

Chemical analysis of DNA sequencing by reducing graphene oxide with the released H ion

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ABSTRACT

The DNA polymer synthesis is the addition of deoxynucleotide triphosphate units by phosphodiester bonds. The nucleotide bases are adenine A, guanine G, cytosine C and thymine T. The sequence of nucleotides bases in the genome is random. The phosphodiester bond is formed between 5 position carbon of one deoxynucleotide triphosphate with the 3 position carbon of another deoxynucleotide triphosphate. The addition reaction releases two unstable phosphate groups. The groups stabilised by joining together to release water molecule. The water molecule is protonated by acid to release H ion. The H ion is captured by pH sensitive carbon compound graphene oxide. The released H ion reduced the graphene oxide resulting in the pH change. The DNA polymer is a double stranded molecule and synthesis was performed on a template strand by complimentary addition of nucleotides, with A complimenting T, and C with G by di and tri hydrogen bonds respectively. The nucleotides in a pre-fixed sequence of A, G, C and T were flown for addition on the complimentary template strand. The addition of nucleotide was sensed by change in pH of sensor with the released H ion. The non addition did not confer any pH change signal in the sensor. The addition sequence of nucleotide in the polymers synthesis is recorded to infer the exact sequence of targeted region of genome. We have investigated an efficient cost effective fast method of DNA sequencing using a chemical computational model. This method provide the sequence of genome without using any dye labelled nucleotide and also no laser optics is required for recording the nucleotide addition signal. The repeat nucleotide sequence signals were in proportion to pH shift. The method can efficiently read the sequence size of upto 200 nucleotides. The computational analysis was performed by in silico synthesis of polymer on a complimentary template strand with capture of H ion on reducing graphene oxide and signal was recorded by measuring the shift in pH.

Keywords: DNA, Nucleotide, Polymer, H ion, pH, Graphene Oxide, Sequencing

INTRODUCTION

Deoxyribo Nucleic Acid DNA sequencing is revolutionary chemical method to read the nucleotide sequence of the genome. The nucleotide sequence is the genetic code for building the functional structure of all living organisms. The DNA is a double stranded self replicating molecule. The double strand structure makes its own copies by unwinding and complimentary synthesis of nucleotide sequence on template strand. The self replicating property of DNA polymer makes the living organism to reproduce the genome in the form of next generation by reproduction. The chemistry of DNA sequencing elucidates each nucleotide of the genome. The chemical method involved fluorescent dye tagged nucleotide addition of dideoxynucleotide triphosphate lacking oxygen at 3 position carbon C with deoxynucleotide triphosphate to terminate further addition of nucleotide and absorption of dye emission wavelength on recorder to provide the signal of nucleotide on the recorder. This method requires a balanced ratio of deoxynucleotide triphosphate and fluorescent dideoxynucleotide triphosphate for synthesis and controlled chain termination to read the nucleotide. This method applied a cascade of chemicals to amplify the DNA strand and perform the concurrent synthesis with controlled termination. The fluorescent dye absorption and emission spectra with laser light to be recorded for reading the nucleotides. Therefore the chemistry of DNA sequencing by polymer

synthesis is to be studied further for fast and economical method. The envisaged method should be chemically validated. The chemical mechanism of polymer formation involves the addition of nucleotide units with the release of adding units and two phosphate groups. The phosphate groups joined together to release water molecule and this water molecule is protonated to form the H ion. The H ion released is sensed by reducing graphene oxide which causes the shift in pH of the sensor. The addition of pre-fixed sequence of flown nucleotide to the template strand is recorded to read the sequence of nucleotides in the synthesizing DNA polymer. The pH meter based reading of nucleotide addition is easily maintained and recorded. The sequence of upto 200 nucleotides in a stretch can easily be read in this chemical method. The capture of H ion by shift in pH sensing graphene oxide on reduction is to be recorded at an optimum temperature of 25 °C to rule out any false signals. The sequence read is utilized for inferring molecular markers, single nucleotide polymorphism SNP and short tandem repeats STR sequence in the targeted site of genome for genetic identification and diversity.

