Pico-Calorimeter Biosensor: a Novel Method for Sequencing of DNA

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ABSTRACT

The essence of biology is a deep understanding of all of the species and their biological mechanisms. Speciation and biological function are primarily determined by the organism's DNA sequence. The development of vastly improved DNA sequence determination for personalized medicine and ecological studies could complete the revolution initiated by the Human Genome Project. The Human Genome Project was essentially accomplished by a reduction in the cost of DNA sequencing by three orders of magnitude. It is desired to reduce the cost by another three orders of magnitude to enable profiling of individuals genome. To achieve this goal, a highly integrated platform will be needed. Described below is a new technique based on sequencing-by-synthesis which has the potential to reduce the cost of genome sequencing to $1000 level/genome, a reduction by 4 orders of magnitude based on the effect of miniaturization on the dramatically decrease of sensor geometries and reagents volumes in addition to the array based detection which aims to run millions of strands simultaneously and leads a handheld device gene sequencer to bring DNA sequencing to every lab and possibly every individual without any prior knowledge in the field. The described method relies on heat, IR and/or PH detection resulted in DNA synthesis.

$$(DNA)_n + dNTP \xleftrightarrow{\text{DNA polymerase}} (DNA)_{n+1} + PPi + \Delta T + \Delta pH$$

These are the physical reaction products in bold in the formula above. The formula represents the incorporation of a nucletode, dNTp, which could be any nucleotide, G: Guanine, A: Adenine
T: Thymine, or C: Cytosine as incorporated into a growing DNA strand. The ΔT in the above reaction is about 22 kT or ~570 meV per nucleotide incorporation, and is measured in accordance with the present invention, as well as ΔpH. The incorporation of the nucleotide in the above reaction is monitored to provide sequence information. The schematic of the system is shown in the Fig. 1.
Towards development of this technique we developed a 3-D simulation model to calculate optimal geometry and amount of DNA needed to have detectable DNA synthesis (heat generation profile is demonstrated in the Fig.2). An IR microscopy test was run to proof of the concept for the ThermoSequencing technique by measuring the heat released during the reaction which confirmed the simulation results (Fig. 3). Then using an ITC (Isothermal Titration Calorimetric) microcalorimeter system, the enthalpy changes in the media due to different environmental and instrumental factors have been investigated which leads to design and fabricate of a pico-calorimeter for gene sequencing followed by a flow-based array of wells each equipped with a pico-calorimeter for DNA sequencing. In addition, other applications of this system would be pursued.
The micrograph in Fig. 4 shows an initial thermal-isolated PDMS microfluidics platform with gate control and in Fig. 5 the graph of ITC microcalorimeter results is shown.

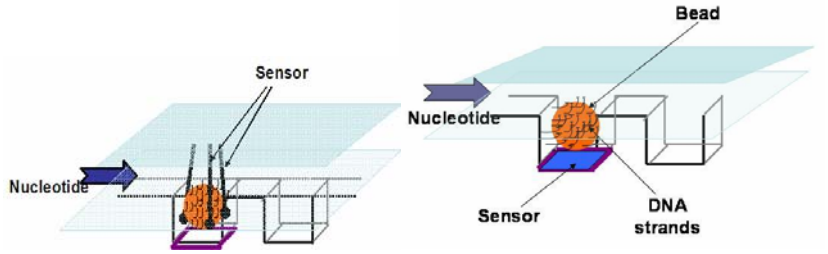


Fig.1, Schematics of the System with DNA immobilized on biotinylated magnetic beads in microfluidic array system

