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STATEMENT OF THE PROBLEM

OBJECTIVES

HYPOTHESES

RESEARCH METHODOLOGY

RESULTS & DISCUSSION

FINDINGS

RECOMMENDATIONS/SUGGESTIONS

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- Schemenner, R.W., Huber, J.C. and Cook, R.L. (1987), "Geographic Differences and the Location of New Manufacturing Facilities," Journal of Urban Economics, Vol. 21, No. 1, pp. 83-104.

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A DNA-BASED ALGORITHM FOR MINIMUM SPANNING TREE PROBLEM USING TEMPERATURE GRADIENT TECHNIQUE

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ABSTRACT

The biological deoxyribonucleic acid (DNA) strand is found to be a promising computing unit. In this paper, the thermodynamic properties of DNA have been utilized along with other biochemical operations to obtain the minimum spanning tree (MST). Actual distance values are represented using the thermodynamic properties of DNA. All possible Euler cycles of the different spanning trees of the problem are first generated. From this generated Euler cycle, the MST is obtained. Moreover, the proposed approach can be adopted to solve many real-life applications like broadcasting and scheduling problems, with necessary modifications.

KEYWORDS

DNA computing, Euler cycle, Euler path, spanning tree, temperature gradient.

INTRODUCTION

Ever since Adleman (1994) has published a paper on molecular computation for solving Hamiltonian Path Problem (HPP), attempts are being made to utilize DNA manipulations for solving computationally difficult problems. Narayanan and Zorbalas (1998) had solved shortest path problem using constant proportional length-based DNA computing technique, in which, a constant increase of DNA strands in length is encoded according to the actual length of the distance. Yamamoto *et al.* (2002a and 2002b) have proposed a concentration-controlled DNA computing for accomplishing a local search for the shortest path problem. Ibrahim *et al*. (2004) have presented another approach for solving shortest path problem using direct-proportional length-based DNA computing, in which the length of the strand goes very long when the weight of the edge goes high. Lee *et al*. (2004) have presented a DNA computing technique based on temperature gradient for solving the TSP problem. Representation of weight information for weighted-graph problem is one of the most important yet challenging problems. Han *et al.* (2008) proposed DNA algorithm for the minimum spanning tree in which the length of the strands depends upon the number of vertices and the weight. In this paper, the melting temperature of a DNA strand is used to represent numerical values. For each vertex and each unique distance, a fixed-length DNA strand has been assigned.

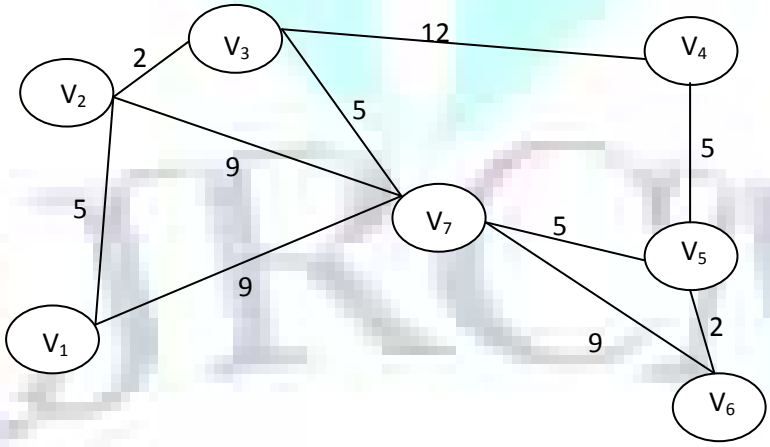
The rest of the paper is organized as follows: Section 2 describes the MST and basic operations on DNA. Section 3 gives the proposed DNA encoding method for the MST. Section 4 presents the complete biomolecular algorithm for solving the MST. Section 5 gives the outcome of the DNA computation and conclusions are given in Section 6.

PRELIMINARIES

MINIMUM SPANNING TREE PROBLEM

Given a connected undirected weighted graph, $G = (V, E, W)$ where V is the set of vertices, E is the set of edges between the vertices and W is a mapping, $W: E \rightarrow R$, R being set of real numbers. Spanning tree of graph G is a subgraph of the graph G which is an undirected tree containing all the vertices. Minimum spanning tree of a connected undirected weighted graph $G = (V,E,W)$, is a spanning tree with minimum weight. In this work, an instance with seven vertices is considered throughout as shown in *Fig.1*. Every vertex represents a particular location and edges represent the roads between the vertices and weight represents the distances between any two vertices. The objective of the problem is to find MST of the graph $G = (V, E, W)$.