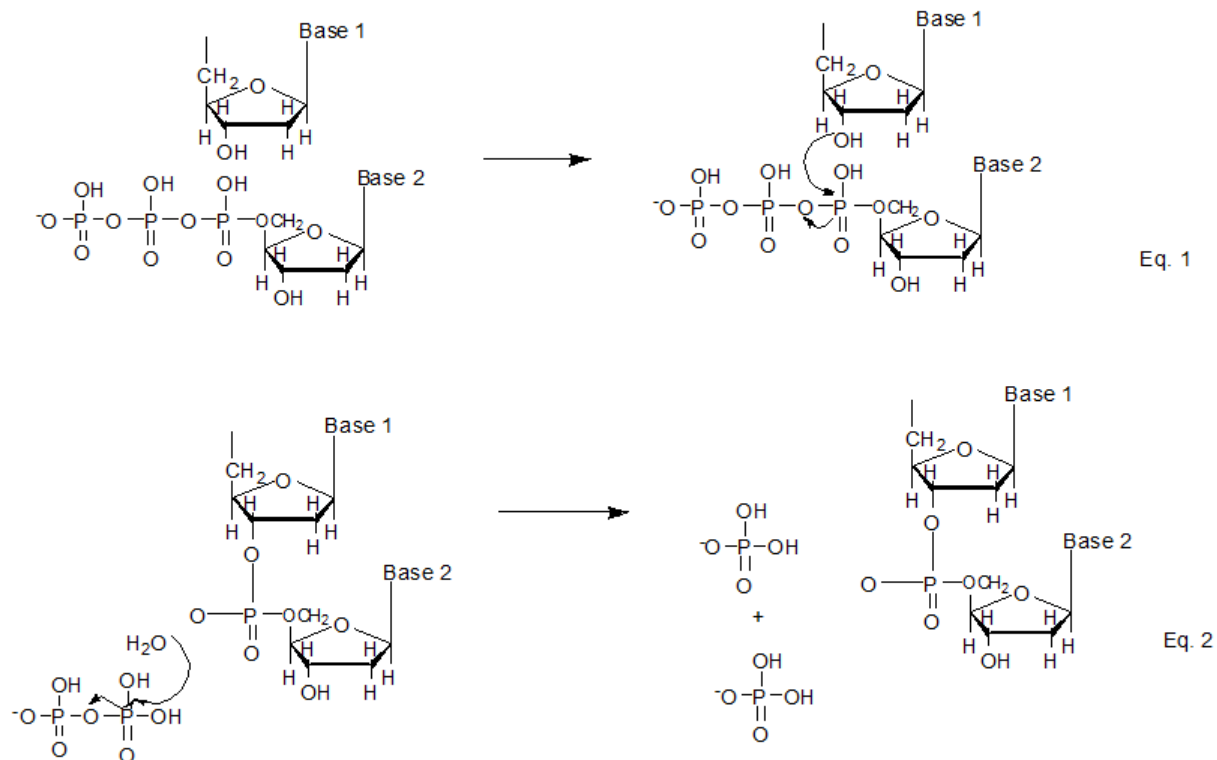
EXPERIMENTAL SECTION

DNA sequence templates were selected for sequencing. The template strand nucleotides sequences were input in the computer system. The nucleotide addition mechanism with their structures and chemical reactions of polymer formation with the by-products were made the supporting input files. The nucleotides are flown for complimentary addition of nucleotide on template strand in pre-fixed order of A, G, C and T. The pH meter with attached recorder in the system provide the shift in pH signal to record the capture of H ion on addition of nucleotide by reducing graphene oxide in the synthesizing DNA polymer chain.

RESULTS AND DISCUSSION

The DNA polymer synthesis is the chemical addition of deoxynucleotide triphosphate dNTP units with release of adding units and two phosphate groups. The phosphate groups joined together to form pyrophosphate and water molecule. The water molecule was protonated to release H ion. The H ion release was captured by shift in pH in the recorder. The sequential flow of deoxynucleotide triphosphate containing nucleotide A, G, T and C was maintained to record the addition of nucleotide to the complementary strand of DNA. The chemical mechanism of DNA polymer synthesis is shown in Figure 1 as per addition of deoxynucleotide triphosphate units [1].

