Second Order Limit Language with Two Cutting Sites

Muhammad Azrin Ahmad, Nor Haniza Sarmin, Wan Heng Fong, Yuhani Yusof, Noraziah Adzhar

Abstract: The study of recombinant conduct of bi-stranded Deoxyribonucleic acid particles with the presence of chose confinement compound and ligase leads to the development of mathematical modelling of splicing system. Therefore, molecular biologists start focusing more on splicing systems. The splicing language that is generated from the splicing system is categorized into inert/adult, transient and limit languages. Recently, the study of the limit language has been extended to the second order limit language. Previous researchers have focused their study on the three categories of splicing language. A normal splicing system with no restriction on the number of cutting site and the properties of the rule result to non-existence of the second request limit language. Within this paper, existence of the second request limit language in a type of splicing framework, namely the Y-G splicing framework is investigated in which there are two cutting sites in the set of rules.

Keywords: splicing system; splicing language; limit language; DNA

I. INTRODUCTION

Deoxyribonucleic acid or DNA works as a hereditary material in human beings and most other organisms [1]. The hereditary effect is performed by its two natural functions: coding for the manufacture of essential nutrients and self-reproduction which transfers information from the parent cells to the offspring cells. Basically, a DNA molecule consists of a phosphate cluster, a sugar cluster and a kind of 4 bases of element base. The knowledge is gathered into four differing types of element base which are Adenine (A), Guanine (G), Cytosine (C) and Thymine (T) [2]. By Watson-Crick complementarity, the only possible pairings between the nitrogenous bases are A with T, C with G and vice versa [1]. Restriction endonuclease, commonly known as restriction enzyme, is isolated from bacteria and it has an ability to cut the DNA at a specific sequence [2].

Revised Manuscript Received on February 05 2019.

Muhammad Azrin Ahmad, Faculty of Industrial Sciences & Technology, Universiti Malaysia Pahang, 26300 LebuhrayaTunRazak, Gambang, Pahang, Malaysia

Nor HanizaSarmin, Department of Mathematical Sciences, Faculty of Science, Universiti Teknologi Malaysia, 81310 Johor Bahru, Johor, Malaysia

Wan Heng Fong, Department of Mathematical Sciences, Faculty of Science, Universiti Teknologi Malaysia, 81310 Johor Bahru, Johor, Malaysia

Yuhani Yusof, Faculty of Industrial Sciences & Technology, Universiti Malaysia Pahang, 26300 LebuhrayaTunRazak, Gambang, Pahang, Malaysia

Noraziah Adzhar, Faculty of Industrial Sciences & Technology, Universiti Malaysia Pahang, 26300 LebuhrayaTunRazak, Gambang, Pahang, Malaysia

A specific site on the DNA molecules is known as a recognition site where the cleavage process is taken place. After the cutting process, two pieces with either sticky finish or blunt finish are shaped. The recombination process then takes place by the presence of an appropriate ligase. The process occurs when the two fragments end with complementary bases and the two fragments are also from the same overhang [3]. This process results in the formation of either new hybrid molecules or the same DNA molecules.

The linkage of the study of recombination of DNA molecules with mathematics has been pioneered by Head [4] utilizing the learning of formal dialect hypothesis and informational macromolecules, namely the numerical displaying of joining framework. The mathematical model of splicing framework, $S=(A,I,B,C)$ comprises of a lot of limited alphabets, $A=\{a,c,g,t\}$, a lot of beginning strings or axioms *I*, and a pair of limited sets namely pattern *B* or *C* which is a triple of *c*, *x*, *d* in A^*, where A^* denotes a pair all strings over an alphabet *A* which is gotten by linking at least zero symbols from *A*. The set of alphabets *A* represents the complementarity bases, [A/T], [C/G], [G/C] and [T/A] respectively [5]. After the cutting process, the molecules that produce $5'$-overhang or gruff end is assigned to design *B* as long as molecules with $3'$-overhang are assigned to pattern *C* [4].

