DNA Recombination: Looking at a Biological Process through the Lens of Knot Theory

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1 Introduction: DNA Recombination

1.1 The Structure of a DNA Molecule

All living cells, without any exception, contain their hereditary information in the form of double stranded molecules of DNA. These molecules are linear for higher living organisms such as humans, but can also form closed loops, as is the case for bateria. DNA is a chain of 4 basic subunits: adenine, thymine, cytosine and guanine. The specific sequence of these four subunits forms the basis for the genetic information, and is termed the **primary** structure of DNA.

Due to the chemical structure of the four basic subunits, DNA takes on a local double helical geometry, which is named the **secondary** structure of DNA. More importantly for this study, DNA molecules can become folded on themselves, much as a phone wire can twist on itself. As a result, DNA can take on a very complex three dimensional structure (Fig. 1)[1], which is termed the **tertiary** structure of DNA.

Figure 1: Tertiary Structure of DNA. DNA can fold and twist on itself, thus having the ability to take on very complex three dimensional structures, [picture obtained from *Berg, Tymoczko and Stryer. Biochemistry. W. H. Freeman. 6th edition. New York. p789*].

1.2 DNA Recombination

This complex tertiary structure can cause DNA to become knotted. If we represent a DNA double helix as a simple thread, we can think of the thread as crossing over and under itself. In the case of bacterial, viral, or mitochondrial DNA, the molecules can become truly knotted as they form closed loops (Fig. 2)[6]. It is impossible to untie the knots these specific DNA molecules form without using a tool to cut the loops and undo the crossings. Molecular biologists use DNA knots to study specific enzymes called recombinases.

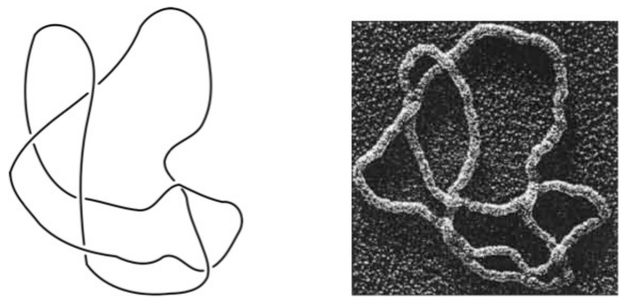


Figure 2: DNA knotting. As DNA takes on a complex three dimensional structure, it can cross over and under itself. In the case of bacterial DNA, the whole molecule forms a closed loop, and is thus truly knotted. These knots are observed experimentally, [picture obtained from *Sumners. Lifting the Curtain: Using Topology to Probe the Hidden Action of Enzymes. Notices of AMS. 1995. 528-37*].

Generally speaking, a recombinase is an enzyme that cuts and pastes DNA segments. Recombinases bind to two DNA segments, forming a productive synapse; they cut each segment, switch them, and paste them back together to obtain recombined DNA. These enzymes are involved in a wide variety of processes, and are of great interest to molecular biologists. There are two main families of DNA recombinases: serine recombinases, and tyrosine recombinases.

Let us consider the basic mechanism of employed by tyrosine recombinases (Fig. 3)[5]. A tyrosine recombinase begins by binding two DNA segments, forming a productive synapse. It then cuts one strand of each segment, exchanges them, and pastes them to the other segment. It then does the same operation on the two other strands. Serine recombinases use a similar mechanism. While these basic mechanisms are well understood, there is much to learn about their details. Molecular biologist can learn more about these by studying how recombinases change DNA knotting configurations.

To understand this, we can represent DNA schematically as a simple thread. We can then represent the product schematically as well. Given a specific knot, we can place a productive synapes of this given substrate, and see that recombination would change the knot inherently (Fig. 4). Molecular biologists can learn very important information about recombinases by studying which types of knots they produce for a given knot substrate. However, identifying the product knots experimentally can be very challenging.

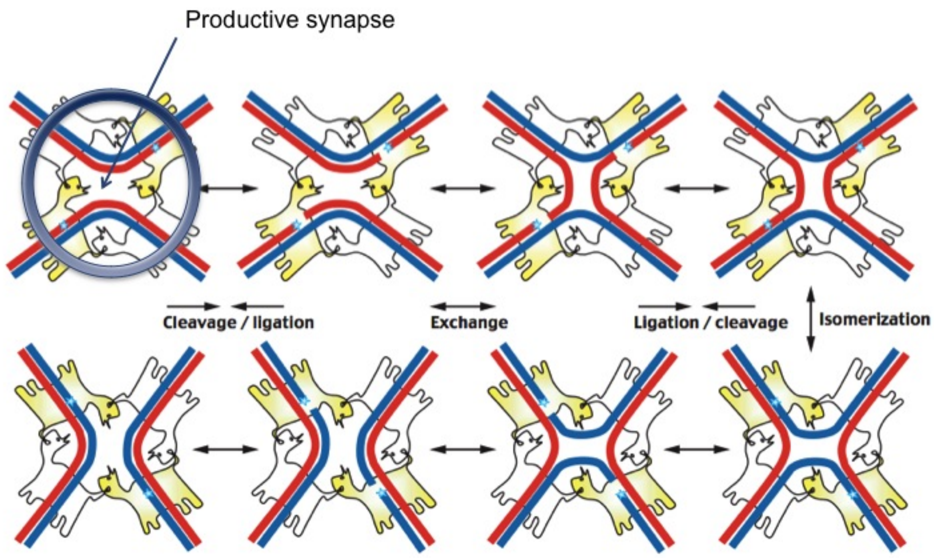


Figure 3: Tyrosine recombinase general mechanism, picture obtained from [*Grindley, Whiteson and Rice. Mechanisms of Site-Specific Recombination. Annual Review of Biochemistry. 2006. 567-601*].

Topological techniques can greatly help molecular biologists by predicting all possible product knots for a given substrate. We were interested in a specific knot substrate, the connected sum of two torus knots. These knots are not only interesting mathematically, but they have also been observed experimentally as the recombination product of the Hin recombinase. This study's goal was to determine all the recombination products for the connected sum of two trefoil knots (Fig. 5). Before presenting the proof, we will introduce the reader to the basics of knot theory, and then the Buck-Flapan model which we used to write our proof.

2 Basics of Knot Theory

In order to better understand DNA knots and DNA recombination, this section introduces the reader to the basics of knot theory. The content covered here includes the definition of knots, ambient isotopy, torus knots, connected sums of two knots and in particular, connected sums of two torus knots, and finally, knots and surfaces.