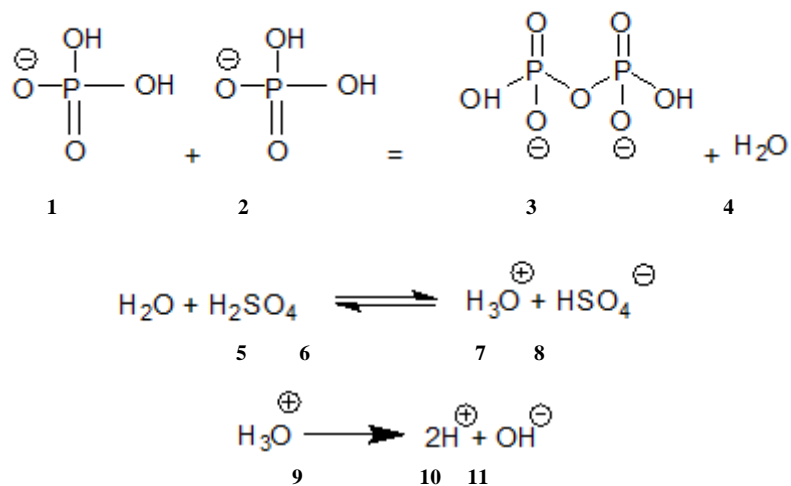
Figure 1:



The by-product of synthesis reaction is two phosphate groups. These groups joined together to form pyrophosphate and water molecule. The water molecule is protonated by acid to release H ion which becomes hydronium ion in

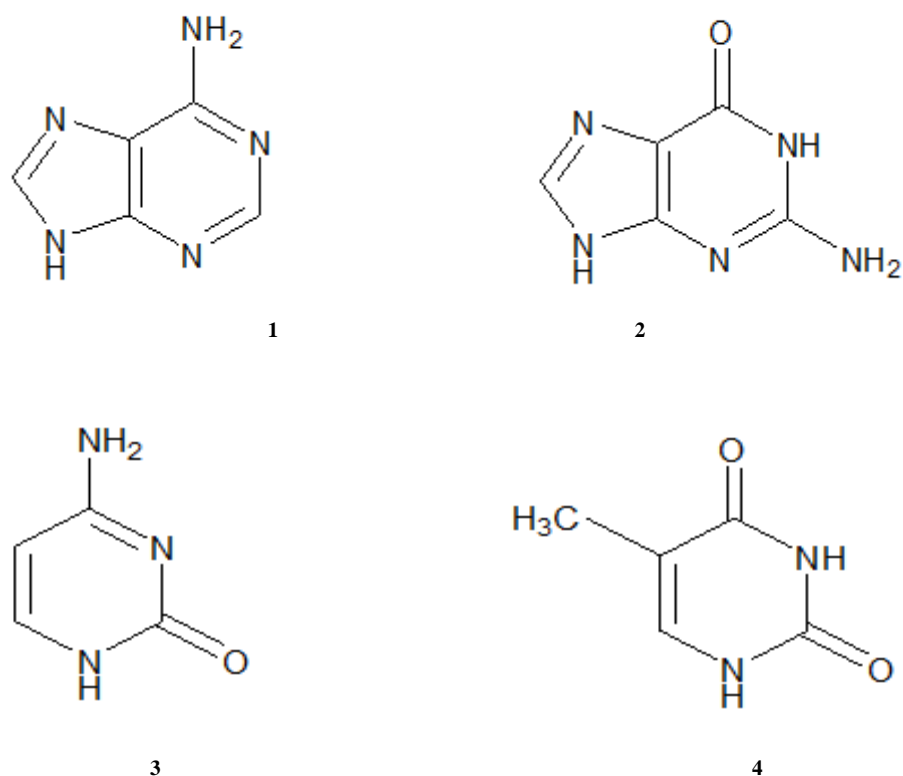
aqueous solution. The chemical mechanism of formation of H ion is shown in Figure 2. The released two phosphate groups (1 and 2) released joined to form dihydrogen diphosphate (3) and water molecule (4). The water molecule (5) is protonated by sulphuric acid (6) in reversible reaction to form hydronium ion (7) in aqueous solution with sulphate ion (8). The hydronium ion (9) is the H ion (10) in aqueous solution containing hydroxide (11).

