# Novel Alternative, Multistranded, Plasmid, and Helical Transitional DNA and RNA Microarrays: The Next Generation of Nucleic Acid Microarrays

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## ABSTRACT

A DNA microarray is considered to be a multiplex research tool used for gene expression studies. It is a two-dimensional array on a substrate surface that permits for the assaying of vast amounts of biomaterials. The novel Alternative, Multistranded, Plasmid, and Helical Transitional DNA and RNA Microarrays have been developed that allows for the immobilization of intact, nondenatured, double-stranded (ds-) DNA, ds-RNA, and alternative and multistranded nucleic acids. It goes beyond the limitations of conventional DNA microarrays. It also allows for the study of transitional changes that occur in the structure of DNA and RNA. Alternative types of DNA, RNA and multistranded nucleic acids are immobilized by a variety of different surface chemistries onto a novel substrate surface. This technology represents the next generation of DNA and RNA microarrays, which will aid in the characterization of nucleic acid structure and function, and accelerate the discovery of drugs that bind to exotic nucleic acids and transitions in DNA and RNA. These novel nucleic acid microarrays can be used to improve the study of DNA and RNA structure, gene expression, drug discovery and development, and treatment of various diseases.

***Keywords:*** Multistranded DNA and RNA, alternative nucleic acids, Z-DNA, DNA microarrays, quadruplex and triplex DNA.

## INTRODUCTION

Advances in molecular biology and informational methods have resulted in the discovery of many genes and genetic factors. Growth in DNA, RNA and protein synthesis and sequencing has improved the advancement of gene, protein and DNA-protein complex discovery. Pharmacogenomics is playing a significant role in the genomics revolution. This new period of growth in genomics, proteomics and epigenetics is accountable for a key transformation in the way we study DNA, RNA, proteins, nucleic acid-ligand, biochemical processes and drug development.

The structure–function relationship of DNA, RNA and protein is critical in order to understand how nucleic acids work. Deciphering the mechanisms of how nucleic acids, nucleic acid-binding proteins and drugs control gene expression is very significant for research scientists. Nucleic acids are no longer thought of as one dimensional molecules. Researchers look upon them as exceedingly energetic molecules that can undergo major structural changes. One such example involves the more conventional right-handed double-stranded (ds-) B-DNA's transition to the alternative left-handed ds-Z-DNA [1-6]. Left-handed Z-DNA is a alternative form of the right-handed ds-B-DNA in which the double helix unwinds to the left in a zig-zag pattern. Gene expression is controlled by DNA base pairs, nucleic acid-binding proteins and by structural changes in DNA [1-5]. The structural polymorphisms of many nucleic acids are reliant on the immediate cellular conditions, viz., proteins, ionic strength and polyamines [1-5]. Local areas of DNA are sufficiently flexible to allow for structural changes that make the most of stacking while reducing adverse steric molecular interactions. Neighboring DNA supercoiling effects fashioned by the transcription process can maintain alternative DNA structural conformations. An example of this occurs with Z-DNA [5]. DNA and RNA can also adopt other alternative structures, such as parallel-stranded DNA, bulged DNA, Holliday intermediates, anisomorphic DNA, slipped-stranded DNA, mispaired DNA, DNA-RNA hybrids, B-DNA/Z-DNA junctions, cruciform structures, catenanes, triplex DNA, quadruplex DNA, and pentaplex DNA and hairpin nucleic acids (Figure 1-4)[1-5]. As a result, the study and experimentation of nucleic acid base pairs, distribution and content alone does not provide investigators with enough knowledge concerning factors that control gene expression. To more fully study the expression of human and non-human genes, and determine new DNA and RNA target sites for new classes of drugs,

scientists should focus more on multistranded DNA and RNA, the characterization of alternative ds- and single-stranded (ss-) DNA and RNA structures, and potential sequences in which nucleic acids undergo structural transitions (Figures 1-4).

## Novel Alternative, Multistranded, Plasmid, and Helical Transitional DNA and RNA Microarrays

DNA microarrays are a multiplex technology used by molecular biologists to characterize genes. Conventional DNA microarray technology is a powerful tool used to analyze genomes and study the patterns of gene expression. This technology has many applications in a variety of different disciplines, ranging from human to non-human and plant life.