There are several models of splicing system, namely Head, Paun [6], Pixton [7], Goode-Pixton [8] and Yusof-Goode (Y-G) [9] splicing system. This research focuses on two issues: a model based on the generation of language, and a model to preserve the biological characteristics of the splicing process [10]. The Y-G splicing system is chosen since our research focuses on the preservation of biological characteristics of splicing process. In addition, the Y-G model has been proven in [9] to present the straightforward conduct of the DNA natural process.

A collection of DNA molecules that is produced when selective enzymes react with the DNA molecules is called the splicing language [4]. For an example, two restriction enzymes are chosen which are *Hpa*II and *HinP*1I where both of them are supplied with the CutSmart® Buffer. The Y-G splicing system consisting of a set of alphabets, $A=\{a,c,g,t\}$, a set of initial strings, $I=\{\alpha ccgg\beta, \gamma gcgc\delta\}$ such that α with β, γ with δ, α' with β', γ' with δ' are complement to each other

*Published By:
Blue Eyes Intelligence Engineering
& Sciences Publication*

where

$\alpha, \beta, \gamma, \delta, \alpha', \beta', \gamma', \delta' \in A^*$ and $R = \{r_1, r_2\}$ is a set of rules where $r_1 = (c; cg, g : c; cg, g)$ and $r_2 = (g; cg, c : g; cg, c)$.

The splicing language of this splicing system is

$$L(S) = I \cup \{\alpha ccgg\alpha', \beta' ccgg\beta, \gamma gcgc\gamma', \delta' gcgc\delta, \alpha ccgc\delta, \gamma gcgg\beta, \alpha ccgc\gamma', \delta' gcgg\beta\}.$$

A few types of splicing languages have been discovered including inert/adult, transient and limit languages [8]. The different kinds of splicing languages were introduced when a few experiments were carried out to verify the existence of splicing language from the biological aspect. According to the molecular experiment [8], a limit language is the remaining molecules after the splicing system has reached its equilibrium points or is completed. Later, the study of limit language has been extended to the study of the second order limit language [11].

Previously, sufficient conditions for the second order limit language have been discussed [12]. In this paper, the details of the formation of the second order limit language are investigated and the focus is on the number of cutting sites that exist in the set of rules. The results are presented in a series of lemmas followed by theorems.

This paper is arranged as pursues: the first section is the introduction, followed by the preliminaries in that fundamental descriptions are presented. The third section discusses the primary aftereffects of this paper. Then, the conclusions upon the findings are presented in the last section.

II. PRELIMINARIES

In this area, some basic explanation of this research then some types of splicing languages used in this paper are given.

The first three basic definitions are related to formal language theory, namely alphabet, string and language.

Definition 2.1 [13] A letters in order, *A*, is a finite, nonempty set of symbols.

Definition 2.2 [13] A string is a limited arrangement of symbols from the letter set.

Definition 2.3 [13] A set of strings all of which are chosen from some A^*, where *A* is a particular alphabet, is called a language.

From the biological point of view, complementarity bases are represented as a set of alphabet, initial strands of dsDNA molecules are represented as a set of initial strings while the dsDNA obtained from the cutting and pasting process is represented as language.

The concatenation between two languages, L_1 and L_2 has been given in [5] where $L_1 L_2 = \{xy \mid x \in L_1, y \in L_2\}$.

Further:

$$L^0 = \varnothing,$$

$$L^{i+1} = LL^i,\ i \geq 0,$$

$$L^* = \bigcup_{i=0}^{\infty} L^i \left(\text{the * - Kleene closure}\right),$$

$$L^+ = \bigcup_{i=1}^{\infty} L^i \left(\text{the * - Kleene closure}\right).$$

In this paper, we focus on Y-G splicing system. Its definition is given in the following:

Definition 2.4 [9] A splicing framework $S = (A, I, R)$ comprises of a lot of letters in order *A*, a pair of starting strings *I* in A^* then a set of rules, $r \in R$ where $r = (u, x, v: y, x, z)$. For $s_1 = \alpha uxv\beta$ and $s_2 = \gamma yxz\delta$ components of *I*, splicing s_1 and s_2 utilizing *r* produces the starting string *I* with $\alpha uxz\delta$ and $\gamma yxv\beta$, displaying in either order where $\alpha, \beta, \gamma, \delta, u, x, v, y$ and $z \in A^*$ are the free monoids produced by *A* with the sequence task and 1 as the integrity component.