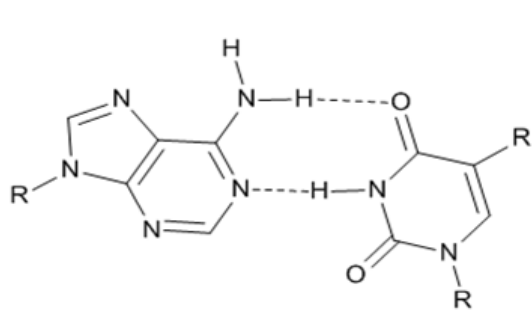
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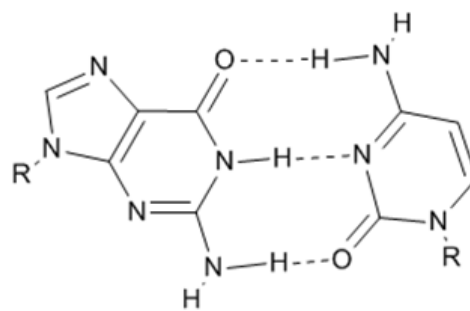
The structure of nucleotides base, base pairing and deoxynucleotide triphosphate is shown in figure 3.

Figure 3: (1) Adenine A (2) Guanine G (3) Cytosine C (4) Thymine T (5) Adenine-Thymine base pair (6) Cytosine-Guanine base pair (7) deoxynucleotide triphosphate

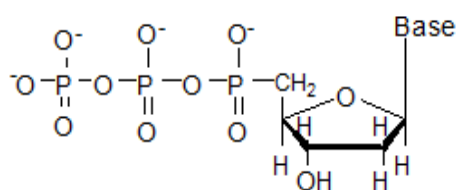




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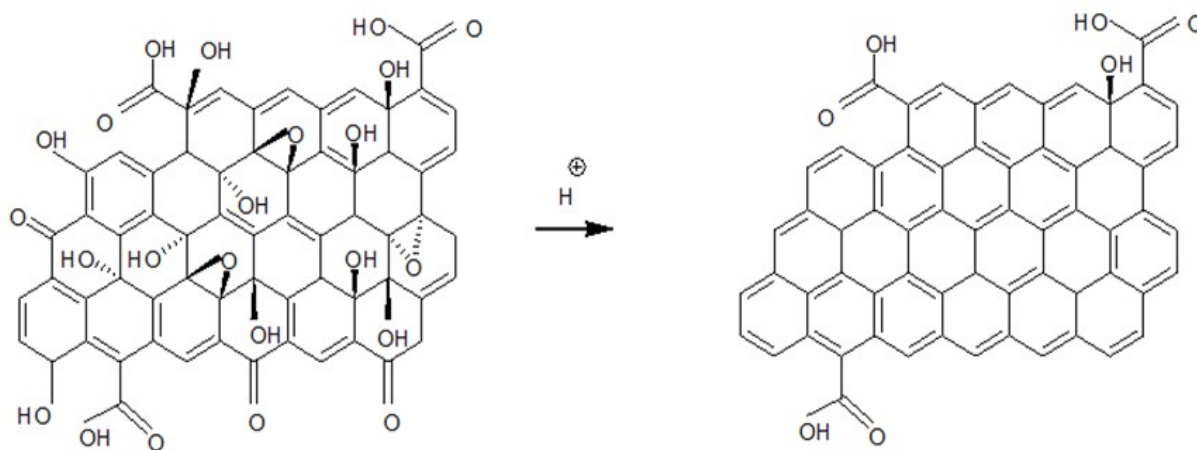
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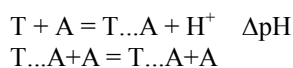
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The H ion released can be sensed by reducing graphene oxide. The reduction of graphene oxide is shown in Figure 4.