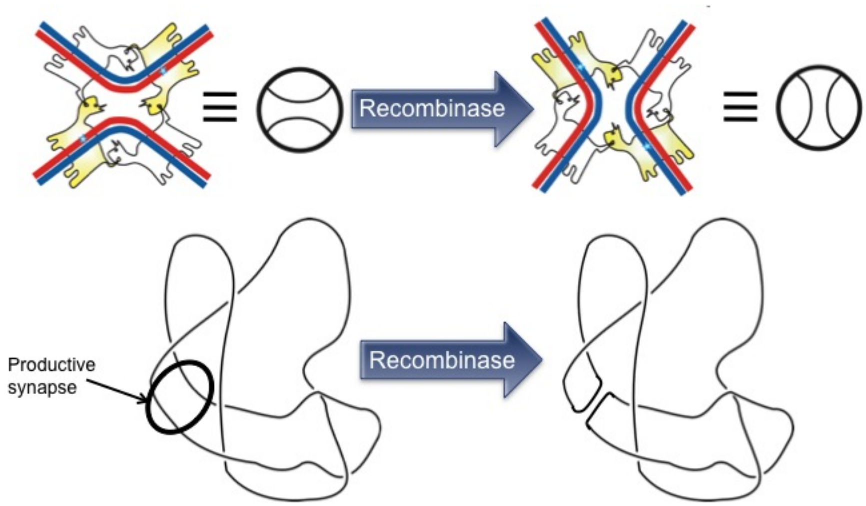


Figure 4: Recombinases and DNA knotting

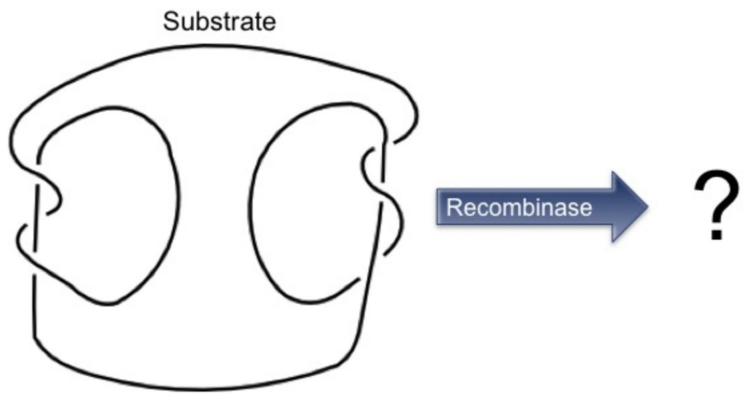


Figure 5: Our problem.

2.1 Knots

What is a knot? Before the formal mathematical definition of knots is stated, it is interesting to first consider how one may construct a knot. Suppose one is given a piece of closed loop shown on the left of Fig.6, and asked to cut it into an open curve by a pair of scissors. Then,the curve can be tied by creating over and under crossings shown in the third picture of Fig.6. If the two ends of the open strand are pasted together to form a closed curve, then a new knot was successfully generated. It is worth noting here that a knot cannot be turned into an open curve again without using any cutting device.

Definition: A **knot** is a closed curve in space that does not intersect itself anywhere.

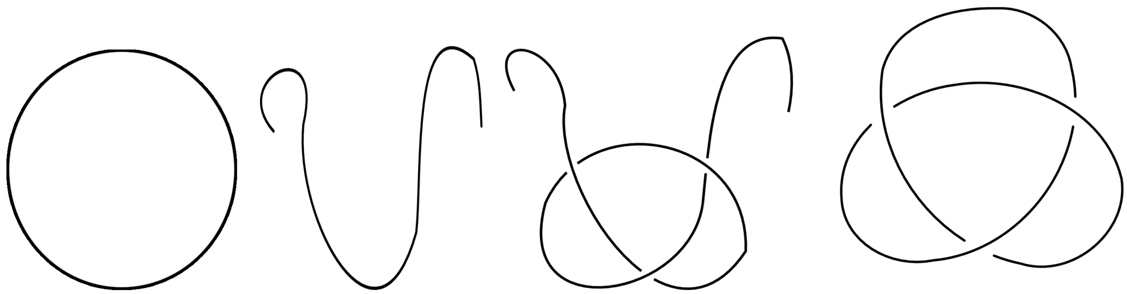


Figure 6: The process of constructing a knot. The fourth picture on the right is a trefoil knot.

2.1.1 Ambient Isotopy

Definition: **Ambient isotopy** is a continuous distortion of a manifold, taking one submanifold to another. Two knots are equivalent if there exists an ambient isotopy between them.

We regard knots as being deformable. Suppose knots are made of silk or rubber, one can imagine the knots as being bent or unbent, twisted or untangled, pulled or pushed, shrunk or stretched as one would like. Such deformations do not change the type of the knot. They only change the planar projections of knots, namely, how the knots are portrayed on a plane. Knot theorists call such deformations **ambient isotopy**. Mathematically speaking, ambient isotopy is a continuous map on the knot complement in $\mathbb{R}^3$. Two knots are equivalent if there exists an ambient isotopy between them. In Fig.7, all three graphs are ambient isotopic. The left-most graph can be achieved by stretching and shrinking some parts of the arcs from the middle knot. Similarly, the right-most knot is obtained by pulling the tiny piece of arc at the bottom to form an extended loop.

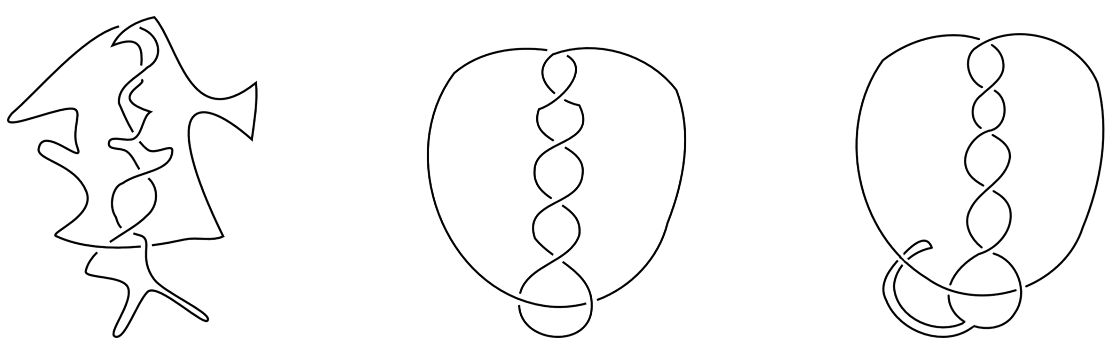


Figure 7: Ambient Isotopy and Knot Equivalence: The above three knots are equivalent because there exist ambient isotopies between them.

The reader should familiarize himself or herself with some specific knot types that knot theorists often study.

Most of them are useful in our analysis as well. We begin by considering the most basic knot, shown on the left-most of Fig.9, which is called **unknot**. Unsurprisingly, this knot type is called unknot because it is just a closed loop, without having crossings along the loop. Knot theorists also term it as trivial knot. We now introduce the reader to a class of knots called **torus knots**, which are the center piece of this study. (See Fig.8, Fig.9b and Fig.9c)

2.1.2 Torus Knots

Definition: A **torus knot** is a knot that lies on an unknotted torus, without crossing over or under itself when it is on the torus.

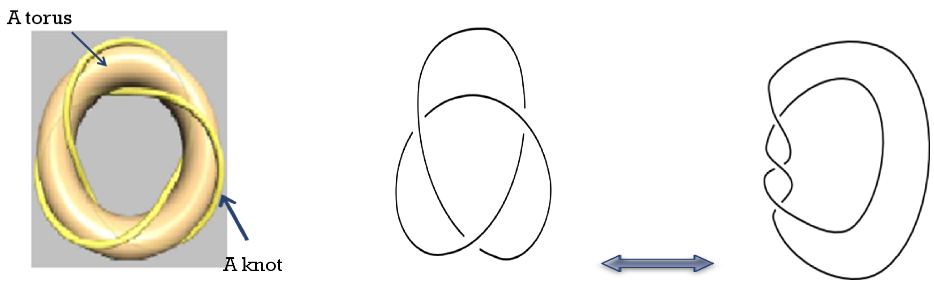
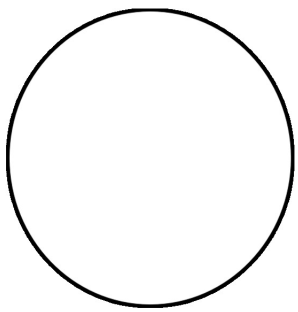


Figure 8: Illustrations of Torus knot T(2,3): The left-most graph best illustrates the torus knot T(2,3) wraps around a torus, [picture obtained from *Knot Spanning-Surface Generator, R. Khardekar and X. R. Chen, CiteSeer*].