FIG. 1: THE GRAPH CORRESPONDING TO 7 VERTICES



CHEMICAL OPERATIONS ON DNA

It is well established that DNA encodes the genetic information of cellular organisms. DNA consists of strands viewed as a chain of nucleotides or bases. The four bases are adenine, guanine, cytosine and thymine, which are abbreviated as A, G, C and T. Each strand, according to chemical convention, has a 5' and 3' ends. Bonding occurs by the pair-wise attraction of bases, and hydrogen bonds are formed between a pair of bases. This forms the base pair (bp). G bonds with C and A bonds with T. Operations can be performed on DNA strands, namely denaturing, annealing, ligation, polymerase chain reaction (PCR), gel electrophoresis and cloning.

DENATURING AND ANNEALING

Denaturing is disintegrating the double-stranded DNA into two single strands by heating the solution. Annealing is the reverse of denaturing. Here, the solution of single strands is cooled (hybridized), allowing complementary strands to become bound together.

LIGATION

In double-stranded DNA, if one of the single strands contains a discontinuity (ie, one nucleotide is not bound to its neighbour) then this can be repaired by DNA ligase.

GEL ELECTROPHORESIS

Gel electrophoresis is a technique used to sort DNA strands by length. Electrophoresis is the movement of molecules in a charged field. DNA carries a negative charge, so it tends to be attracted to the anode. If the strands are allowed to move in a gel, strands move at a rate that is proportional to their length. Longer strands move slowly than the shorter strands, because of the porous nature of the gel.

TEMPERATURE GRADIENT GEL ELECTROPHORESIS (TGGE)

TGGE is a gel electrophoresis method, which operates by correlating the melting characteristic of a DNA strand to its electro-migration. Electrophoresis starts with double-stranded molecules. The DNA starts to melt at a certain temperature resulting in a fork-like structure. In this conformation, the migration is slowed down compared with a completely double-stranded DNA fragment. DNA fragments of the same size but different sequence as the melting temperature strongly depends on the base sequence. Thus, TGGE not only separates molecules, but also gives additional information about melting behaviour and stability. TGGE is an extremely sensitive method, and so it can detect even point mutation. TGGE is a sequence-dependent and size-independent method.

PCR

Polymerase chain reaction or PCR is another method for amplifying DNA. PCR is a process that quickly amplifies the amount of DNA in a given solution. Each cycle of the reaction doubles the quantity of each strand, giving an exponential growth in the number of stands.

DENATURE TEMPERATURE GRADIENT PCR (DTG-PCR)

This is a modified PCR method, in which the denaturation temperature changes with each cycle. The cycle begins with the temperature of 70^{o}C as the denaturation temperature. Then, the denaturation temperature is gradually increased by 18^{o}C for every cycle. This process is carried out until it reaches to 95^{o}C. The temperature 95^{o}C is maintained for the rest of the cycle. The other procedures for amplifying the DNA strands are the same as PCR.

MELTING TEMPERATURE

The melting temperature (Tm) of an oligonucleotide is the temperature at which 50% of the oligonucleotide and its perfect complement are in duplex. In this work, two methods are employed to calculate the melting temperature. One is the GC content method, in which the content of G and C are the main factors for determining the melting temperature. GC content method is applicable for strands longer than 50 nucleotides. The second method is the nearest-neighbour (NN) model, in which the thermal stability of a DNA strand is calculated based on the identity and orientation of neighbouring base pairs. This method is accurate for DNA strands up to 108 bp.

EULER GRAPH AND EULER CYCLE

In a graph G, a Euler path is a path that visits each edge exactly once. A Euler cycle is a Euler path that starts and ends on the same vertex.

SPANNING TREE

A spanning tree T of a connected, undirected graph G is a tree composed of all the vertices and some (or perhaps all) of the edges of G. Informally, a spanning tree of G is a selection of edges of G that form a tree spanning every vertex.