Two types of splicing languages are discussed in this paper, namely transient and limit languages. Experimentally, a splicing language is called transient if a set of strings is eventually used up and disappear in a given system. Other than that, a splicing language is a limit language (first order limit language) where it is the set of words that are predicted to appear when the system reaches its equilibrium point. The definition of an extension of the first order limit language, namely the second order limit language, is defined below.

Definition 2.5 [11] Let *L*(*S*) be a splicing language of a splicing framework, *S* and $L_1(S)$ is the first order limit language. A splicing language is called a second order limit language, $L_2(S)$ if the set of strings produced in $L_2(S)$ is distinct from the set of strings of *L*(*S*) in which $L_2(S) \cap L(S) = \varnothing$ and $L_1(S) \not\subset L_2(S)$.

The next definition gives the characteristic on a string of double-stranded DNA (dsDNA) that will be discussed throughout this paper.

Definition 2.6 A string *I* of dsDNA is claimed to be palindromic if the sequence from the left to the right facet of the higher single strand upto the sequence from the right to the left facet of the lower single strand.

III. MAIN RESULTS

In this section, 5 lemmas and 2 theorems on the existence of the second order limit language in Y-G splice system with 2 cutting sites are given. Within the following theorem, a Y-G splice system associating a rule that has 2 cutting sites is considered. A few cases are considered due to the palindromic properties of the set of molecules.

Published By:
Blue Eyes Intelligence Engineering
& Sciences Publication

Theorem 1

Let $S=(A,I,R)$ be a Y-G splicing system where *A* is a set of alphabets, $I=\{s_1,s_2\}$ is a set of initial strings that has two cutting sites and $R=\{r\}$ is a set of rules. Then, there exists a second order limit language.

Proof

Case 1: Suppose that the recognition site of the rules is palindromic such that $s_1=\alpha axbaxb\beta,\ s_2=\gamma axbaxb\delta$ and $r=(a;x,b:a;x,b)$ where *a* and*b* are complement to each other and $\alpha,\beta,a,b,x\in A^*$. Therefore, some strings of the splicing language is generated as follows:

$$\{\alpha axbaxb\beta,\gamma axbaxb\delta\}\xrightarrow{r} I\cup\left\{\begin{array}{l}\alpha axb\beta,\alpha axb\alpha',\beta' axb\beta,\alpha axbaxb\alpha',\alpha axbaxb\beta,\\ \beta' axbaxb\beta,\alpha axbaxbaxb\beta,\alpha axbaxbaxb\alpha',\\ \beta' axbaxbaxb\beta,\gamma axb\delta,\gamma axb\gamma',\delta' axb\delta,\gamma axbaxb\gamma',\\ \gamma axbaxb\delta,\delta' axbaxb\delta,\gamma axbaxbaxb\delta,\gamma axbaxbaxb\gamma',\\ \delta' axbaxbaxb\delta,\alpha axb\delta,\alpha axb\gamma',\beta' axb\delta,\alpha axbaxb\gamma',\\ \alpha axbaxb\delta,\beta' axbaxb\delta,\alpha axbaxbaxb\delta,\alpha axbaxbaxb\gamma',\\ \beta' axbaxbaxb\delta,\beta' axbaxbaxb\gamma',\cdots\end{array}\right\}.$$

The resulted splicing language above is denoted as $L(S)$. The second order limit language, $L_2(S)$ is obtained when the splicing takes place for the second time and the resulted strings are distinct from $L(S)$. Thus, $L_2(S)$ can be generalized in the following form:

$$L_2(S)=\left\{\begin{array}{l}\alpha a(xba)^* xb\alpha',\beta' a(xba)^* xb\beta,\alpha a(xba)^* xb\beta,\\ \gamma a(xba)^* xb\gamma',\delta' a(xba)^* xb\delta,\gamma a(xba)^* xb\delta,\\ \alpha a(xba)^* xb\gamma',\beta' a(xba)^* xb\delta,\alpha a(xba)^* xb\delta,\\ \gamma a(xba)^* xb\beta\end{array}\right\}.$$