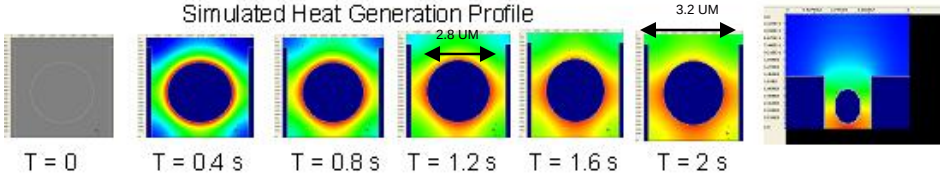
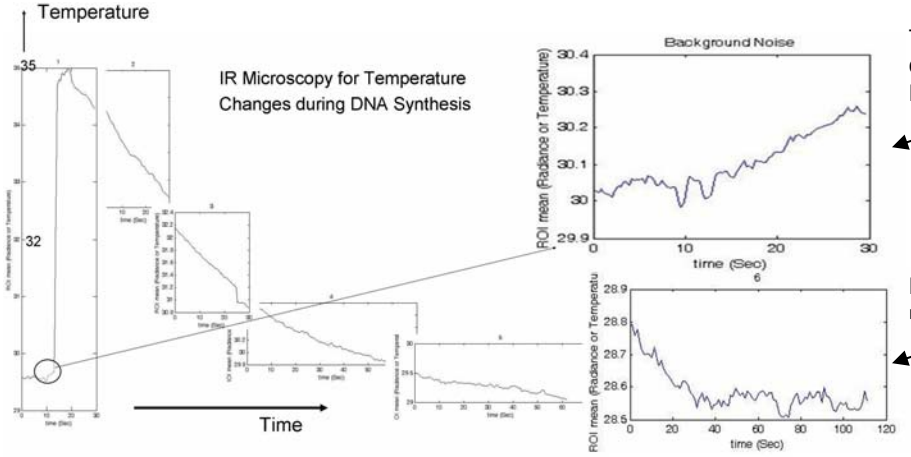


Fig.2. 3-D simulation results of heat profile during the DNA incorporation



The background environmental noise of the DNA solution

Return back to the DNA noise level steady state

Fig.3. IR Microscopy experiment's result; the instantaneous (~2 sec) change of temperature detected by IR microscope during adding 200 uL dNTP, 100mM (50uL each) to the solution of 4 uL of 65-mer ss-DNA and 20 uL Klenow Fragment Exo- Polymerase and 200 uL of Mg2+ Buffer.

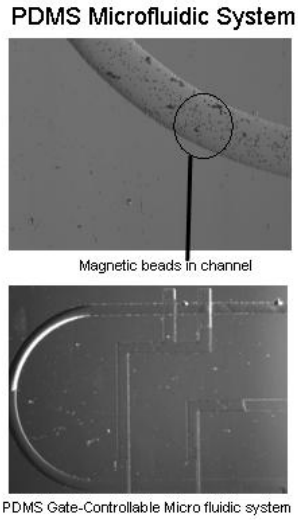


Fig.4. Micrograph of PDMS Gate Controllable Microfluidics System (2.8 um magnetic beads in the channel)

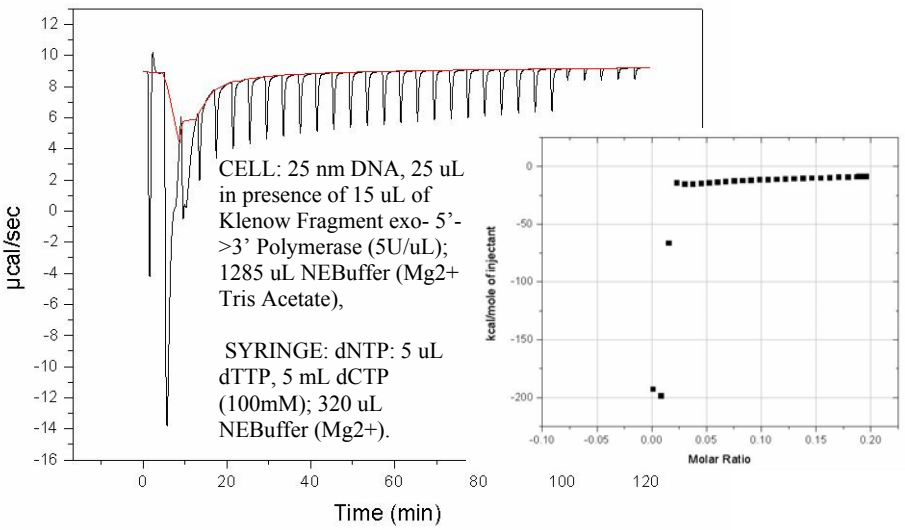


Fig.5. ITC Microcalorimeter Results {# of Injection: 30, Injection duration: 20 Sec each, Time Interval between two consequence injection: 240 sec, Syringe Volume: 340 uL, Cell Volume: 1.40004 uL)}.