We want to introduce a potent new method that goes beyond customary DNA microarrays and signifies the next generation of DNA and RNA, namely, the new Alternative, Multistranded, Plasmid, and Helical Transitional DNA and RNA Microarrays (Figures 1-4) [1-2]. These microarrays anchor unaltered, intact, nondenatured (i.e., low- and/or high-molecular-weight) nucleic acids using diverse surface chemistries onto a novel substrate surface. The microarray permits for the characterization of DNA and RNA structural transitions, and testing drugs for their ability to combine with nucleic acids under diverse conditions. The intact, unbroken nucleic acids can be placed onto the substrate surface directly (i.e., presynthesized) or slowly synthesized. Some of the nucleic acids include triplex DNA, quadruplex DNA, pentaplex DNA, ds-DNA, ds-RNA, B-DNA, A-DNA, C-DNA, Z-DNA, A-RNA, Z-RNA, cruciform structures, DNA-RNA hybrids, parallel-stranded DNA, peptide nucleic acid (PNA), locked nucleic acid (LNA), and DNA, RNA, peptide aptamers [1,2,5]. This original advance will allow for the development of many new types of microarrays (e.g., Z-DNA, triplex DNA, quadruplex DNA, and Z-RNA). Most notably, unlike conventional DNA microarrays that immobilize ss-DNA, our microarrays immobilize intact, nondenatured DNA and RNA molecules.

Employing our novel microarrays, nucleic acids can be immobilized by different mechanisms, viz., free-floating partially immobilized, semi-free-floating partially immobilized and fully immobilized nucleic acids (Figure 3). These microarrays can be anchored by an assortment of noncovalent attachments, such as, nylon, and nitrocellulose [1,2,5]. Covalent immobilization can be engaged to produce better anchoring of the DNA or RNA molecules, such as, thiol-modified DNA, amino-terminal DNA, disulfide-modified DNA, and phosphorylated DNA, just to name a few. These exotic nucleic acids can also be covalently grafted to unmodified glass surfaces. Manufacturing parameters of multistranded and alternative microarrays depend on many factors, such as length and width of nucleic acids, structural transitions of nucleic acids (e.g., right-handed B-DNA to left-handed Z-DNA), base pair sequences, specific and nonspecific binding of nucleic acids to the solid support, *in situ* synthesis or mechanical deposition of prefabricated nucleic acids, ink jetting or spotting of DNA or RNA, and various user end protocols (e.g., surface blocking, hybridization protocols, drug-DNA interactions, drug-nucleic acid-protein interactions) [1-2].

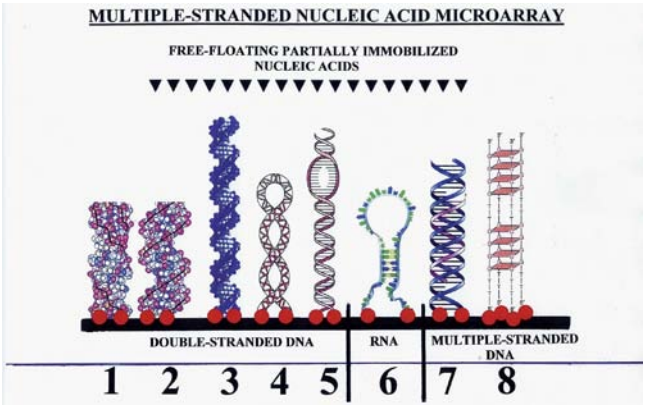


Figure 1: Multiple-stranded Nucleic Acid Microarrays. Eight different types of nucleic acid molecules are immobilized to the substrate surface (i.e., free-floating partially immobilized nucleic acids). This figure reveals three categories of nucleic acids, namely, ds-DNA, RNA and multiple-stranded DNAs.

The DNAs and RNAs employed in the next generation of nucleic acid microarrays can position DNAs from 1- to 90-mers in length. The larger (i.e., longer and/or especially wider) the molecules, the fewer spotted nucleic acids per slide (i.e., low-density multistranded DNA or RNA microarrays: spotting diameter greater than 75µm). B-DNA and Z-DNA permit for more spotted DNA molecules per slide [e.g., high-density microarrays: spotting diameter smaller than 75µm (1 x 3 inch slide increases spotting numbers)]. The distance between spotting areas of nucleic acids can range from 45 to 155µm.