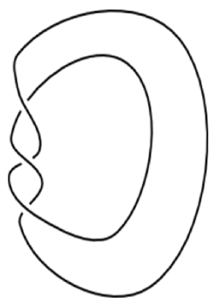
The left-most picture in Fig.8 best illustrates the definition of a torus knot. The shallow yellow part indicates a torus surface and the golden rope wrapping around it indicates the knot that is wrapping around this torus. Therefore, a knot that lies on an invisible torus that does not cross under or over itself is called a **torus knot**. Knot theorists often denote a torus knot by $T(p, q)$ with $p, q \in \mathbb{Z}$. Here, p represents the number of times that the rope wraps around an invisible torus longitudinally, that is, in a longer way; q represents the number of times that the rope wraps around an invisible torus meridionally, that is, in a shorter way. Thus, the trefoil knot is also a $T(2, 3)$ torus knot in the sense that the the knot wraps around a torus meridionally three times and longitudinally twice. If the reader imagines that the torus disappears, it should then be clear to visualize how the middle knot picture in Fig.8 was obtained.

Moreover, up to ambient isotopy, the middle knot of Fig.8 can be deformed by moving around the knot arcs to obtain a new knot projection, which is shown in the right-most picture in Fig.8. These two knots are equivalent. We base our study on the right-most knot projection of Fig.8, as it facilitates our understanding about the surfaces of this specific knot.

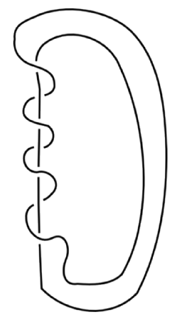
Similarly, Fig.9c listed in Fig.9 is a $T(2,7)$. Knots can be as complicated as the fourth and fifth knots listed in Fig.8. These two specific knots are called **pretzel knot** and **clasp knot**, respectively. They are important in our studies, because they turn out to be parts of the product knots that we obtain after DNA recombination by using the new substrate that we define in our problem. Pretzel knots and clasp knots are complicated knot types. Their characteristics are beyond the scope of this paper. The reader can refer to *The Knot Book*[4] to learn more about them. After considering the basic knot types, we introduce the reader to **connected sums** of two knots.



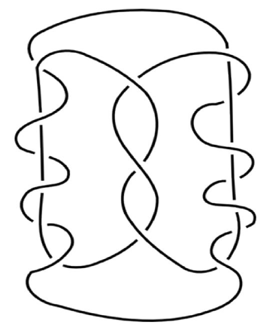
(a) An unknot



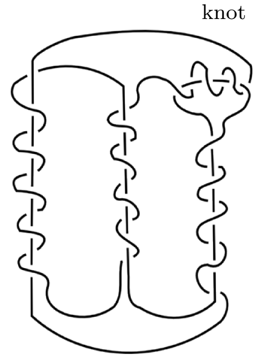
(b) A $T(2,3)$ knot



(c) A $T(2,7)$ knot



(d) A pretzel knot



(e) A clasp knot

Figure 9: Pictures of knots

2.1.3 Connected Sums

Definition: Mathematically speaking, **connected sum** of two knots is an operation, whose effect is to join two given manifolds together near a chosen spot on each.

The right graph in Fig.10 may seem quite familiar, as it appears to consist of one $T(2,3)$ to the left and one reversed version of $T(2,3)$ to the right. Indeed, this new knot is called the connected sum of two $T(2,3)$ torus knots. Mathematically speaking, a connected sum is an operation, whose effect is to join two manifolds near two chosen points on each. Here, we restrict the definition to only apply to knots, instead of higher dimensional manifolds.

A connected sum of two knots can be easily constructed. The reader first needs to find two chosen points on each knot. He or she can then cut both knots at the chosen spots, and add a "bridge" from both chosen spots to connect two knots together. Up to ambient isotopy, the reader can pull wide apart the arcs to form a better-looking knot projection on a plane. The process is illustrated in Fig.11 with a specific knot type $T(2,3)$. However, this process is applicable to any arbitrary knot connected sums. Note, this is a simplified version of connecting two knots together.

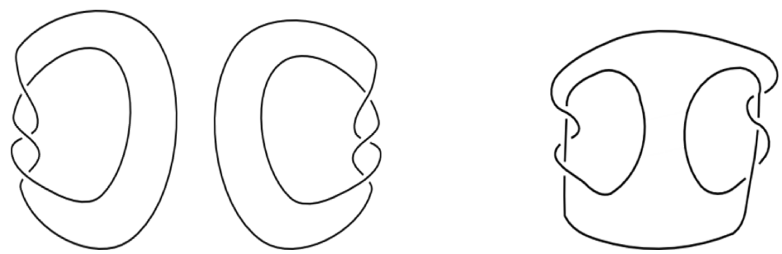


Figure 10: T(2, 3) + T(2,3) = T(2,3)#T(2,3)

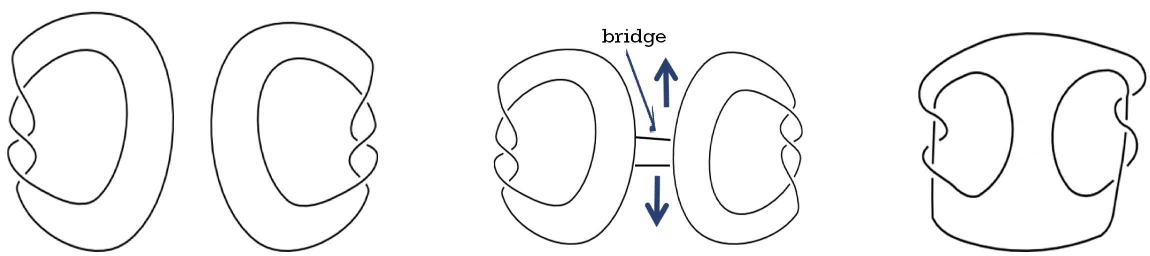


Figure 11: How we construct the given knot projection of T(2,3)#T(2,3) by adding a new bridge and pull wide apart the bridge.

The right-most graph in Fig.11 is one of the most important graphs in our study, because it forms the new substrate knot that we define in our study. This graph shows up regularly in the later sections of this paper. We denote it as a $T(2,3)\#T(2,3)$.

2.2 Surfaces

2.2.1 Surfaces with Boundary

So far, this paper has introduced some important knot types by visualizing planar knot projections. This next section introduces the concept of **surfaces**. Surfaces are objects that are well understood mathematically, and knot theorists can use this knowledge to study and distinguish different knots. As a result, surfaces are widely used in knot theory, and prove to be particularly helpful to study questions such as the one set out in this study.