Case 2: Suppose that the crossing site of the rules are palindromic such that $s_1=\alpha cabd\beta,\ s_2=\gamma cabd\delta$ and $r=(c;ab,d:c;ab,d)$ where a with b, c with c' and d with d' are complement to each other and $\alpha,\beta,\gamma,\delta,a,b,c,d,\alpha',\beta',\gamma',\delta',c',d'\in A^*$. Therefore, some strings of the splicing language is generated as follows:

$$L(S)=I\cup\left\{\begin{array}{l}\alpha cabd\beta,\alpha cabc\alpha',\beta' d' abd\beta,\alpha cabdcabc'\alpha',\alpha cabc' d' abd\beta,\\ \beta' d' abdcabd\beta,\alpha cabdcabc' d' abd\beta,\alpha cabdcabc' d' abc'\alpha',\\ \alpha cabc' d' abdcabd\beta,\alpha cabc' d' abdcabc'\alpha',\beta' d' abdcabc' d' abd\beta,\\ \beta' d' abc' d' abdcabd\beta,\gamma cabd\delta,\gamma cabc'\gamma',\delta' d' abd\gamma,\gamma cabdcabc'\gamma',\\ \gamma cabc' d' abd\delta,\delta' d' abdcabd\delta,\gamma cabdcabc' d' abd\delta,\gamma cabdcabc' d' abc'\gamma',\\ \gamma cabc' d' abdcabd\delta,\gamma cabc' d' abdcabc'\gamma',\delta' d' abdcabc' d' abd\delta,\\ \delta' d' abc' d' abdcabd\delta,\cdots\end{array}\right\}.$$

The second order limit language, $L_2(S)$ is generated below:

$$L_2(S)=\begin{Bmatrix}\alpha c(abdc\cup abc'd')^* abc'\alpha', \beta'd'(abc'd'\cup abdc)^* abd\beta, \alpha c(abc'd'\cup abdc)^* abd\beta,\\ \gamma c(abdc\cup abc'd')^* abc'\gamma', \delta'd'(abc'd'\cup abdc)^* abd\delta, \gamma c(abc'd'\cup abdc)^* abd\delta,\\ \alpha c(abdc\cup abc'd')^* abc'\gamma', \beta'd'(abc'd'\cup abdc)^* abd\delta, \alpha c(abc'd'\cup abdc)^* abd\delta,\\ \gamma c(abc'd'\cup abdc)^* abd\beta\end{Bmatrix}.$$

Case 3: Suppose that the rule is non-palindromic such that $s_1=\alpha axdaxd\beta,\ s_2=\gamma axdaxd\delta$ and $r=(a;x,d:a;x,d)$ where $\alpha,\beta,\gamma,\delta,a,d\in A^*$. Therefore, the splicing language is generated as follows:

$$L(S)=I\cup\begin{Bmatrix}\alpha axd\beta, \gamma axd\delta, \alpha axdaxd\delta, \gamma axdaxd\beta,\\ \alpha axdaxdaxd\delta, \gamma axdaxdaxd\beta\end{Bmatrix}.$$

Thus, $L_2(S)$ is listed in the following general form:

$$L_2(S)=\begin{Bmatrix}\alpha a(xda)^* xd\beta, \gamma a(xda)^* xd\delta,\\ \alpha a(xda)^* xd\delta, \gamma a(xda)^* xd\beta\end{Bmatrix}. \square$$

The second hypothesis, a Y-G splicing framework that has 2 guidelines of 2 cutting sites is considered. In proving the existence of the second order limit language, five lemmas are used. The crossing sites in these lemmas have different characteristics.Then, four cases are discussed in each lemma i.e. the first rule is connected to the first string and the 2nd guideline is applied to the second string, both rules are applied on the first string and the second rule is applied on the second string, the first rule is applied on the first string and both rules are applied on the second string, and both rules are applied on both strings.

Lemma 1

Let $S=(A,I,R)$ be a Y-G splicing framework where *A* is a set of letters in order, $I=\{s_1,s_2\}$ is a pair of starting strings that has two cutting sites and $R=\{r_1,r_2\}$ is a set of rules, given that $r_1=(a;x,b:a;x,b)$ and $r_2=(c;x,d:c;x,d)$ where *a* with *b* and *c* with *d*are complement to each other and $\alpha,\beta,\gamma,\delta,a,b,c,d,\alpha',\beta',\gamma',\delta'\in A^*$. Therefore, a crossing site of the set of rules is palindromic and identical. Then, the second order limit language exists.