The DNAs and RNAs can be anchored at one or both of their 3'- or 5'-helical ends, namely, free-floating partially immobilized or semi-free-floating partially immobilized molecules (Figure 3). Free-floating partially anchored, or semi-free-floating partially immobilized DNA or RNA can be placed onto the substrate surface (e.g., amino-terminated DNA grafted onto an aldehyde surface: covalent attachment), by conjugating the end of one, two, three, four, five, or more of their strands with biotin, or other chemical factors, and functionalizing the substrate surface with avidin, or other factors. These immobilized molecules can now move freely: rotate and bend while being noncovalently or covalently anchored to the microarray

surface (Figures 1-3). Using more strands of the helix to secure the nucleic acids results in better immobilization to the solid surface.

An additional anchoring technique can be employed: nucleic acids can be positioned onto the substrate surface as free-floating partially immobilized or semi-free-floating partially immobilized molecules, with a completely dissimilar anchoring method, namely, a short piece of fully anchored DNA [1]. The fully anchored nucleic acid relies on a very short piece of DNA lying completely on its side, therefore, reacting with the poly-L-lysine coated microarray surface. The anchored DNA can be exposed to many different pretreatments, such UV light, or other factors, and thereby covalently linked to the solid support. The anchored nucleic acids can range from approximately 1- to 20-mers [1].

Another anchoring mechanism can be used which permits for a different approach towards studying nucleic acids and developing pharmaceuticals. The full immobilized DNAs or RNAs are placed entirely on their sides (e.g., nonfree-floating), for example, the negative charges of the ds-B-DNA sugar-phosphate backbone intermingle with the positive charges of the functionalized substrate surface (e.g., nylon sheets, nitrocellulose papers, poly-L-lysine coated glass or polystyrene plates) (Figure 1-4).

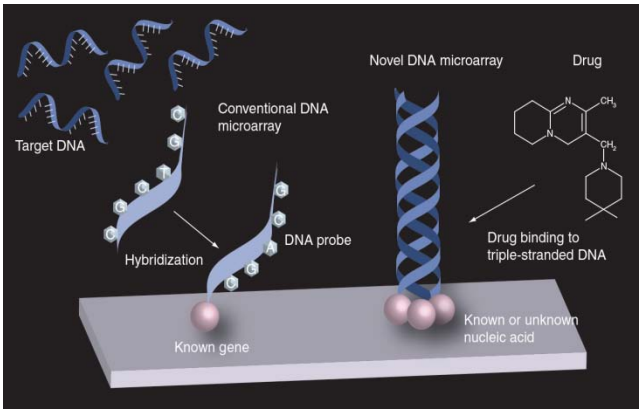


Figure 2 : Comparison Between Conventional DNA (Hybridization) Microarrays and the Novel Microarrays.

Unbroken, closed circular ds-DNA plasmids (with or without genes placed inside of them) can as well be placed on the microarray surface by using 0.2 to 10% of the plasmid molecule. This piece is anchored to the microarray surface by a novel procedure involving a minute sequence (e.g., approximately 15-mers or more) of triplex DNA (e.g., triple-helix specific stabilizing compound: benzoquinoxaline) and/or quadruplex DNA molecules.

Our group has also created a proprietary microarray solid support surface (substrate surface). It drastically improves the immobilization of DNAs and RNA molecules [1,2,5]. The microarray is fabricated in a way to both increase surface area and enhance binding of the DNA and RNA molecules to the microarray. Treating the glass or plastic surface and/or exposing it to different intensities of UV light, or other factors, under highly specific conditions, increases the immobilization of DNAs and RNAs. This happens in spite of the orientation of the nucleic acid molecules. This novel substrate surface increases the competence of the microarray, which allows for extended protocols without DNA and RNA molecules falling off the solid support. Partial genes, completes genes, native nucleic acids, synthetic polynucleotides, nucleic acid-ligand complexes, nucleic acid-protein complexes or nucleic acid-protein-ligand complexes can be anchored to the microarray. These different alternative nucleic acid molecules can then be extensively characterized under extremely precise environmental conditions. Keeping the free-floating partially immobilized nucleic acids from sticking to the unfunctionalized or functionalized solid support (e.g., poly-L-lysine, aminosilanated) directly depends on the specific surface chemistry (e.g., glass or plastic) [1,2,5].