Definition: A **surface** is a two dimensional topological manifold.

This definition seems technical. To better illustrate, we may consider a good example of a surface is the glaze of a doughnut, instead of the solid doughnut itself. Both graphs in Fig.12 are good representatives of surfaces of a knot. The left one is the surface of a sphere while the right one is the torus surface. Some surfaces have boundaries as shown in Fig.12. However, some surfaces have boundary and are called **surfaces with boundary**.

Definition: A **surface with boundary** is a closed surfaces with more than one holes in it. In the other word, It is a surface with a number of open discs removed from the closed surface.

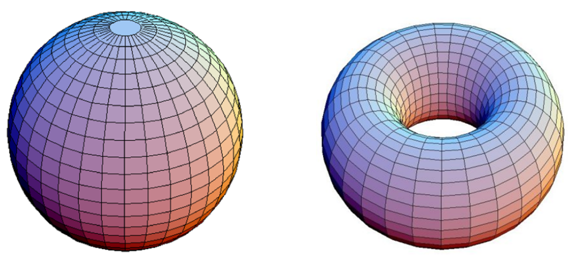


Figure 12: Two examples of surfaces without boundary. Left graph indicates a sphere surface; right indicates a torus surface. [Pictures were made using *Mathematica*]

Fig.13 best illustrates a torus with boundary. The open disc is removed from the torus surface. The black highlighted closed loop indicates the **boundary component** of this surface. If looking from another perspective, the boundary component of this surface is an unknot.

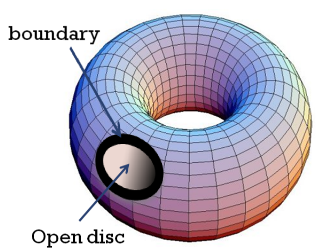


Figure 13: One example of surfaces with boundary. [Picture was made using *Mathematica*]

2.2.2 Spanning Surface and Planar Surface with Twists

We study a specific type of surface **spanning surface** in detail.

Definition: A **spanning surface** is a surface whose boundary component is a knot.

Every knot as a spanning surface, but a spanning surface is not unique. Fig.14 illustrates one spanning surface (represented by the grey area) that we can assign to a trefoil knot. To answer our question about the recombination products, we consider a **planar surface with twists** for the substrate knot $T(2,m)$ # $T(2,n)$.

Definition: A **planar surface with twists** is a surface that lies in the plane with twists which are allowed to live outside the plane of the surface. For $T(2,m)$ # $T(2,n)$, we considered two twisted bands connected together. (Fig.15)

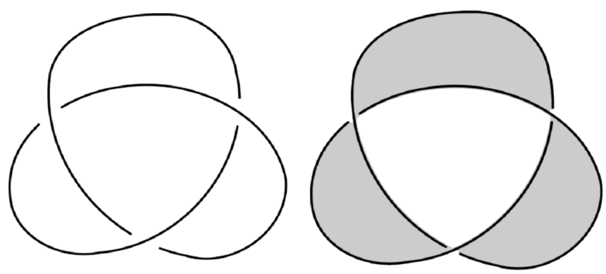


Figure 14: A spanning surface of the trefoil knot: a spanning surface for the trefoil knot. It is a planar surface with twist in this case.

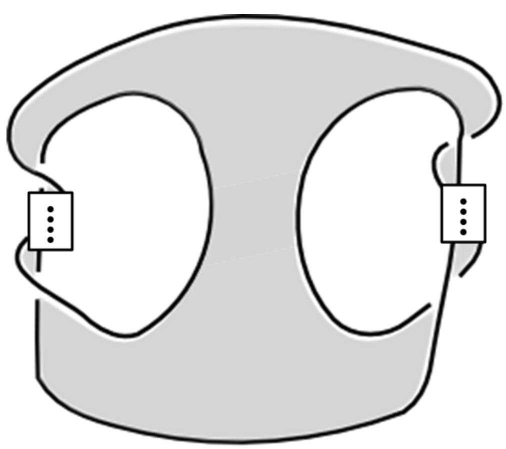


Figure 15: Spanning surface for $T(2,m)\#T(2,n)$.

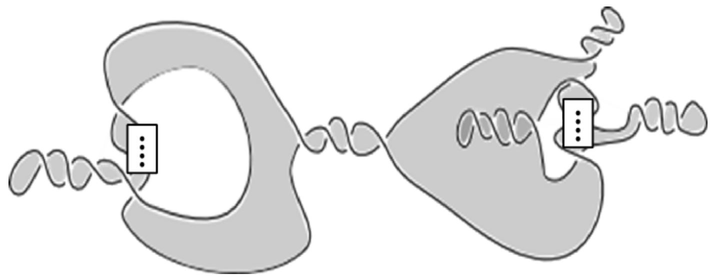


Figure 16: A more general Spanning surface for $T(2,m)\#T(2,n)$.

However, this surface may not be an accurate representation of DNA molecules in solution. In reality, DNA knots can have supercoils at different locations, even though its basic knotting configuration remains the same. Therefore, we considered a more general spanning surface. (Fig.16)

Even though the spanning surface looks complicated, it is still a planar surface with twists. The spanning surface is very important in our analysis. It provides a way to visualize the 3-dimensional supercoiled structure of DNA knot. As such, it is a tool that helps create a model to study DNA recombination systematically in full generality. The spanning surface shown in Fig.16 thus plays an important role in our proof, which will be discussed in the later sections.

3 Buck and Flapan's Work

Our problem and its analysis are an extension of Buck and Flapan's previous work[3],[2]. They developed a methodology to study the products arising from DNA recombination. In this previous work, they considered substrates to be an unknot, an unlink or a torus knot of the form $T(2,m)$, and categorized families of their recombination products. In this paper, we will refer to their methodology as the Buck-Flapan model. Their model developed mathematical assumptions based on biological evidence, to analyze DNA recombination using topological techniques. To study our problem, which was to determine all recombination product knots of a connected sum of two torus-2 knots, we used the methodology developed in the Buck-Flapan model.

3.1 Terminology

We will be using the following terminology and notation for the rest of the paper.

1. First, let J denote the substrate which is $T(2,m)\#T(2,n)$ in our problem, where $m \neq n \forall m,n \geq 3$ (Fig.17).

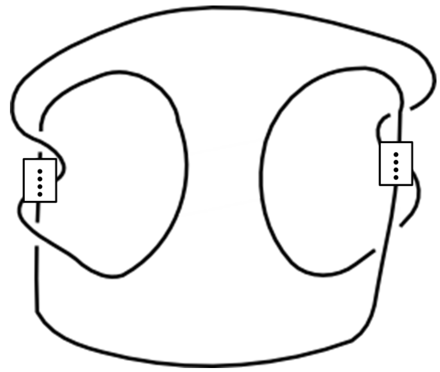


Figure 17: Substrate knot in our problem $T(2,m)\#T(2,n)$.

2. Second, let B denote the recombinase complex, which intersects the substrate, J, at precisely four points (two crossover sites) (Fig.18). Also, we denote $C = cl(\mathbb{R}^3 - B)$.

Figure 18: Productive synapse B: B can be of the following two forms. The outer circle represents the boundary of the recombinase.

3. Third, let D denote the spanning surface with boundary J, which is a planar surface with twists, topologically equivalent to two twisted bands connected (Fig.19).