PROPOSED DNA ENCODING METHOD FOR MST

Consider an undirected graph as shown in *Fig.1*. There are seven Vertices (V_i ,*i* = 1 to 7). In this work, for each vertices a unique vertex strand (vs) with fixed length DNA single strand of length 20 bases having similar melting temperature is synthesized by the sequence generator. For each distance, a unique distance strand (*ds*) with fixed length DNA single strand of length 20 bases with varying melting temperature is also generated. For smaller distance, a DNA sequence with lower melting temperature and for longer distance a DNA sequence with higher temperature is assigned. The DNA sequence for each vertex and for each unique distance is shown in Table 1 and in Table 2. For clarity, the *ds* is represented in lower case. These sequences are generated using the DNA sequence generator software available (MC 2009).

TABLE 1: VERTEX SEQUENCES FOR SEVEN VERTICES

Vertex (V_i)	20-mer sequence (5'-3')	GC%	GC method T_m(^{o}C)	NN method T_m(^{o}C)
V_1	GAAGCCTACT GTACTCTGCT	50	52	52
V_2	GAAGGATACT GGACGCTCTT	50	52	52
V_3	AAAGGGCGTC TTTTAACGGA	50	52	52
V_4	GAAGGATACT GGACGCTGAT	50	52	52
V_5	TATGCGGATT TGGAGGGTGA	50	52	53
V_6	GGTGAACCAA GGTGAACCAA	50	52	52
V_7	GCGTCTCGAC GTAATGTTGA	50	52	52

TABLE 2: FOUR DISTANCE STRANDS

Distance	20-mer sequence (5'-3') (*ds*)	GC%	GC method T_m(^{o}C)	NN method T_m(^{o}C)
2	atgtgaaaatagaaattaag	20	39	40
5	gcatttagtttcagatagat	30	44	44
9	ttactccgcgtaacctatcc	50	52	51
12	agggacaccagcgcggcgaa	70	60	62

TABLE 3: DNA SEQUENCE FOR EDGES

No.	Edge	DNA sequence(5'-3')
1	$V_1 \rightarrow V_2$	GAAGCCTACT GTACTCTGCT gcatttagtttcagatagat GAAGGATACT
2	$V_1 \rightarrow V_7$	GAAGCCTACT GTACTCTGCT ttactccgcgtaacctatcc GCGTCTCGAC
3	$V_2 \rightarrow V_1$	GGACGCTCTT gcatttagtttcagatagat GAAGCCTACT GTACTCTGCT
4	$V_7 \rightarrow V_1$	GTAATGTTGA ttactccgcgtaacctatcc GAAGCCTACT GTACTCTGCT
5	$V_2 \rightarrow V_3$	GGACGCTCTT atgtgaaaatagaaattaag AAAGGGCGTC
6	$V_3 \rightarrow V_2$	TTTTAACGGA atgtgaaaatagaaattaag GAAGGATACT
7	$V_7 \rightarrow V_3$	GTAATGTTGA gcatttagtttcagatagat AAAGGGCGTC
8	$V_3 \rightarrow V_7$	TTTTAACGGA gcatttagtttcagatagat GCGTCTCGAC
9	$V_3 \rightarrow V_4$	TTTTAACGGA agggacaccagcgcggcgaa GAAGGATACT
10	$V_4 \rightarrow V_3$	GGACGCTGAT agggacaccagcgcggcgaa AAAGGGCGTC
11	$V_4 \rightarrow V_5$	GGACGCTGAT gcatttagtttcagatagat TATGCGGATT
12	$V_5 \rightarrow V_4$	TGGAGGGTGA gcatttagtttcagatagat GAAGGATACT
13	$V_6 \rightarrow V_5$	GGTGAACCAA atgtgaaaatagaaattaag TATGCGGATT
14	$V_5 \rightarrow V_6$	TGGAGGGTGA atgtgaaaatagaaattaag GGTGAACCAA
15	$V_7 \rightarrow V_5$	GTAATGTTGA gcatttagtttcagatagat TATGCGGATT
16	$V_5 \rightarrow V_7$	TGGAGGGTGA gcatttagtttcagatagat GCGTCTCGAC
17	$V_7 \rightarrow V_6$	GTAATGTTGA ttactccgcgtaacctatcc GGTGAACCAA
18	$V_6 \rightarrow V_7$	GGTGAACCAA ttactccgcgtaacctatcc GCGTCTCGAC
19	$V_2 \rightarrow V_7$	GGACGCTCTT ttactccgcgtaacctatcc GCGTCTCGAC
20	$V_7 \rightarrow V_2$	GTAATGTTGA ttactccgcgtaacctatcc GAAGGATACT

