Creation of a DNA Sequencing Chip through Covalent Bonding Between DNA Molecule and Surface Functional Groups

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Goal: The current methods for genome sequencing are expensive, labor intensive, and time consuming. In response to such disadvantages, one of the overall goals on the laboratory-wide level is the creation of a cost-effective sequencing method. The particular aspect of the project is the construction of the DNA chip and development of the surface chemistry, in other words, the anchoring of a strand of DNA long enough to be measured by the atomic force microscopy (AFM) onto the slide.

Introduction

While the price to sequence a human genome has decreased three orders of magnitude in the last fifteen years, it is still a figure that inhibits the use of genome sequencing for ordinary medical purposes. If a DNA sequencing method could be devised that would lower the cost of sequencing, many practical applications would emerge. Individuals could obtain a full personal genome sequence to give to their doctors, drastically increasing the power of preventive medicine. Doctors will be better able to diagnose and treat individuals already affected by disease than they would without the genomic profile of a patient. Drugs could be tailored to a specific individual, making them more effective and reducing side effects.

Another advantage to an affordable sequencing method is that it would make genome sequences easily and readily available. Therefore, more researchers than are currently working in the field can study genomes and increase the number of genomes available for comparison between individuals, promoting a better understanding of disease.

Basic Methods

The aims of the large-scale project are going to be accomplished through force spectroscopy of a single DNA m differences in
We aim to create the DNA chip by covalently linking the DNA to the slide, through interactions between molecul using covaler
Once the DNA chip is created, it can be used in the larger project to sequence genomic DNA. A magnetic fluo M tip and, as

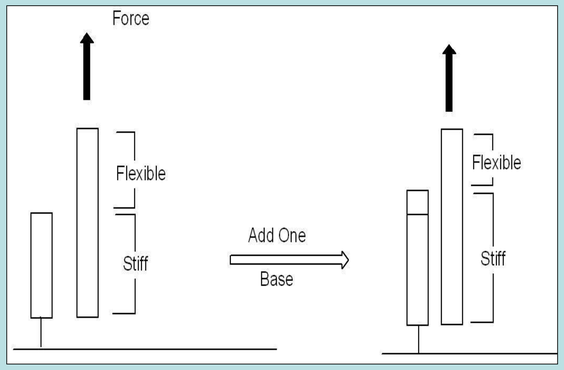


Figure 1: Diagram of the Sequencing Techniques Using the Mechanical Properties of DNA:
Single nucleotide addition is used to determine the composition of a DNA fragment through force spectroscopy.

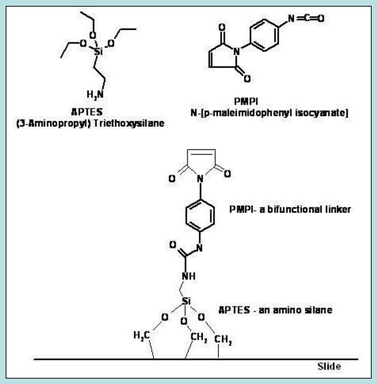


Figure 2: Linker Molecules for DNA Primer Attachment:
These molecules were used to link the 20-mer DNA primer to the substrate slide.

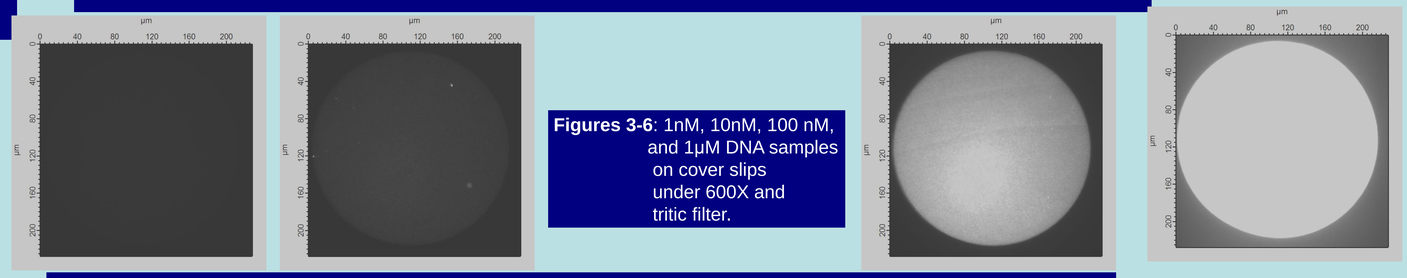
Procedure:

Attaching the DNA primer to the slide will be accomplished as pictured below in Figure 2. A primer of single-stranded DNA will be attached to a substrate slide through coating of the slide with an amino-silane, followed by reaction with a bifunctional linker molecule, and a thiol-terminated nucleotide.

→ Clean substrate slide with a highly oxidative treatment (70% H_2SO_4, 30% H_2O_2), wash, dry with N_2
→One cover slip per reaction vessel → 5 ml toluene, 50 µL (3-Aminopropyl) Triethoxysilane,
→Cover and wait forty-five minutes
→Individually remove and rinse immediately with toluene, ethanol, and methanol
→Place in oven at 120° C in a dry vial. Anneal in oven for at least forty-five minutes
→Place coverslip in solution of 0.001 g N-[p-maleimidophenyl isocyanate] and 15 mL acetonitrile.
→Protect solution from light and wait two hours, wash with acetonitrile, then methanol
→Attach primer DNA:
- → Reagents: 10 uM DNA Primer (100 uL)
  100 mM PBS with 1 M NaCl + 15 mg TCEP (10 mL total)
- → Dilute DNA to appropriate concentrations using TCEP solution
- → Place 50 uL of DNA/TCEP solution on cover slip and let incubate for 45 minutes
- → Rinse with ethanol, dry with nitrogen
- → Place in 3mM ethanolic solution of MHA for one hour
- → Rinse with ethanol and dry with nitrogen

Results:

The success of this procedure to attach the primer DNA was characterized by exposing the cover slips to fluorescent TAMRA DNA prime
The increasing brightness, as well as evidence of photo-bleaching, indicates that DNA is indeed attaching to the surface.



Figures 3-6: 1nM, 10nM, 100 nM, and 1µM DNA samples on cover slips under 600X and tritic filter.

Conclusions and Future Directions:

Further experiments need to be completed in order to ensure that DNA is only attaching to the PMPI molecule, and not the surface of the s
The eventual goal is to create an array of DNA primers, utilizing all possible combinations and arrangements of nucleotides. This array of