3.2 Assumptions of the Buck-Flapan Model

We now introduce the reader to the three assumptions developed by Buck and Flapan to study DNA recombination from a topological perspective. These assumptions ensure that the product knots that are predicted topologically are always grounded in reality, supported by biological evidence.

Assumption 1:

The first assumption of the Buck-Flapan model concerns the interior of the productive synapse B. This

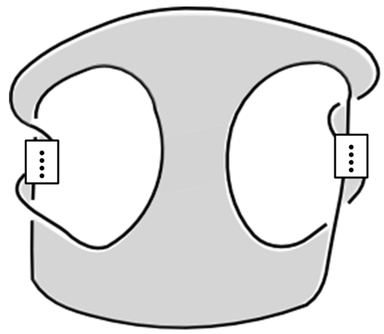


Figure 19: A spanning surface for $T(2,m)\#T(2,n)$.

assumption states that multiple crossings inside B are not allowed. The only allowed case is to have two parallel threads of J inside B with exactly four points in $J \cap \partial B$.

This assumption is based on evidence from recent crystal structures that show that productive synapses do not trap such multiple crossings. Thus, only one possible picture is possible for the interior of B, which is having two parallel segments of DNA running through B.(See Fig.20 and Fig.21)

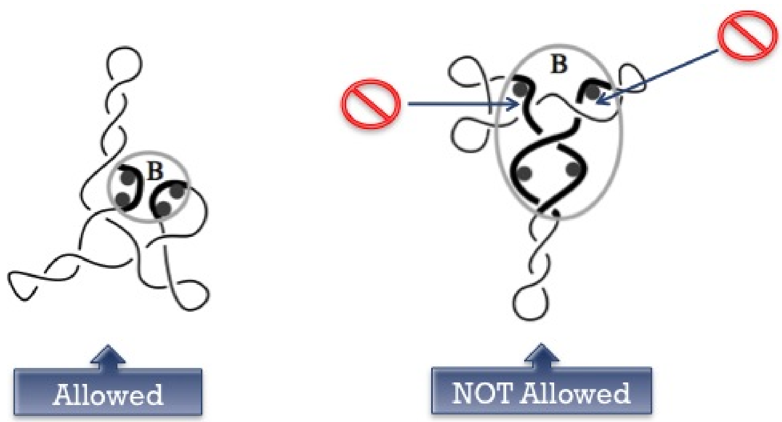


Figure 20: Assumption 1. Parallel segments in B are allowed in B, but multiple crossings are not

Figure 21: General allowed forms for B by assumption 1.

Assumption 2:

This assumption is related to restrictions outside B. Since where B intersects D determines the product knot type, assumption 2 is an important factor in restricting the types of product knots.

Assumption 2 states that J has a planar spanning surface D such that $D \cap \partial B$ consists of two arcs which are co-planar and, as Buck and Flapan describe it, "$D \cap C$ is unknotted rel ∂B." In other words, recombination cases where D is not planar prior to recombination are strictly not allowed. This means that cases where the substrate becomes doubly knotted prior to recombination are forbidden.

These are the implications of assumption 2:

1. B does not pierce through the spanning surface D in a nontrivial way. This implies that $D \cap \partial B$ cannot contain a circle in addition to the 2 segments of J required by **Assumption 1**.
2. No nontrivial knots are trapped outside B assuming B is fixed. This refers to the fact that substrate cannot knot itself before recombination. Indeed, if such doubly knotted cases were allowed, the surface would no longer be planar before recombination, which is forbidden by this assumption.

Biologically, since the opposing strands of the supercoiled DNA are close together, the restriction that B does not pierce through D is valid since the probability of this happening is low. In addition, if a nontrivial knot could be trapped outside B, those forms of product knots that might arise from such recombination process are not supported experimentally or by numerical simulations.

Assumption 3:

Assumption three of the Buck-Flapan model concerns the actions of the recombinases. These are important because they directly determine the knotted products that can finally be obtained.

There are two types of recomninases that are discussed here: serine recombinase and tyrosine recombinase. Serine recombinase is able to carry multiple rounds of DNA recombination, and tyrosine recombinase only facilitates one round of recombination. The recombination process takes the shape of horizontal twists and vertical twists. It is important to note that twists can be positive or negative, and thus recombination can either add twists to a given knot, or also delete twists by introducing twists of opposite sign to the ones present in the knot prior to recombination.

1. Serine Recombinase.

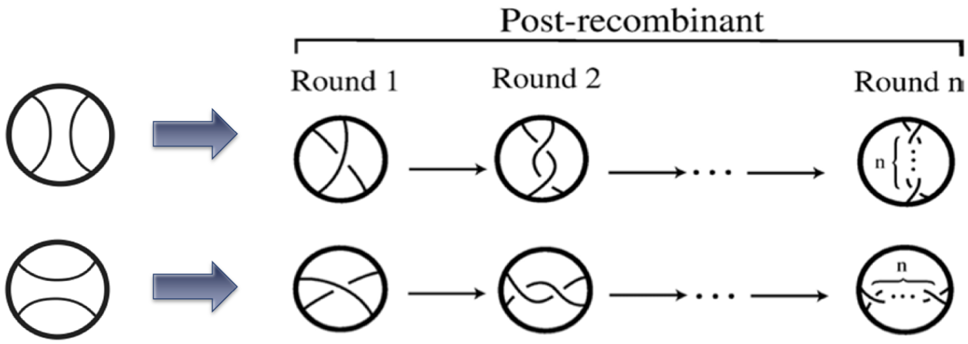


Figure 22: The process of Serine Recombinase.

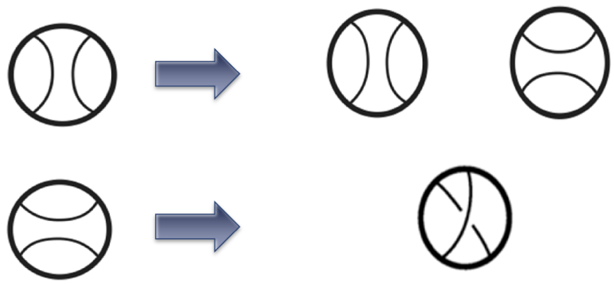


Figure 23: The process of Serine Recombinase.

Fig.22 illustrates the possible products after n rounds of DNA recombination by serine recombinases. If the recombination process starts with the substrate in the upper-left position of Figure 9, with two arcs of DNA segments in vertical parallel shape, one round of recombination is equivalent to adding a new **vertical** crossing in the productive synapse. After n rounds within a given productive synapse B, the productive synapse contains n **vertical** crossings. Similarly, if the recombination process starts with the lower-left substrate in Fig.22, with two arcs of DNA segments in **horizontal** parallel shape, one round of recombination is equivalent to adding a new **horizontal** crossing in the productive synapse. After n rounds, the productive synapse contains n horizontal crossings.

2. Tyrosine Recombinase.

Fig.23 displays the possible products arisen from tyrosine recombinase. As mentioned above, tyrosine recombinases distinguish themselves from serine recombinases in the sense that they only facilitate one round of DNA recombination. Thus, the DNA recombination for a tyrosine recombinase is quite simple and does not yield as many interesting results.

4 Proof of the Recombination Products of a New Substrate $J = T(2,m)\#T(2,n)$

Our study aims at determining the product knots arising from the actions of recombinase on $T(2,m)$ # $T(2,n)$, where $m \neq n \forall m, n \geq 3$.

