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Ordered DNA fragmentation using soft lithography and amplification for next generation sequencing

NaHyun Cho^{1,2}, Sara Goodwin² Jonathan Sokolov¹ and William Richard McCombie² ¹Stony Brook University, USA ²Cold Spring Harbor Laboratory, USA

Purrent Next Generation Sequencing (NGS) technology starts with randomly fragmented DNA from whole genomic DNA. Because of this randomness, all DNA fragments need to sequence massive parallel reads in order to know the whole sequencing. In this study, we try to cut DNA fragments into 10-15 kbps using soft lithography technology because one of the NGS platforms is Pacific Biosciences' RS, able to read larger size fragments, up to 15 kilo base pairs, quickly. Also, as an ultimate goal, we will try to keep the DNA fragments in the orders from the surface, so the DNA reads do not need to be sequenced several times. In previous studies, we stretched DNA on PMMA (Poly Methyl Methacrylate) substrate and the stretched DNA could be linearly cut with soft lithography by applying DNase I enzyme. After cutting the DNA on the substrate, in order to sequence the DNA fragments with NGS technology, the DNA fragments are taken from the surface and placed in a solution base. We dissolved the PMMA substrate and fragmented DNA fragments together and separated the DNA fragments using a Phenol-Chloroform Isoamyl (PCI) extraction procedure. The principle of separating DNA with PCI mixture is based on solubility differences between organic and aqueous liquids. DNA is a negatively charged, hydrophilic bio-polymer because of its negatively charged phosphate groups. On the other hand, PMMA is a noncharged polymer that is dissolved in chloroform. By dissolving the PMMA surface, it is possible to separate DNA from the surface using liquid-liquid phase separation (Organic phase: Aqueous phase). For the data processing, confocal microscopy was used to take images of cut DNA on the PMMA surface. Gel electrophoresis and bioanalyzer were conducted to confirm the distribution of the DNA fragments. Finally, PacBio RS II which is the one of the long-read Next-Generation sequencing platforms was used to confirm quality and quantity of the fragmented DNA from surfaces.

Biography

NaHyun Cho is a PhD candidate in Materials Science and Engineering at Stony Brook University in New York. She previously earned a BS in Engineering Chemistry and a ME in Materials Science and Engineering from at Stony Brook University. She spent two and half years in the field as a research engineer with LG Electronics in Seoul, South Korea, working in their crystalline solar cell R&D Center. Her current area of research is ordered DNA fragmentation on surfaces for next-generation sequencing. In order to further develop her research focus, she is a visiting student at both Cold Spring Harbor Laboratory and Brookhaven National Laboratory in New York.

nahyun.cho@stonybrook.edu

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