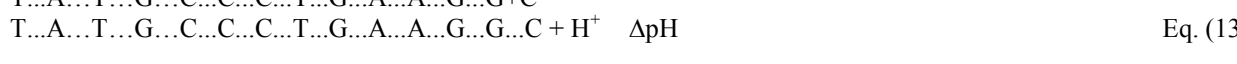
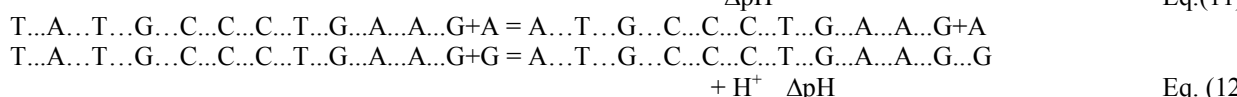
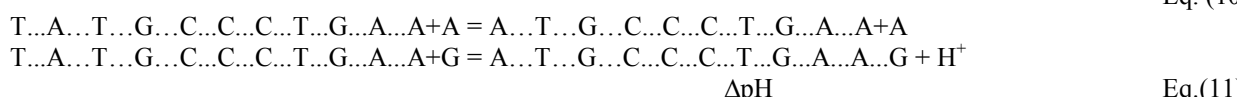
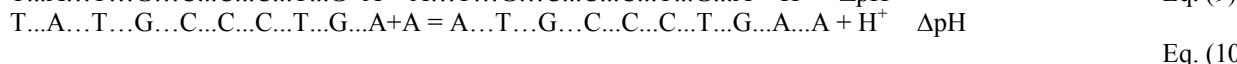
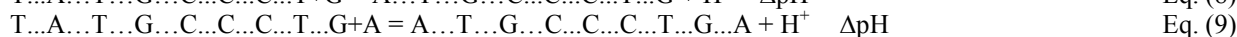
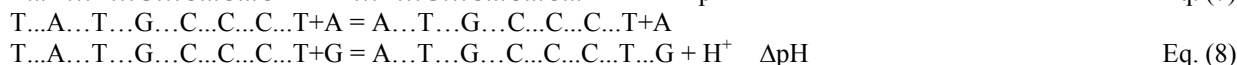
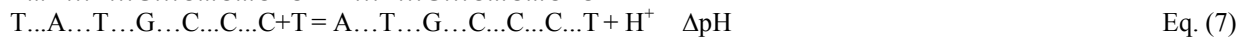
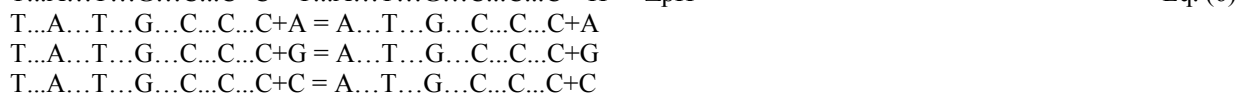
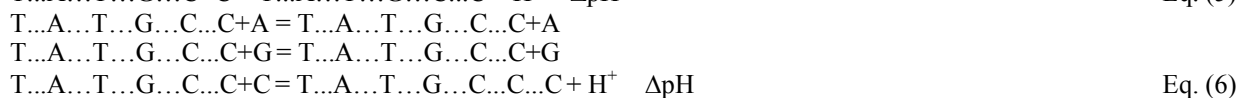
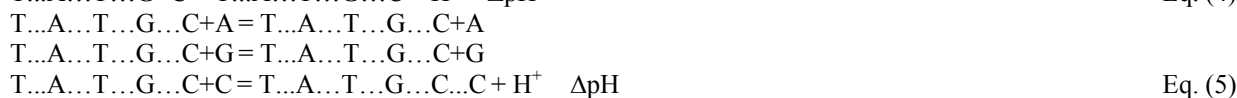
Figure 4:



The H ion reduces the graphene oxide. The addition of H ion shifts the pH to acidic side. The graphene oxide is soluble at high pH and aggregates are formed at low pH. This property of graphene oxide is applied in the measurement of released H ion by measuring shift in pH signal during DNA polymer synthesis [2]-[7]. The flow of nucleotides base is controlled in the sequence of A, G, C and T. The addition of nucleotide is sensed by shift in pH, non-addition make a blank shift and repetitive addition by proportional shift. The Equations of reading of adding nucleotide to the complementary template strand by capture of H ion resulting in shift of pH on reduction of graphene oxide sensor is:



Eq. (1)



The equations 1-13 described the sequencing of 13 nucleotide base units in addition to initial nucleotide with the release of H ion captured by shift in pH by reducing graphene oxide. The cycle moves on the addition of nucleotide base on the complimentary template strand. The template strand of upto 200 nucleotides is sequenced by this method. The template strand is enzymatically fragmented from the genomic DNA for error free short strand sequencing. The pH shift of 14 to 1 corresponds to synthesis cycle of 13 nucleotide bases from the initial base. The shift in pH cycle goes on to read the nucleotide base addition in the synthesis cycle. The pH shift by reduction of graphene oxide with released H ion during polymer synthesis is shown in Table 1.