Theorem: *Suppose that assumptions 1, 2 and 3 hold for a particular serine recombinase-DNA complex with substrate J. If J is $T(2,m)\#T(2,n)$, where $m \neq n \forall m, n \geq 3$ then the only possible products knots are either in the family illustrated below Fig.24 or a connect sum of either $T(2,m)$ or $T(2,n)$ with product knots in Buck and Flapan's paper.*[3]

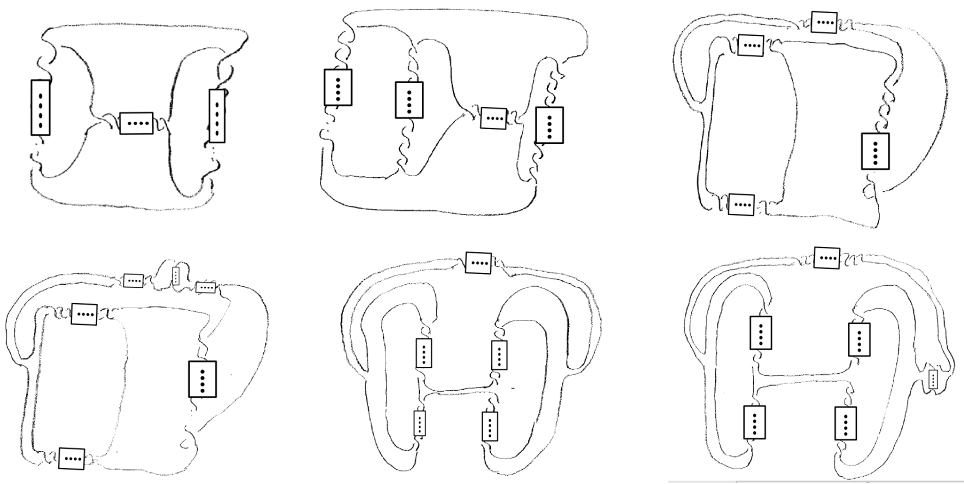


Figure 24: Different product knot types arising from serine recombination process.

The rest of this section will prove the theorem above.

4.1 Abstract Surface D_A

We choose an equivalent abstract surface D_A with boundary J. (See Fig.25) The abstract surface D_A helps us to determine various places that ∂B can intersect D. D_A is a planar surface with arcs. The spanning surface D can be obtained by replacing the neighborhood of each arc in D_A by a half-twisted band and removing the top and bottom of ends of the band. The boundary of D_A is composed of two inner circles and one outer circle. There are two types of arcs on D_A: one has both ends on the same circle, and the other has both ends on two different circles (Fig.25).

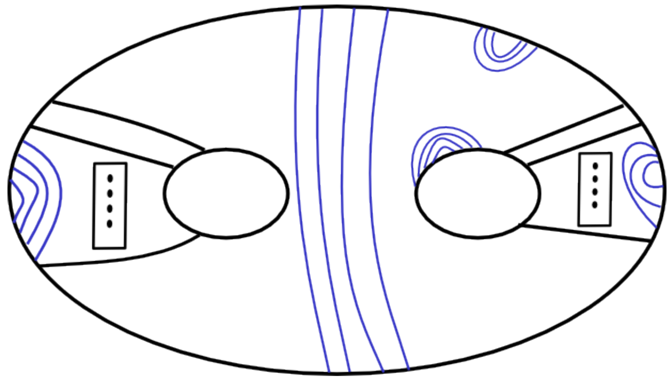


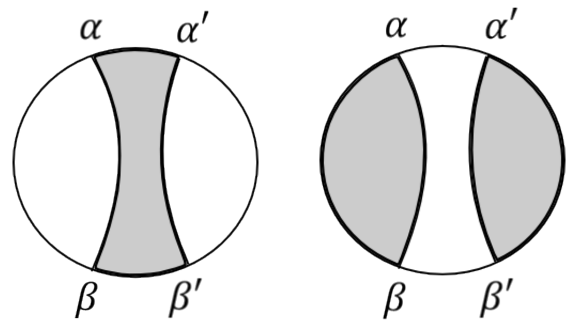
Figure 25: The abstract surface D_A for the more general spanning substrate knot shown in Fig.16

Note that in an abstract surface, arcs with two ends on the same circle represent trivial crossings in a knot (for example in Fig.16). This is because replacing the neighborhood of each arc with its two ends on the same circle with a half-twisted band and removing the top and bottom ends of the band, we obtain under and over crossings extending from the component of boundary J. It is obvious that arcs of this type represent trivial crossings in the knot under consideration. However, arcs with ends on different circles represent nontrivial twists, which cannot be undone.

4.2 Two subcases to divide our problem: F_1 and F_2

We now divide our problem into two more manageable subcases. Depending on where ∂B intersects D, and according to assumptions 1, 2, there are the two basic forms for the interior of B, which we term F_1 and F_2 as shown in Fig. 26. Indeed, by assumption 1, only two basic forms are allowed for the interior of B. There are only two possible ways to represent the spanning surface in these two forms, as there are only two ways of symmetrically coloring the regions inside B to properly represent D. For clarity, we will label the points of intersection between ∂B and J as α, $\alpha^{'}$, β, and $\beta^{'}$. We can think of $B \cap D \cap C$ as a sphere which cuts through the spanning surface D. In doing so, two arcs of $\partial B \cap D$ either cut a strip or two disks from D, which correspond to the basic forms F_1 and F_2.(Fig.26)

F_1 and F_2 will now be considered as two separate subcases. Recombination products for unknots and single torus knots have been determined previously. If in the connect sum considered here, B is placed on a single of the torus knots of the connect sum, the products are equivalent of a connected sum between a knot $T(2, n)$ with a product arising from recombination on a $T(2, m)$ knot.



(a) F_1 (b) F_2

Figure 26: Basics forms F_1 and F_2.

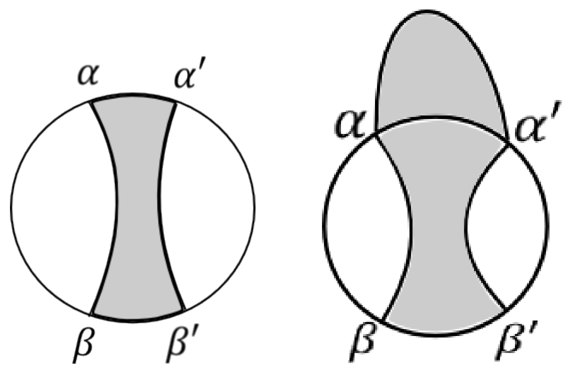
4.3 Basic Form F_1 and Product Knots

Product knots arising from subcase F_1 are solved here. To fully solve this case, we first branch into two sub-categories, named $F_1(a)$ and $F_1(b)$, based on two different surfaces we acquired by removing $D \cap \partial B$ in D. Then, given our general surface (Fig.16), we consider all possible places where this basic form F_1 may be placed. Finally, we list all the nontrivial product knots and links brought about by the recombination process.