Proof

Case 1: Suppose r_1 applies on $s_1=\alpha axbaxb\beta$ and r_2 applies on $s_2=\gamma cxdcxd\delta$. The second order limit language, $L_2(S)$ is generated as follows:

$$L_2(S)=\begin{Bmatrix}\alpha a(xba\cup xdc)^* xb\alpha', \beta'a(xba\cup xdc)^* xb\beta, \alpha a(xba\cup xdc)^* xb\beta,\\ \gamma c(xdc\cup xba)^* xd\gamma', \delta'c(xdc\cup xba)^* xd\delta, \gamma c(xdc\cup xba)^* xd\delta,\\ \alpha a(xba\cup xdc)^* xd\gamma', \beta'a(xdc\cup xba)^* xd\delta, \alpha a(xdc\cup xba)^* xd\delta,\\ \gamma c(xba\cup xdc)^* xb\beta\end{Bmatrix}.$$

Case 2: Suppose r_1,r_2 apply on $s_1=\alpha axbcxd\beta$ and r_2 applies on $s_2=\gamma cxdcxd\delta$. The second order limit language $L_2(S)$ is generated as follows:

$$L_2(S)=\begin{Bmatrix}\alpha a(xbc\cup xda\cup xdc)^* xb\alpha', \beta'c(xbc\cup xda\cup xdc)^* xd\beta, \alpha a(xbc\cup xda\cup xdc)^* xd\beta,\\ \gamma c(xdc)^* xd\gamma', \delta'c(xdc)^* xd\delta, \gamma c(xdc)^* xd\delta, \alpha a(xbc\cup xda\cup xdc)^* xd\gamma',\\ \beta'c(xbc\cup xda\cup xdc)^* xd\delta, \alpha a(xbc\cup xda\cup xdc)^* xd\delta, \gamma c(xbc\cup xda\cup xdc)^* xd\beta\end{Bmatrix}.$$

Case 3: Suppose r_1 applies on $s_1=\alpha axbaxb\beta$ and r_1,r_2 apply on $s_2=\gamma axbcxd\delta$. The second order limit language, $L_2(S)$ is generated as follows:

$$L_2(S)=\left\{\begin{array}{l}\alpha a(xba)^* xb\alpha',\beta'c(xba)^* xd\beta,\alpha a(xba)^* xd\beta,\gamma c(xbc\cup xda\cup xdc)^* xd\gamma',\\ \delta'c(xbc\cup xda\cup xdc)^* xd\delta,\gamma c(xbc\cup xda\cup xdc)^* xd\delta,\alpha a(xbc\cup xda\cup xdc)^* xd\gamma',\\ \beta'c(xbc\cup xda\cup xdc)^* xd\delta,\alpha a(xbc\cup xda\cup xdc)^* xd\delta,\gamma c(xbc\cup xda\cup xdc)^* xd\beta\end{array}\right\}.$$

Case 4: Suppose r_1 and r_2 apply on both $s_1=\alpha axbcxd\beta$ and $s_2=\gamma axbcxd\delta$. The second order limit language, $L_2(S)$ is generated as follows:

$$L_2(S)=\left\{\begin{array}{l}\alpha a(xbc\cup xda\cup xdc)^* xb\alpha',\beta'c(xbc\cup xda\cup xdc)^* xd\beta,\alpha a(xbc\cup xda\cup xdc)^* xd\beta,\\ \gamma a(xbc\cup xda\cup xdc)^* xb\gamma',\delta'c(xbc\cup xda\cup xdc)^* xd\delta,\gamma a(xbc\cup xda\cup xdc)^* xd\delta,\\ \alpha a(xbc\cup xda\cup xdc)^* xb\gamma',\beta'c(xbc\cup xda\cup xdc)^* xd\delta,\alpha a(xbc\cup xda\cup xdc)^* xd\delta,\\ \gamma a(xbc\cup xda\cup xdc)^* xd\beta\end{array}\right\}.$$

The proofs for Lemma 2 – Lemma 5 are not given since they are similar to Lemma 1.