The edge(road) sequence between the two vertices V_i and V_j has been created to contain three strands. The first strand is a complement strand of rear $|vs|/2$ bases of oligonucleotide of V_i. The second strand is the complement of distance strand. The third strand is a complement strand of front $|vs|/2$ bases of oligonucleotide V_j. If V_i is V_1, then the first strand of edge sequence is a complement strand of 20 bases of oligonucleotide V_1. If V_j is V_1, then the third strand is a complement strand of 20 bases of oligonucleotide V_1. Table 3 shows the edge strands generated for all the 20 edges of the graph shown in *Fig.1*.

PROPOSED BIO-MOLECULAR ALGORITHM FOR SOLVING MST

Applying chemical operations on DNA and with massive parallelism inherent in DNA computing, the MST can be obtained in polynomial time. The molecular algorithm for solving MST requires 7 steps. In the first step, the initial pool of vertex strands and the road strands are synthesized and allowed to hybridize to their complement strands. The concentration of the economical road strands is increased as the rate of biochemical reactions depending on the reaction rate constants and the reactant concentration.

In the second step, all the strands which have the starting and ending vertex strand as V_1 are amplified by PCR operation. During the first PCR cycle, vertex strand corresponding to V_1 is used as primer, whereas the DNA strands complementary to vertex strand V_1 is also used as primer from the second PCR cycle. In step 3, the generated strands are allowed to run in a gel electrophoresis operation. The strands having length

$$\left[(2n-1)*|vs|+(2n-2)*|ds|\right]_{\text{bp}}$$

are separated. Each strands having length $\left[(2n-1)*|vs|+(2n-2)*|ds|\right]$bp represents one possible Euler cycle of a spanning tree having visiting all the vertices, and visiting all the roads connecting these vertices exactly twice and reaching the vertex V_1. In the example given in *Fig.1*, the length of the Euler cycle of a spanning tree strand will be 500bp, given $|vs| = 20$, $|ds| = 20$ and $n = 7$.

In the fourth step, affinity purification is carried out to make sure that the strands have all the vertex strands exactly once. For carrying out affinity separation, the separated strands of length $\left[(2n-1)*|vs|+(2n-2)*|ds|\right]_{\text{bp}}$ are amplified with 5'-biotinylated V_1 as a primer. After amplification, a biotinylated complement DNA sequence of the first robot ($\overline{V_1}$) is used as the filter to attract strands of V_1 and the filtering is done. The remaining strands after filtering are removed. The same operations are carried out with other filter probes having the complement sequence of V_i (i = 2 to n). The resultant strands will be strands involving all robots with length 500bp. Every Euler cycle of a spanning tree visits 2n-1 vertices and 2n-2 edges of a problem with n vertices. Out of these 2n-2 edges, n-1 edges connecting all the vertices must present exactly twice. Even though the number of bases is the same for all the separated strands, there is a possibility for visiting particular edge more than twice. To remove such strands again affinity purification is carried out with each edge sequence. Complement DNA sequence of a particular edge sequence is used to attract the corresponding edge sequence. From the attracted strands it is checked for the presence of reverse direction edge strand also. For example if the edge sequence $V_1 \rightarrow V_2$ is used to attract the strands having this sequence, from the attracted strands it is to be checked for the presence of $V_2 \rightarrow V_1$ edge sequence. The strand which is not attracted by the reverse direction edge strand can be removed. A possible single strand covering all vertices and the presence of n-1 distinct edges each appearing twice is given in *Fig.4*.

GAAGCCTACTGTACTCTGCTgcatttagtttcagatagatGAAGGATACTGGACGCTCTTattgaaaatagaaattaagAAAGGGCGTCTTTTAACGGAgcatttagtttcagatagatGCGTCTCGACGA
ATGTTGAgcatttagtttcagatagatTATGCGGATTTGGAGGGTGAgcatttagtttcagatagatGAAGGATACTGGACGCTGATgcatttagt
ttcagatagatTATGCGGATTTGGAGGGTGAatgtgaaaatagaaattaagGGTGAACCAAGGTGAACCAAatgtgaaaatagaaattaagTATGCGGATTTGGAGGGT
GAgcatttagtttcagatagatGCGTCTCGACGTAATGTTGAgcatttagtttcagatagatAAAGGGCGTCTTTTAACGGAatgtgaaaatagaaattaagGAAGGATACTGGACGC
TCTTgcatttagtttcagatagatGAAGCCTACTGTACTCTGCT