4.3.1 Subcategories $F_1(a)$ and $F_1(b)$

Recall that according to assumption 2, we define our spanning surface D with boundary J ($T(2,m)\#T(2,n)$) as two closed twisted bands connected by a band. Under this circumstance, $D \cap \partial B$ contains two parallel arcs ($\alpha\alpha^{'}$ and $\beta\beta^{'}$) on the same plane and the rest of the surface is unknotted relative ∂B. We call this case when each arc of $D \cap \partial B$ cuts D into a strip basic form 1, labeled F_1 (See Fig.26).

We further divide our basic form F_1 into two specific sub-cases, labeled as $F_1(a)$ and $F_1(b)$. (Fig.27) In $F_1(a)$, removing the strip $\alpha\alpha^{'}\beta^{'}\beta$ returns a connected surface. In $F_1(b)$, removing the strip $\alpha\alpha^{'}\beta^{'}\beta$ returns a disk and a connected surface separately. The product knots of these two sub-cases are different, and thus we need to separately consider them.



(a) $F_1(a)$ (b) $F_1(b)$

Figure 27: Two Subcases of $F_1(a)$ and $F_1(b)$.

4.3.2 All possible locations for B on D for both subcategories $F_1(a)$ and $F_1(b)$

Given subcategories $F_1(a)$ and $F_1(b)$, we consider all possible locations for B on D. We defined a general abstract space D_A^G with trivial twists along the arcs above. In the following section, we use a simple abstract surface D_A^S in which trivial twists are removed (see Fig.28). Indeed, we can remove these twists without changing its knot type. The only way that these trivial twists may matter to our study is if they are contained in the surface where $D \cap \partial B$. However, up to isotopy, we can always slide B along the twist to a piece of arc that is untangled with any twists. Under this circumstance, it is sufficient to simply work with D_A^S, because all the trivial twists contained in D_A^G should not impact the recombination process, and thus are independent of the product knots.

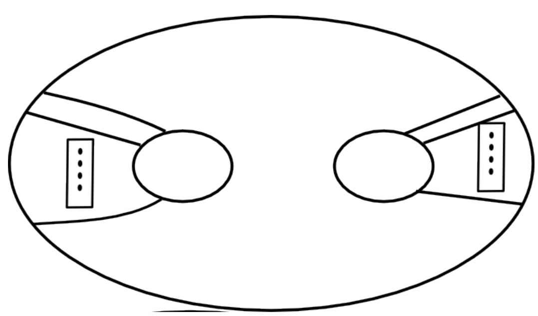


Figure 28: A Simple Abstract Surface D_A^S

Given our simple abstract surface D_A^S, we can have three different places at which we will obtain the basic form $F_1(a)$, up to isotopy[1].(See Fig.29)

From left to right in Fig.29:

[1]Please see appendix for the detailed drawings of the product knots.

1. We can have arc $\alpha\alpha^{'}$ on one outer circle component and arc $\beta\beta^{'}$ on another inner circle component.

2. We can have each arc $\alpha\alpha^{'}$ and $\beta\beta^{'}$ attached to two separate inner circle component.

3. We can have each arc attaching to two different places on the same outer circle component.

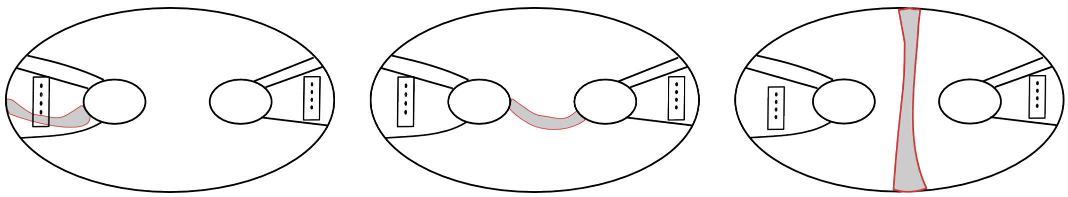


Figure 29: Three places to obtain the basic form $F_1(a)$.

4.3.3 Product Knots of $F_1(a)$:

1. In Case I, based on different types of twists contained in the strip of $D \cap \partial B$, we obtain product knots of subfamilies of [1] a torus knot, [2] connected sum of two torus knots, [3] connected sum of a torus knot with a clasp knot. (See Illustration.)

2. In Case II, based on different types of twists contained in the strip of $D \cap \partial B$, we obtain product knots of subfamilies of [1] a torus knot, [2] a pretzel knot, and [3] an unknown knot. (See illustration.)

3. In Case III, based on different types of twists contained in the strip of $D \cap \partial B$, we obtain product knots of subfamilies of [1] two separate torus knots, [2] connected sum of two torus knots, and [3] three connected torus knots. (See illustration.)

4.3.4 Product Knots of $F_1(b)$:

For $F_2(b)$ to occur at different places on $C \cap D$, it is equivalent of considering different places for both $\alpha\beta$ and $\alpha^{'}\beta^{'}$ to be on the same circular boundary of the abstract surface D_A^S. As argued above, up to isotopy, where $\alpha\beta$ and $\alpha^{'}\beta^{'}$ attach to a given circular boundary of D_A^S does not alter the product knots. For the outer circular boundary of D_A^S, there is only one way for $\alpha\beta$ and $\alpha^{'}\beta^{'}$ to attach to it. (See the picture on the left of Fig.31) For each of the inner circular boundary of D_A^S, there are two ways. (See the picture on the right of Fig.31) The second case where $\alpha\beta$ and $\alpha^{'}\beta^{'}$ wrap around the other inner circular boundary cannot occur. (See Fig.30) This is because it does not satisfy the requirement of $F_2(b)$, which specifies that $D \cap \partial B$ cuts off a strip and a disk. Thus, there are in total two cases to consider for $F_2(b)$.

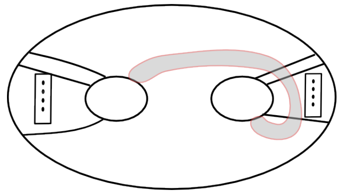


Figure 30: This case cannot occur.

1. In Case I, no matter where B intersects $C \cap D$, we obtain product knots of subfamilies [1] an unknot and the original substrate knot. and [2] a connect sum of $T(2,m)\#T(2,n)\#T(2,p)$, where p is the total number of times of recombination within B.

2. In Case II, we obtain product knots of subfamilies [1] an unknot and the original substrate knot, and [2] an unknown knot (The clasp knot as referred in Buck and Flapan's paper)

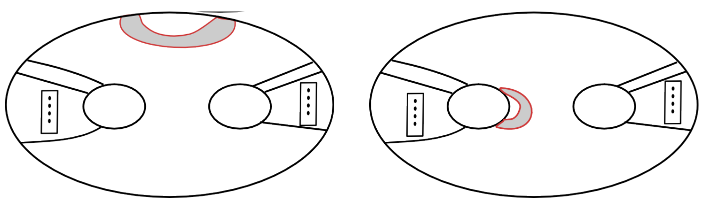


Figure 31: Two places to obtain the basic form $F_1(b)$.