Lemma 2

Let $S=(A,I,R)$ be a Y-G splicing system where *A* is a set of alphabets, $I=\{s_1,s_2\}$ is a set of initial strings that has two cutting sites and $R=\{r_1,r_2\}$ is a set of rules, given that $r_1=(a;x,b:a;x,b)$ and $r_2=(c;y,d:c;y,d)$ where *a* with *b* and *c* with *d* are complement to each other and $\alpha,\beta,\gamma,\delta,a,b,c,d,\alpha',\beta',\gamma',\delta'\in A^*$. Therefore, the crossing site of the set of rules is palindromic and non-identical where $x\neq y$. Then, the second order limit language exists.

Moreover, Lemma 3 and Lemma 4 discuss about crossing site of the set of rules which is non-palindromic.

Lemma 3

Let $S=(A,I,R)$ be a Y-G splicing system where *A* is a set of alphabets, $I=\{s_1,s_2\}$ is a set of initial strings that has two cutting sites and $R=\{r_1,r_2\}$ is a set of rules, given that $r_1=(a;x_1x_2,b:a;x_1x_2,b)$ and $r_2=(c;x_1x_2,d:c;x_1x_2,d)$ where *a* with *b* and *c* with *d* are complement to each other and $\alpha,\beta,\gamma,\delta,a,b,c,x_1,x_2,d,\alpha',\beta',\gamma',\delta',x_1',x_2'\in A^*$. Therefore, the crossing site of the set of rules is non-palindromic, i.e, x_1 is not complement to x_2, and is identical. Then, the second order limit language exists.

Lemma 4

Let $S=(A,I,R)$ be a Y-G splicing system where *A* is a set of alphabets, $I=\{s_1,s_2\}$ is a set of initial strings that has two cutting sites and $R=\{r_1,r_2\}$ is a set of rules, given that $r_1=(a;x_1x_2,b:a;x_1x_2,b)$ and $r_2=(c;y_1y_2,d:c;y_1y_2,d)$ where *a* with *b* and *c* with *d* are complement to each other and $\alpha,\beta,\gamma,\delta,x_1,x_2,y_1,y_2,a,b,c,d,\alpha',\beta',\gamma',\delta',x_1',x_2',y_1',y_2'\in A^*$. Therefore, crossing site of the set of rules is non-palindromic such that x_1 and x_2 are not complement to y_1 and y_2 respectively and is non-identical where $x_1x_2\neq y_1y_2$. Then, the second order limit language exists.

Next, Lemma 5 discusses about crossing sites of the set of rules which are palindromic and non-palindromic.

In addition, Lemma 5 concentrate on a set of rules that contain both palindromic and non-palindromic cutting sites.

Lemma 5

Let $S=(A,I,R)$ be a Y-G splicing system where *A* is a set of alphabets, $I=\{s_1,s_2\}$ is a set of initial strings that has two cutting sites and $R=\{r_1,r_2\}$ is a set of rules, given that $r_1=(a;x_1x_2,b:a;x_1x_2,b)$ and $r_2=(c;y_1y_2,d:c;y_1y_2,d)$ where *a* with *b*, *c* with *d* and x_1 with x_2 are complement to each other and $\alpha,\beta,\gamma,\delta,x_1,x_2,y_1,y_2,a,b,c,d,\alpha',\beta',\gamma',\delta',x_1',x_2',y_1',y_2' \in A^*$. Therefore, crossing site of the first rule is palindromic and the second rule is non-palindromic such that y_1 is not complement to y_2. Then, the second order limit language exists.

Theorem 2

Let $S=(A,I,R)$ be a Y-G splicing system where *A* is a set of alphabets, *I* is a set of initial strings with two cutting sites and *R* is a set of two different rules where it is either palindromic or non-palindromic or both. Then there exists a second order limit language.