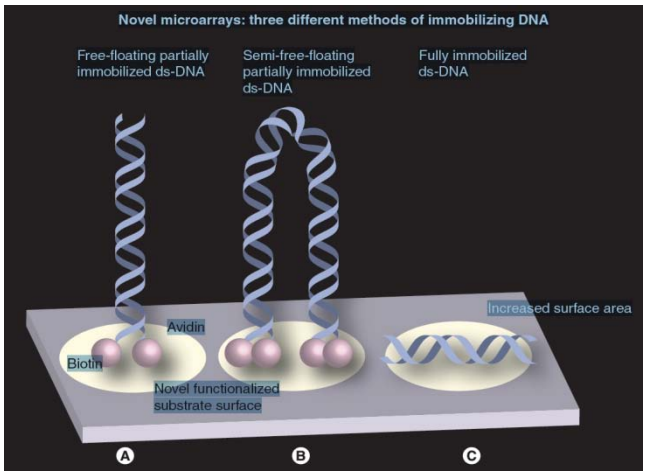


Figure 3 : Novel Microarrays: Three Different Methods for Immobilizing DNA.

## CONCLUSION

The novel Alternative, Multistranded, Plasmid, and Helical Transitional DNA and RNA Microarrays [1,2,5] will allow for the study of physical changes in nucleic acids, the mapping of gene-regulatory networks, DNA and RNA sequences, RNA (DNA) interference studies, comparative genomics, hybridization conditions, methylation analysis, RNA editing, DNA (RNA or peptide) aptamer studies, gene expression, translation modifiers [e.g., microRNAs (mi-RNA)], DNA (RNA) drug interactions and ss- or multistranded nucleic acid-protein-drug interactions, and the effects of SNPs on DNA

structure. The next generation microarrays will permit for a more whole mapping of gene-regulatory networks by focusing on genetic elements that have one or more segments of multistranded nucleic acids, alternative nucleic acids and regions that undergo helical transitions.

The alternative, multistranded and helical transitional DNA and RNA microarrays hold immense promise for individualizing medical therapy for a variety of human and non-human diseases. This technological approach represents a major development in tools and data analysis methods, which will translate into more dynamic research approaches. Our novel technology will eventually allow researchers to perform highly specific genome-wide scanning for conventional and alternative nucleic acid structure and polymorphisms that are connected to disease risk and/or drug response. It will provide a platform for experimentation with other non-conventional molecules such as PNA, LNA, RNAi and miRNAs. Ultimately, this will result in the selection of the best drug therapies and appropriate dosages based on the patient's genome, and not therapies based on drugs given to the general population.

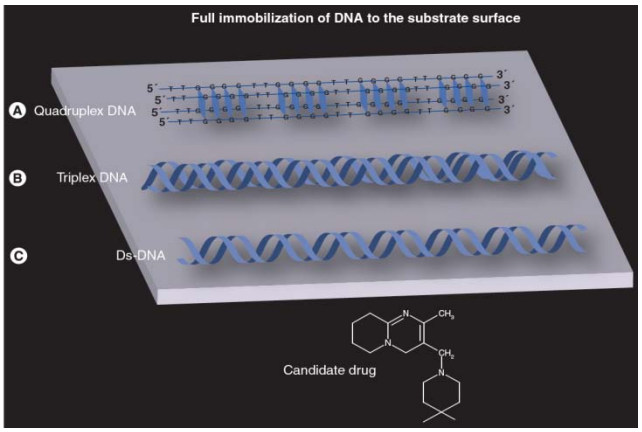


Figure 4: Full Immobilization of Conventional (e.g., ds-DNA), and Alternative DNA Molecules (e.g., Quadruplex DNA and Triplex DNA). This figure shows how the novel substrate surface can be used for experimentation with a drug under different environmental conditions.

We believe that these new microarrays represent an innovative molecular biological technique. These next generation DNA and RNA microarrays allow high-throughput drug screening of huge numbers of alternative and multistranded DNA and RNA structures. The novel Alternative, Multistranded, Plasmid, and Helical Transitional DNA and RNA Microarrays represents the next generation of nucleic acid nanotechnology. These microarrays will permit investigators to make the most of all genomic, proteomic and pharmaceutical entities. By going beyond conventional ds-DNA-based microarrays and characterizing exotic structures such as Z-DNA, triplex DNA and quadruplex DNA, scientists can now target exotic nucleic acids that regulate genetic factors [1,2,5]. Researchers using this microarray can take a new approach towards studying the underlying genetic factors that regulate human diseases, such as cancer, diabetes, infectious diseases and AIDS.

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