4.3.5 Finger Pulling Case

We now consider the case where the interior of B takes on form F_2 (See Fig.32). In this case, $\alpha\alpha$' and $\beta\beta$' lie in J, and the space delimited by $\alpha\alpha$'$\beta\beta$' is empty. To find all the possible recombinant products, we consider where the segments $\alpha\alpha$' and $\beta\beta$' can be in J. We begin by observing that if, for example, $\alpha\alpha$' is on an arc between two crossings in J, then we can slide $\alpha\alpha$' to any location on this arc to obtain an equivalent product after recombination. Thus, we can define arcs in J that have the property that if $\alpha\alpha$' or $\beta\beta$' are placed on one of them, then the segments can be slid across the arc and yield an equivalent product after recombination. In other words, arcs are segments of J between two crossings with no crossing on the arc. We define 4 types of these arcs as shown on Fig. 31. It is important to note that we do not need to categorize the long top and bottom arcs as an arc type. Having part of one of these two arcs inside of B would be a special case of having a segment of arc type 3 or 4 inside B, where the number of crossings between the arc from 3 or 4 and either of the long segments would be 0. If we consider all the possible pairs of types of arcs, we will thus consider all the possible cases of form F_2.

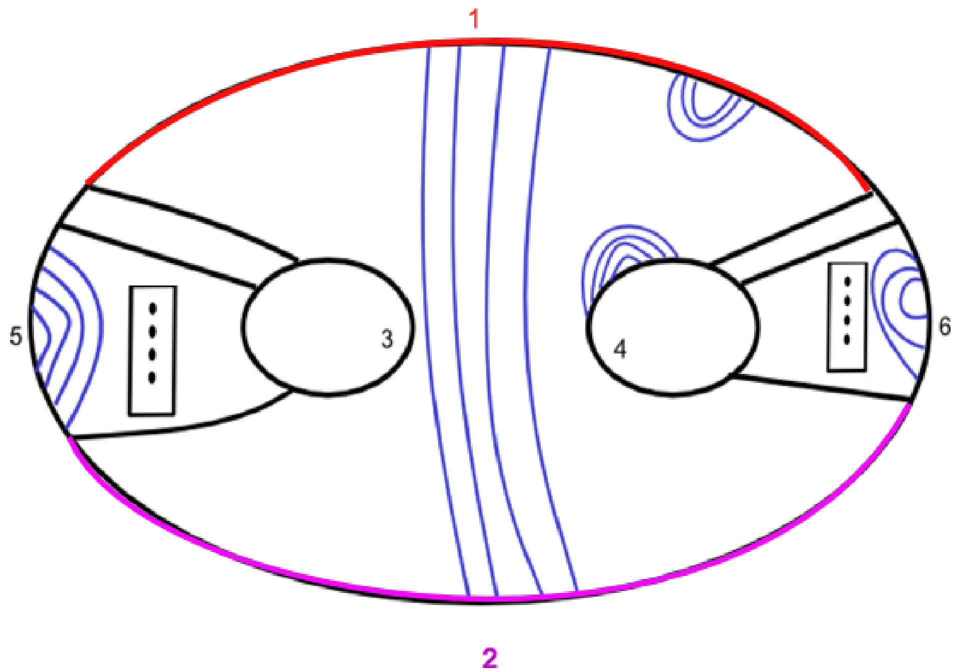


Figure 32: General Abstract Surface with Different Arcs Labeled.

It is important to note that in considering this case, we are interested in studying the general abstract surface D_A, where twists are potentially introduced in J. We notice that in the case of form F_2, these additional twists can only impact on the recombinant product if $\alpha\alpha$' or $\beta\beta$' lies on the contour of one of these twists. Indeed, if they do not, then the twists can be undone after recombination by a Reidemeister move I. For generality, we will consider for each type of arc the cases where $\alpha\alpha$' and $\beta\beta$' on such twists. We now consider all possible cases of where $\alpha\alpha$' and $\beta\beta$' can be, and determine the recombinant products.

1. Case 1: $\alpha\alpha$' and $\beta\beta$' are on arcs 3 and 4.

2. Case 2: $\alpha\alpha$' and $\beta\beta$' are on arcs 5 and 6.

3. Case 3: $\alpha\alpha$' and $\beta\beta$' are on arcs 3 and 6 (or 4 and 5).

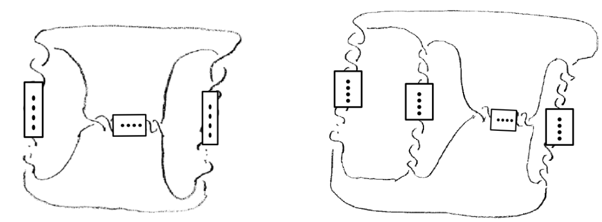


Figure 33: Products of Case 1

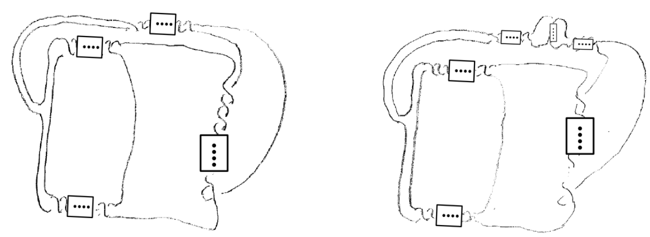


Figure 34: Products of Case 2

5 Generalization

<u>Theorem:</u> *Suppose that assumptions 1, 2 and 3 hold for a particular serine recombinase-DNA complex with substrate J being* $\#_{i=2}^{N}T(2,m_i)$*; also suppose that we assign a spanning surface D, which is a planner surface with twists for J, the only possible products are product knots of T(2,*m_{N-1}*) # T(2,*m_N*) # (*$\#_{i=2}^{N-2}T(2,m_i)$*).*

By induction, we can extend our result to a connect sum of N torus-2 knots: $\#_{i=2}^{N}T(2,m_i)$ (Fig.36). For a connect some of N torus-2 knots, where one knot connects to another does not topologically change the knot type. For instance, shrinking the torus-2 knots, we can slide them along other turus-2 knots connected to them, creating different projections of the same knot, $\#_{i=2}^{N}T(2,m_i)$. This property of the connect sum allows us to generalize to N connect sum of torus-2 knots. If we allow recombination to happen in any two

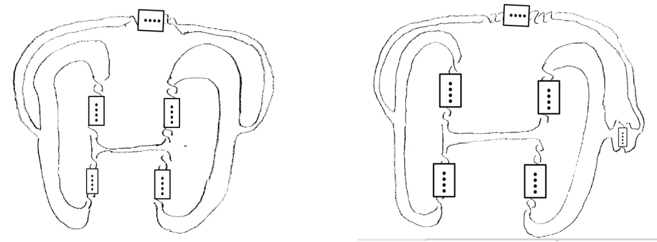


Figure 35: Products of Case 3

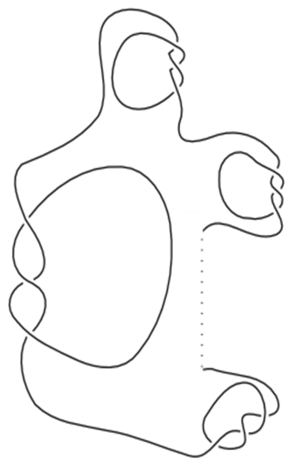


Figure 36: A connect sum of N torus-2 knots

torus-2 knots within the connect sum, types of the product knots are independent of which two torus-2 knots are involved in recombination. Because of the nice property of the connect sum, for the products knots that arise from any two torus-2 knots, there always exists an equivalent projection for the connect sum of the rest of the $(N-2)$ numbers of torus-2 knots, such that the type of product knots are: product knots of T(2,m_{N-1}) # T(2,m_N) # $(\#_{i=2}^{N-2}T(2,m_i))$.

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