Proof

From Lemma 1 to Lemma 5, Y-G splicing systems involving a set of rules that is palindromic and identical, palindromic and non-identical, non-palindromic and identical, non-palindromic and non-identical, and a set of rules that contains a string with palindromic sequence and another string with non-palindromic sequence, are considered respectively. For each lemma, four cases are discussed i.e. the first rule is applied to the first string and the second rule is applied to the second string, both rules are applied on the first string and the second rule is applied on the second string, the first rule is applied on the first string and both rules are applied on the second string, and both rules are applied on both strings. Since all lemmas produce a second order limit language, therefore, it has been proven that Y-G splicing systems involving a set of initial strings with two cutting sites and a set of two different rules where it is either palindromic or non-palindromic or both, produced a second order limit language. Thus, the proof is complete.

IV. CONCLUSIONS

In summary, it has been shown that the second order limit language is proven to exist regardless of the number of rules involved (at most two rules) giving that there are two cutting sites in the set of rules for the splicing system. In the first theorem, when a rule is involved, three cases has been considered to study the existence of the second order limit language where the recognition site of the set of guidelines is palindromic, the crossing site of the set of rules is palindromic and the set of rules is non-palindromic. Five lemmas have been presented to prove the second theorem, where two rules are considered. These lemmas are divided into five different characteristics which are the set of guidelines is palindromic and indistinguishable, the set of guidelines is palindromic and non-indistinguishable, a set of rules is non-palindromic and identical, the set of rules is non-palindromic and non-identical, and a rule is palindromic and the other one is non-palindromic. Biologically, the second order limit language for a specific case has been proven to exist in the mathematical modelling of the laboratory experiment.

ACKNOWLEDGEMENT

The first author is indebted to Universiti Malaysia Pahang for the financial funding through RDU1703278. The second and third authors want to thank MOE and Research Management Centre (RMC), UniversitiTeknologi Malaysia (UTM) for the financial funding through UTM Research University Grant Vote No. 13H18.

REFERENCES

1. Tamarin, R. H. (2001).*Principles of Genetics*, 7th Ed., The Mac-Graw Hill Companies, USA.
2. Alcamo, I. E. (2001).*DNA Technology The Awesome Skill*, 2nd ed., Harcourt/Academic Press, USA.
3. Dwyer, C. and Lebeck, A. (2008).*Introduction to DNA Self Assembled Computer Design*, Boston, Artech House, Inc., London.
4. Head T.“Formal Language Theory and DNA: An Analysis of The Generative Capacity of Specific Recombinant Behaviors.”*Bulletin of Mathematical Biology* 49, (1987): 737 – 759.
5. Paun, Gh., Rozenberg, G. and Salomaa, A. (1998).*DNA Computing: New Computing Paradigms*, Springer-Verlag Berlin Heidelberg, New York, London.
6. Paun, Gh.“On the Splicing Operation.”*Discrete Applied Mathematics* 70, (1996): 57 – 59.
7. Pixton, D.“Regularity of Splicing Languages.”*Discrete Applied Mathematics* 69, (1996): 101-124.
8. Goode, E. and Pixton, D. “Splicing to the Limit,” in *Aspects of Molecular Computing*, edited by N. Janoska, Gh. Paun and G. Rozenberg, Springer Berlin Heidelberg, New York, London (2004).
9. Yusof, Y., Sarmin, N. H., Goode, T. E., Mahmud, M and Fong, W. H.“An Extension of DNA Splicing System.”In *Proceedings - 2011 Sixth International Conference on Bio-Inspired Computing: Theories and Applications*, p. 246. 2011.
10. Karimi, F., Sarmin N. H. and Fong, W. H.“Some Sufficient Conditions for Persistent Splicing Systems.”*Australian Journal of Basic and Applied Sciences* 5,no. 1 (2011): 20 – 24.
11. Ahmad, M. A., Sarmin, N. H., Fong. W. H. and Yusof, Y.“An Extension of First Order Limit Language.” In*AIP Conference Proceedings* 1602,p. 627. 2014.
12. Ahmad, M. A., Sarmin, N. H., Fong. W. H. and Yusof, Y.“A Comparison of Second Order and Non-Second Order Limit Language Generated by Yusof-Goode Splicing System.”*Jurnal Teknologi* 72, no. 1 (2015): 27 – 31.
13. Linz, P. (2006). An Introduction to Formal Languages and Automata, Jones and Barlett Publisher, USA.