



## Immobilization of Reconstituted Bacteriophage lambda DNA on the Solid Support

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### Abstract:

The world of life science research has changed dramatically over the past decade. Researchers now rely on miniaturized systems with tiny cards or chips that contain virtual laboratories on a microscopic scale. Results are achieved in minimum time using smaller amounts of reagents. The use of life processes and materials was perfected in millions of years by nature for bioengineering, to develop devices for following applications to improve quality of human life. This is the latest area of Research and Development. DNA, the blueprint of life, has taken centre stage in biological research during the past few decades. The precise positioning of micro structured surfaces is of immense importance for the realization of Bio-MEMS and Bio-nano-devices. Bacteriophage lambda has been used for the current research work. The current work aimed to view and study the functioning of genetic material *in vitro* by Immobilization of double stranded DNA on a solid support, here Gold plated silicon wafer and gold plated microelectrode plates are used as solid surface. The work explored the key factors that influence oligonucleotide DNA assembly and surface occupancy behavior and the interferences involved during the covalent binding of reconstituted thiol tethered double stranded Bacteriophage Lambda DNA on the surface of gold microelectrodes. Atomic Force Microscope (AFM) was used to study the affect of cleaning on the surface of gold.

**Key Words:** DNA (DeoxyriboNucleic Acid), Bacteriophage lambda, Bionanodevices.

### Introduction:

Among the myriad of biomolecules nucleic acids play key role in sustaining life. As a genetic material it directs all the processes of life in a very systematic manner with high fidelity. Nucleic acids like proteins are essential for life. It is genetic material in all forms of the living organisms. The term nucleic acid comes from the fact that they are found in nucleus referring to both RNA and DNA. The discovery of DNA is one of the outstanding milestones in the biology because it finally solved the problem of how the living organisms store the information necessary to control

the activities of life and pass this information to subsequent generations. Biological function of DNA is to code for proteins that are expressed in the form of genetic information. With the advent of technology Scientists are trying to assign a new role to DNA which nature has never intended for this molecule.

### Bacteriophage Lambda:

Bacteriophage Lambda is extensively studied virus, a temperate phage of E.Coli, an extra cellular plasmid. This virus is harmless to man and therefore makes an excellent source of DNA. The DNA of the phage is double stranded and is in the linear form phage particle is 48.5kb pairs. At each end are short single stranded Cohesive ends of 5'projections of 12 nucleotides which are complementary in sequence 5'-GGG CGG CGA CCT-3', 5'-AGG TCG CCG CCC-3' are present. In recombination process the cohesive ends of Lambda DNA associate to form cos site. DNA portion of cos sites is rich in Guanine Cytosine (GC) bases.

Then current work relies on the principle that the Thiol group has an affinity towards the gold particles. This property of affinity is used for immobilization of DNA on the Solid Surface.

### Materials and Methods:

#### *The Basic materials used in the study include:*

**Primers-** Primer-I 5'GGGCGGCGACCT 3', Primer-II 5'AGGTCGCCGCC 3', Non-methylated Bacteriophage Lambda DNA, Calf Intestinal Phosphorylase, Polynucleotide Kinase and T<sub>4</sub> DNA Ligase.

### Experimental Procedure:

#### *Storage of thiolated Oligonucleotide Primers:*

Thiol modified oligonucleotide Primers should be kept either in inert atmosphere or solution containing DTT (0.01M) to avoid oxidative disulfide formation.

#### *Modification of the Primers:*

The oligonucleotide primers were Phosphorylated using Polynucleotide kinase and the Bacteriophage

Lambda was Dephosphorylated, using Phosphatase enzyme.

***Hybridization and Ligation:***

The oligonucleotide primers and the cos sites of bacteriophage lambda DNA were hybridized and ligated using DNA ligase enzyme.

***Purification of the Oligo nucleotide Primers:***

Oligonucleotide primers were treated with DTT (Dithiothreitol) to avoid oxidative disulfide formation. Impurities of primers were removed by Gel Exclusion Chromatography.

***Preparation of the Bacteriophage lambda:***

The impurities like buffer ions, agarose, enzymes were removed from Bacteriophage Lambda DNA solution by using Phenol-Chloroform extraction method and Nucleo-Trap Purification.

***Agarose Gel Electrophoresis-*** The presence of purified product has been confirmed by Agarose (0.5%) gel electrophoresis. This method would separate the DNA bands based on their molecular weight and purity factor.

