

OFFICIAL ABSTRACT and CERTIFICATION

Gel and Electric Field-Based Desorption of DNA from PMMA-Coated Silicon Surfaces to Optimize Sequencing Accuracy

Elizabeth Korn

Plainview-Old Bethpage John F. Kennedy High School, Plainview NY, U.S.A.

Following the success of the Human Genome Project in 2003, DNA sequencing has been applied successfully to carrier screening, detection of inherited disorders, and DNA library preparation. Unfortunately, current sequencing methods are limited to DNA fragments a few kilobases long and result in many inaccuracies due to random fragmentation and the repetitive nature of DNA. One method being explored to combat this inaccuracy is DNA combing, in which a substrate is slowly pulled out of a DNA solution, depositing DNA molecules linearly on its surface, allowing for controlled cutting of DNA to then be sequenced in an orderly fashion. Polymethyl methacrylate (PMMA)-coated silicon wafers have been successfully used for DNA combing and cutting; however, they have presented issues in the removal of DNA for subsequent replication and sequencing. The goal of this study was to determine an effective method of desorbing DNA from PMMA-coated silicon wafers, specifically using agarose gels and an electric field. Some samples of DNA-dipped PMMA-coated silicon wafers incubated in an oven, while others sat in a well with buffer and an electric field, with and without a gel, with the hope of removing the DNA. Each sample was photographed before and after DNA was desorbed and the percent change in DNA on the samples was quantified. With 97.9% DNA removed, the use of an agarose gel and electric field (at .39 V/mm for 15 minutes) provides a significant increase in DNA desorption, overall improving the applicability of the DNA combing and cutting technique to DNA sequencing.

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