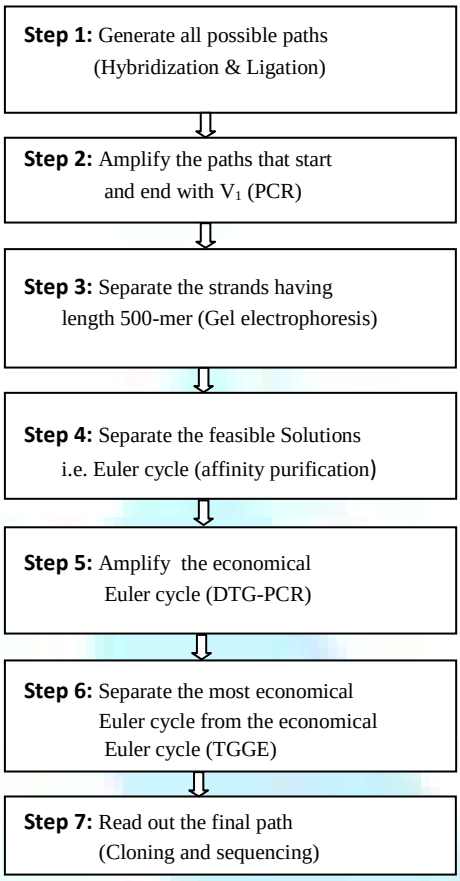
FIG.4 EULER CYCLE SINGLE STRAND INVOLVING ALL VERTICES STRANDS WITH THEIR DISTANCE

In the fifth step, the economical Euler cycle strands having minimum distance has to be separated from the possible Euler cycle strands. The resultant strands of the affinity purification are the strands having same length. They start from V_1 visiting all vertices exactly once and end with V_1. Even though all the feasible strands have the same length, the base composition is different for each strand. For each road strand a low melting temperature is assigned for smaller distance and high melting temperature is assigned for longer distance. Using this variation in the thermodynamic characteristics of each strand, the economical Euler cycle strand gets amplified more by DTG-PCR. The resultant strands from DTG-PCR will have the economical Euler cycle strand occupying the major part in the solution which can be detected easily.

In the sixth step, the most economical Euler cycle paths are separated from the economical paths, by the TGGE. Since all the strands have the same length, they cannot be separated by normal gel electrophoresis operation. Since each strand has different melting temperature, TGGE method is used for separation. TGGE is a gel electrophoresis method which is based on the correlation of the melting characteristic of a DNA strand to its electromigration. TGGE is an extremely sensitive method, which can detect even point mutation. The most economical Euler cycle path is found in one major band and the others are in minor band.

In the last step, the DNA strands from the major band were cloned and sequenced, which has the minimum distance awakening schedule i.e. MST. The algorithm for solving MST is shown as flowchart in *Fig.5*.

FIG.5: FLOWCHART FOR FINDING EULER CYCLE WITH MINIMUM



OUTCOME OF DNA COMPUTATION

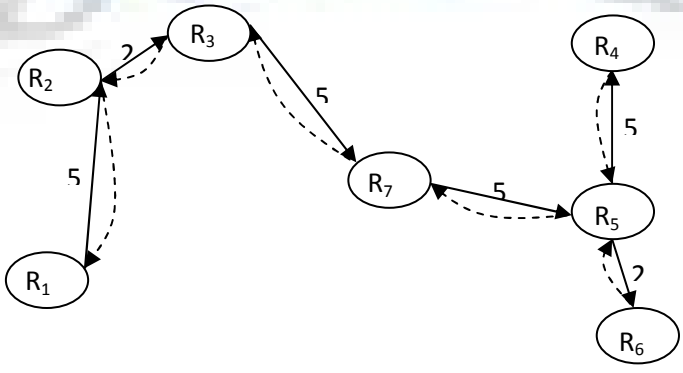
The proposed method gives all possible Euler cycles of the spanning trees of the graph shown in *Fig.1*. During the operation DTG-PCR, the strands representing the economical spanning tree with minimum weight has the minimum temperature. These strands gets amplified more in number than the other strands. These strands occupy the major band in the TGGE operation. The few possible economical Euler cycles of a spanning tree strands involving all the vertices with $2n-2$ edges are shown in Table 4. The temperature for each strand is calculated by the software tool (OPC 2009).