Table 1: The pH shift and nucleotide read during DNA polymer synthesis

S. No.	pHi	pHf	ΔpH	Nucleotide Read
1.	14	13	1	2
2.	13	12	1	3
3.	12	11	1	4
4.	11	10	1	5
5.	10	9	1	6
6.	9	8	1	7
7.	8	7	1	8
8.	6	5	1	9
9.	5	4	1	10
10.	4	3	1	11
11.	3	2	1	12
12.	2	1	1	13

The investigated chemical method of DNA sequencing is envisaged on computational model of mechanism of DNA polymer synthesis with the efficiency of reading the 13 nucleotides by shift in pH cycle of 14 to 1 on reduction of

graphene oxide by released H ion during polymer synthesis. The DNA sequencing is a complex chemical method to read the each nucleotide of the genome sequence of any organism. DNA sequencing elucidates the sequences of nucleotides responsible for phenotype variation, genetic diversity, individual identification and disease diagnostics. The method of synthesis with deoxynucleotide triphosphate and terminating synthesis with dideoxynucleotide triphosphate provide the error free reading of nucleotides [8]-[10]. The sequencing chemistry has been investigated for error free long read of nucleotides [11]-[12]. The computational analysis is the best envisaged method for implementing DNA sequencing in laboratory work. The nucleotide units are the monomer of the polymer synthesis. The joining nucleotides are in the form of deoxynucleotide triphosphate groups combining together by phosphodiester bond. The phosphodiester bond formation releases two phosphate groups. These groups joined together to form diphosphate and water molecule. The water molecule is protonated by sulphuric acid and hydronium ion is released in aqueous solution. The hydronium ion is combination of H ion and hydroxide. The adding nucleotides are flown in sequence of A, G, C and T. The addition of nucleotide on the complimentary template releases H ion. The H ion capture signals the addition of nucleotide base. The carbon compound graphene oxide has known properties of sensing pH change by reduction with H ion. The released H ion is captured by graphene oxide. The graphene oxide is very sensitive to H ion addition and voltage of 29mV/pH is reported³. The synthesis started to one pre-added nucleotide on the complimentary strand. The next addition started with the right hit of nucleotide flown in sequential order of A, G, C and T. In case the first nucleotide added to the synthesis then flow cycle again initiate with the starting nucleotide. The hit and addition is sensed by shift in pH by reduction of graphene oxide. The addition cycle goes on where the hit of nucleotide is random and any of the four nucleotides can be added depending on the complimentary template strand sequence. The addition is indicated by shift in pH signal and non addition indicated by a blank signal. The repeat addition results in proportional signal of pH shift. The method developed can read the error free sequence of short strand of nucleotides upto 200 bases. The shift in pH cycle moves on from 14 to 1 and then next cycle for signalling the addition of H ion. The earlier sequencing methods require fluorescent dye labelled nucleotide bases. The addition of fluorescent dyes need to be captured by light absorption and emission spectra. The emission spectra have to be signalled by detector and reaction products has to run in optical capillary. The ratio of synthesizing deoxynucleotide triphosphate and synthesis terminator dideoxynucleotide triphosphate has to be controlled for the desired length of sequencing. This all make the method complex and expensive. The computational designed method in this study is quite simple and economical. It is a pH meter based DNA sequencing method. It can be designed and tested in the laboratory at very low input cost. The nucleotide addition sequence to complimentary template strand by sequential flow of free nucleotide bases has to be controlled meticulously. The addition of nucleotides is signalled by measuring the shift in pH on reducing graphene oxide sensor with released H ion, is also to be monitored carefully to rule out any false addition.

CONCLUSION

We have computationally designed a chemical model to sequence the nucleotides of genome by capturing the released H ion during the DNA polymer synthesis with reducing graphene oxide. The shift in pH signal the adding nucleotide and this combined with pre-fixed sequential flow of nucleotide bases on the complimentary template strand read the sequence. The sequencing cycle of 13 nucleotides on pH shift of 14 to 1 is shown in this study. The sequencing cycles goes on to read the random addition of nucleotides on the complimentary template strand upto 200 nucleotide bases. The sequencing characterizes the short tandem repeat STR sequences and single nucleotide polymorphism SNP molecular markers on the target site of genome. The molecular markers are designed for genetic identification and diversity studies [13]-[14]. We further propose to explore the chemical mechanism of DNA polymer synthesis for design of molecular markers and their biochemical analysis for human genetic identification and diversity studies.

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