***Cleaning and Activation of Surface-***

Prior to immobilization of DNA the gold and the microelectrode surface was cleaned to create resist free ambience. For the effectual immobilization experiment, the gold substrate and the microelectrode surface was activated by treating with strong oxidizing agent (Pirhana Solution)

***Immobilization of sample-***

The immobilization was carried out by two methods DNA oligonucleotide was immobilized by covalently attaching it on microelectrode surface [1, 2, 3, 5, 9, 10, 30] One suitable model system for fundamental studies consists of thiol-containing probes that are immobilized through self-assembly to gold surfaces, as recently employed by several investigators (Bamdad, 1997; Levicky et al., 1998; Herne and Tarlov, 1997; Peterlinz et al., 199).

***Immobilization on Gold Surface:***

Gold coating on silicon was done at the Thin Film Lab of CSIO (Figure 1) Immobilization of the DNA was done on Gold plated Silicon Wafer to study the extent of Immobilization and surface occupancy behavior. The immobilization on the Microelectrode Surface was done by Drop Method and attempting to immobilize the DNA precisely in between the two gold plated microelectrodes. The drop method was

done under two conditions i.e. under Normal and Humid conditions. The two differ in that in the latter wet conditions are maintained through out the experiment whereas in the former case it is not taken into consideration whether the sample is in dry state or wet state.

***Visualization of the samples:***

The samples were visualized using DAPI dye under inverted Fluorescent Microscope (Carl-Zeiss, Germany). Figure 2.

***Spectrophotometric Estimation of DNA:***

The DNA was estimated by taking Optical Density readings at 260nm wavelength using Spectrophotometer. DNA shows absorption maxima around 260 nm due to the presence of Purine and Pyrimidine bases. The presence of protein moieties is confirmed by taking the ratio of readings at 260nm to that of 280nm. This gives the index of purity of DNA which is 1.

***Results and Discussion:***

The research was mainly focused towards the immobilization of DNA. The immobilization procedures need to be standardized before its application. DNA could be immobilized by various protocols using non-covalent and covalent interactions. The main concern of the research work done is to use the affinity of thiol moiety for the Gold (Herne and Tarlov 1997). The thiolated 3' end of the reconstituted Bacteriophage Lambda DNA was successfully immobilized on the surface of gold and microelectrode surface. There is both specific and non-specific immobilization of DNA on the microelectrode surface.

The position of DNA immobilization is shown in the below figures Figures showing slide without immobilization and immobilization under Normal conditions by Droplet method. The immobilized DNA cannot be moved using optical tweezers in droplet method as the sample is completely dry. It is possible for DNA immobilized under humid conditions by Deposition method where the sample remains wet through out the experiment.

Only thiol treated DNA was expected to be there on surface of microelectrodes but other contaminants were also present. These prevent the access of DNA to the gold surface. These impurities may be protein by nature as Ligase enzyme was added.

Fig 1: Immobilization on Gold Surface

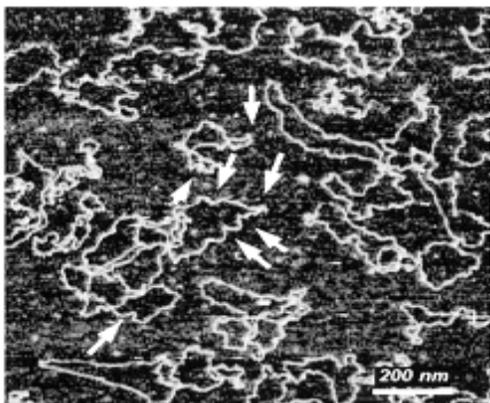
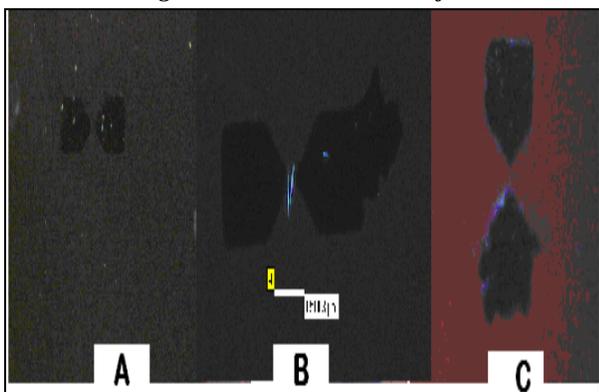


Fig 2: DNA on electrode surface



A-Without Immobilization, B,C-Immobilized DNA

#### Conclusion:

From the methods used for immobilization it can be conferred that in the dry conditions the receding meniscus results in the aggregation of DNA along with the impurities and formation of Dendrite structures. When humid conditions are maintained more of the DNA is on the electrodes. Sometimes it appears in between the Microelectrode surface. The current work can also be extended to test the Electrical conductivity of  $\lambda$ -DNA using DNA covalently bonded to gold electrodes. If the results are positive this could be an important mile stone in the development of nano-devices.

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