TABLE 4: FEW EULER CYCLE PATHS FOR THE GRAPH SHOWN IN FIG.1

Few Euler cycles of the spanning trees for the graph shown in Fig.1	T_m(°C) GC Method	T_m(°C) NN Method	GC content (%)	Distance
V1→V2→V3→V7→V5→V4→V5→V6→V5→V7→V3→V2→V1 (minimum spanning tree of the graph in *Fig.1*)	79	83	38	48
V1→V7→V1→V2→V3→V4→V5→V6→V5→V4→V3→V2→V1	81	84	43	70
V1→V7→V2→V7→V3→V4→V3→V7→V6→V7→V5→V7→V1	83	85	48	98
V1→V7→V2→V7→V5→V6→V5→V4→V3→V4→V5→V7→V1	82	85	46	84
V1→V2→V3→V4→V5→V7→V6→V7→V5→V4→V3→V2→V1	82	84	44	76

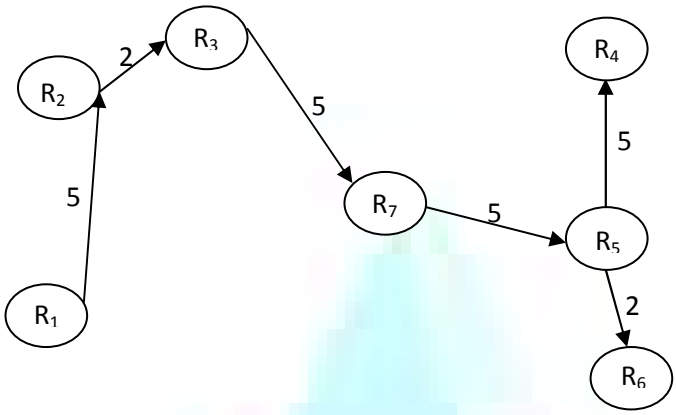
TGGE method is used to identify the most economical Euler cycle of a spanning tree from the economical Euler cycle of a spanning tree. Since all the strands have the same length, conventional gel electrophoresis method is not applicable. So, from the DTG-PCR product TGGE can detect the most economical Euler cycle by forming one major band and other minor bands.The major band can be cloned and sequenced, which corresponds to the most economical Euler cycle $V_1 \rightarrow V_2 \rightarrow V_3 \rightarrow V_7 \rightarrow V_5 \rightarrow V_4 \rightarrow V_5 \rightarrow V_6 \rightarrow V_5 \rightarrow V_7 \rightarrow V_3 \rightarrow V_2 \rightarrow V_1$ for the graph in *Fig.1*. The obtained strand from TGGE is the most economical Euler cycle path having distance 48 as shown in *Fig.6*.

FIG.6: MOST ECONOMICAL EULER CYCLE OF A SPANNING TREE FOR THE GRAPH SHOWN IN FIG.1WITH MINIMUM DISTANCE 48



From the obtained most economical Euler cycle the minimum spanning tree with distance 24 as shown in *Fig.7* can be obtained by removing the duplicate road sequence. This minimum spanning tree for the graph shown in *Fig.1*.

FIG.7: MINIMUM SPANNING TREE FOR THE GRAPH SHOWN IN FIG.1 WITH MINIMUM DISTANCE 24



CONCLUSION

In this paper, a DNA encoding method has been developed and an algorithm for solving the MST has been designed. In this encoding method, the melting temperature of a DNA strand is used to represent numerical values. In this work, for each vertex and each unique distance, a fixed-length DNA strand has been assigned and concentration of the economical edge strand is increased so that it drives the generation of the most economical Euler cycle. Furthermore, DGT-PCR helps to amplify the most economical Euler cycle of the spanning tree that has the minimum temperature and TGGE helps to detect the most economical Euler cycle of the spanning tree from the economical Euler cycles. Furthermore, real values for distance can be employed with the thermodynamic properties of the DNA. The DNA strands have been shown to be successful in solving the MST and this, in future, will help to overcome the limitations of electronic computer, namely storage, speed and miniaturization. A computer with DNA strands shall be benign to the environment. The possibility of representing numerical data in a DNA sequence paves the way for solving many more numerical optimization problems.

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