Simulations and Experiments for DNA Sequencing by Denaturation

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***Short Abstract* — A new DNA sequencing method called sequencing by denaturation (SBD) is reported here. DNA templates are immobilized on a surface to be sequenced in massive parallel. A Sanger sequencing reaction with fluorescently-labeled dideoxynucleotides is performed followed by denaturation. By analyzing the change in fluorescence while each dideoxy-terminated DNA fragments denatures sequentially, the DNA sequence can be determined. Thermodynamic simulations and a base-calling algorithm for SBD have been developed. The instrumentation has been constructed to demonstrate the experimental proof-of-concept of SBD. This technology can be applied to the systems analysis of cellular regulation through high-throughput genotyping and gene expression.**

***Keywords* — sequencing by denaturation, SBD, denaturation, DNA sequencing, thermodynamics, fluorescence imaging.**

I. Introduction

GENOME sequencing of many individuals is required for many applications including studies of complex diseases and personalized medicine. Although several next-generation sequencing technologies have been introduced, the cost and time to sequence a human genome is still too much for routine sequencing of genomes due to the complexity and throughput of each method.

Here we describe a novel high-throughput sequencing method called sequencing by denaturation (SBD) [1]. In SBD, standard Sanger sequencing reactions with fluorescent dideoxynucleotides are performed on templates immobilized on a surface. Instead of separation through gel electrophoresis, single-base separation is obtained by analyzing the melting profile through fluorescence measurements as the labeled dideoxy-terminated fragments are denatured sequentially from the templates. By detecting the change in fluorescence on the surface, DNA sequence could be read out after processing.

II. Methods

A simulation is conducted to evaluate the feasibility of SBD. Denaturation profiles of random DNA templates were simulated using a thermodynamic model. A base-calling algorithm was developed to analyze the data. Data curves similar to the traditional electropherograms were generated by taking the negative derivatives of the denaturation profiles. In order to resolve the overlapping curves, negative derivative curve was fit to the sum of Gaussian curves to deconvolve it into the melting temperatures of its component curves. By reading out the order of the melting temperatures of these components, the DNA sequence was determined.

An integrated system was established to perform SBD experimentally. The system consists of a custom-built reaction chamber, a fluidic system, thermoelectric modules for temperature control, and a microscope for fluorescence detection. Denaturation curves of individual DNA fragments were measured on the surface using this system. The denaturation profiles were analyzed using the base-calling algorithm described above to evaluate the feasibility of SBD.

III. Results

Simulations of the melting curves of 1000 randomized 20-base sequences showed that the melting temperatures of short oligonucleotides at lengths 12~32 increase monotonically. This property can be used to sequence DNA with an error rate of 4% with a 20-base read length.

Experimentally, the melting curves of the first 21~27 bases of "CCATCAGTCATGTACGAAGTCAGTCAT" were measured using the instrument constructed. The melting temperatures are well separated and single-base resolution was achieved. By combining the 21mer and 25mer, 22mer and 26mer, and 24mer and 27mer for the measurement of denaturation profiles, the sequencing of 7 bases has been simulated experimentally. Using the base-calling algorithm developed above, the sequence was determined correctly, which demonstrates how DNA sequencing can be determined by SBD.

IV. Conclusion

A novel DNA sequencing method called sequencing by denaturation is developed and evaluated. Because the process only requires heating for denaturation, this method is several orders of magnitude less expensive in reagent cost than other next-generation DNA sequencing methods. This technology can be useful for the studies of cell regulation through high-throughput sequencing of genomes, gene expression analysis, and genotyping.

References

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