$\gamma a_1 a_1 a_2 a_2 b_1 \left(b_1 b_2 b_2 a_1 \cup a_1 a_2 a_2 b_1\right)^n b_1 b_2 b_2 \beta,$$

where $n = k + i,\ i = 0,\ 1,\ 2, \ldots, k$.

To show the two stages splicing languages are persistent, the pattern with same crossing should be considered. According to definition of persistent if $y = a_1 a_2$ be the sub segment of $\alpha a_1 a_1 a_2$, that is crossings of $\alpha a_1 a_1 a_2 a_2 b_1 b_1 b_2 b_2 \beta$. This sub segments $y = a_1 a_2$ contains an occurrence of the crossing of a site in $\alpha a_1 \left(a_1 a_2 a_2 b_1 \cup b_1 b_2 b_2 a_1\right)^k a_1 a_2 a_2 \alpha'$, as well as crossing of the rest of the splicing languages that have a pattern of $\left(a_1, a_1 a_2, a_2 : a_1, a_1 a_2, a_2\right)$. By using the same approach, the persistency of two stages splicing languages, which have a pattern of $\left(b_1, b_1 b_2, b_2 : b_1, b_1 b_2, b_2\right)$, is proven. Hence, the set of two stages splicing languages is persistent.

In the next theorem, the persistency of two stages splicing languages is proven with respect to Y-G splicing system consisting two initial strings (with two cutting sites) and two rules with palindromic disjoint crossing sites.

Theorem2: The set of two stages splicing languages, that is produced by Y-G splicing system consisting two initial strings (with two cutting sites) and two rules with disjoint crossing sites and palindromic sequences, is persistent.□

Proof: Assume $S = \left(A, I, R\right)$ be a Y-G splicing system that consists the two initial strings $s_1, s_2 \in I$ and two rules $r_1, r_2 \in R$. Thus, the rules $r_1, r_2 \in R$ are presented as $r_1 = \left(a_1, a_1 a_2, a_2 : a_1, a_1 a_2, a_2\right)$ and $r_2 = \left(b_1, b_1 b_2, b_2 : b_1, b_1 b_2, b_2\right)$, respectively where a_1 is complementary with a_2, b_1 is complementary with b_2 and vice- versa, $a_1, a_2, b_1, b_2 \in A^*$. Therefore, for the persistency of the generating splicing languages at two stages with respect to recognition sites of initial strings four cases need be considered.Based on the number of cutting sites of initial strings four cases need to be considered.

Case 1: r_1 is used on s_1 and r_2 is used on s_2.
Case 2: r_1 is used on s_1 and both r_1 and r_2 are used on s_2
Case 3:both r_1 and r_2 are used on s_1 and r_2 is used on s_2.
Case 4:both r_1 and r_2 are used on s_1 and s_2.

Since the cases of **Theorem 2**have compatibility with **Lemma 5**, **Lemma 6**, **Lemma 7** and **Lemma 8**, respectively, thus the proof of this theorem can exactly be resulted from the above proven lemmas.

The following corollary can be directly resulted from **Theorem 2** and definition of permanent, which indicates the set of the above two stages splicing languages is also permanent.

Corollary 2: The set of two stages splicing languages, that is produced by Y-G splicing system consisting two initial strings (with two cutting sites) and two rules with disjoint crossing sites and palindromic sequences, is permanent.

In the following, a biological example is provided concerning **Lemma 6**, which indicates the validating of the theorem in a real sense.

Example 1: Suppose that $S = \left(A, I, R\right)$ is a Y-G splicing system where $A = \{[A/T], [G,C], [C/G], [T/A]\}$ and the set of splicing rule R consists restriction enzymes namely $FspB$I and $PspL$I, which their cleavage patterns are represented as $\begin{matrix} 5'...C\blacktriangledown TAG..3' \\ 3'...GAT\blacktriangle C...5' \end{matrix}$ and $\begin{matrix} 5'...C\blacktriangledown GTACG..3' \\ 3'...GCATG\blacktriangle C...5' \end{matrix}$, respectively.Assume $s_1 = \begin{matrix} 5'...MMMCTAGCTAGNNN...3' \\ 3'...MMMGATCGATCNNN...5' \end{matrix}$ and $s_2 = \begin{matrix} 5'...XXXCGTACGCGTACGYYY...3' \\ 3'...XXXGCATGCGCATGCYYY...5' \end{matrix}$ are two initial DNA fragments in I, where $M, N, X, Y \in A$. Therefore, according to **Lemma 6**, the resulting two stages recombinant DNA strandsare persistent.

Conclusion

In this paper, the concept of two stages splicing languages is introduced and its persistent and permanent aspects are investigated by providing somelemmas, theorems and corollaries. In the first four lemmas (**Theorem 1**), the persistency of two stages DNA splicing languages with respect to two initial strings (with two cutting sites) and two rules with disjoint non-palindromic crossing sites are presented. The difference of these lemmas is on applying the rules on initial strings. In the last four lemmas (**Theorem 2**), the persistency of two stages DNA splicing languages are investigated based on two initial strings (with two cutting sites) and two rules with palindromic disjoint crossing sites. All in all, if the crossing sites of the rules are disjoint and the whole sequences of the rules are palindromic or only the crossing sites of the rules are non-palindromic, the two stages DNA splicing languages, which are produced by Y-G splicing system, are persistent as well as permanent.

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